Published in "Neuron 74(2): 246–260 , 2012"
which should be cited to refer to this work.

Timing to Perfection: The Biology of Central and Peripheral Circadian Clocks

Urs Albrecht[1,*]
[1]Department of Biology, Unit of Biochemistry, University of Fribourg, Chemin du Musée 5, CH-1700 Fribourg, Switzerland
*Correspondence: urs.albrecht@unifr.ch

The mammalian circadian system, which is comprised of multiple cellular clocks located in the organs and tissues, orchestrates their regulation in a hierarchical manner throughout the 24 hr of the day. At the top of the hierarchy are the suprachiasmatic nuclei, which synchronize subordinate organ and tissue clocks using electrical, endocrine, and metabolic signaling pathways that impact the molecular mechanisms of cellular clocks. The interplay between the central neural and peripheral tissue clocks is not fully understood and remains a major challenge in determining how neurological and metabolic homeostasis is achieved across the sleep-wake cycle. Disturbances in the communication between the plethora of body clocks can desynchronize the circadian system, which is believed to contribute to the development of diseases such as obesity and neuropsychiatric disorders. This review will highlight the relationship between clocks and metabolism, and describe how cues such as light, food, and reward mediate entrainment of the circadian system.

Introduction

Circadian clocks generate self-sustaining, cell-autonomous oscillations with a time period of approximately 24 hr (circa diem, approximately one day). Such oscillations are thought to have evolved in response to the daily light/dark rhythms, which are associated with food availability; it is believed that the internalization of the 24 hr rhythms of light and dark made it advantageous to the organism to predict daily recurring events even when conditions remained constant (e.g., constant darkness). Hence, organisms that are able to take advantage of the daily variations in light by staying in tune with the environmental light/dark cycle outgrow organisms that cannot; this growth difference has been conclusively shown in cyanobacteria (Ouyang et al., 1998).

In multicellular organisms such as mammals, organs form a hierarchically structured circadian system, with the brain and the liver serving an important coordinating function. This system has been optimized for adaptation and survival (Figure 1A). Because individual cells contain circadian clocks (Balsalobre et al., 1998), these individual oscillators need to be synchronized within the tissue. In turn, tissues are kept in a stable phase-relationship with each other to render clock information useful for the entire multicellular organism. To build such a coherent circadian system, cellular clocks must be able to respond to a stimulus (e.g., input from other cells), integrate the phase information regarding when the stimulus occurred into their molecular intracellular clock mechanism, and transfer clock information to other cells (output) (Figure 1B). This organization is schematically repeated at the systems level where signals, such as light detected by the retina (input, external environment), are transmitted to the suprachiasmatic nuclei (SCN) (clock). There, light signals are integrated to adjust the information about time (see below). Subsequently, this elicits a change in the onset of certain behaviors and tissue activities (output) (Figure 1B). Conversely, tissue signals representing the internal environment may return information to the clock (Figure 1B, purple arrows). Thus, the hallmarks of organization in a circadian timing system are the perception of the environmental input, integration of time-related information into the autonomous circadian clock device, transmission of adjusted timing information to metabolic and physiological processes, and subsequent feedback of tissue information (Eskin, 1979). The circadian system must continuously adapt to and synchronize with the environment and the body's internal signals in order to organize individual cellular clocks and combine tissue subnetworks into a coherent functional network that regulates behavior and physiology.

In the following sections, I will review advances made in understanding the central and peripheral components of this clockwork mechanism, and discuss critical factors from the environment (light and food) that serve as signals to synchronize the circadian system. Particular attention will be paid to the interplay between the circadian clock and metabolism for internal clock synchronization. Finally, I will discuss the implications of proper clock synchronization for human health and disease.

Molecular Clockwork

The molecular mechanisms that drive circadian oscillations in mammalian cells have been revealed during the last decade. The two main processes that form the foundation of these rhythms are the oscillating posttranslational modifications of proteins (e.g., phosphorylation) and the transcriptional-translational feedback loop (TTL) (Figure 2A). The TTL comprises of a positive and a negative limb that are interconnected (the blue and purple lines in Figure 2A). In the positive limb of the mammalian system, the transcriptional activator protein BMAL (isoforms 1 and 2) (Hogenesch et al., 1998; Shi et al., 2010) dimerizes with CLOCK (or NPAS2 in brain tissue) (Gekakis et al., 1998; Reick et al., 2001), and this heterodimer binds to the E-box promoter

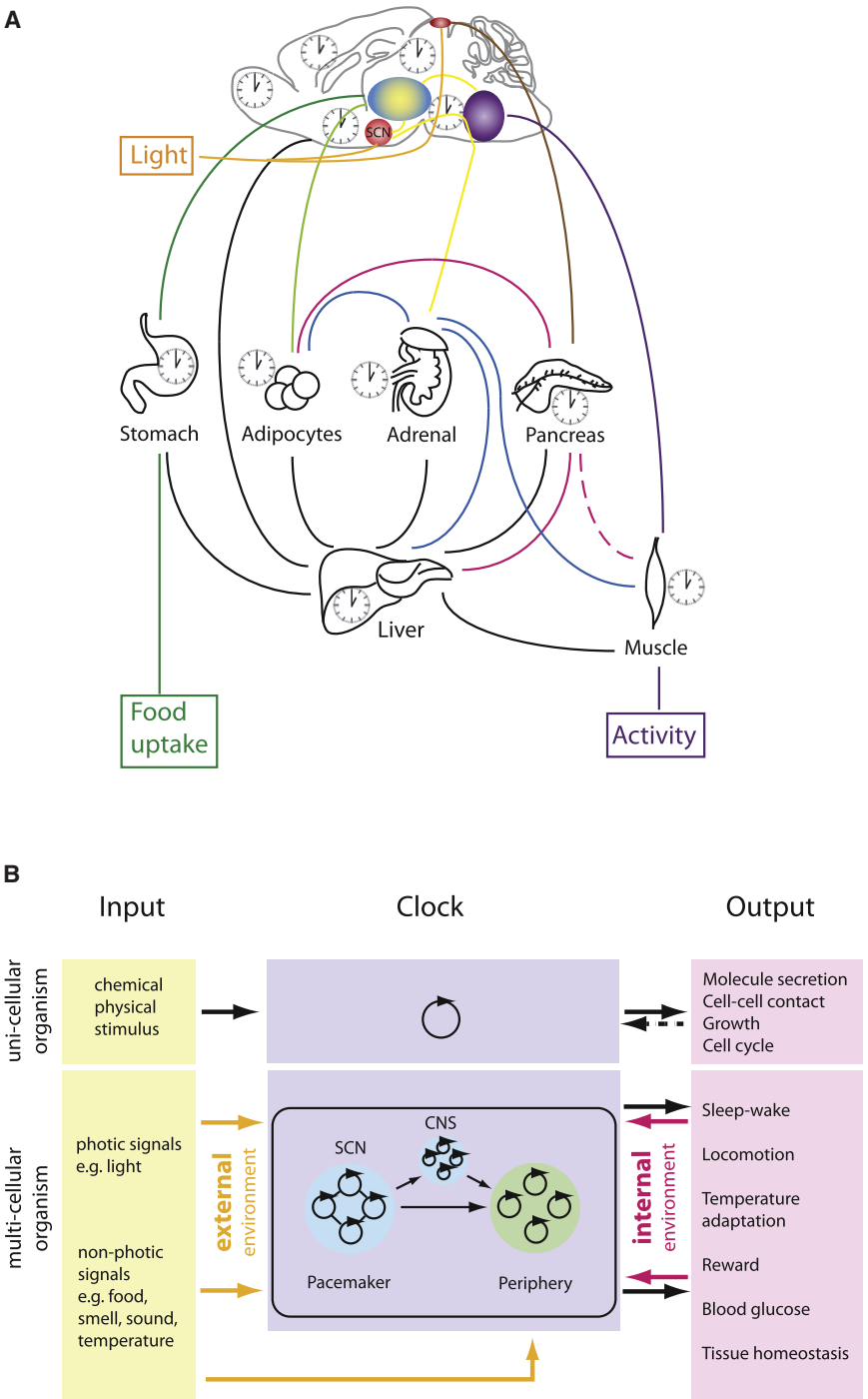

Figure 1. Organization of the Circadian System

(A) Circadian clocks are found in all cells of various organs. The master clock is located in the SCN (red circle) of the brain and synchronizes the other central clocks that are related to metabolic and reward integration (yellow-blue shaded oval) and motor coordination (purple oval) via direct and indirect pathways (yellow lines). The SCN and the pineal clock (red oval) are sensitive to light (orange lines). Hormonal signaling between organs is shown: melatonin (brown line); ghrelin (dark green); leptin (light green); insulin/glucagon (pink, hatched line = insulin only) and adrenaline (blue). Metabolic signaling between organs is shown: carbohydrates, fatty acids, and amino acids (black). Purple: neuronal connections between the brain, spinal cord and muscles.

(B) Subdivision of the circadian system: input to the clock, clock mechanism, and clock output. This division can be made at the cellular level (top) as well as at the systemic level (bottom). Synchronization between cellular clocks becomes an issue in multicellular systems, such as in organs and in entire organisms (see A).

elements (CACGTG) present in clock and clock-controlled genes (CCGs). The clock genes *Period* (isoforms *Per1* and *Per2*) (Zheng et al., 2001) and *Cryptochrome* (isoforms *Cry1* and *Cry2*) (van der Horst et al., 1999), when activated in this manner, constitute the negative portion of the TTL. The mRNA of these genes is translated in the cytoplasm, and the resulting proteins form heterodimers that eventually enter the nucleus to inhibit transcription by binding to the BMAL/CLOCK (or NPAS2) complex (Kume et al., 1999). The PER/CRY multimers recruit a PSF/Sin3-HDAC complex, shutting down transcription by deacetylating histones 3 and 4 (Duong et al., 2011). Other histone-modifying enzymes, such as JARID1a, also seem to influence circadian transcription (DiTacchio et al., 2011). The positive and negative limbs are connected by nuclear receptors from the REV-ERB and ROR families. These receptors are transcriptionally regulated by the positive limb and activate (ROR) or inhibit (REV-ERB) transcription of the *Bmal* (Preitner et al., 2002; Sato et al., 2004, Cho et al., 2012), *Npas2* (Crumbley et al., 2010), and *Clock* (Crumbley and Burris, 2011) genes (Figure 2, blue), thereby modulating their own activators. This process is fine tuned by the PER2 protein, which interacts with REV-ERBα (Schmutz et al., 2010) to synchronize the negative and positive limbs of the TTL (Figure 2A, purple arrow).

