Interconnections between circadian clocks and metabolism

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Circadian rhythms evolved through adaptation to daily light/dark changes in the environment; they are believed to be regulated by the core circadian clock interlocking feedback loop. Recent studies indicate that each core component executes general and specific functions in metabolism. Here, we review the current understanding of the role of these core circadian clock genes in the regulation of metabolism using various genetically modified animal models. Additionally, emerging evidence shows that exposure to environmental stimuli, such as artificial light, unbalanced diet, mistimed eating, and exercise, remodels the circadian physiological processes and causes metabolic disorders. This Review summarizes the reciprocal regulation between the circadian clock and metabolism, highlights remaining gaps in knowledge about the regulation of circadian rhythms and metabolism, and examines potential applications to human health and disease.

To adapt to daily environmental changes caused by our Earth's rotation, most organisms on the planet evolved near-24-hour cycles of behavioral, physiological, and metabolic rhythms (1). In addition to the entrained environmental stimuli, the internal timekeeping system of the circadian clock has evolved to anticipate external changes (2, 3). These conserved rhythms synchronize internal biological and behavioral processes to the external temporal environment, presumably providing organisms with selective advantages for survival. However, over the past century, modern industrialized society has profoundly changed our external environment (4). For example, the boundaries between day and night have been blurred by electric light and travel across different time zones. Disrupted circadian rhythms are highly associated with metabolic disorders (5). Conversely, obesity induced by overeating or overnutritional environment leads to circadian remodeling (6, 7). Understanding the reciprocal regulation of circadian rhythm and metabolism may provide mechanistic insights into circadian physiology and advance new chronotherapy approaches and therapeutic targets for metabolic disorders.

Intrinsic circadian clock machinery and metabolism

"Circadian rhythm" refers to an innate, endogenous, and entrainable rhythm whose cycle is approximately 24 hours. This rhythm is retained under constant conditions, in the absence of any external or environmental cue that entrains the circadian clock (referred to as zeitgebers), including light, temperature, eating patterns, exercise, and social interactions. More generally, a biological rhythm synchronized by light/dark cycles is known as a diurnal rhythm,

which may or may not be a circadian rhythm (depending on whether it is independent of light/dark cycles). Organisms most active in the daytime (such as humans) are referred to as diurnal, whereas nocturnal organisms (such as mice and rats) sleep primarily during the light phase. Owing to the power of genetic manipulation and the ability to carefully and (relatively) inexpensively control housing conditions, mechanistic studies of circadian rhythms in mammals have largely used mice as models, which presents translational challenges to human chronomedicine since people are diurnal. Moreover, metabolic differences between rodents and humans need to be taken into account. For example, rodents feed frequently and have fast metabolic rates, such that 24 hours of fasting could result in a profound loss of body weight (up to 20%) and an even greater percentage loss of body fat (8).

Molecular circadian clock machinery. The notion of intrinsic clock machinery was first posited in 1729 by the discovery that, in constant darkness, the leaves of the mimosa plant retained their daily pattern of opening and closing, indicating that this rhythm was programmed and not just a response to natural light (9). Two centuries later, in 1971, the genetic foundation of circadian rhythms was pioneered by Ron Konopka and Seymour Benzer (10). They reported that mutations at a single genetic locus produced abnormal circadian rhythms in the behavior of *Drosophila melanogaster* (10). In 1984, the laboratories of Hall, Rosbash, and Young cloned the first circadian rhythm gene, *period* (*Per*), in the fruit fly (11–15), and 10 years later Takahashi and colleagues discovered circadian locomotor output cycles kaput (*Clock*), the first mammalian circadian clock gene, in mice (16).

Interestingly, *Per* encodes a protein that represses its own transcription, resulting in daily *Per* rhythm, while CLOCK activates the transcription of *Per*, forming a negative-feedback loop that is highly conserved from flies to humans (17). In mammals, two basic helix-loop-helix (bHLH) transcriptional activators, CLOCK and brain and muscle ARNT-like 1 (ARNTL1; also known as BMAL1 and MOP3), form a heterodimer and bind to
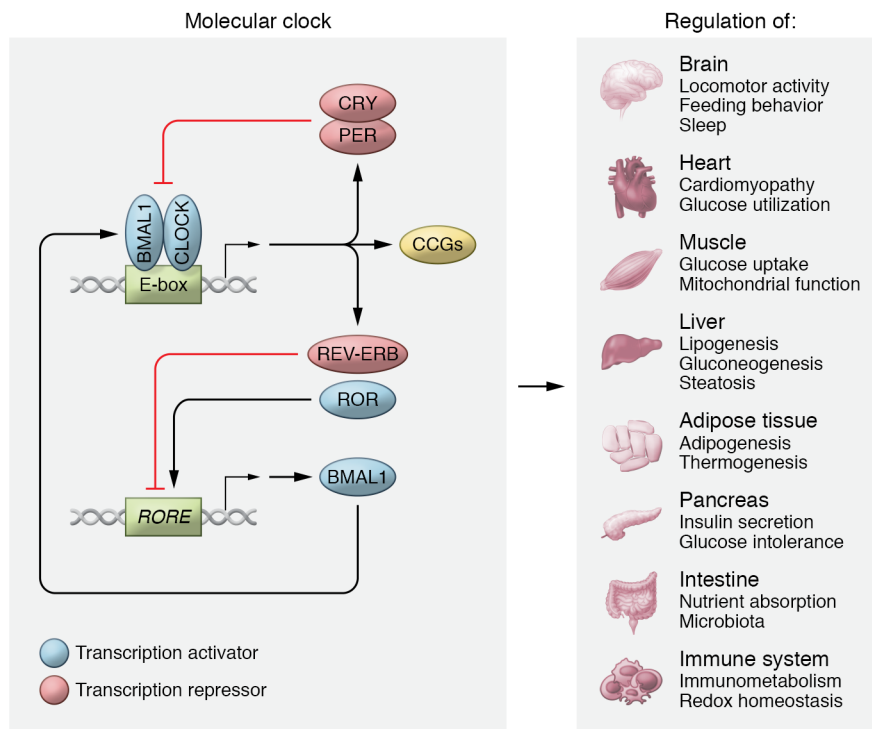
Figure 1. Intrinsic circadian clock machinery and metabolism. Core circadian clock genes, including transcription activators and repressors, form an autoregulatory feedback loop present in most cells to regulate their circadian physiology. The figure summarizes the major metabolic functions regulated by core circadian clock genes in indicated tissues based on loss- and gain-of-function studies from genetically modified animal models.

