Cell

Leading Edge
Review

The Meter of Metabolism

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The circadian system orchestrates the temporal organization of many aspects of physiology, including metabolism, in synchrony with the 24 hr rotation of the Earth. Like the metabolic system, the circadian system is a complex feedback network that involves interactions between the central nervous system and peripheral tissues. Emerging evidence suggests that circadian regulation is intimately linked to metabolic homeostasis and that dysregulation of circadian rhythms can contribute to disease. Conversely, metabolic signals also feed back into the circadian system, modulating circadian gene expression and behavior. Here, we review the relationship between the circadian and metabolic systems and the implications for cardiovascular disease, obesity, and diabetes.

"*It don't mean a thing if it ain't got that swing*."
—Edward Kennedy "Duke" Ellington (1899–1974)

What would music be without rhythm? So much in music depends upon timing, as do many aspects of our daily lives, including the rhythms of eating, fasting, sleep, and wakefulness. These behaviors, although seemingly second nature, are now recognized to be governed by an intricate system of internal molecular clocks. These clocks coordinate biological processes to maintain synchrony with the environmental cycles of light and nutrients. It has been known for many years that numerous aspects of metabolism exhibit daily rhythmicity, including many types of circulating and intracellular metabolites, feeding-related hormones, and ingestive behaviors. Many of these rhythms are driven, at least in part, by circadian clocks. However, it has become clear that this is not a simple, linear relationship. Rather, metabolism and circadian clocks are tightly interlocked: clocks drive metabolic processes, and various metabolic parameters affect clocks, producing complex feedback relationships (Figure 1).

The mechanism underlying circadian rhythmicity is composed of a set of interlocking transcription/translation feedback loops that result in cascades of gene expression with 24 hr periodicity (for a more detailed review of this mechanism, see Lowrey and Takahashi, 2004) (Figure 2). At the core of this mechanism in mammals is the heterodimeric transcription factor complex of CLOCK and BMAL1, which activates transcription of the *Period* (*Per*) genes and *Cryptochrome* (*Cry*) genes via E-box enhancer elements in their promoters. The products of these genes interact to form complexes with each other. They also interact with other proteins such as casein kinase Iε. The PER/CRY repressive complex eventually translocates into the nucleus to inhibit CLOCK/BMAL1 transactivation activity, resulting in the repression of the *Per* and *Cry* genes. Removal of the repressor complex, at least in part through ubiquitination and degradation by the proteasome (Siepka et al., 2007), eventually relieves repression, thereby allowing the negative feedback loop to start again.

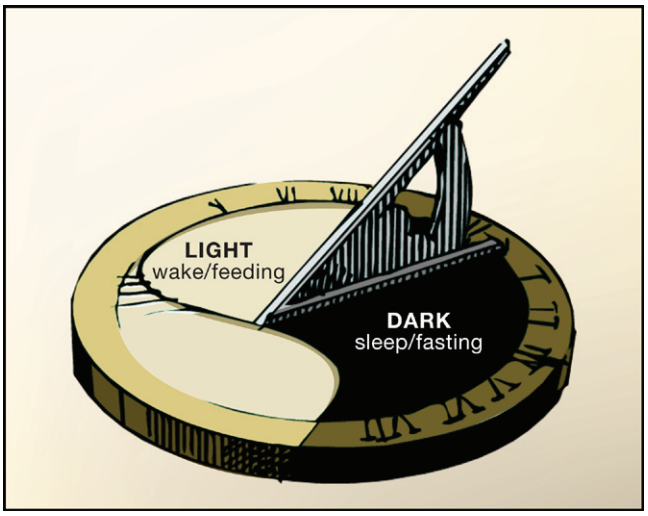

Figure 1. The Sleep/Wake and Fasting/Feeding Cycles
Genetic and molecular evidence suggest that within both the central nervous system (CNS) and peripheral tissues, coregulation of the daily alteration between the sleep/wake and fasting/feeding cycles reflects coupling of both behavioral and metabolic pathways. Because the availability of food and the risk of predators are tied to the environmental cycle of light and darkness, these interlinked cycles may have provided selective advantages. Although this review focuses primarily on the interdependence of circadian and metabolic systems, it is important to note that the circadian system also impacts sleep, the restriction of which is sufficient to lead to abnormalities in metabolic homeostasis.