The nicotinamide phosphoribosyltransferase (NAMPT) gene, a CCG that feeds back on the clock mechanism, codes for the rate-limiting enzyme for adenine dinucleotide (NAD^+) synthesis in the mammalian salvage pathway of nicotinamide (Figure 2A, brown arrows). NAD^+ functions as a metabolic oscillator and regulates the core clock machinery via SIRT1 (Nakahata et al., 2009; Ramsey et al., 2009), which is a histone deacetylase, to modulate transcriptional activity of the clock (Asher et al., 2008; Nakahata et al., 2008). Hence, metabolic processes affecting levels of NAD^+, representing the internal environment, feed back on the

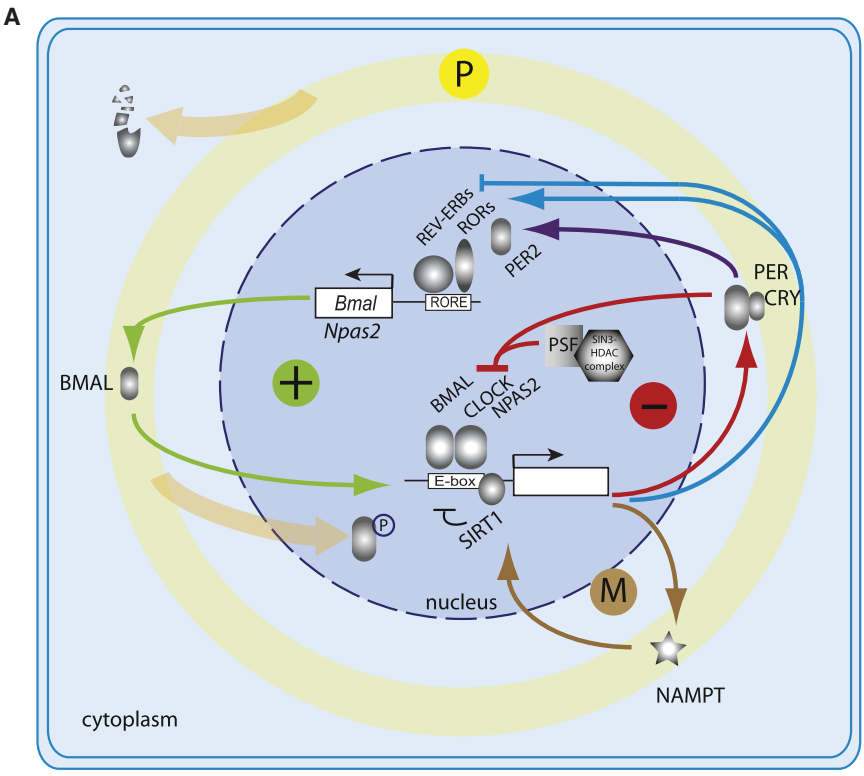

Figure 2. Molecular Circadian Clock Mechanisms in a Cell

(A) The clock mechanism consists of two main parts: (1) a transcriptional-translational feedback loop (TTL) consisting of a positive (green +) and a negative (red –) limb and (2) oscillating post-translational modification of gene products in the TTL (yellow circle marked with P), which regulate degradation and/or nuclear localization of these proteins (orange arrows).

The positive and negative limbs are intertwined via clock protein-driven nuclear receptors (blue lines) and their interactions with PER2, a component of the negative limb (purple arrow). A metabolic oscillator (brown M) is driven by the TTL and feeds back on it via SIRT1 (for details, see text).

(B) Promoter elements in clock controlled genes (CCGs). The CCGs are regulated either directly or indirectly. (1) Direct regulation via BMAL/CLOCK binding at E-boxes or REV-ERB/ROR binding at RORE-elements and (2) Indirect regulation via binding of clock-regulated PAR-ZIP factors (e.g., DBP) on D-elements, or via protein-protein interactions between PER2 and nuclear receptors (hatched line) at nuclear receptor elements (NREs) such as ROREs.

clock mechanism, illustrating that the elements of clock output can affect the clock itself (Figure 1B, purple arrows).

The NAMPT promoter is modulated by clock components via E-box elements. However, not all CCGs are driven in a circadian fashion by this mechanism. Promoter analysis and systems-biological approaches have revealed that nuclear receptor elements (NREs) are an additional transcriptional module that underlies mammalian circadian clocks (Ueda et al., 2002) (Figure 2B). The binding of nuclear receptors (e.g., REV-ERBα, PPARα, and Glucocorticoid receptor) to such promoter elements is modulated by PER2 (Schmutz et al., 2010) or Cryptochromes (CRY) (Lamia et al., 2011) (Figure 2B, hatched line). Furthermore, D-box elements have been recognized as the third important factor that regulates circadian transcription (Ueda et al., 2005). These elements are occupied by PAR-Zip transcription factors (e.g., Dbp) that are themselves under the control of E-box-mediated transcription, and therefore, they modulate CCGs indirectly in a circadian manner (Lavery et al., 1999) (Figure 2B).

Transcriptional regulation is not the sole mechanism responsible for the generation of circadian oscillations. In mammalian cells, circadian oscillations in gene expression are largely unperturbed by cell division (Nagoshi et al., 2004), and mammalian clocks are resistant to large changes in transcription rate (Dibner et al., 2009). Posttranslational events that modulate protein half-life and subcellular localization appear to contribute significantly to circadian oscillations (Figure 2A, orange arrows). Various kinases and phosphatases regulate the speed, precision, and function of the circadian clock (reviewed in Vanselow and Kramer, 2010). Recent studies have implicated a number of microRNAs (miRNAs) (Cheng et al., 2007) and several RNA-binding protein complexes in the regulation of circadian polyadenylation, splicing, RNA stabilization, and degradation (reviewed in Pegoraro and Tauber, 2008). Thus, the regulation of circadian rhythms in a cell is controlled by multiple processes involving the expression of genes, from DNA to RNA to protein.

SCN, Retinal Input, and Other Clocks in the Brain

In mammals, the circadian timing system is composed of virtually as many clocks as there are cells in the body. A significant question is how all these clocks are synchronized to one another and whether a primary pacemaker governing the multitude of

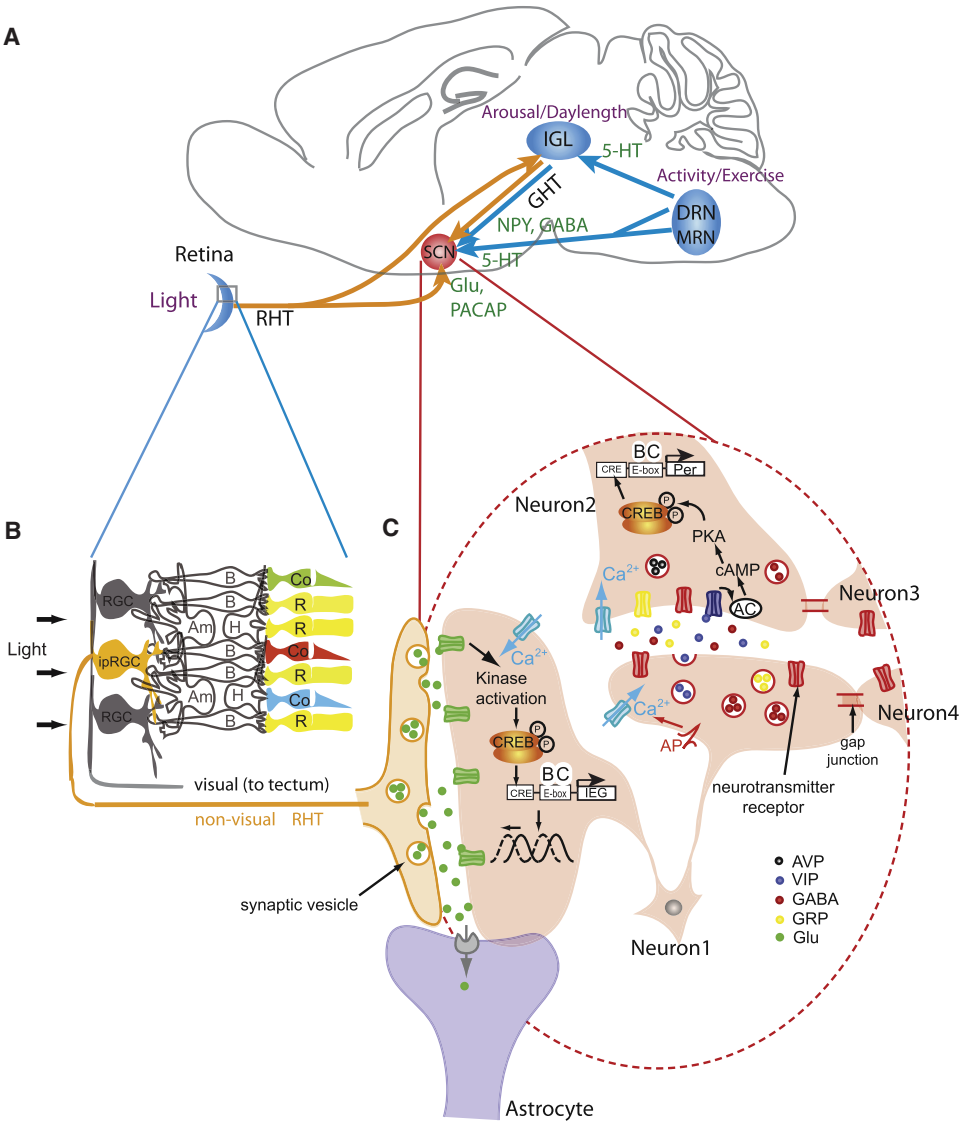

Figure 3. Schematic Representation of Light Input and Intercellular Signaling Mechanisms in the SCN

(A) Main afferent pathways to the SCN in the rat. Orange arrows: photic input; blue arrows: nonphotic input to the SCN; 5HT, serotonin; DRN, dorsal raphe nucleus; IGL, intergeniculate leaflet; GABA, gamma-aminobutyric acid; GHT, geniculohypothalamic tract; Glu, glutamate; MRN, median raphe nucleus; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating peptide; RHT, retinohypothalamic tract; SCN, suprachiasmatic nuclei.

(B) Schematic representation of the retina. Intrinsically photosensitive retinal ganglion cells (ipRGCs) project to the SCN via the retinohypothalamic tract (RHT) to transmit light information. Rods (R); cones (Co); horizontal cells (H); bipolar cells (B); amacrine cells (Am) and regular retinal ganglion cells (RGC) transmit information about color and shape to the tectum.

(C) Signal transduction pathways in the SCN. Light input to the SCN: orange RHT synapse releasing glutamate (Glu, green), leading to kinase activation and expression of immediate early genes (IEG) resulting in a phase shift of the circadian clock. Glutamate is eventually cleared from the synaptic cleft via glutamate transporters (gray) on astrocytes (light purple). Intercellular signaling: vasointestinal peptide (VIP, purple dots); arginine vasopressin (AVP, black dots); gamma-aminobutyric acid (GABA, red dots); gastrin-releasing peptide (GRP, yellow dots) and gap junctions are involved (for details see text). AC, adenylate cyclase; AP, action potential; cAMP, cyclic adenosine-mono-phosphate; CRE, cAMP response element; CREB, CRE binding protein; PKA, protein kinase A (modified from Dibner et al., 2010, and Welsh et al., 2010).

clocks exists. Ablation and transplantation experiments have revealed such a pacemaker in the hypothalamus. It is located in nuclei just above the optic chiasm and is hence termed the suprachiasmatic nuclei (SCN). The SCN are important for rhythmic hormone secretion and locomotor activity (Lehman et al., 1987) and being at the top of the hierarchical organization of the circadian timing system (Figures 1A and 3A). As such they serve as a central conductor orchestrating the other clocks and thus entraining the circadian system to the environmental light/dark cycle.

Light information is perceived primarily via intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina, which express the photopigment melanopsin. These cells send photic information directly to the SCN via the RHT (Figure 3B). The

monosynaptic RHT fibers terminate in the ventrolateral part of the SCN, directly onto neurons that express vasoactive intestinal polypeptide (VIP). Light stimulation of the retina during the subjective night leads to the release of the neurotransmitters glutamate (Glu) and pituitary adenylate cyclase-activating protein (PACAP) at the terminal synapses of the RHT, and the signal is then propagated to the SCN (Figures 3B and 3C) (reviewed in Ecker et al., 2010). This leads to the activation of several signaling pathways that evoke chromatin remodeling and the induction of immediate early genes and clock genes (reviewed in Golombek and Rosenstein, 2010). As a consequence, the circadian clock phase is changed, and this alteration can be readily observed (e.g., a change in the onset of wheel running activity in rodents, reviewed in Antle et al., 2009).

Retinal neurons of the RHT signal only to a small subset of SCN cells, which then transmit retinal information to their neighboring cells. This, together with the observation that expression of neuropeptides within the SCN is not homogeneous, showed that the SCN are a network of functionally and phenotypically differentiated cells (reviewed in Antle and Silver, 2005). These individual cellular oscillators are coupled to produce a consistent circadian oscillation within the SCN. The mechanisms involved include neurochemical signals such as vasoactive intestinal polypeptide (VIP), arginine vasopressin (AVP), GABA, ghrelin-releasing peptide (GRP), and gap junctions (Figure 3C) (reviewed in Welsh et al., 2010).