E-box element to activate the expression of the transcriptional repressors cryptochromes 1 and 2 (CRY1/2), periods 1, 2, and 3 (PER1/2/3), and REV-ERBα/β (also known as nuclear receptor subfamily 1 group D members 1 and 2 [NR1D1/2]) (17–19). CRY and PER repress CLOCK-BMAL1–dependent transcription (20-22). REV-ERBα and REV-ERBβ repress *Bmal1* transcription via recruitment of nuclear receptor corepressor (NCoR) and histone deacetylase 3 (HDAC3) (23–25). Additionally, retinoic acid receptor–related orphan receptors α and γ (RORα/γ) are induced by CLOCK-BMAL1 and function as transcriptional activators to positively on *Bmal1* expression in competition with REV-ERBα/β on ROR response elements (ROREs) (1, 19). Overall, the circadian clock oscillator uses multiple mechanisms to drive the temporally restricted gene expression pattern.

Core circadian clock genes and metabolism. To understand the connection between circadian rhythm and metabolism, genetically modified mouse strains targeting circadian clock genes have been generated and phenotypically characterized. It is important to recognize that these targeted genes are transcription factors, and as such, their genetic loss affects both their role in the circadian clock and their more general function as direct regulators of gene expression. Figure 1 and Table 1 summarize the effects, described below, of knockout of these clock components on biological rhythms and metabolic function.

Bmal1. BMAL1 is the core clock component whose single knockout in a mouse model results in complete loss of rhythmicity (26). *Bmal1*-null mice have altered activity, reduced total activity levels on light/dark cycles, and complete abolition of behavioral circadian rhythms in constant darkness (26). *Bmal1*-knockout mice also show impaired gluconeogenesis, display various symptoms of early aging, and have reduced lifespan and body weight (27, 28). Intriguingly, constitutive expression of *Bmal1* in brains of

whole-body *Bmal1*-knockout mice restored the circadian rhythms of wheel-running activity, but activity levels and body weight were still lower than in WT mice (29). By contrast, re-expression of *Bmal1* in the skeletal muscle of *Bmal1*-null mice normalized activity levels and body weight but not the circadian rhythm of behavior (29), suggesting distinct tissue-specific functions of BMAL1.

Tissue-specific knockout of *Bmal1* has provided insight into its specific functions. Pancreatic β cell–specific *Bmal1*-knockout mice have impaired glucose tolerance and decreased insulin secretion, resulting in hyperglycemia without affecting the circadian rhythms of activity and feeding/fasting cycles (30). Liver-specific *Bmal1*-knockout mice have higher triglyceride and cholesterol accumulation in the liver and plasma, which is rescued by liver-specific *Bmal1* re-expression (31, 32). Adipocyte-specific deletion of *Bmal1* increases adipose tissue mass and body weight gain as a result of reduced energy expenditure and increased daytime food intake (33). In cardiomyocyte-specific *Bmal1*-knockout mice, hearts decrease glucose utilization, and the mice exhibit early-onset development of dilated cardiomyopathy and early mortality (34, 35). Skeletal muscle–specific *Bmal1*-knockout mice show impaired insulin-stimulated glucose uptake and decreased glucose oxidation, but circadian rhythms of locomotor activity are normal (36, 37). *Bmal1* also modulates immunometabolism, as macrophage-specific *Bmal1* knockout impairs mitochondrial function and exacerbates succinate dehydrogenase–mediated mitochondrial production of reactive oxygen species (38). In summary, these studies indicate that BMAL1 is not only a key regulatory signal in the suprachiasmatic nucleus (SCN) regulating overall rhythmic behavior, but also drives local tissue-specific metabolic functions.

Clock and Npas2. The *Clock* gene was the first gene identified to have a critical role in mammalian circadian rhythms (16, 39).