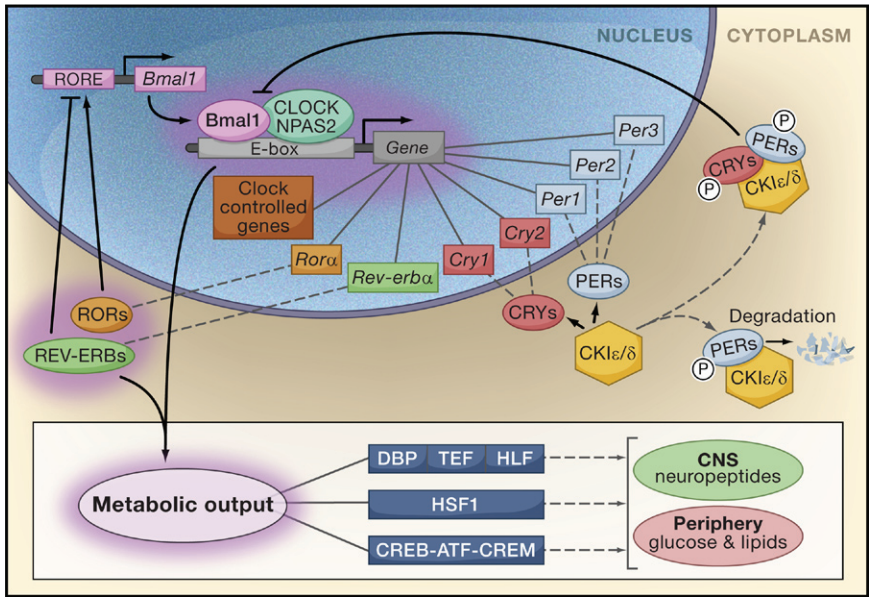

Figure 2. Building Blocks of the Molecular Clock

The discovery of the core components of the circadian clock was first revealed through forward genetics showing that the clock is encoded by a transcription-translation feedback loop that oscillates with a periodicity of 24 hr in pacemaker neurons and peripheral cells. Subsequent analyses have identified robust outputs of the clock on metabolic pathways in liver, fat, and muscle, suggesting convergence of circadian and metabolic pathways at the transcriptional level. The core mammalian clock is comprised of the heterodimeric activators CLOCK and BMAL1 that activate transcription of the genes encoding the repressors PERIOD (PER) and CRYPTOCHROME (CRY). An interlocked regulatory loop directs alternating activation and repression of *BMAL1* expression by the nuclear receptors RORα and REV-ERBα, respectively, via binding at the ROR enhancer elements (ROREs) in the *BMAL1* promoter. Several other metabolically active nuclear receptors and coactivators have been identified as modulators of BMAL and CLOCK, including PPARγ and PGC1α. Physiological outputs of the clock within both neural and peripheral metabolic tissues may originate either directly through the core clock proteins (shaded circles) or through the rhythmic expression of transcription factors (dark blue) that are modulated by the clock. DBP, Albumin D-binding protein; TEF, thyrotroph embryonic factor; HLF, hepatocyte leukemia factor; HSF-1, heat shock factor 1; CREB, cyclic AMP response element-binding protein; ATF, activating transcription factor; CREM, cyclic adenosine monophosphate response element modulator.

This core negative feedback loop is modulated by other interlocking feedback loops. The best characterized of these loops involves the orphan nuclear receptors REV-ERBα and RORα, which drive rhythmic *Bmal1* expression. The *Bmal1* promoter contains ROR enhancer elements (ROREs) that can be bound by RORα and REV-ERBα: RORα activates transcription, whereas REV-ERBα represses transcription. The *Bmal1* rhythmicity is driven by a rhythmic change in RORE occupancy by RORα and REV-ERBα. This alternating promoter occupancy occurs because REV-ERBα levels are robustly rhythmic, a result of direct transcriptional activation of the *Rev-erbα* gene by CLOCK/BMAL1 (Preitner et al., 2002).