The SCN are not the only structure in the brain displaying daily oscillations. Nuclei in the thalamus and hypothalamus, amygdala, hippocampus, habenula, and the olfactory bulbs show such oscillations (reviewed in Guilding and Piggins, 2007). The most robust rhythms, beyond those observed in the SCN, are found in the olfactory bulbs and tissues that have neuroendocrine functions. These brain areas include the arcuate nucleus (ARC), the paraventricular nucleus (PVN), and the pituitary gland.

Studies in intact animals have documented that signals from the SCN can synchronize populations of weakly coupled or non-coupled cells in the brain, and neuronal projections between these different, non-SCN brain regions may assist in maintaining circadian rhythms via neuronal circuits (Colwell, 2011). These circuits are critical not only for keeping circadian oscillations constitutive but also for regulating physiology and behavior, such as the integration of metabolic information and reward-driven behaviors that occur within a 24 hr time period (see below).

Peripheral Clocks and Metabolism

Peripheral circadian clocks, such as those that are found in the liver, are influenced by the autonomic nervous system and by systemic cues including body temperature, hormone metabolites, and feeding/fasting cycles (see Figure 1). Although the SCN serves as the master synchronizer of the entire system, food intake can uncouple peripheral clocks from control by the SCN. Through changes in feeding schedule, the phase relationship between the central clock in the SCN and the clocks in the liver can be altered (Damiola et al., 2000), suggesting that changes in metabolism caused by alterations in feeding rhythm may affect the circadian system.

Genome-wide transcriptome profiling studies have provided support for the view that a tight connection exists between metabolism and the circadian system (reviewed in Duffield, 2003). According to these studies, about 15% of all genes display daily oscillations in their expression; a large fraction of these genes encode for important regulators of carbohydrate, lipid, and cholesterol metabolism as well as for regulators of detoxification mechanisms. Among the regulatory genes identified were transcription factors that serve as output regulators for the circadian clock. In the liver, these include transcription factors of the PAR bZip family such as DBP, TEF, and HLF (Gachon et al., 2006) that bind to D-elements (Figure 2), the PAR bZip-related repressor E4BP4 (Mitsui et al., 2001), the Krüppel-like factors KLF10 (Hirota et al., 2010a) and KLF15 (Jeyaraj et al., 2012), and nuclear receptors (Yang et al., 2006). All of these transcription factors identified are known to regulate genes involved in metabolism.

Systemic and Local Regulation of Metabolism

The timing of metabolism can be influenced by the circadian system via systemic cues emanating from the SCN or through local oscillators in peripheral tissues (Figure 1). Discrimination between these two routes has been made possible by comparing whole transcriptome analyses of mice with or without functional liver clocks (Kornmann et al., 2007). Six out of seven oscillating transcripts ceased to fluctuate when local circadian oscillators were arrested, indicating that they depend on local oscillators in liver cells as opposed to systemic signals from the SCN. These genes may be termed ''locally clock-controlled output genes'' (Asher and Schibler, 2011). The remaining single oscillating mRNA transcript continued to fluctuate in a daily manner with few changes in phase, amplitude, or magnitude. These systemically driven liver clock genes most likely include immediate early genes, which can convey rhythmic signals to core clock genes in hepatocytes and are consequently involved in the synchronization of liver clocks and genes directly involved in rhythmic liver physiology and metabolic functions (centrally clock controlled output genes, Asher and Schibler, 2011). Candidates for the synchronization of the various body clocks in mammals are heat-shock transcription factor 1 (HSF1) (Reinke et al., 2008) and serum response factor 1 (SRF1).

The signaling pathways involved in the phase entrainment of peripheral clocks are numerous and are just beginning to be unraveled. To distinguish between SCN-dependent and feeding-dependent regulators, the kinetics of feeding-induced phase adaptation have been studied. Because the reversal of feeding rhythms sends conflicting feeding messages and SCN signals to peripheral organs, the effects of feeding rhythms on phase adaptation in peripheral clocks have been studied either in the absence of SCN-dependent glucocorticoid signaling or of nutrient-dependent signaling. Ablation of glucocorticoid receptor (GR) in the liver and inversion of feeding time have revealed that GR and poly (ADP-ribose) polymerase 1 (PARP-1), respectively, participate in the phase resetting of liver clocks. While GR signaling is SCN dependent (Le Minh et al., 2001), PARP1 is a feeding-dependent regulator (Asher et al., 2010).

Nutrient Sensors and Circadian Clock Components

To establish communication between circadian clocks and metabolism, sensors affecting both systems exist as outlined

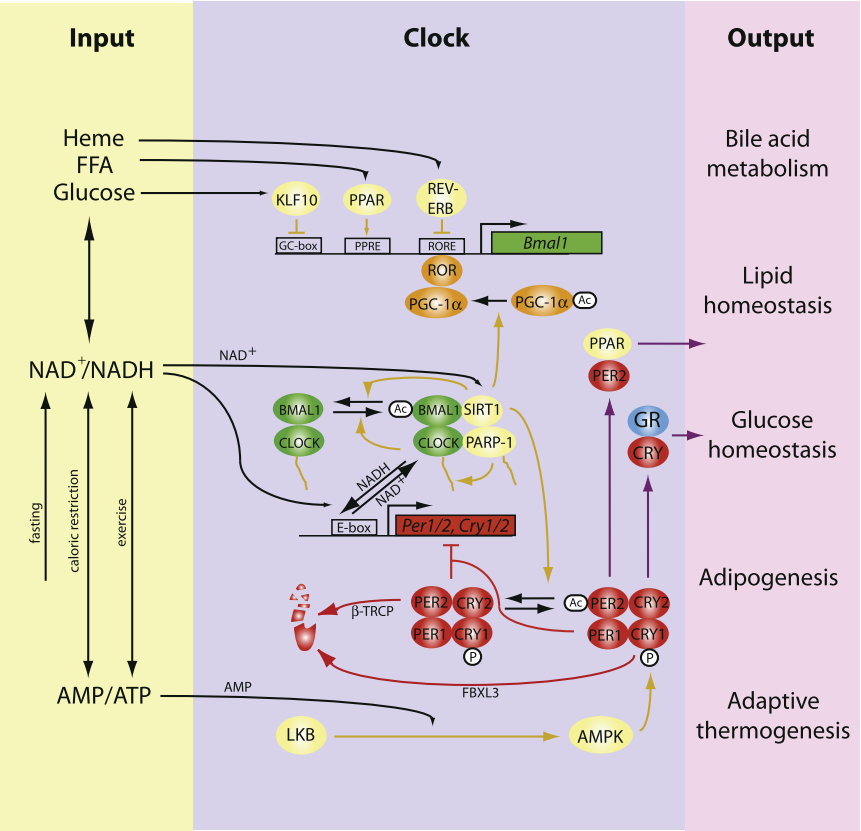

Figure 4. Metabolic Regulators and Circadian Clock Components

A schematic representation of the interactions between metabolic input signals (yellow box) and metabolic regulators (yellow, orange) with clock (blue box) components of the positive limb (green) and the negative limb (red) of the circadian clock mechanism in the liver. Metabolic regulators affected by input signals are represented in yellow. Krüppel-like factor 10 (KLF10), the PPARs and REV-ERBs regulate *Bmal1* expression by binding to the corresponding recognition sequences in the *Bmal1* promoter. Levels of NAD^+ influence SIRT1 activity, which deacetylates BMAL1, PER2, and PGC-1α proteins. This leads to PGC-1α-mediated coactivation of *Bmal1* expression together with ROR. Deacetylated PER2 is marked by β-TRCP (F-Box protein of ubiquitin ligase complexes) for degradation. Deacetylated BMAL1 (which can be acetylated by CLOCK) leads to reduced binding potential to E-boxes. The ratio of NAD^+/NADH modulates this binding. Oscillating PARP1 activity regulated by an unknown factor modulates CLOCK via ribosylation (orange tail). Liver kinase B (LKB) phosphorylates AMPK when the AMP/ATP ratio increases. Then, AMPK phosphorylates CRY1, thereby targeting it for degradation by FBXL3 (an F-box protein of ubiquitin ligase complexes). Interactions involving metabolic regulators are represented by yellow arrows. Metabolic output is indicated by the PER2/PPAR and CRY/glucocorticoid receptor (GR) complexes (Purple arrows). Ac = acetyl group and p = phosphate group (modified from Asher and Schibler, 2011).

below. These may include redox sensors, AMP/ATP ratio sensors, glucose sensors, and fatty acid sensors (Figure 4).

Redox Sensors

The first evidence for an involvement of redox state in circadian clock regulation came from biochemical experiments that revealed the sensitivity of CLOCK/BMAL1 and NPAS2/BMAL1 to the $NAD(P)^+$/NAD(P)H ratio when binding to their cognate E-box sequence (Rutter et al., 2001). Whereas the reduced forms of NADH and NADPH stimulate binding, the oxidized forms NAD^+ and $NADP^+$ strongly inhibit binding. Because NAMPT (the enzyme driving the NAD^+ salvage pathway) is transcriptionally regulated by the circadian clock, NAD^+ levels oscillate daily in the cytosol and most likely in the nucleus as well (Nakahata et al., 2009; Ramsey et al., 2009). Disruption of NAD^+ oscillation by mutations to NAD^+ hydrolase CD38 alters behavioral and metabolic circadian rhythms (Sahar et al., 2011). Specifically, CD38-deficient mice display shortened circadian periodicity and alterations in plasma levels of amino acids. These data illustrate the importance of oscillating NAD^+ levels and highlight its importance in amino acid regulation. Oscillating NAD^+ levels also have indirect involvement in the regulation of brain function because several amino acids, such as tryptophan, tyrosine, glutamate, aspartate, glycine, and GABA, are either precursors of neurotransmitters or themselves neurotransmitters. Although NAD^+ is modulated by many additional processes (e.g., glycolysis, fatty acid synthesis), its regulation via NAMPT reinforces coupling between the circadian clock mechanism and NAD^+-dependent metabolic pathways.

In addition to the BMAL1/CLOCK and BMAL1/NPAS2 heterodimers that can serve as sensors for the $NAD(P)^+$/NAD(P)H ratio, the NAD^+-dependent enzymes SIRT1 and PARP-1 may also link this metabolic ratio to the circadian clock. Levels of the enzyme SIRT1, which deacetylates histones and several transcription factors (Blander and Guarente, 2004), fluctuate throughout the day (Asher et al., 2008), and its activity may change as well (Nakahata et al., 2008). This enzyme physically interacts with BMAL1/CLOCK heterodimers, leading to a rhythmic deacetylation of BMAL1, histone H3 (Nakahata et al., 2008), and PER2 (Asher et al., 2008). As a result, the stability and/or activity of these proteins may be affected and lead to changes in circadian gene expression. Interestingly, SIRT1 also affects the activity of other transcription factors such as PPARα (Purushotham et al., 2009) and coactivator PGC-1α (Rodgers et al., 2005), highlighting another avenue for the modulation of circadian gene expression and metabolism in the liver. Specifically, PGC-1α appears to be tightly linked to the circadian clock mechanism because it is expressed in a circadian fashion and serves as a coactivator of ROR (Liu et al., 2007), an activator of several clock components (see above and Figures 2 and 4). Furthermore, PGC-1α is a coactivator of FOXO1, which is part of a fasting-inducible switch that modulates gluconeogenesis (Liu et al., 2008). These relationships illustrate how circadian mechanisms and energy homeostasis could be related. Another

potential NAD^+ sensor is PARP-1 (Asher et al., 2010), a feeding-dependent factor implicated in the phase entrainment of peripheral oscillators. Illustrating a possible way to orchestrate feeding-induced phase changes and glucose homeostasis, PARP1 acts by binding to FOXO1 and attenuating the transactivation potential of the latter (Sakamaki et al., 2009).