Table 1. Whole-body genetically modified mouse models and related metabolic phenotypes

Genetic modification	Circadian impact	Major metabolic impact	Refs.
Bmal1 KO	Impaired in LD, completely lost in DD	Impaired gluconeogenesis, early aging, reduced lifespans, and less fat and muscle mass	26–28
Clock$^{\Delta 19/\Delta 19}$	Impaired in DD	Hepatic steatosis, obesity, hypertriglyceridemia and hyperglycemia	28, 30, 43–45
Clock KO	Retain rhythmic		46
Clock/Npas2 KO	Impaired in DD		50
Rev-erbα KO	Retain rhythmic	Abnormal lipid, glucose, cholesterol, and bile acid metabolism, lost normal rhythms of body temperature	66–69
Rev-erbα/β KO	Impaired in DD	Abnormal lipid, glucose, cholesterol, and bile acid metabolism, hepatic steatosis	23, 24
Rora KO	Retain rhythmic	Reduced levels of adiposity and hepatic triglyceride, inflammation, and insulin resistance under HFD	87–89
Rorc KO	Retain rhythmic	Reduced levels of lipid in liver and blood, cholesterol, and bile acid pool size	57, 58
Per2 KO	Retain rhythmic	Impaired ability to use carbohydrates, decreased body weight	108, 111
Per1/Per2 KO	Impaired in DD		106
Cry1 KO	Retain rhythmic	Resistance to HFD-induced obesity	116
Cry1/2 KO	Impaired in DD	Glucose intolerance and constitutively high levels of circulating corticosterone	114, 118

Positional cloning and transgenic rescue studies identified a *Clock* point mutation (*Clock*$^{\Delta 19}$) leading to altered circadian behavior (39, 40). This single point mutation within the intron between exons 18 and 19 caused aberrant mRNA splicing with resultant loss of 51 amino acids that comprise a putative transcriptional regulatory domain (41). This mutant CLOCK$^{\Delta 19}$ protein could form heterodimers with BMAL1 but failed to activate transcription (42). Compared with WT mice, *Clock*$^{\Delta 19/\Delta 19}$ double-mutant mice display metabolic disorders, including hepatic steatosis, obesity, hypertriglyceridemia and hyperglycemia, and increased absorption of lipids throughout the day (28, 30, 43–45).

Interestingly, although *Clock*$^{\Delta 19/\Delta 19}$ double-mutant mice display abnormal circadian behavior in constant darkness (16, 39), mice with whole-body knockout of *Clock* (*Clock*$^{-/-}$) display robust behavioral rhythmicity, although they do have altered responses to light (46). Neuronal PAS domain–containing protein 2 (NPAS2) is a paralog of CLOCK that can form a heterodimer with BMAL1 (47, 48). Like *Clock*-null mice, *Npas2*-null mice retain robust circadian rhythms, suggesting a potential complementary effect between CLOCK and NPAS2 (49). Indeed, mice with double knockout of CLOCK and NPAS2 (*Clock*$^{-/-}$ *Npas2*$^{-/-}$) exhibit arrhythmic locomotor behavior in constant darkness (50). However, *Npas2* and *Clock* do not equally contribute to circadian regulation. *Npas2* is mainly expressed in the brain, and *Clock* is highly expressed in the peripheral tissues (51). *Npas2*-null mice adapt slowly to restricted feeding (52), while peripheral oscillators are arrhythmic without *Clock* (53).

Rev-erbs. REV-ERBα (54) and REV-ERBβ (55) are closely related members of the nuclear receptor superfamily. In most nuclear receptors, activation function 2 (AF2) is required to interact with coactivators and activate gene transcription (56), but REV-ERBs lack AF2 at the C-terminal end of the ligand-binding domain. As a result, REV-ERBs are thought to be unable to activate transcription and act as constitutive repressors of transcription (57). Recent studies have indicated that REV-ERBs repress gene expression in two ways: First, they compete with the transcriptional activators RORs at sites known as ROREs (58, 59), passively blocking activation. Second, REV-ERBs actively repress gene expression from these sites by recruiting the NCoR-HDAC3 corepressor complex (25, 60, 61). This active repression function requires two REV-ERB moieties, binding as closely spaced monomers or cooperatively as a homodimer (62). In addition to direct DNA binding, some REV-ERBα binding activity occurs via tethering. One example of such indirect binding was revealed through the function of a REV-ERBα mutant that lacks the DNA-binding domain (60). In this case, REV-ERBα was tethered to DNA through hepatocyte nuclear factors (HNFs). Since HNFs, particularly HNF4 and HNF6 (63–65), are specific to the liver, this example demonstrates how lineage-specific factors can facilitate tissue-specific genomic binding of transcriptional modifiers like REV-ERBα.