This model of the molecular mechanism of the mammalian circadian clock has been developed relatively recently—the first mammalian clock genes were not cloned until 1997 (Antoch et al., 1997; King et al., 1997; Sun et al., 1997; Tei et al., 1997). Thus, much of the early work in circadian rhythms in mammals focused on behavioral assays such as locomotor activity. These studies found that the clock that controlled behavioral circadian rhythms was localized in the suprachiasmatic nucleus (SCN) in the hypothalamus (Ralph et al., 1990). It took the identification of core clock components that cycled at the mRNA level to provide "universal" markers for examination of rhythmicity in many tissues. The development of transgenic animals containing luciferase reporter constructs driven by the promoters of cycling clock genes was particularly instrumental in showing that circadian clocks existed in tissues throughout the body (Yamazaki et al., 2000; Yoo et al., 2004). The function of these peripheral clocks in most cases remains to be defined, but, as discussed below, it is likely that these clocks are important for driving local rhythms that are physiologically relevant for each specific tissue. The system appears to be arranged in a hierarchical manner with the SCN acting as the "master pacemaker" in mammals. The SCN drives rhythmic behavior and coordinates the many peripheral clocks through as yet poorly defined humoral and neural signals as well as indirectly by modulating activity and food intake (Figure 3). This complex interplay between the CNS and periphery in driving circadian rhythms is reminiscent in many ways of the metabolic system. The peripheral metabolic tissues respond in various ways to metabolic signals, and the CNS coordinates these peripheral events through direct control and indirect means such as regulation of food intake and energy expenditure.

Linking Circadian Rhythms to Metabolic Control

The pervasive circadian control of metabolism is exemplified by microarray studies that have examined gene expression profiles throughout the circadian cycle in the mammalian liver, skeletal muscle, and brown and white adipose tissue (Akhtar et al., 2002; Kita et al., 2002; McCarthy et al., 2007; Panda et al., 2002; Reddy et al., 2006; Storch et al., 2002; Ueda et al., 2002; Zvonic et al., 2006). The numbers of genes judged to be rhythmic ranged from 3% to 20% in the different microarray studies of gene expression profiles, suggesting that a large proportion of the transcriptomes in these tissues are under circadian control. Among the rhythmic genes identified, many have roles in biosynthetic and metabolic processes, including cholesterol and lipid metabolism, glycolysis and gluconeogenesis, oxidative phosphorylation, and detoxification pathways. Importantly, the rate-limiting enzymes in many of these pathways are under circadian control (Panda et al., 2002), suggesting that the clock's influence on these processes may be even broader than indicated by the numbers of rhythmic genes. The idea that these circadian oscillations in gene expression are physiologically meaningful is also supported by data showing that rhythmic genes in different tissues have varying degrees

of overlap, with different rhythmic gene patterns in the different tissues (McCarthy et al., 2007; Panda et al., 2002; Storch et al., 2002; Ueda et al., 2002; Zvonic et al., 2006). This suggests that rhythmic gene expression is regulated in a tissue-specific manner, enabling each cell/tissue to appropriately carry out its unique function.

Similar results were also found in a comprehensive analysis of nuclear receptor mRNA profiles measured throughout the daily cycle in liver, skeletal muscle, and brown and white adipose tissues (Yang et al., 2006). Of the 49 nuclear receptor mRNAs analyzed, more than half were rhythmic, with some variation between the profiles in the different tissues. The broad rhythmicity of this class of transcription factors, which interact with dietary lipids and fat-soluble hormones as ligands, suggests a rather direct link between nutrient-sensing pathways and the circadian control of gene expression.

Considering these circadian gene expression patterns, it is difficult to infer whether the rhythmic patterns are driven directly by the core circadian clock mechanism or instead are driven indirectly by the rhythmic feeding profiles in the animals being tested (Figure 4). Because many metabolic genes are affected by the availability of nutrients, rhythmic feeding is likely to generate many such rhythms. In addition, rhythmic activity of animals affects body temperature, which again may contribute to driving rhythms in gene expression. This issue becomes even more complicated when one considers that the clocks located in many peripheral tissues such as the liver are rapidly entrained to food availability. Therefore, many possible signals may control rhythmic gene expression in the liver, including the central SCN clock, the mysterious "food-entrainable oscillator" (see below) in the brain (through some type of systemic signal), the local endogenous liver clock (which is coupled to the SCN clock), or food availability (either through signals from the food or through food entrainment of the liver clock).