Nuclear Receptors as Sterol, Fatty Acid, and Glucocorticoid Sensors

Several organic molecules serve as ligands for nuclear receptors, which regulate specific genes in response to ligand binding. Therefore, these receptors control the development, homeostasis, and metabolism of an organism. Of the 49 nuclear receptors, 20 have been reported to display a circadian pattern of mRNA expression in the liver, 19 in white adipose tissue, 18 in brown adipose tissue, and seven in muscle (Yang et al., 2006). The receptors that display these circadian patterns include various isoforms of PPAR, REV-ERB, ROR, and TR. Some of these receptors, such as the REV-ERBs and the RORs, are directly involved in the modulation of the core clock circuitry (Figure 2) and may interact with clock components including PER2 (Schmutz et al., 2010) and CRY (Lamia et al., 2011). Other nuclear receptors, including LXR and FXR, can either stimulate or repress genes that produce molecular ligands; one example is the regulation of *Cyp7a1*. This gene encodes for the rate-limiting enzyme that converts cholesterol to bile acids (Peet et al., 1998), possibly affecting the intracellular levels of sterol compounds that suppress the transactivational activities of the core clock factors RORα and RORγ in the liver.

Fatty acids and their intermediates are natural ligands for PPARs. PPARs regulate adipocytes and insulin sensitivity (PPARγ), modulate the fatty acid oxidation system in mitochondria (PPARα), and regulate cell proliferation, differentiation, and migration in wound healing and inflammatory processes (PPARδ). The isoforms PPARα and PPARγ have been shown to interact (directly or indirectly) with PER2 (Grimaldi et al., 2010; Schmutz et al., 2010), leading to a time-of-day-dependent modulation of lipid metabolism (Figure 4) (Grimaldi et al., 2010). In addition, PER3 appears to form a complex with PPARγ, leading to reduced transactivation potential of this nuclear receptor. Accordingly, an increase in adipose tissue and a decrease in muscle tissue were observed in *Per3*-deficient mice (Costa et al., 2011). Interestingly, PER2 appears to regulate gamma interferon production in natural killer cells (Liu et al., 2006), pointing to a potential modulatory function of PER2 for PPARδ.

Regulation of glucose homeostasis involves glucocorticoids and its receptor. A recent study reported that the clock components of the cryptochrome (*Cry*) family interact with GR and modulate glucose homeostasis (Figure 4) (Lamia et al., 2011). This interaction reduces GR activation potential for the expression of the phosphoenolpyruvate caboxykinase 1 gene (*Pck1*)—a gene that encodes the rate-limiting enzyme in gluconeogenesis (PEPCK). Accordingly, *Cry*-deficient cells increased *Pck1* expression in response to dexamethason (a synthetic glucocorticoid). In contrast the NF-κB signaling pathway, through which glucocorticoids modulate inflammation, was not affected. This indicates a separation of CRY function in the gluconeogenic and inflammatory pathways of glucocorticoid action (Lamia et al., 2011). Therefore, modulation of CRY levels may be a potential therapeutic strategy to reduce the side-effects of glucocorticoids on metabolism (i.e., hyperglycemia and diabetes) during anti-inflammatory treatment.

Glucose and AMP/ATP Sensors

Application of high glucose concentrations to fibroblast cell cultures leads to acute transcriptional repression of the *Per1*, *Per2*, and *Bmal1* genes, thereby synchronizing fibroblast clocks (Hirota et al., 2002). This is reminiscent of glucocorticoid or glucocorticoid analog synchronization of cell cultures (Balsalobre et al., 2000), with the difference being that they induce *Per1* and *Per2* gene expression that leads to a repression of their own transcription and subsequent synchronization of all cells within hours. Glucose appears to upregulate TIEG1 (KLF10), a negatively acting zinc-finger transcription factor (Hirota et al., 2002). It binds to two GC-rich elements in the *Bmal1* promoter and thereby represses *Bmal1* transcription. In vitro experiments have shown that siRNA-mediated knockdown of TIEG1/KLF10 causes period shortening of cellular bioluminescence rhythms driven by *Bmal1*-luciferase and *Per2*-luciferase reporters (Hirota et al., 2010a). Interestingly, *Tieg1/Klf10* is regulated by BMAL1/CLOCK and thus appears to be part of a feedback loop involving the circadian clock and glucose levels (Guillaumond et al., 2010) (Figure 4). Accordingly, glucose absorbed with food or generated by gluconeogenesis will stimulate *Tieg1/Klf10* expression and reduce the expression of *Bmal1* and genes encoding for enzymes involved in gluconeogenesis. In line with this notion is the observation that *Klf10* knockout mice display postprandial and fasting hyperglycemia, although curiously, this has only been observed in male mice. However, KLF10 is implicated in circadian lipid and cholesterol homeostasis in females (Guillaumond et al., 2010). Collectively, it appears that TIEG1/KLF10 is a transcriptional regulator that links the circadian clock to energy metabolism in the liver.

One measure of metabolic state is the ratio between AMP and ATP. Once the ratio increases (high AMP levels), cells reduce the activity of ATP-consuming pathways and increase the activity of ATP-generating pathways. A major sensor for the AMP/ATP ratio is adenosine monophosphate-dependent protein kinase (AMPK), which becomes activated when AMP binds to its γ-subunit. This binding elicits a structural change in the AMPK catalytic α-subunit, making it a substrate for liver kinase B1 (LKB1). LKB1 then phosphorylates a threonine in the α-subunit of AMPK, leading to activation of AMPK (Carling et al., 2011).

It appears that AMPK impacts circadian clock mechanisms in various ways. It can directly phosphorylate CRY1, leading to destabilization and degradation of this core clock protein (Lamia et al., 2009) and consequently affecting the negative limb of the circadian clock mechanism (Figure 4). The activity of AMPK kinase also appears to modulate PER2 protein stability via an indirect mechanism involving casein kinase 1ε (CK1ε). AMPK phosphorylates S389 of CK1ε, enhancing CK1ε-mediated phosphorylation of PER2, which in turn leads to accelerated degradation of PER2. Accordingly, a lack of the catalytic subunit α-2 of AMPK would lead to an accumulation of PER2, which has been observed in *Ampkα2* knockout mice (Um et al., 2007). Taken together, it appears that AMPK is another potential regulator of the coupling between metabolism and the circadian clock.

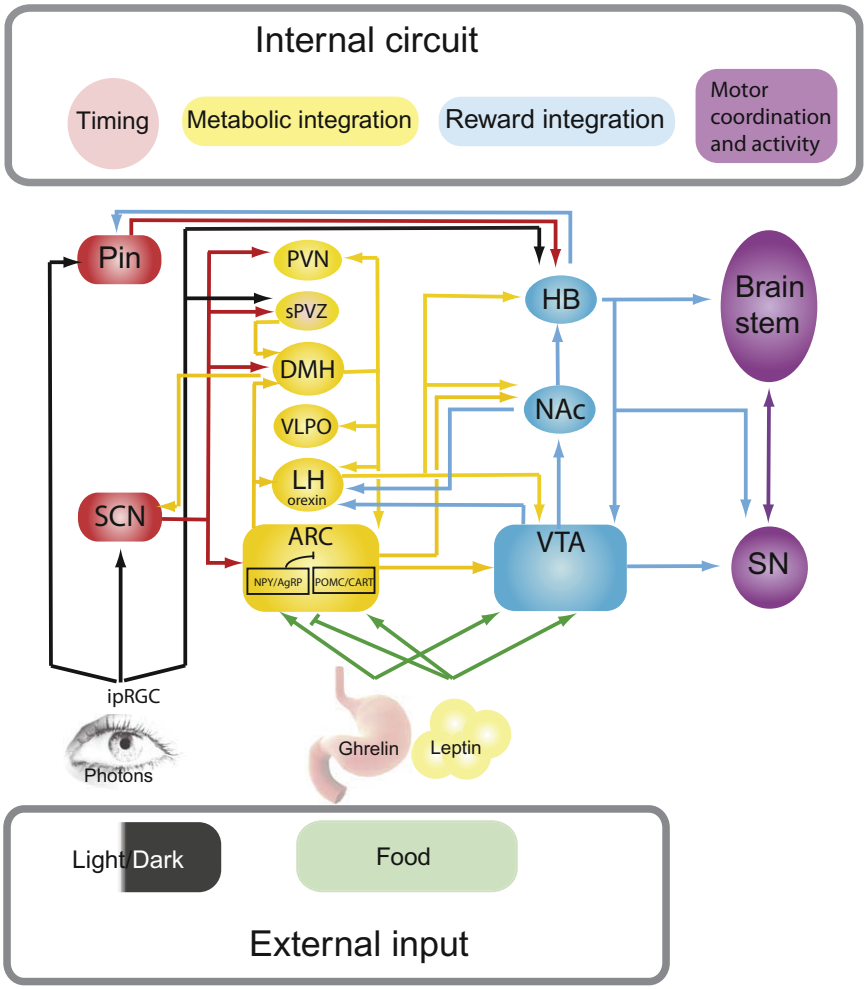

Figure 5. Major Neural Pathways Integrating Light and Feeding Signals

Light signals are directly transmitted to three entities in the brain (black arrows): (1) Structures important for timing (red), such as the SCN and Pin; (2) structures important for metabolic integration (yellow), such as the sPVZ; and (3) structures important for reward integration (blue), such as the HB. Light information is indirectly transmitted from the SCN and Pin (red arrows). The SCN projects to areas important for metabolic integration including the PVN, sPVZ, DMH, and ARC. The Pin transmits light information to the HB. Feeding signals (leptin and ghrelin) primarily affect two entities in the brain (green arrows): (1) the ARC, which is important for the metabolic integration of feeding signals (yellow), and (2) the VTA, which is important for the integration of reward (blue). The structures important for metabolic integration (yellow) and reward integration (blue) exchange information with each other (yellow and blue arrows) and can affect the SCN and Pin timing centers (red). Light and feeding signals combine and contribute to motor coordination and activity (purple).

ARC, arcuate nucleus; DMH, dorsomedial hypothalamus; HB, habenula; ipRGC, intrinsically photosensitive retinal ganglion cell; LH, lateral hypothalamus; NAc, nucleus accumbens; Pin, pineal gland; PVN, paraventricular nucleus; SCN, suprachiasmatic nuclei; SN, substantia nigra; sPVZ, subparaventricular zone; VLPO, ventrolateral preoptic nucleus; VTA, ventral tegmental area.

The interplay between the clock and metabolism is not only apparent at the cellular level, but also at the systemic level. This is discussed in the next sections.

Integration of Light, Food, and Reward Signals in the Midbrain for Dynamic Entrainment of the Circadian System

Light Signals

Areas in the brain responsible for metabolic integration (the PVN, sPVZ, DMH, and ARC) and reward integration (HB) receive direct light signals from ipRGCs (Figure 5, black arrows), as revealed by retrograde labeling (Qu et al., 1996) and transgenic ganglion cell tracing (Hattar et al., 2006). Light information also reaches these areas indirectly via the SCN and the pineal gland (Pin) (Figure 5, red arrows) (Morin, 2007). These findings illustrate that environmental light information can reach areas deep in the brain and potentially affect regulation of metabolism and reward integration simultaneously. To some degree, feeding and reward may be coupled by the light/dark cycle, and 24 hr oscillations may be maintained in these brain areas to ensure proper coordination of physiology in the organism (see below).

Light information also indirectly reaches peripheral organs including the adrenal glands, the liver, and the pancreas. The SCN distribute a rhythmic signal to all tissues of the body via hormones and the autonomous nervous system (Buijs et al., 1998). The SCN's control of glucocorticoid secretion is thought to be an important example of SCN influence on peripheral clocks. Light can indirectly activate the adrenal gland via the SCN to affect gene expression and glucocorticoid release (Ishida et al., 2005). Thus, the adrenal circadian clock is entrained by light and the adrenal clock gates glucocorticoid production in response to adrenocorticotropic hormone (ACTH) (Oster et al., 2006). Furthermore, nocturnal light affects clock gene expression in the liver via the SCN and the autonomic nervous system (Cailotto et al., 2009). Light also directly affects the pineal gland, in which melatonin synthesis takes place. Light that is applied during the dark phase results in a suppression of melatonin secretion. Interestingly, melatonin receptors are present in the pancreas, and the rhythms of insulin secretion by β-cells can be phase-shifted by the introduction of melatonin (Mulder et al., 2009). This implies that light influences pancreatic insulin secretion via the suppression of nocturnal melatonin. This suggests an indirect influence of light on the mechanisms of glucose homeostasis, supporting the finding that melatonin signaling affects insulin secretion (Mühlbauer et al., 2009). The importance of light input for regulating physiological mechanisms that impact human well-being is underscored by the fact that light therapy

can be very effective for the treatment of certain mood and eating disorders, such as seasonal affective disorder (Prasko, 2008) and night eating disorder (Goel et al., 2009).