REV-ERBα is an important metabolic regulator and modulates lipid, glucose, cholesterol, and bile acid metabolism, as well as adipogenesis and inflammation (66–69). Heme, a substance precursive to hemoglobin, has been identified as a direct ligand for REV-ERBα and REV-ERBβ (70). Although the physiological role of this interaction remains to be fully understood, heme suppresses hepatic gluconeogenic gene expression and glucose output through REV-ERBα–mediated gene expression (70, 71). Deficiency of both *Rev-erb* genes led marked hepatic steatosis and profoundly altered circadian wheel-running behavior and lipid homeostasis (23, 24). Although REV-ERBα is required for adipocyte differentiation in cell culture (72–74), adipose tissue mass is increased via fibroblast growth factor 21 (FGF-21) signaling in mice lacking REV-ERBα (75, 76). *Rev-erbα*–null mice were also shown to lose normal rhythms of body temperature, brown adipose tissue (BAT) activity, and improved cold tolerance at 5 pm, suggesting that REV-ERBα contributes to thermogenic oscillation (77). In skeletal muscle, *Rev-erbα* deficiency led to reduced mitochondrial content and oxidative function, upregulated autophagy, and compromised exercise capacity (78). *Rev-erbα* mutation (DBD domain deletion) and *Rev-erbβ* deletion in SCN GABAergic neurons disrupted the rhythms of hepatic glucose production in mice (79). Collectively, these studies using tissue-specific genetic models suggest tissue-specific functions of REV-ERBs in mice. Emerging studies are actively dissecting whether the metabolic effects of tissue-specific functions of REV-ERBs are mediated cell-autonomously or by modulation of systemic signals (80–82).
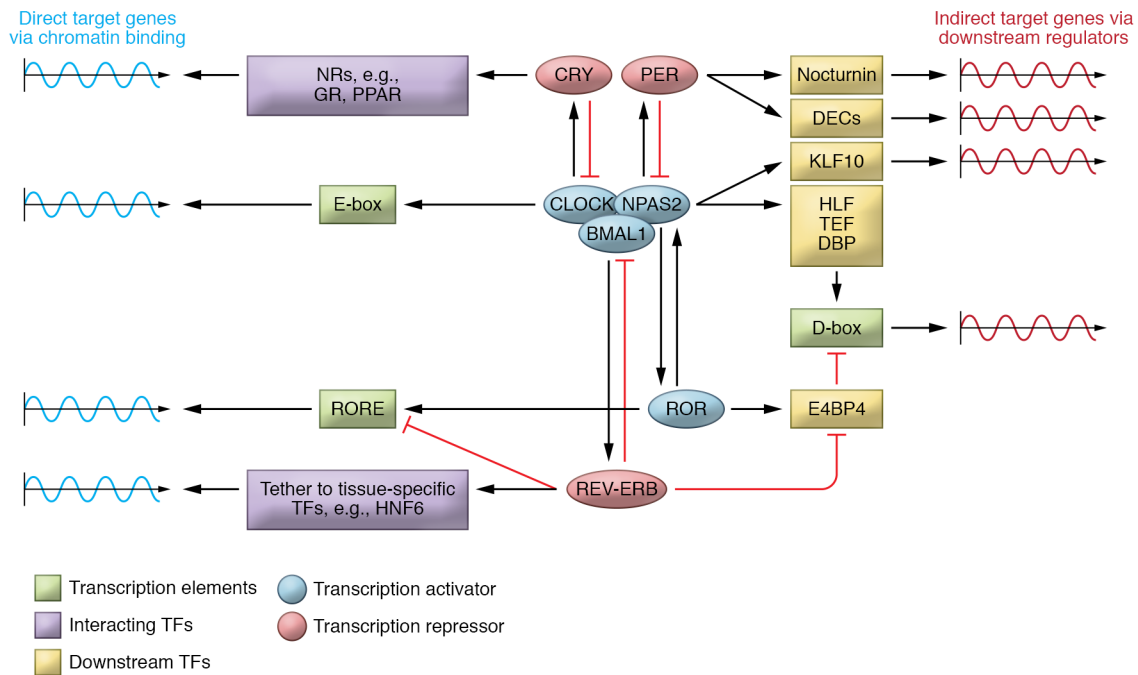
Figure 2. Mechanisms of core clock component–specific regulation of target genes. Core clock components independently, or forming protein complexes, bind to specific chromatin regions to directly regulate circadian gene expression (left). Each core clock component indirectly mediates circadian gene expression via downstream TFs (right). GR, glucocorticoid receptor; NRs, nuclear receptors.

Rors. The three RORs (RORα, RORβ, and RORγ) are members of the nuclear receptor superfamily. They were named based on sequence similarities to the retinoic acid receptor (83–85), but they share DNA-binding specificity with REV-ERBs (58, 59). Their endogenous ligands remain controversial, but they may be activated by oxysterols as well as being constitutively activated through the ligand-independent recruitment of transcriptional coactivators (86). Both RORα and RORγ have been implicated in the control of energy homeostasis and regulation of lipid and glucose metabolism.

Deficiency of *Rora*, but not *Rorc*, in mice fed an obesogenic high-fat diet (HFD) led to reduced levels of adiposity and hepatic triglyceride levels, inflammation, and insulin resistance in comparison with WT mice (87–89). Multiple organs contributed to this phenotype. In skeletal muscle, compared with WT mice, increased levels of AKT and phosphorylated AKT and enhanced glucose uptake were observed in *Rora*-deficient mice (89). Upon *Rora* knockout, genes related to lipid synthesis were downregulated in the liver, and inflammatory genes were also downregulated in white adipocyte tissue (87, 88). However, a previous study reported that RORα-null mice had increased triglyceride accumulation and lipogenic gene expression in the liver (90), leading researchers to revisit the function of RORs in liver. Liver-specific double knockout of *Rora/Rorc* shows the overactivation of the INSIG2/SREBP lipogenic response at the peak expression of RORs in mice (5 am) and exacerbation of diet-induced hepatic steatosis, demonstrating an important circadian rhythmic consideration for metabolic studies (91). In BAT, *Ucp1* and other thermogenic genes were upregulated upon *Rora* knockout. Consistently, primary brown adipocytes from *Rora*-deficient mice displayed a higher metabolic rate (87).