To address such issues, a conditional transgenic mouse has been generated in which the circadian clock can be reversibly and specifically disabled only in the liver, keeping the rest of the circadian system intact (Kornmann et al., 2007). Tetracycline-responsive, hepatocyte-specific overexpression of *Rev-erbα* in these mice causes constitutive repression of *Bmal1* transcription when the tetracycline analog doxycycline is absent, resulting in the loss of clock function. Normal *Bmal1* expression and normal hepatic circadian clock function is restored by feeding the mice doxycycline. Microarray analysis of the livers of the doxycycline-fed mice revealed 351 circadian transcripts, with significant overlap with the previous liver microarray data sets. However, after removal of doxycycline from the food, the great majority of these rhythmic genes no longer cycled in the liver, indicating that these genes are normally driven by the local liver clock and not by rhythmic systemic signals. Interestingly, 31 genes were still observed to have robust circadian patterns of expression in the absence of doxycycline, suggesting that this small subset of rhythmic liver genes is driven by some systemic signal independent of the liver clock. Thus, the control of metabolically relevant hepatic gene expression is complex, most likely responding to both external and cell-autonomous signals to maintain appropriate temporal coordination of these metabolic pathways.

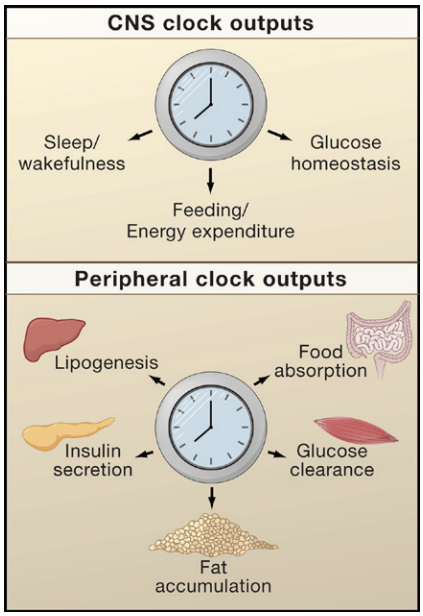

Figure 3. Central Pacemaker and Peripheral Clocks

The master pacemaker encoding the mammalian clock resides within the SCN, although clock genes are also expressed in other regions of the brain and in most peripheral tissues. Emerging evidence suggests that peripheral tissue clocks are synchronized through humoral, nutrient, and autonomic wiring and that the cell-autonomous function of the clock is important in pathways involved in fuel storage and consumption. A hierarchical model indicates that all peripheral clocks are subordinate to the SCN. However, more recent work suggests that peripheral clocks play a broader role than previously realized in health and disease.

Food Can Entrain Circadian Clocks

The circadian/metabolic relationship is complicated by the fact that many tissues and cell types contribute to the metabolic state. These tissues and cell types all contain endogenous circadian oscillators that communicate with each other in ways that are not well understood. With regard to the circadian system, the clock in the hypothalamic SCN is thought to be the "master clock" and is entrained by an external cue (zeitgeber): light from the eyes via the retinohypothalamic tract. This clock drives behavioral rhythms such as that of locomotor activity and coordinates the many peripheral clocks so that they maintain proper phase relationships with each other. It is now known that peripheral clocks can themselves also be entrained by various stimuli, with feeding being a dominant zeitgeber for many. In rodents that are given a restricted food (RF) paradigm where food is only available for a few hours during the day (a time when nocturnal rodents do not normally eat), the phase of many peripheral clocks shift by as much as 180° within about a week (Damiola et al., 2000; Stokkan et al., 2001). The liver clock entrains particularly rapidly to food availability, with large phase shifts within 2 days of an altered feeding schedule.