Feeding Signals

A second external input to the metabolic and reward systems of the brain are feeding signals. These signals affect the homeostatic control of feeding as it relates to the regulation of energy balance (Figure 5, metabolic integration), and they also regulate hedonic aspects of feeding (Figure 5, reward integration) (reviewed in Lutter and Nestler, 2009).

Circulating hormones, such as ghrelin and leptin, relay information about peripheral energy levels to the brain and control feeding homeostasis (Figures 1A and 5). Ghrelin is secreted in anticipation of a regularly scheduled mealtime by the oxyntic gland cells in the stomach, which leads to activation of ghrelin receptors expressed primarily on NPY/AgRP (Agouti-related-peptide) neurons within the arcuate nucleus (Figure 5, ARC). This process promotes feeding behavior (reviewed in Zigman and Elmquist, 2003) and an increase in locomotor activity that is termed ''food anticipatory activity'' (FAA). Because ghrelin administration affects clock phase in the SCN in vitro and advances wheel-running behavior following food deprivation, it appears that ghrelin not only affects the metabolic integration centers of the brain but also the circadian system (Yannielli et al., 2007) that regulates FAA activity. Oxyntic cells coexpress ghrelin and the circadian clock proteins PER1 and PER2 in a circadian fashion, and *Per1/2* double mutant animals lack ghrelin expression (LeSauter et al., 2009). This implies an involvement of the molecular clock mechanism in circadian regulation of ghrelin production and/or release. Because mice lacking ghrelin receptors display reduced FAA, and mice mutant in the *Per2* gene show no FAA (Feillet et al., 2006), it is conceivable that there is a food-entrainable oscillator (FEO) in ghrelin-secreting stomach cells. This stomach FEO could partially affect clocks in the ARC of the brain. Additional FEOs in other tissues can be envisioned, such as in the liver and the brain, and these could potentially act via leptin or other feeding-related hormones including NPY and PYY (peptide YY).

Leptin synthesized and secreted by white adipose tissue suppresses food intake and stimulates metabolic processes that dissipate excess energy storage (reviewed in Zigman and Elmquist, 2003). Circadian oscillations in leptin have been observed in the plasma of rats (Sukumaran et al., 2010) and may activate leptin receptors in a time-dependent fashion. Leptin receptors are expressed in the ARC (Figure 5) in neurons that also express pro-opiomelanocortin (POMC) and cocaine-amphetamine-regulated transcript (CART), as well as in NPY- and AgRP-expressing neurons. Activation of leptin receptors in POMC/CART neurons stimulates the activity of these neurons and suppresses feeding while increasing metabolic rate. In contrast, activation of leptin receptors in NPY/AgRP neurons inhibits the activity of the proappetite NPY/AgRP neurons (Saper et al., 2002), reinforcing the suppression of food intake and opposing the effects of ghrelin (see above). Interestingly, leptin stimulates the transcription factor STAT3, which in conjunction with the transcription factor Nhlh2 regulates transcription of prohormone-converting enzymes 1 (PC1) and 2 (PC2) (Fox and Good, 2008). These enzymes are involved in the conversion of POMC to various hormones, such as ACTH, and various types of melanocyte-stimulating hormones (MSH) (reviewed in Mountjoy, 2010). Central administration of α-MSH reduces appetite and increases energy expenditure (Cone, 2006) via its actions on melanocortin receptors (Mc3r and Mc4r) (Cone, 2006). Lack of Mc3r in mice leads to reduced FAA under restricted feeding conditions, and the expression of the clock genes *Npas2* and *Per2* in the cortex is also reduced (Sutton et al., 2008). These observations are consistent with the reported reduction or absence of FAA in mutant mice that lack clock components *Npas2* or *Per2*, respectively (Dudley et al., 2003; Feillet et al., 2006). Collectively, it appears that circadian leptin in the serum binds to its receptors in a time-dependent fashion, thereby activating neurons in the ARC and modulating transcription of target genes in a 24 hr cycle. However, the details of how this is achieved are still a matter of investigation.

Reward Signals

There is surmounting evidence to support the theory that the consumption of both food and drugs of abuse converge on a shared pathway within the limbic system that mediates motivated behaviors (reviewed in Simerly, 2006). Much of the research has focused on the mesolimbic dopamine pathway because common drugs of abuse increase dopamine signaling from nerves that originate in the ventral tegmental area (VTA) and project to the nucleus accumbens (NAc), which is part of the striatum (Figure 5) (Nestler and Carlezon, 2006). An increase in dopaminergic transmission is thought to occur either by direct action of drugs on dopaminergic neurons (cocaine, nicotine) or indirectly by inhibition of GABAergic interneurons in the VTA (alcohol, opiates). In addition, the peptide neurotransmitter orexin, which is expressed in a subset of neurons in the lateral hypothalamus (LH) that have projections to the VTA, is also implicated in mediating drug-induced activation of dopaminergic neurons in the VTA (Borgland et al., 2006). Interestingly, the activation of orexin neurons appears to be under circadian control (Marston et al., 2008), linking arousal and drug-induced behavior by the circadian clock mechanism.

Natural rewards such as food induce similar responses in the mesolimbic dopamine pathway (Kelley and Berridge, 2002). Presentation of palatable food induces the release of dopamine into the NAc, which in turn promotes the animal's behavioral attempts to obtain food rewards via increased arousal and psychomotor activation. It appears that taste receptors are not required for this process to occur (de Araujo et al., 2008), but orexin neurons in the LH may be activated during feeding, which consequently causes the release of orexin directly onto VTA dopamine neurons (Figure 5) (Zheng et al., 2007). In transgenic orexin neuron-ablated mice, FAA was reduced in conjunction with attenuated expression of clock genes (*Npas2*, *Bmal1*, *Per1*) in the forebrain (Akiyama et al., 2004). This finding indicates a relationship between orexin and the circadian clock. Furthermore, daily fluctuations of orexin in the cerebrospinal fluid are maintained in rats housed under constant dark conditions. Moreover, lesions of the SCN in rats ablated circadian rhythms of orexin-A (Zhang et al., 2004). These findings indicate that orexin levels are regulated by the circadian clock. However, whether orexin expression is regulated by circadian components or is under indirect control of the circadian clock is not known.

Leptin and ghrelin also exert effects on the motivation to obtain food through their regulation of mesolimbic dopamine signaling in the VTA (Figure 5). Dopamine neuron firing in the VTA is inhibited by leptin receptor activation (Fulton et al., 2006) whereas blocking leptin signals in the VTA increases locomotor activity and food intake (Hommel et al., 2006). These data are consistent with the finding that basal secretion and feeding-stimulated release of dopamine can be decreased by leptin in the NAc of rats (Krügel et al., 2003). Imaging studies in human subjects confirm the involvement of the mesolimbic dopamine (DA) system in leptin's actions (Farooqi et al., 2007). A recent study indicates that leptin receptor-expressing neurons in the lateral hypothalamus (LH) that coexpress neurotensin mediate the physiological actions of leptin. These specialized neurons innervate local orexin neurons and the VTA neurons in the mesolimbic DA system (Leinninger et al., 2011). Removing the leptin receptor from these LH neurons causes mice to have orexin neurons that are unresponsive to fasting and diminished amphetamine responses in the mesolimbic DA system, resulting in reduced locomotor activity in these animals (Leinninger et al., 2011). These observations indicate that leptin may impact orexin neurons and the mesolimbic DA system to control energy balance. In contrast to leptin, ghrelin administration in rodents stimulates the release of dopamine into the NAc via activation of its receptors in the VTA (Jerlhag et al., 2007), mimicking the process that is observed in humans (Malik et al., 2008).

Components of the circadian clock modulate dopamine levels in the NAc of mice via direct regulation of monoamine oxidase A, a key enzyme in dopamine degradation. This finding implies that the circadian clock is involved in the regulation of the reward system (Hampp et al., 2008). As a consequence, the efficiency of dopaminergic signaling in the mesolimbic dopaminergic system is modulated by dopamine degradation caused by the circadian clock. Accordingly, the amount of dopamine, which is released from the VTA, in the NAc in response to leptin- or ghrelin- induced signaling is modulated in a time-dependent fashion. In this manner, a systemic integration of time and food signals is achieved, balancing energy homeostasis. This concept is also illustrated by the finding that the regulation of dopaminergic transmission and reward is altered in mice mutant for the gene *Clock* and associated with increased expression and phosphorylation of tyrosine hydroxylase (TH) (McClung et al., 2005), the rate-limiting enzyme for dopamine synthesis. Additionally, these mutants show elevated leptin levels (Turek et al., 2005), which may be responsible for the elevated TH activity, because leptin increases the synthesis and activity of TH (Fulton et al., 2006). As a consequence, these animals probably have elevated dopamine levels contributing to the mania-like behavior (Roybal et al., 2007) and the increased firing rate of VTA dopaminergic neurons observed in these animals (Mukherjee et al., 2010).

Desynchrony of the Circadian System: Implications for Human Diseases

The circadian system is strongly entwined with metabolism (see above, Dallmann et al., 2012), organizing it in a temporal fashion that optimizes the organism's performance over the day's 24 hr. Concurrently, this organization ensures tissue homeostasis by keeping various physiological processes in balance. Perturbations of the circadian system caused by rotating shift work, frequent transmeridian flights and stress lead to de-synchronization of the various body clocks. This is likely to be a confounding factor that favors the development of diseases such as metabolic syndrome (obesity, diabetes, cardiovascular problems) and neurological disorders. In these disorders, energy uptake and expenditure, and neuronal activation and inhibition become imbalanced.

Studies in humans suggest that disruption of daily metabolic rhythms is an exacerbating factor in the metabolic syndrome (Gallou-Kabani et al., 2007). Shift-work and sleep deprivation are known to dampen rhythms in growth hormone and melatonin, reduce insulin sensitivity, and elevate circulating cortisol levels (Spiegel et al., 2009). These changes favor weight gain, obesity, and development of metabolic syndrome. Recently, forced circadian desynchronization (a simulation of shift work) in humans was shown to impact on neuroendocrine control of glucose metabolism and energetics (Scheer et al., 2009). Participants subjected to the shift-work protocol showed increased blood pressure, inverted cortisol rhythms accompanied by hypoleptinemia and insulin resistance (Scheer et al., 2009). Interestingly, patients with diabetes display dampened rhythms of glucose tolerance and insulin secretion (Boden et al., 1999), indicating that the relationship between circadian disruption and metabolic pathologies is bidirectional (Figure 1B, pink arrows). This suggests that circadian disruption may lead to a vicious cycle contributing to the augmentation and progression of metabolic syndrome. The importance of a functional circadian system for metabolic homeostasis is evident from studies in mice with disrupted clock function (see above). For example, *Clock* mutant mice exhibit a reduced metabolic rate and obesity (Turek et al., 2005) and further show impaired glucose tolerance, reduced insulin secretion, and defects in size and proliferation of pancreatic islets (Marcheva et al., 2010).

Metabolic disorders, eating disorders and obesity are often associated with mood disorders in humans (McIntyre, 2009). This association is paralleled in a mouse model in which the *Clock* gene has been mutated. These animals display metabolic problems and obesity (Turek et al., 2005) and a behavior reminiscent of mania in bipolar disorder patients (Roybal et al., 2007) (see above). As with metabolic syndrome, chronic shift-work may favor the development of mood disorders (Scott, 2000), probably due to a misalignment of rhythms in body temperature, melatonin, and sleep (Hasler et al., 2010). Conversely, individuals that suffer from mood disorders benefit from strict daily routines including strictly followed bed- and mealtime (Frank et al., 2000). These routines probably help to entrain and synchronize the plethora of clocks in the body to maintain the integrity of the circadian system and physiology (Hlastala and Frank, 2006).