Like RORα, RORγ plays important roles in the metabolic regulation of multiple organs. *Rorc*-deficient mice show decreased adipocyte sizes and high insulin sensitivity with improved control of circulating free fatty acids compared with WT controls. HFD-fed *Rorc*-deficient mice are also protected from hyperglycemia and insulin resistance (92). Consistently, *Rorc* expression in the adipose stromal vascular fraction from obese human subjects is positively correlated with adipocyte size and negatively correlated with adipogenesis and insulin sensitivity (92). In the liver, both whole-body *Rorc*-knockout and liver-specific *Rorc*-knockout mice display reduced levels of lipid in liver and blood, reduced cholesterol, and reduced bile acid pool size (93, 94). RORγ is highly expressed in skeletal muscle and controls the expression of genes that regulate muscle and fat mass, and modulates the production of reactive oxygen species (95). An isoform of RORγ, called RORγt, is unique to inflammatory Th17 lymphocytes (96), although its metabolic function has not been characterized.

Pers and Crys. *Period*, the first clock gene to be identified, has three homolog genes (*Per1*, *Per2*, and *Per3*) in mammals (97–100). Cryptochrome (*Cry*) has two homologs, *Cry1* and *Cry2* (101). Although PERs and CRYs lack a DNA-binding domain and therefore are very unlikely to directly bind to DNA, they form a heterodimer that moves into the nucleus upon phosphorylation by casein kinase 1 (CK1), and inhibit the transcriptional activity of BMAL1-CLOCK heterodimer (102–105).

Per1- or *Per2*-deficient mice, but not *Per3*-deficient mice, display disrupted locomotor activity rhythms in extended exposure to constant darkness. Interestingly, the circadian disruption observed in *Per1/Per3* and *Per2/Per3* double-knockout mice resembles rhythms of circadian disruption of *Per1-* or *Per2*-defi-
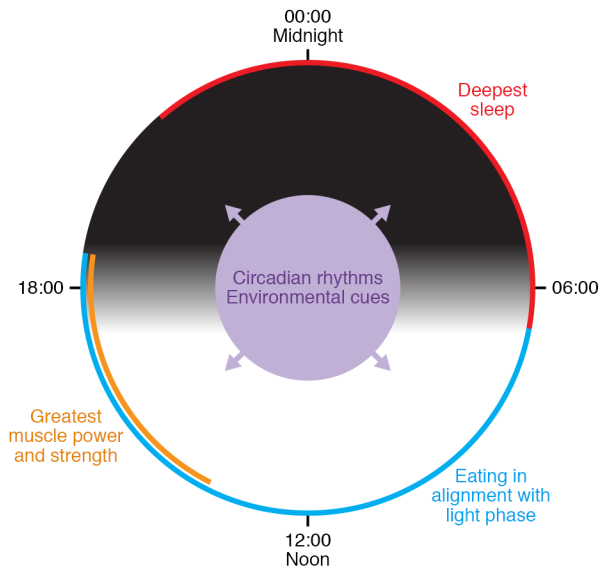
Figure 3. Interactions between circadian rhythms and human physiology. Light/dark cycles synchronize the intrinsic clock to a 24-hour cycle. In human physiology, alignment of wake/sleep cycles, eating patterns, and exercise schedule to certain circadian phases leads to beneficial metabolic outcomes, while misalignment exacerbates or causes metabolic disorders.

cient mice, while *Per1/Per2* double-knockout mice are immediately arrhythmic in constant darkness (106). PERs have different functions in the regulation of metabolism (106). *Per1*-deficient mice display elevated blood pressure involving a mechanism of renal sodium reabsorption (107). Rhythms of glucocorticoid secretion and diurnal feeding rhythms are disrupted in normal chow– and HFD-fed *Per2*-deficient mice, resulting in decreased body weight gain in mice (108). Moreover, *Per2*-deficient mice exhibit increased oxidative capacity in white adipocyte tissue and enhanced adipocyte differentiation of cultured fibroblasts (109), and *Per1/Per2*–deficient mice have different circadian accumulation patterns of triacylglycerol compared with WT mice (110). *Per2* deficiency in ischemic hearts impairs carbohydrate utilization for oxygen-efficient glycolysis (111). *Per3*- or *Per1/Per2/Per3*–deficient mice also gained more diet-induced body weight, suggesting an opposing function to *Per2* (112), while another study indicated that *Per2* knockout alters the body composition, with both increased adipose and decreased muscle tissue (113).

Mice lacking *Cry1* or *Cry2* alone display a phase-accelerated or a phase-delayed free-running period of locomotor activities, respectively, but the circadian rhythms are still robust. *Cry1/Cry2* double-knockout mice show arrhythmic behavior in constant darkness (114). Mice deficient in *Cry1*, but not mice deficient in *Cry2*, are resistant to HFD-induced obesity (115). HFD-fed *Cry1/Cry2* double-knockout mice rapidly gain weight and display hyperinsulinemia (116). CRY regulates glucose homeostasis through several

mechanisms. CRY represses gluconeogenesis by inhibiting protein kinase A–mediated phosphorylation of cAMP response element–binding protein (CREB) during fasting via blocking of glucagon-mediated increases in intracellular cAMP concentration (117). CRY1 and CRY2 interact with glucocorticoid receptor (GR) in a ligand-dependent manner. Knockout of *Cry1/Cry2* results in glucose intolerance and constitutively high levels of circulating corticosterone (118). Moreover, SREBP1c downregulates gluconeogenesis via CRY1-mediated FOXO1 degradation to regulate hyperglycemia in *db/db* mice (119).