An interesting example of food's effect on the liver peripheral clock comes from studies of the common vole *Microtus arvalis*, which normally has an ultradian pattern of activity with short bouts of feeding every 2–3 hr (van der Veen et al., 2006). These animals have circadian rhythms of gene expression in their SCN but not in their livers. However, if the voles are given food on a circadian time schedule, or if their activity becomes circadian (this happens when they are given a running wheel), their livers now exhibit circadian rhythms of gene expression. Mouse livers do not show this flexibility; if a mouse is given ultradian exposure to food, their livers remain rhythmic, although

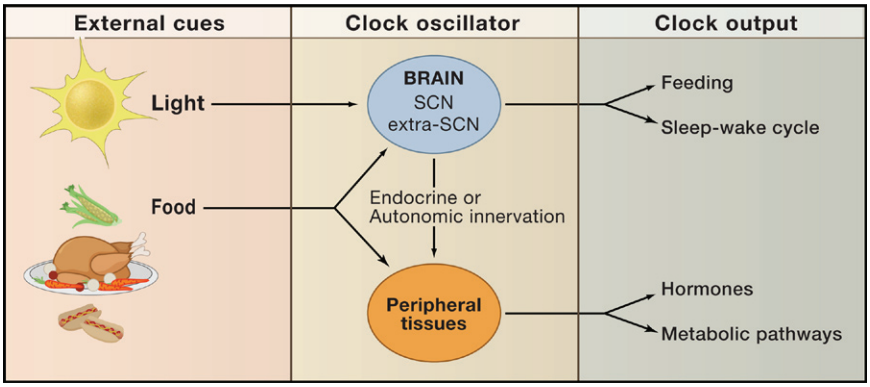

Figure 4. External Cues and Clock Outputs
The predominant external cue (zeitgeber) of the SCN clock is light. Clocks in peripheral tissues such as the liver also can be entrained by food. Nutrient and hormonal cues may also affect the period and phase characteristics of the master clock neurons, although little is known about how metabolic signals are communicated to the SCN. Outputs of both the SCN and peripheral clocks impact behavioral and metabolic responses such as feeding, sleep-wakefulness, hormone secretion, and metabolic homeostasis. Figure adapted from Ramsey et al., 2007.

the amplitude is somewhat dampened. These findings suggest that the rhythmicity of the liver is important for proper metabolic function and that the voles have evolved a system to allow them to normally suppress the circadian aspect of liver function to adapt to their ultradian pattern of food intake.

What are the signals that arise from feeding that entrain the liver clock and other peripheral oscillators? They could be food itself, food-induced metabolites, or hormones whose secretion is controlled by feeding or its absence. The mechanisms involved in the regulation of hepatic circadian oscillators are not yet fully understood, but several observations provide some insights into how this system may work. In cultured cell assays, many types of stimuli can induce or reset circadian clocks through regulation of clock gene expression (Balsalobre et al., 1998, 2000a, 2000b). These factors include forskolin, phorbol esters, glucose, and glucocorticoids, indicating that activation of many common signaling pathways can converge to influence the circadian clock. For example, in Rat-1 fibroblast cultures, insulin causes an acute induction of *Per1* mRNA production (Balsalobre et al., 2000b). Addition of a glucose bolus to the culture medium causes a downregulation of *Per1* and *Per2* mRNA levels and induces rhythmic gene expression patterns (Hirota et al., 2002). In this culture system, the downregulation of *Per* expression by glucose appears to be indirect (it is blocked by inhibition of transcription or translation) and seems to depend on glucose metabolism rather than glucose itself. Interestingly, it was recently reported that leptin causes upregulation of *Per2* and *Clock* gene expression in mouse osteoblasts, which have endogenous circadian clocks (Fu et al., 2005). Another example of clock regulation can be found in flies where the metabolic transcription factor FOXO has also been shown to modulate expression of clock genes and locomotor activity behavior (Zheng et al., 2007). Thus, multiple signals may be involved in the entrainment of clocks, including nutrients (sterols, lipids, and/or carbohydrates), humoral signals (insulin, glucocorticoid, and perhaps incretin), and possibly even signals from vagal efferents that travel from autonomic centers to the liver (Cailotto et al., 2008; Kalsbeek et al., 2004; Pocai et al., 2005).