One of the mood disorders related to misalignment between environmental external and body internal rhythms is seasonal affective disorder (SAD). It is characterized by depressive symptoms that occur during the winter (Magnusson and Boivin, 2003). Because light therapy is an efficient method for the treatment of SAD (Terman and Terman, 2005) it is hypothesized that light, which suppresses melatonin secretion by the pineal gland (Figure 1A), may entrain the circadian system via this humoral

pathway and by resetting clock phase in the SCN (see above) and may synchronize humoral and neuronal signaling in the brain. However, the mechanism of how light mediates the beneficial effects for the treatment of mood disorders is not completely understood.

A dysfunctional circadian system can affect mood-related behaviors as evidenced by genetic alterations in clock genes of mice. A mutation in the *Clock* gene is accompanied by a spectrum of behavioral abnormalities including mania and hyperactivity (Roybal et al., 2007). Additionally, these animals as well as animals mutant in the *Per* genes display altered sensitization to, and preference for, drugs of abuse such as cocaine (Abarca et al., 2002; McClung et al., 2005) and alcohol (Dong et al., 2011; Spanagel et al., 2005). Clock gene mutations appear to affect the dopaminergic system (see above, Hampp et al., 2008; Roybal et al., 2007), but also other neurochemical systems appear to be affected. Expression of the glutamate transporter *Eaat1* is reduced in *Per2* mutant mice, leading to decreased uptake of glutamate by astrocytes and increased extracellular glutamate levels. Moreover, the increased alcohol consumption observed in these animals was reversed by acamprosate (Spanagel et al., 2005) a pharmacological agent believed to reduce abnormally high glutamatergic activity.

The interactions between circadian clocks and neurochemical mechanisms in the brain appear to be complex and may illustrate the pleiotropy of clock genes. This pleiotropy may be key to temporally coordinate and couple mechanistically unrelated physiological pathways such as weight and mood regulation, which once uncoupled may favor development of obesity and depression.

Perspectives

Because the circadian system appears to be involved in a number of diseases including obesity and depression, it may be an entry point for the development of treatments for these diseases. As highlighted in this review, light and food can significantly impact the circadian system; this implies that lifestyle affects human health via the circadian system with nutrition, movement and light exposure as the key elements involved.

Nuclear receptors, kinases, and molecules such as melatonin are a part of the circadian system. It is thus plausible that pharmacological agents acting on these components or mimicking their actions may serve as a conduit to ameliorate health problems associated with the circadian clock.

One modulator of kinase activity is lithium, which inhibits GSK3β activity. This kinase regulates clock components such as REV-ERBα (Yin et al., 2006) and PER2 (Iitaka et al., 2005), lengthens the circadian period (Li et al., 2012), and thus may relay the beneficial effects of lithium in the treatment of depressive disorders (Johnsson et al., 1983). However, its precise mechanism of action is not understood. Another potential pharmacological target to alter the circadian clock is casein kinase 1δ (CK1δ). Application of a CK1δ inhibitor (PF-670462) to wild-type mice lengthened circadian period accompanied by nuclear retention of the clock protein PER2. This treatment lengthened the period in a phase specific manner, selectively extending the duration of PER2-mediated transcriptional feedback (Meng et al., 2010). This suggests that CK1δ inhibition might be effective in increasing the synchronization of disrupted circadian oscillators offering an avenue for therapeutic treatment of diseases caused by disrupted or desynchronized circadian rhythms. Recently, longdaysin, a molecule that targets three kinases, CK1α, CK1δ, and ERK2, was discovered during a large-scale chemical screen. Longdaysin inhibition of CK1α reduced PER1 phosphorylation and degradation. As a consequence, the clock periods in human cells and in zebrafish embryos became longer, pointing to a therapeutic potential of longdaysin in manipulating and synchronizing circadian clocks (Hirota et al., 2010b).

Another approach to modulate clock activity is through delivery of substances that modulate the action of nuclear receptors. For example GSK4112 (SR6452) competes with heme for the binding to proteins of the REV-ERB family to modulate adipogenesis in mice (Kumar et al., 2010) and SR9011 as well as SR9009 regulate circadian behavior and metabolism (Solt et al., 2012). Synthetic molecules binding to proteins of the ROR family have been identified as well (Kumar et al., 2011; Wang et al., 2010); however, their action on the circadian clock and on diseases related to metabolism or mood disorders has not been established and is currently under investigation.

The circadian system is undoubtedly involved in a spectrum of disorders including metabolic and mood disorders. Circadian dysfunction can be either a contributing factor or a consequence of disease. Therefore, targeting the circadian clock for strengthening homeostatic mechanisms may be a promising therapeutic aim. This may be achieved either by pharmacological agents or by strengthening the clock via natural input such as light and feeding. Circadian pharmacology has just witnessed its dawn and holds a strong future given the promise of newly discovered agents and their effective modes of action.

ACKNOWLEDGMENTS

I apologize for not being able to include all of the relevant studies due to space limitations. I thank Drs. Jean-Luc Dreyer, Jürgen Ripperger, and Gurudutt Pendyala for comments on the manuscript. Funding provided by the Swiss National Science Foundation, the State of Fribourg, the Swiss International Cooperative Program, and the Velux Foundation is gratefully acknowledged.

REFERENCES

Abarca, C., Albrecht, U., and Spanagel, R. (2002). Cocaine sensitization and reward are under the influence of circadian genes and rhythm. Proc. Natl. Acad. Sci. USA *99*, 9026–9030.

Akiyama, M., Yuasa, T., Hayasaka, N., Horikawa, K., Sakurai, T., and Shibata, S. (2004). Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. Eur. J. Neurosci. *20*, 3054–3062.

Antle, M.C., and Silver, R. (2005). Orchestrating time: arrangements of the brain circadian clock. Trends Neurosci. *28*, 145–151.

Antle, M.C., Smith, V.M., Sterniczuk, R., Yamakawa, G.R., and Rakai, B.D. (2009). Physiological responses of the circadian clock to acute light exposure at night. Rev. Endocr. Metab. Disord. *10*, 279–291.

Asher, G., and Schibler, U. (2011). Crosstalk between components of circadian and metabolic cycles in mammals. Cell Metab. *13*, 125–137.

Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., Mostoslavsky, R., Alt, F.W., and Schibler, U. (2008). SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell *134*, 317–328.

Asher, G., Reinke, H., Altmeyer, M., Gutierrez-Arcelus, M., Hottiger, M.O., and Schibler, U. (2010). Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. Cell *142*, 943–953.

Balsalobre, A., Damiola, F., and Schibler, U. (1998). A serum shock induces circadian gene expression in mammalian tissue culture cells. Cell *93*, 929–937.

Balsalobre, A., Brown, S.A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H.M., Schütz, G., and Schibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. Science *289*, 2344–2347.

Blander, G., and Guarente, L. (2004). The Sir2 family of protein deacetylases. Annu. Rev. Biochem. *73*, 417–435.

Boden, G., Chen, X., and Polansky, M. (1999). Disruption of circadian insulin secretion is associated with reduced glucose uptake in first-degree relatives of patients with type 2 diabetes. Diabetes *48*, 2182–2188.

Borgland, S.L., Taha, S.A., Sarti, F., Fields, H.L., and Bonci, A. (2006). Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. Neuron *49*, 589–601.

Buijs, R.M., Hermes, M.H., and Kalsbeek, A. (1998). The suprachiasmatic nucleus-paraventricular nucleus interactions: a bridge to the neuroendocrine and autonomic nervous system. Prog. Brain Res. *119*, 365–382.

Cailotto, C., Lei, J., van der Vliet, J., van Heijningen, C., van Eden, C.G., Kalsbeek, A., Pévet, P., and Buijs, R.M. (2009). Effects of nocturnal light on (clock) gene expression in peripheral organs: a role for the autonomic innervation of the liver. PLoS ONE *4*, e5650.

Carling, D., Mayer, F.V., Sanders, M.J., and Gamblin, S.J. (2011). AMP-activated protein kinase: nature's energy sensor. Nat. Chem. Biol. *7*, 512–518.

Cheng, H.Y., Papp, J.W., Varlamova, O., Dziema, H., Russell, B., Curfman, J.P., Nakazawa, T., Shimizu, K., Okamura, H., Impey, S., and Obrietan, K. (2007). microRNA modulation of circadian-clock period and entrainment. Neuron *54*, 813–829.

Cho, H., Zhao, X., Hatori, M., Yu, R.T., Barish, G.D., Lam, M.T., Chong, L.W., Ditacchio, L., Atkins, A.R., Glass, C.K., et al. (2012). Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. Nature.

Colwell, C.S. (2011). Linking neural activity and molecular oscillations in the SCN. Nat. Rev. Neurosci. *12*, 553–569.

Cone, R.D. (2006). Studies on the physiological functions of the melanocortin system. Endocr. Rev. *27*, 736–749.

Costa, M.J., So, A.Y., Kaasik, K., Krueger, K.C., Pillsbury, M.L., Fu, Y.H., Ptacek, L.J., Yamamoto, K.R., and Feldman, B.J. (2011). Circadian rhythm gene period 3 is an inhibitor of the adipocyte cell fate. J. Biol. Chem. *286*, 9063–9070.

Crumbley, C., and Burris, T.P. (2011). Direct regulation of CLOCK expression by REV-ERB. PLoS ONE *6*, e17290.

Crumbley, C., Wang, Y., Kojetin, D.J., and Burris, T.P. (2010). Characterization of the core mammalian clock component, NPAS2, as a REV-ERBalpha/ROR-alpha target gene. J. Biol. Chem. *285*, 35386–35392.

Dallmann, R., Viola, A.U., Tarokh, L., Cajochen, C., and Brown, S.A. (2012). The human circadian metabolome. Proc. Natl. Acad. Sci. USA *109*, 2625–2629.

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev. *14*, 2950–2961.

de Araujo, I.E., Oliveira-Maia, A.J., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., Nicolelis, M.A., and Simon, S.A. (2008). Food reward in the absence of taste receptor signaling. Neuron *57*, 930–941.

Dibner, C., Sage, D., Unser, M., Bauer, C., d'Eysmond, T., Naef, F., and Schibler, U. (2009). Circadian gene expression is resilient to large fluctuations in overall transcription rates. EMBO J. *28*, 123–134.

Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu. Rev. Physiol. *72*, 517–549.

DiTacchio, L., Le, H.D., Vollmers, C., Hatori, M., Witcher, M., Secombe, J., and Panda, S. (2011). Histone lysine demethylase JARID1a activates CLOCK-BMAL1 and influences the circadian clock. Science *333*, 1881–1885.

Dong, L., Bilbao, A., Laucht, M., Henriksson, R., Yakovleva, T., Ridinger, M., Desrivieres, S., Clarke, T.K., Lourdusamy, A., Smolka, M.N., et al. (2011). Effects of the circadian rhythm gene period 1 (per1) on psychosocial stress-induced alcohol drinking. Am. J. Psychiatry *168*, 1090–1098.

Dudley, C.A., Erbel-Sieler, C., Estill, S.J., Reick, M., Franken, P., Pitts, S., and McKnight, S.L. (2003). Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. Science *301*, 379–383.

Duffield, G.E. (2003). DNA microarray analyses of circadian timing: the genomic basis of biological time. J. Neuroendocrinol. *15*, 991–1002.

Duong, H.A., Robles, M.S., Knutti, D., and Weitz, C.J. (2011). A molecular mechanism for circadian clock negative feedback. Science *332*, 1436–1439.

Ecker, J.L., Dumitrescu, O.N., Wong, K.Y., Alam, N.M., Chen, S.K., LeGates, T., Renna, J.M., Prusky, G.T., Berson, D.M., and Hattar, S. (2010). Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. Neuron *67*, 49–60.