Core clock component–specific regulation of metabolic cycles. In addition to the common regulation of the core circadian clock interlocking feedback loop, groups of rhythmic genes are specifically regulated by certain core clock components. Consistently, knockouts of these components share some common phenotypes, including disrupted locomotor activities in constant dark and tumorigenesis in mice with chronic jet lag (120), but each knockout model also has component-specific metabolic outcomes as discussed above. To explore the underlying mechanisms of component-specific regulation, the genome occupancy of core clock components was determined, revealing that only a small proportion of binding sites are shared among all of the core components (25, 121). Further genome-wide rhythmic enhancer mapping using global run-on sequencing (GRO-Seq) identified that each phase of enhancers and downstream gene transcription is regulated by distinct core clock transcription factors (TFs) (122).

As summarized in Figure 2, the BMAL1-CLOCK complex can bind to E-box motif (123, 124), while REV-ERBs, competing with RORs, bind on RORE motifs to regulate the expression of target genes whose enhancers or promoters contain these motifs (58, 59, 122). In addition to the interaction with BMAL1-CLOCK, CRY1 broadly interacts with multiple nuclear receptors and modulates specific gene expression (125). In addition to competing with ROR on ROREs, REV-ERBs can be tethered by cell type–specific TFs and regulate the rhythmic expression of another specific group of genes involved in metabolism (60). The studies described above provide mechanisms of how core clock components bind in diverse ways on chromatin and directly regulate the oscillating expression of their target genes. Core clock components can also indirectly regulate their target genes via downstream TFs. For example, BMAL1 activates the oscillating expression of *Hlf*, *Tef*, and *Dbp* (which encode TFs in the PAR bZIP family) to indirectly regulate the expression of rhythmic

Table 2. Effects of environmental cues on biological rhythms and metabolism in humans

Environmental cue	Major metabolic impact	Refs.
Circadian misalignment (shift work, jet lag, sleep disorder)	Abnormal glucose and lipid metabolism, decreased insulin sensitivity, increased inflammation, and adverse cardiovascular consequences	229–235
Eating patterns (early meal, intermittent fasting, time-restricted feeding)	Affect levels of plasma glucose, triglyceride, BMI, GLP-1, insulin, blood pressure, body weight, B cell responsiveness, oxidative stress, fat oxidation, and cholesterol	193, 236–241
Physical activity (scheduled exercise)	Affects oxygen consumption, respiratory exchange ratio, heart rate, blood glucose levels, and body temperature	193, 197, 242, 243

GLP-1, glucagon-like peptide-1; BMI, body mass index.

genes whose regulatory elements contain D-box (123, 126, 127). REV-ERBs repress another D-box–binding transcription repressor, *E4bp4*, which subsequently regulates the rhythmic expression of E4BP4 target genes (122). These downstream TFs of each core clock component either independently or collaboratively regulate the rhythmic expression of circadian output genes.

Environmental cues regulating biological rhythms and metabolism

Biological rhythms are the natural cycle of change in the body regarding biological processes, which are regulated by intrinsic circadian clock machinery and adjusted (entrained) by external environmental inputs and social cues, including light/dark cycles, nutrients, feeding schedule, and exercise time (128–130). Figure 3 and Table 2 summarize the effects of these zeitgebers on biological rhythms and metabolic function.

Follow the light. Photosensitive organisms are biologically programmed to follow light/dark cycles in order to entrain hormone production and bodily function. In mammals, the daily light/dark cycles entrain the circadian clock of the brain, specifically the SCN of the hypothalamus, to the 24-hour period in our environment. It has been suggested that disruption of circadian rhythms caused by abnormal light/dark cycles, including artificial light at night and the usage of screens with shorter-wavelength lights, results in metabolic disorders (131, 132). Night-shift workers display dyslipidemia, increased postprandial serum glucose and insulin (133), and increased circulating levels of several biomarkers of metabolic syndrome and inflammation (134). Moreover, night-shift workers and people with long working hours have a high risk of obesity and diabetes (135–142). Experimental animal models have been used to demonstrate that circadian misalignment causes metabolic disturbances. Here, we will discuss the effect of constant bright light (LL), alternating dim and bright light (dLL), and wavelengths of light on metabolism, including obesity, insulin resistance, and hepatic steatosis (143, 144).

Mice exposed to LL become behaviorally arrhythmic, and their SCNs become desynchronized (145). The disruption of the peripheral clock was also observed in LL-exposed mice. Mice exposed to LL developed obesity and hepatic steatosis, which was paralleled by an altered miRNA profile targeting the core clock gene *Rev-erbα* (143). When an obesogenic diet is superimposed on LL, mice display a reduced amplitude of rhythms in the SCN and a complete abolishment of circadian rhythms of feeding pattern, energy expenditure, and insulin sensitivity (146). During early development, the circadian system experiences a critical adjustment and is vulnerable to altered lighting conditions. During lactation, short-term LL in pups caused a loss of rhythmicity, a reduction in vasoactive intestinal polypeptide–positive (VIP-positive) and arginine vasopressin–positive (AVP-positive) cells in the SCN, a reduction of PER1 expression in the SCN, reduced body weight gain, and loss of daily rhythms in plasma glucose and triglycerides (147). These rhythmic metabolic disorders could not be restored in conditions of alternating light and dark (LD) after lactation (147). In adult rats, LL downregulated plasma melatonin (which is absent in most mouse models; refs. 148–150) and systematically induced complete circadian rhythmic suppression of body temperature and locomotor activity, but

this circadian disruption could be restored within 1 week in constant darkness (DD) (151).