Nutrient Signaling and Circadian Components

Many transcription factors that are known to respond to food or metabolites, such as nuclear receptors, also regulate components of the circadian clock. As mentioned above, a recent comprehensive survey of nuclear receptor mRNA profiles in several metabolic tissues in mice revealed that more than half of the known nuclear receptors exhibit rhythmic mRNA expression profiles (Yang et al., 2006). These receptors, which sense various lipids, vitamins, and fat-soluble hormones, are known mediators of metabolism. Direct links between some of these rhythmic nuclear receptors and the core clock components have been demonstrated. Therefore, these nuclear receptors may be part of the pathway by which food can entrain the liver clock (Ramsey et al., 2007).

The orphan nuclear receptors RORα and REV-ERBα are themselves considered part of the clock mechanism and contribute to an interlocking feedback loop that controls *Bmal1* transcription (Akashi and Takumi, 2005; Preitner et al., 2002; Sato et al., 2004). Interestingly, a critical role has recently been demonstrated for the peroxisome proliferator-activated receptor (PPAR) nuclear receptor coactivator PGC-1α in circadian regulation (Liu et al., 2007). PGC-1α is a not a nuclear receptor itself but is a transcriptional coactivator that regulates nuclear receptor activity and is a regulator of genes important in oxidative phosphorylation. PGC-1α stimulates *Bmal1* expression through coactivation of ROR proteins. Liver-specific knockdown of PGC-1α results in arrhythmicity, suggesting that this protein is required for normal clock function in this tissue. This protein is also of particular interest because it is inducible—sensitive to a variety of environmental signals including nutritional status, activity, and temperature—and is thought to regulate adaptive energy metabolism in multiple tissues (Leone et al., 2005; Lin et al., 2005). PGC-1α therefore may be a key component that couples metabolic signals with clock function.

Other genes have been implicated in clock regulation. For example, *Bmal1* is also transcriptionally regulated by the nuclear receptor PPARα, a key regulator of lipid metabolism (Canaple et al., 2006; Inoue et al., 2005). The glucocorticoid receptor has also been linked to the clock mechanism by the demonstration that glucocorticoids acutely induce *Per1* in cell culture and can reset peripheral clocks in vivo (Balsalobre et al., 2000a; Reddy et al., 2007). In addition, in vascular tissue, RARα and RXRα have been shown to bind directly to CLOCK and the closely related NPAS2 (MOP4) protein in a hormone-dependent manner, causing inhibition of CLOCK(NPAS2)/BMAl1 activity (McNamara et al., 2001). Two recent studies raise interest in the role of the histone deacetylase SIRT1 in the integration of circadian and metabolic transcription networks (Asher et al., 2008; Nakahata et al., 2008). *Sirt1* is an ortholog

of the yeast *Sir2* gene, a key factor in the longevity response to caloric restriction. SIRT1 has now been shown to interact directly with CLOCK and to deacetylate BMAL1 and PER2. Further studies will be necessary to delineate the physiological intersection between these sirtuin proteins and circadian pathways and the potential implications for the coordination of feeding, activity, and metabolic homeostasis.

Another possibility that has not been well studied is that CLOCK, NPAS2, BMAL1, or the PERs may themselves act directly as sensors for some feeding related signal. These proteins all contain PAS domains, an ancient sensory domain conserved throughout all kingdoms (reviewed in Gu et al., 2000; Kewley et al., 2004). PAS domains detect redox state, hypoglycemia, oxygen balance, and xenobiotic metabolism. Signals are transduced by PAS domains to the rest of the protein to induce changes in the functional state of the protein. Do these PAS-containing clock proteins have the ability to sense their environment and adjust their transcriptional activity accordingly? There are two interesting observations suggesting that this may be the case.