Eskin, A. (1979). Identification and physiology of circadian pacemakers. Introduction. Fed. Proc. *38*, 2570–2572.

Farooqi, I.S., Bullmore, E., Keogh, J., Gillard, J., O'Rahilly, S., and Fletcher, P.C. (2007). Leptin regulates striatal regions and human eating behavior. Science *317*, 1355.

Feillet, C.A., Ripperger, J.A., Magnone, M.C., Dulloo, A., Albrecht, U., and Challet, E. (2006). Lack of food anticipation in Per2 mutant mice. Curr. Biol. *16*, 2016–2022.

Fox, D.L., and Good, D.J. (2008). Nescient helix-loop-helix 2 interacts with signal transducer and activator of transcription 3 to regulate transcription of prohormone convertase 1/3. Mol. Endocrinol. *22*, 1438–1448.

Frank, E., Swartz, H.A., and Kupfer, D.J. (2000). Interpersonal and social rhythm therapy: managing the chaos of bipolar disorder. Biol. Psychiatry *48*, 593–604.

Fulton, S., Pissios, P., Manchon, R.P., Stiles, L., Frank, L., Pothos, E.N., Maratos-Flier, E., and Flier, J.S. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. Neuron *51*, 811–822.

Gachon, F., Olela, F.F., Schaad, O., Descombes, P., and Schibler, U. (2006). The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. Cell Metab. *4*, 25–36.

Gallou-Kabani, C., Vigé, A., and Junien, C. (2007). Lifelong circadian and epigenetic drifts in metabolic syndrome. Epigenetics *2*, 137–146.

Gekakis, N., Staknis, D., Nguyen, H.B., Davis, F.C., Wilsbacher, L.D., King, D.P., Takahashi, J.S., and Weitz, C.J. (1998). Role of the CLOCK protein in the mammalian circadian mechanism. Science *280*, 1564–1569.

Goel, N., Stunkard, A.J., Rogers, N.L., Van Dongen, H.P., Allison, K.C., O'Reardon, J.P., Ahima, R.S., Cummings, D.E., Heo, M., and Dinges, D.F. (2009). Circadian rhythm profiles in women with night eating syndrome. J. Biol. Rhythms *24*, 85–94.

Golombek, D.A., and Rosenstein, R.E. (2010). Physiology of circadian entrainment. Physiol. Rev. *90*, 1063–1102.

Grimaldi, B., Bellet, M.M., Katada, S., Astarita, G., Hirayama, J., Amin, R.H., Granneman, J.G., Piomelli, D., Leff, T., and Sassone-Corsi, P. (2010). PER2 controls lipid metabolism by direct regulation of PPARγ. Cell Metab. *12*, 509–520.

Guilding, C., and Piggins, H.D. (2007). Challenging the omnipotence of the suprachiasmatic timekeeper: are circadian oscillators present throughout the mammalian brain? Eur. J. Neurosci. *25*, 3195–3216.

Guillaumond, F., Gréchez-Cassiau, A., Subramaniam, M., Brangolo, S., Peteri-Brünback, B., Staels, B., Fiévet, C., Spelsberg, T.C., Delaunay, F., and Teboul, M. (2010). Kruppel-like factor KLF10 is a link between the circadian clock and metabolism in liver. Mol. Cell. Biol. *30*, 3059–3070.

Hampp, G., Ripperger, J.A., Houben, T., Schmutz, I., Blex, C., Perreau-Lenz, S., Brunk, I., Spanagel, R., Ahnert-Hilger, G., Meijer, J.H., and Albrecht, U. (2008). Regulation of monoamine oxidase A by circadian-clock components implies clock influence on mood. Curr. Biol. *18*, 678–683.

Hasler, B.P., Buysse, D.J., Kupfer, D.J., and Germain, A. (2010). Phase relationships between core body temperature, melatonin, and sleep are associated with depression severity: further evidence for circadian misalignment in non-seasonal depression. Psychiatry Res. *178*, 205–207.

Hattar, S., Kumar, M., Park, A., Tong, P., Tung, J., Yau, K.W., and Berson, D.M. (2006). Central projections of melanopsin-expressing retinal ganglion cells in the mouse. J. Comp. Neurol. *497*, 326–349.

Hirota, T., Okano, T., Kokame, K., Shirotani-Ikejima, H., Miyata, T., and Fukada, Y. (2002). Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured Rat-1 fibroblasts. J. Biol. Chem. *277*, 44244–44251.

Hirota, T., Kon, N., Itagaki, T., Hoshina, N., Okano, T., and Fukada, Y. (2010a). Transcriptional repressor TIEG1 regulates Bmal1 gene through GC box and controls circadian clockwork. Genes Cells *15*, 111–121.

Hirota, T., Lee, J.W., Lewis, W.G., Zhang, E.E., Breton, G., Liu, X., Garcia, M., Peters, E.C., Etchegaray, J.P., Traver, D., et al. (2010b). High-throughput chemical screen identifies a novel potent modulator of cellular circadian rhythms and reveals CKIα as a clock regulatory kinase. PLoS Biol. *8*, e1000559.

Hlastala, S.A., and Frank, E. (2006). Adapting interpersonal and social rhythm therapy to the developmental needs of adolescents with bipolar disorder. Dev. Psychopathol. *18*, 1267–1288.

Hogenesch, J.B., Gu, Y.Z., Jain, S., and Bradfield, C.A. (1998). The basic-helix-loop-helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. Proc. Natl. Acad. Sci. USA *95*, 5474–5479.

Hommel, J.D., Trinko, R., Sears, R.M., Georgescu, D., Liu, Z.W., Gao, X.B., Thurmon, J.J., Marinelli, M., and DiLeone, R.J. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron *51*, 801–810.

Iitaka, C., Miyazaki, K., Akaike, T., and Ishida, N. (2005). A role for glycogen synthase kinase-3beta in the mammalian circadian clock. J. Biol. Chem. *280*, 29397–29402.

Ishida, A., Mutoh, T., Ueyama, T., Bando, H., Masubuchi, S., Nakahara, D., Tsujimoto, G., and Okamura, H. (2005). Light activates the adrenal gland: timing of gene expression and glucocorticoid release. Cell Metab. *2*, 297–307.

Jerlhag, E., Egecioglu, E., Dickson, S.L., Douhan, A., Svensson, L., and Engel, J.A. (2007). Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. Addict. Biol. *12*, 6–16.

Jeyaraj, D., Scheer, F.A., Ripperger, J.A., Haldar, S.M., Lu, Y., Prosdocimo, D.A., Eapen, S.J., Eapen, B.L., Cui, Y., Mahabeleshwar, G.H., et al. (2012). Klf15 orchestrates circadian nitrogen homeostasis. Cell Metab. *15*, 311–323.

Johnsson, A., Engelmann, W., Pflug, B., and Klemke, W. (1983). Period lengthening of human circadian rhythms by lithium carbonate, a prophylactic for depressive disorders. Int. J. Chronobiol. *8*, 129–147.

Kelley, A.E., and Berridge, K.C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. J. Neurosci. *22*, 3306–3311.

Kornmann, B., Schaad, O., Bujard, H., Takahashi, J.S., and Schibler, U. (2007). System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. PLoS Biol. *5*, e34.

Krügel, U., Schraft, T., Kittner, H., Kiess, W., and Illes, P. (2003). Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. Eur. J. Pharmacol. *482*, 185–187.

Kumar, N., Solt, L.A., Wang, Y., Rogers, P.M., Bhattacharyya, G., Kamenecka, T.M., Stayrook, K.R., Crumbley, C., Floyd, Z.E., Gimble, J.M., et al. (2010). Regulation of adipogenesis by natural and synthetic REV-ERB ligands. Endocrinology *151*, 3015–3025.

Kumar, N., Kojetin, D.J., Solt, L.A., Kumar, K.G., Nuhant, P., Duckett, D.R., Cameron, M.D., Butler, A.A., Roush, W.R., Griffin, P.R., and Burris, T.P. (2011). Identification of SR3335 (ML-176): a synthetic RORα selective inverse agonist. ACS Chem. Biol. *6*, 218–222.

Kume, K., Zylka, M.J., Sriram, S., Shearman, L.P., Weaver, D.R., Jin, X., Maywood, E.S., Hastings, M.H., and Reppert, S.M. (1999). mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell *98*, 193–205.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., et al. (2009). AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science *326*, 437–440.

Lamia, K.A., Papp, S.J., Yu, R.T., Barish, G.D., Uhlenhaut, N.H., Jonker, J.W., Downes, M., and Evans, R.M. (2011). Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. Nature *480*, 552–556.

Lavery, D.J., Lopez-Molina, L., Margueron, R., Fleury-Olela, F., Conquet, F., Schibler, U., and Bonfils, C. (1999). Circadian expression of the steroid 15 alpha-hydroxylase (Cyp2a4) and coumarin 7-hydroxylase (Cyp2a5) genes in mouse liver is regulated by the PAR leucine zipper transcription factor DBP. Mol. Cell. Biol. *19*, 6488–6499.

Le Minh, N., Damiola, F., Tronche, F., Schütz, G., and Schibler, U. (2001). Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. EMBO J. *20*, 7128–7136.

Lehman, M.N., Silver, R., Gladstone, W.R., Kahn, R.M., Gibson, M., and Bittman, E.L. (1987). Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. J. Neurosci. *7*, 1626–1638.

Leinninger, G.M., Opland, D.M., Jo, Y.H., Faouzi, M., Christensen, L., Cappellucci, L.A., Rhodes, C.J., Gnegy, M.E., Becker, J.B., Pothos, E.N., et al. (2011). Leptin action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy balance. Cell Metab. *14*, 313–323.

LeSauter, J., Hoque, N., Weintraub, M., Pfaff, D.W., and Silver, R. (2009). Stomach ghrelin-secreting cells as food-entrainable circadian clocks. Proc. Natl. Acad. Sci. USA *106*, 13582–13587.

Li, J., Lu, W.Q., Beesley, S., Loudon, A.S., and Meng, Q.J. (2012). Lithium impacts on the amplitude and period of the molecular circadian clockwork. PLoS ONE *7*, e33292.

Liu, J., Malkani, G., Shi, X., Meyer, M., Cunningham-Runddles, S., Ma, X., and Sun, Z.S. (2006). The circadian clock Period 2 gene regulates gamma interferon production of NK cells in host response to lipopolysaccharide-induced endotoxic shock. Infect. Immun. *74*, 4750–4756.

Liu, C., Li, S., Liu, T., Borjigin, J., and Lin, J.D. (2007). Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism. Nature *447*, 477–481.

Liu, Y., Dentin, R., Chen, D., Hedrick, S., Ravnskjaer, K., Schenk, S., Milne, J., Meyers, D.J., Cole, P., Yates, J., 3rd., et al. (2008). A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. Nature *456*, 269–273.

Lutter, M., and Nestler, E.J. (2009). Homeostatic and hedonic signals interact in the regulation of food intake. J. Nutr. *139*, 629–632.

Magnusson, A., and Boivin, D. (2003). Seasonal affective disorder: an overview. Chronobiol. Int. *20*, 189–207.

Malik, S., McGlone, F., Bedrossian, D., and Dagher, A. (2008). Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab. *7*, 400–409.

Marcheva, B., Ramsey, K.M., Buhr, E.D., Kobayashi, Y., Su, H., Ko, C.H., Ivanova, G., Omura, C., Mo, S., Vitaterna, M.H., et al. (2010). Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature *466*, 627–631.

Marston, O.J., Williams, R.H., Canal, M.M., Samuels, R.E., Upton, N., and Piggins, H.D. (2008). Circadian and dark-pulse activation of orexin/hypocretin neurons. Mol. Brain *1*, 19.

McClung, C.A., Sidiropoulou, K., Vitaterna, M., Takahashi, J.S., White, F.J., Cooper, D.C., and Nestler, E.J. (2005). Regulation of dopaminergic

transmission and cocaine reward by the Clock gene. Proc. Natl. Acad. Sci. USA *102*, 9377–9381.