Like LL, dLL exposes mice to light over the course of the 24-hour day, but also provides a temporal cue for a 24-hour day via different light intensity between day and night. Compared with LL, dLL has lesser impacts on circadian rhythms. Interestingly, compared with LD controls, both LL- and dLL-exposed mice display increased body mass and reduced glucose tolerance, but caloric intake and total daily activity output are not affected (152). In the dLL-exposed mice, rhythms of *Per1* and *Per2* in the hypothalamus were attenuated, similarly to those of REV-ERB genes in the liver and adipose tissue (153).

In addition to LL, acute exposure to light in the night or a different wavelength also affects biological rhythms and metabolism. Very short exposures to nocturnal light inhibit melatonin release, alter clock gene expression, and increase *c-Fos* expression in the SCN, and this effect is wavelength dependent: blue light has the greatest effect, whereas red light has no effect (154–157). Therefore, light as a predominant zeitgeber entraining the clock in the SCN is a major contributor to maintenance of organismal metabolic homeostasis.

You are what you eat. Diet composition is another important factor that affects the circadian clock. HFD disrupts circadian rhythms of locomotor and feeding activity in mice, with greater rhythmic expression of clock genes in fat than in liver (158). Rhythmic transcriptome profiling identified a genome-wide reprogramming of the clock in the liver (159). Using GRO-Seq to map HFD-specific circadian enhancers and quantify HFD-specific transcription rates, the DNA binding motifs for peroxisome proliferator–activated receptor (PPAR) and SREBP were shown to be enriched. Further functional studies revealed an unexpected synchronization of two opposing lipid processes, lipid synthesis and oxidation, at a similar time of the day. The synchronization could be a maladaptive response to the overnutrition environment (160).

Ketogenic diets (KDs) are high-fat, adequate-protein, very-low-carbohydrate diets that induce fatty acid oxidation as an energy source and lead to the synthesis of ketone bodies. This diet is used to treat epilepsy in children (161), to induce weight loss (162–164), and to decrease the risk of heart disease (165, 166). In mice, KD induced a profound circadian remodeling in the liver and gut in a tissue-specific manner. KD drastically alters BMAL1 target genes in the liver, but not the gut, while highly diurnal rhythms of PPARα are only observed in the gut (167). A low-calorie diet, which is known to boost fat metabolism and lifespan, enhances the magnitude of cyclic expression of circadian clock genes in *Drosophila* (168). These results highlight the intricate reciprocal relationship between metabolism and food content–regulated peripheral clocks.

Eat on time. Meal time is known to be a dominant zeitgeber for peripheral tissue clocks (such as the clock in the liver) (81, 169). Eating during the active phase has healthy consequences for metabolism, while mistimed eating leads to metabolic disorders (170, 171). Restricting feeding to the sleep phase (here referred to as reverse-phase feeding [RPF]) uncouples circadian oscillators in peripheral tissues (including liver, kidney, and heart) from the SCN (169) and desynchronizes peripheral clocks (172). Coupling

RPF with HFD exacerbates increased adiposity, decreased glucose tolerance, and dyslipidemia, a metabolic profile often observed in subjects with night-eating syndrome (173). Human epidemiological studies suggest that skipping breakfast is associated with high risks of developing obesity and related metabolic disorders (174–177). Early nocturnal meal skipping in mice, equivalent to breakfast skipping in humans, disturbs the peripheral clock, increases lipid synthesis, and favors body mass gain (178).

Conversely, restricting mice to HFD feeding in the active phase without reducing caloric intake prevents weight gain and metabolic disturbances, including hyperglycemia, insulin resistance, hepatic steatosis, and hypercholesterolemia (179–181). Even in mice lacking core circadian clock genes, time-restricted feeding (TRF) during the active phase (from zeitgeber time points ZT 13 to ZT 22) can prevent HFD-induced metabolic disorders (182). In humans, because of the variation among time-restricted feeding protocols regarding eating time and period, it is unclear whether TRF contributes to weight loss, but TRF showed beneficial metabolic outcomes in several independent studies (183–188).

Exercise with a schedule. Exercise is a crucial intervention in the prevention and treatment of metabolic disorders (189). Scheduled exercise has been shown to entrain circadian rhythms in skeletal muscle (190–192). However, the optimal timing of exercise for preventing the effects of disrupted circadian rhythm and maximizing the health benefits is still largely unknown. Several studies have indicated that exercise performance shows diurnal rhythmicity (193). A recent study in mice indicated that exercise causes circadian remodeling involving carbohydrate exhaustion, usage of alternative energy sources, and adaptation of systemic energy expenditure (194). More world records have been broken by athletes in early evening, as strength, power, and endurance are increased in the early evening compared with early morning (195–197). Interestingly, when the training period exceeded 12 weeks, individuals who exercise in the evening gained more muscle mass than individuals who exercise in the morning (198).

In addition to the above zeitgebers, other external environmental and internal physiological cues, including temperature (199, 200), alcohol (201), aging (202), sexual phenotype (203, 204), cancer (205), microbiota (206, 207), and oxygen levels (208–211), impose significant impacts on biological rhythms and chronometabolism. Moreover, the interactions among these timing cues can collaboratively entrain peripheral clocks. For example, nutrient catabolism, maintenance of body temperature, and exercise are tightly linked to oxygen consumption (208, 209). Heart attack and obstructive sleep apnea caused by metabolic disorders lead to hypoxia (210, 211). These changes in oxygenation affect the circadian clock in an HIF-1α–dependent manner (208–211).