The first example comes from experiments suggesting that cellular redox status is capable of altering CLOCK/BMAL1 and NPAS2/BMAL1 activity (Rutter et al., 2001). The reduced forms of the nicotinamide adenine dinucleotide cofactors, NADH and NADPH, were shown to stimulate the DNA-binding activity of these clock protein heterodimers, whereas the oxidized cofactors inhibited DNA-binding activity. The conversion from active to inactive forms of CLOCK/BMAL1 (or NPAS2/BMAL1) occurs over a very narrow range of reduced to oxidized NAD ratios, making this a very sensitive switch. Therefore, changes in feeding that alter cellular redox status could use such a mechanism to rapidly affect circadian clock activity. The in vivo importance of this mechanism as part of the food-entrainment pathway still remains to be directly demonstrated. In agreement with this model for regulating circadian clock activity, addition of lactate (which should increase the amount of reduced NAD) to neuroblastoma cells does cause NPAS2/BMAL1 activation (Rutter et al., 2001). However, the addition of pyruvate (which should also increase the reduced form of NAD) to Rat-1 fibroblasts does not increase the relative levels of *Per1* or *Per2* (direct targets of CLOCK/BMAL1 and NPAS2/BMAL1) (Hirota et al., 2002). Clearly, additional experiments are needed to determine whether redox status is an important aspect of circadian regulation by food.

In the second example, NPAS2 was demonstrated to bind to heme and may use this cofactor as a gas sensor (Dioum et al., 2002). In the heme-bound form, the DNA-binding activity of NPAS2 was inhibited by carbon monoxide (CO) in a dose-dependent manner. This effect was distinct from the redox-sensing regulation described above, which does not require bound heme. The physiological role for CO sensing as a clock input is unclear, but NPAS2 is known to be important for regulating expression of the rate-limiting enzyme for heme biosynthesis (aminolevulinic acid synthase; ALAS). This NPAS2 function may form a feedback loop that communicates the status of heme metabolism to the clock mechanism (Dioum et al., 2002; Kaasik and Lee, 2004). Interestingly, recent studies of Rev-ERBα have suggested that heme is an endogenous ligand, raising the possibility that heme may play a central role in coordinating circadian function (Raghuram et al., 2007; Yin et al., 2007).

Nutrient Signals and SCN Oscillators

Feeding signals entrain peripheral clocks with great efficacy, whereas the central SCN clock appears to be largely refractory to these signals (Damiola et al., 2000; Stokkan et al., 2001). However, there is evidence that the SCN can receive and respond to signals from feeding under at least some conditions.

In cases where timed feeding is also coupled with caloric restriction, effects on the rat SCN can be observed by phase shifts in locomotor activity, body temperature, and melatonin rhythms (Challet et al., 1997). Entrainment to light-dark cycles is also altered (as measured both in activity rhythms and molecular rhythms in the SCN) (Mendoza et al., 2005b). In addition, light effects on the SCN are altered during times of low glucose availability (Challet et al., 1999). The rat SCN can also entrain to regular scheduled feeding (without caloric restriction), but this occurs much more slowly, taking nearly 12 weeks to reach stable entrainment (as compared to the 3 weeks required for rats under caloric restriction conditions) (Castillo et al., 2004). The reward aspects of food seem to be important in SCN entrainment, since rat SCNs can entrain to rhythmic palatable diet exposure on a background of constant feeding (ad lib) of regular diet in constant dark conditions (Mendoza et al., 2005a). Finally, under conditions of constant light that cause the rat circadian rhythms to become arrhythmic, restricted feeding was capable of rescuing both locomotor activity rhythms and *Per2* mRNA rhythms in the SCN (Lamont et al., 2005). Similar effects of restricted feeding on locomotor activity were previously also observed in hamsters maintained in constant light (Mistlberger, 1993).

Very little is known about how metabolic signals communicate with the SCN. The nature of the signals is unknown, and it is not clear whether this is a direct or indirect regulation. Receptors for leptin and ghrelin are present on SCN cells (Guan et al., 1997; Yi et al., 2006; Zigman et al., 2006), so it is possible that these important metabolic peptides signal directly to these neurons. Indeed, administration of ghrelin to SCN slices or SCN explants in vitro caused phase shifts in *Per2::luc* reporter gene expression (Yannielli et al., 2007). However, administration of ghrelin to wild-type mice only caused phase shifts after 30 hr of food deprivation, whereas intraperitoneal injection of ghrelin did not cause phase shifts in ad lib fed wild-type mice.