McIntyre, R.S. (2009). Managing weight gain in patients with severe mental illness. J. Clin. Psychiatry *70*, e23.

Meng, Q.J., Maywood, E.S., Bechtold, D.A., Lu, W.Q., Li, J., Gibbs, J.E., Dupré, S.M., Chesham, J.E., Rajamohan, F., Knafels, J., et al. (2010). Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. Proc. Natl. Acad. Sci. USA *107*, 15240–15245.

Mitsui, S., Yamaguchi, S., Matsuo, T., Ishida, Y., and Okamura, H. (2001). Antagonistic role of E4BP4 and PAR proteins in the circadian oscillatory mechanism. Genes Dev. *15*, 995–1006.

Morin, L.P. (2007). SCN organization reconsidered. J. Biol. Rhythms *22*, 3–13.

Mountjoy, K.G. (2010). Functions for pro-opiomelanocortin-derived peptides in obesity and diabetes. Biochem. J. *428*, 305–324.

Mühlbauer, E., Gross, E., Labucay, K., Wolgast, S., and Peschke, E. (2009). Loss of melatonin signalling and its impact on circadian rhythms in mouse organs regulating blood glucose. Eur. J. Pharmacol. *606*, 61–71.

Mukherjee, S., Coque, L., Cao, J.L., Kumar, J., Chakravarty, S., Asaithamby, A., Graham, A., Gordon, E., Enwright, J.F., 3rd, DiLeone, R.J., et al. (2010). Knockdown of Clock in the ventral tegmental area through RNA interference results in a mixed state of mania and depression-like behavior. Biol. Psychiatry *68*, 503–511.

Mulder, H., Nagorny, C.L., Lyssenko, V., and Groop, L. (2009). Melatonin receptors in pancreatic islets: good morning to a novel type 2 diabetes gene. Diabetologia *52*, 1240–1249.

Nagoshi, E., Saini, C., Bauer, C., Laroche, T., Naef, F., and Schibler, U. (2004). Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. Cell *119*, 693–705.

Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., Guarente, L.P., and Sassone-Corsi, P. (2008). The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell *134*, 329–340.

Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., and Sassone-Corsi, P. (2009). Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science *324*, 654–657.

Nestler, E.J., and Carlezon, W.A., Jr. (2006). The mesolimbic dopamine reward circuit in depression. Biol. Psychiatry *59*, 1151–1159.

Oster, H., Damerow, S., Kiessling, S., Jakubcakova, V., Abraham, D., Tian, J., Hoffmann, M.W., and Eichele, G. (2006). The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. Cell Metab. *4*, 163–173.

Ouyang, Y., Andersson, C.R., Kondo, T., Golden, S.S., and Johnson, C.H. (1998). Resonating circadian clocks enhance fitness in cyanobacteria. Proc. Natl. Acad. Sci. USA *95*, 8660–8664.

Peet, D.J., Turley, S.D., Ma, W., Janowski, B.A., Lobaccaro, J.M., Hammer, R.E., and Mangelsdorf, D.J. (1998). Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. Cell *93*, 693–704.

Pegoraro, M., and Tauber, E. (2008). The role of microRNAs (miRNA) in circadian rhythmicity. J. Genet. *87*, 505–511.

Prasko, J. (2008). Bright light therapy. Neuroendocrinol. Lett. *29* (*Suppl 1*), 33–64.

Preitner, N., Damiola, F., Lopez-Molina, L., Zakany, J., Duboule, D., Albrecht, U., and Schibler, U. (2002). The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell *110*, 251–260.

Purushotham, A., Schug, T.T., Xu, Q., Surapureddi, S., Guo, X., and Li, X. (2009). Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metab. *9*, 327–338.

Qu, T., Dong, K., Sugioka, K., and Yamadori, T. (1996). Demonstration of direct input from the retina to the lateral habenular nucleus in the albino rat. Brain Res. *709*, 251–258.

Ramsey, K.M., Yoshino, J., Brace, C.S., Abrassart, D., Kobayashi, Y., Marcheva, B., Hong, H.K., Chong, J.L., Buhr, E.D., Lee, C., et al. (2009). Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science *324*, 651–654.

Reick, M., Garcia, J.A., Dudley, C., and McKnight, S.L. (2001). NPAS2: an analog of clock operative in the mammalian forebrain. Science *293*, 506–509.

Reinke, H., Saini, C., Fleury-Olela, F., Dibner, C., Benjamin, I.J., and Schibler, U. (2008). Differential display of DNA-binding proteins reveals heat-shock factor 1 as a circadian transcription factor. Genes Dev. *22*, 331–345.

Rodgers, J.T., Lerin, C., Haas, W., Gygi, S.P., Spiegelman, B.M., and Puigserver, P. (2005). Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature *434*, 113–118.

Roybal, K., Theobold, D., Graham, A., DiNieri, J.A., Russo, S.J., Krishnan, V., Chakravarty, S., Peevey, J., Oehrlein, N., Birnbaum, S., et al. (2007). Mania-like behavior induced by disruption of CLOCK. Proc. Natl. Acad. Sci. USA *104*, 6406–6411.

Rutter, J., Reick, M., Wu, L.C., and McKnight, S.L. (2001). Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. Science *293*, 510–514.

Sahar, S., Nin, V., Barbosa, M.T., Chini, E.N., and Sassone-Corsi, P. (2011). Altered behavioral and metabolic circadian rhythms in mice with disrupted NAD+ oscillation. Aging (Albany NY) *3*, 794–802.

Sakamaki, J., Daitoku, H., Yoshimochi, K., Miwa, M., and Fukamizu, A. (2009). Regulation of FOXO1-mediated transcription and cell proliferation by PARP-1. Biochem. Biophys. Res. Commun. *382*, 497–502.

Saper, C.B., Chou, T.C., and Elmquist, J.K. (2002). The need to feed: homeostatic and hedonic control of eating. Neuron *36*, 199–211.

Sato, T.K., Panda, S., Miraglia, L.J., Reyes, T.M., Rudic, R.D., McNamara, P., Naik, K.A., FitzGerald, G.A., Kay, S.A., and Hogenesch, J.B. (2004). A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron *43*, 527–537.

Scheer, F.A., Hilton, M.F., Mantzoros, C.S., and Shea, S.A. (2009). Adverse metabolic and cardiovascular consequences of circadian misalignment. Proc. Natl. Acad. Sci. USA *106*, 4453–4458.

Schmutz, I., Ripperger, J.A., Baeriswyl-Aebischer, S., and Albrecht, U. (2010). The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. Genes Dev. *24*, 345–357.

Scott, A.J. (2000). Shift work and health. Prim. Care *27*, 1057–1079.

Shi, S., Hida, A., McGuinness, O.P., Wasserman, D.H., Yamazaki, S., and Johnson, C.H. (2010). Circadian clock gene Bmal1 is not essential; functional replacement with its paralog, Bmal2. Curr. Biol. *20*, 316–321.

Simerly, R. (2006). Feeding signals and drugs meet in the midbrain. Nat. Med. *12*, 1244–1246.

Solt, L.A., Wang, Y., Banerjee, S., Hughes, T., Kojetin, D.J., Lundasen, T., Shin, Y., Liu, J., Cameron, M.D., Noel, R., et al. (2012). Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. Nature, in press. Published online March 29, 2012. 10.1038/nature11030.

Spanagel, R., Pendyala, G., Abarca, C., Zghoul, T., Sanchis-Segura, C., Magnone, M.C., Lascorz, J., Depner, M., Holzberg, D., Soyka, M., et al. (2005). The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. Nat. Med. *11*, 35–42.

Spiegel, K., Tasali, E., Leproult, R., and Van Cauter, E. (2009). Effects of poor and short sleep on glucose metabolism and obesity risk. Nat. Rev. Endocrinol. *5*, 253–261.

Sukumaran, S., Xue, B., Jusko, W.J., Dubois, D.C., and Almon, R.R. (2010). Circadian variations in gene expression in rat abdominal adipose tissue and relationship to physiology. Physiol. Genomics *42A*, 141–152.

Sutton, G.M., Perez-Tilve, D., Nogueiras, R., Fang, J., Kim, J.K., Cone, R.D., Gimble, J.M., Tschöp, M.H., and Butler, A.A. (2008). The melanocortin-3 receptor is required for entrainment to meal intake. J. Neurosci. *28*, 12946–12955.

Terman, M., and Terman, J.S. (2005). Light therapy for seasonal and nonseasonal depression: efficacy, protocol, safety, and side effects. CNS Spectr. *10*, 647–663, quiz 672.

Turek, F.W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E., Laposky, A., Losee-Olson, S., Easton, A., Jensen, D.R., et al. (2005). Obesity and metabolic syndrome in circadian Clock mutant mice. Science *308*, 1043–1045.

Ueda, H.R., Chen, W., Adachi, A., Wakamatsu, H., Hayashi, S., Takasugi, T., Nagano, M., Nakahama, K., Suzuki, Y., Sugano, S., et al. (2002). A transcription factor response element for gene expression during circadian night. Nature *418*, 534–539.

Ueda, H.R., Hayashi, S., Chen, W., Sano, M., Machida, M., Shigeyoshi, Y., Iino, M., and Hashimoto, S. (2005). System-level identification of transcriptional circuits underlying mammalian circadian clocks. Nat. Genet. *37*, 187–192.

Um, J.H., Yang, S., Yamazaki, S., Kang, H., Viollet, B., Foretz, M., and Chung, J.H. (2007). Activation of 5′-AMP-activated kinase with diabetes drug metformin induces casein kinase Iepsilon (CKIepsilon)-dependent degradation of clock protein mPer2. J. Biol. Chem. *282*, 20794–20798.

van der Horst, G.T., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J., Verkerk, A., Eker, A.P., van Leenen, D., et al. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature *398*, 627–630.

Vanselow, J.T., and Kramer, A. (2010). Posttranslational regulation of circadian clocks. In Teh Circadian Clock, U. Albrecht, ed. (New York: Springer), pp. 79–104.

Wang, Y., Kumar, N., Nuhant, P., Cameron, M.D., Istrate, M.A., Roush, W.R., Griffin, P.R., and Burris, T.P. (2010). Identification of SR1078, a synthetic agonist for the orphan nuclear receptors RORα and RORγ. ACS Chem. Biol. *5*, 1029–1034.

Welsh, D.K., Takahashi, J.S., and Kay, S.A. (2010). Suprachiasmatic nucleus: cell autonomy and network properties. Annu. Rev. Physiol. *72*, 551–577.

Yang, X., Downes, M., Yu, R.T., Bookout, A.L., He, W., Straume, M., Mangelsdorf, D.J., and Evans, R.M. (2006). Nuclear receptor expression links the circadian clock to metabolism. Cell *126*, 801–810.

Yannielli, P.C., Molyneux, P.C., Harrington, M.E., and Golombek, D.A. (2007). Ghrelin effects on the circadian system of mice. J. Neurosci. *27*, 2890–2895.

Yin, L., Wang, J., Klein, P.S., and Lazar, M.A. (2006). Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock. Science *311*, 1002–1005.

Zhang, S., Zeitzer, J.M., Yoshida, Y., Wisor, J.P., Nishino, S., Edgar, D.M., and Mignot, E. (2004). Lesions of the suprachiasmatic nucleus eliminate the daily rhythm of hypocretin-1 release. Sleep *27*, 619–627.

Zheng, B., Albrecht, U., Kaasik, K., Sage, M., Lu, W., Vaishnav, S., Li, Q., Sun, Z.S., Eichele, G., Bradley, A., and Lee, C.C. (2001). Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. Cell *105*, 683–694.

Zheng, H., Patterson, L.M., and Berthoud, H.R. (2007). Orexin signaling in the ventral tegmental area is required for high-fat appetite induced by opioid stimulation of the nucleus accumbens. J. Neurosci. *27*, 11075–11082.

Zigman, J.M., and Elmquist, J.K. (2003). Minireview: From anorexia to obesity—the yin and yang of body weight control. Endocrinology *144*, 3749–3756.