Conclusions and perspective

Molecular circadian biology originated with a genetic screen in *Drosophila* (identifying the *Per* mutant), and then extended to mammals through genetic screens in mice. As core clock components have been uncovered, circadian whole-body gene-knockout mouse models have revealed the function of the molecular clock in regulating circadian behavior, including sleep/wake and feeding/fasting cycles, as well as maintenance of metabolic homeostasis. Emerging core circadian gene tissue-specific knockout mouse

models and state-of-the-art "omics" analysis across different tissues under various environmental stimuli have shed light on tissue-specific circadian clocks. The recognition that multiple TFs function in the core clock, and that each of these has thousands of genomic DNA binding sites, suggests that the concept of "clock output genes" needs to be revised. Each of the core clock genes contributes directly to individual gene regulation in addition to its role in the reciprocal and homeostatic regulation of other clock genes by transcriptional-translational feedback loops that define the clock itself.

Interorgan rhythmic communications. Future studies are expected to further determine the interorgan rhythmic communications and how they are integrated to perform physiological functions. Multidirectional interorgan interactions, including those between the nervous system and peripheral metabolic organs as well as between metabolic organs, are essential for adaption to external cues and maintain whole-body energy homeostasis. The nervous system coordinates whole-body metabolism not only by direct innervation of the target tissues but also by the production of neurohormones (212). Peripheral organs perform intercellular signaling in an autocrine, paracrine, or endocrine manner (213). Interorgan communications have been explored in feeding, fasting, cold exposure, and exercise conditions (214–220). However, how tissue metabolism is linked and gated to specific temporal windows, and how this coordinated communication and coherence among tissue clocks are remodeled in response to environmental stimuli, need further investigation.

Intraorgan rhythmic communications. In addition to interorgan communication, intraorgan communication has attracted increasing attention due to the improved technique and computing methods of single-cell sequencing (221–225). We recently discovered that the disruption of clocks in hepatocytes via deletion of the core clock genes *REV-ERBα* and *REV-ERBβ* remodels the rhythmic enhancers, transcriptomes, and metabolomes of multiple cell types within the liver (81). These results suggest rhythmic communication of time signals between different cell types within an organ to coordinately perform a given physiological function. Interestingly, even within the same cell type, hepatocyte disruption of REV-ERBα/β regulates target gene expression in a liver zonation–dependent manner. Consistent with these findings, another independent study indicated that core circadian clock genes are expressed in a non-zonated pattern, but the rhythmicity of some oscillating genes is zonation dependent (226), suggesting that the microenvironment, including intraorgan communication, plays an important role in their rhythmic expression. Future studies would be important to determine the underlying mechanism and physiological consequences of intraorgan communication.

Circadian versus non-circadian functions of clock genes. Tissue-specific knockout mouse models have been used to partially solve the above questions, but also raise another question: is it the rhythmicity or the expression level of these core clock genes that is important for circadian regulation? In *Bmal1* whole-body knockout mice, constitutive re-expression of BMAL1 in brain and muscle tissues partially rescues the disrupted rhythmic behavior (29), suggesting that the rhythmicity of the expression level of *Bmal1* mRNA is not essential for rhythmic behavior. This could be due to the post-transcriptional effects of an intact PER/CRY rhythm, but

this needs to be determined. Yet in *Bmal1*-deficient mice, rhythmic re-expression of BMAL1 in the liver and skin cannot restore most of the rhythmic gene expression (227, 228), suggesting that additional signals are essential in these tissues. Comparison of rhythmic and constitutive re-expression of these core circadian genes with similar mean expressions across the day in tissue-specific knockout mouse models could be useful for dissecting the respective role of gene rhythmicity and gene expression level.

Non-canonical rhythmic regulators. In addition to canonical or core clock regulators that are critical to rhythmic biology, there are also non-canonical regulators that either collaboratively or independently regulate various rhythmic processes. For example, diet-induced obesity leads to a profound circadian remodeling in the livers of mice despite only modest changes in expression of all core clock genes (158, 160). Similarly in human muscle, TRF induces a massive circadian remodeling of amino acids and other metabolites without perturbing core clock gene expression (193). These studies suggest there are additional non-canonical rhythmic regulators mediating the circadian remodeling. Interestingly, knockout of REV-ERBs in livers of adult mice disrupts rhythmicity of other core clock genes, yet the majority of circadian output genes retained their rhythms, indicating the role of non-canonical regulators of these genes (81). Moreover, TRF protects mice lacking circadian clocks from diet-induced obesity and metabolic syndrome, also suggesting functional roles for non-canonical regulators (182). Identification of the mechanism and functions of non-canonical rhythmic regulators

could provide insights into the molecular hierarchy of circadian regulation and provide targets for chronotherapy.

Last but not least, expanding our knowledge of the reciprocal regulation of circadian rhythm and metabolism is valuable for boosting the circadian clocks to improve quality of life, including chrono-nutritional and chronopharmacological approaches. Determination of the optimal times to rest, eat, exercise, and administer drugs throughout the day to restore the perturbed circadian machinery in shift workers and individuals with sleep disorders will ultimately contribute to improving the outcome of metabolic disorders.

Acknowledgments

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