Neuropeptide Y (NPY) is a potent appetite transducer, and its secretion exhibits both ultradian and circadian rhythms. The relative levels of anorexigenic (leptin) and orexigenic (ghrelin) hormones participate with the circadian clock in the hypothalamus in the coordination of the temporal patterns of NPY secretion. During restricted feeding paradigms, the daily pattern of NPY shifts along with the locomotor activity to anticipate the food availability (reviewed in Kalra and Kalra, 2004; Sindelar et al., 2005). However, in addition to being an "output" of the SCN clock, NPY also acts as an "input" to the SCN, involved in communicating nonphotic signals (reviewed in Yannielli and Harrington, 2004). In addition, both histaminergic and serotonergic signaling pathways that influence feeding and energy metabolism in the hypothalamus have been shown to modulate both SCN oscillations and sleep (Challet, 2007; Masaki et al., 2004). Therefore, these signaling systems appear to be involved in some sort of feedback loop to link feeding and metabolic state to the SCN.

A Food-Entrainable Oscillator

Understanding of the effects of food on the circadian system is complicated by the presence of another somewhat mysterious oscillator, the food-entrainable oscillator (FEO). This oscillator controls circadian rhythms of food-anticipatory behavior and, as its name suggests, is entrainable by food. When rodents are maintained on a restricted feeding regimen (for example, food is available for only a few hours during the middle of the day), within a few days they show increased activity and other behavioral changes shortly before the time that food becomes available. This rhythmic anticipatory activity has the hallmarks of bona fide circadian rhythms. Namely, the rhythms are maintained for several days of total food deprivation (i.e., they "free run" in constant conditions), they show phase-dependent phase shifts with transients after a shift in mealtime, and they are subject to limits of entrainment (for example, rats can only entrain to food cycles between 22 and 31 hr in length) (reviewed in Stephan, 2002). Despite these similarities to the SCN oscillator, the FEO is anatomically distinct, because SCN lesions do not abolish the rhythmic food-anticipatory behavior. The anatomical location of the FEO is still not clear. Multiple lesion studies (ablation of the adrenals, hypothalamic structures, hippocampus, amygdala, and nucleus accumbens, among others) have failed to abolish this activity.

Although some consideration has been given to the idea that the FEO might reside within the digestive system itself, most studies point to a CNS location (Stephan, 2002). Recently, the dorsal medial hypothalamic (DMH) nucleus has received much attention as a possible oscillator site because of observations from two independent lines of experimentation. Mice lacking a functional *Per2* gene (but not those lacking *Per1*) lose food-anticipatory activity rhythms, suggesting that *Per2* is a critical component of the FEO system (Feillet et al., 2006). Examination of *Per2* expression throughout the brain revealed a robust rhythm in the DMH only when mice were entrained to restricted feeding conditions (Mieda et al., 2006). Furthermore, the DMH rhythm of *Per2* persisted through 2 days of total food deprivation. Recent neurotoxic DMH lesions resulted in significant losses of food-anticipatory rhythms (as measured by general cage activity, sleep, and body temperature), also supporting DMH as the location for the FEO (Gooley et al., 2006). However, robust food-anticipatory rhythms were maintained in DMH-lesioned animals in an independent study that examined a different behavioral readout (food bin approaches) (Landry et al., 2007). The difficulty in conclusively identifying the location of the FEO has raised the possibility that the FEO may not reside in a single structure but may rather be distributed among many sites (Davidson, 2006).

Effects of Circadian Control on Metabolism

In view of the fact that metabolic networks are under extensive circadian control at the levels of transcription, translation, and posttranslational mediated signaling, does the integration of circadian and metabolic cycles confer adaptive advantage and optimize energy utilization? Answers to this question have begun to emerge from studies in *Cyanobacteria* and the plant *Arabidopsis thaliana*. Observations in these model organisms indicate that synchrony of endogenous circadian period length with the environment may play a direct role in survival and optimize functions ranging from photosynthesis to reproduction (Dodd et al., 2005; Ouyang et al., 1998). *Cyanobacteria* strains with circadian periods similar to that of the light-dark cycle had a higher relative fitness than those with periods different from the environmental light cycle. Likewise, plants in which internal period length is aligned with the external light cycle display improved carbon fixation, growth, and reproduction. Those plants in which period length is discordant with the light cycle display reduced fertility and longevity. Interestingly, although circadian clocks have not been identified in yeast, these organisms exhibit robust metabolic cycles when cultured in nutrient-poor conditions (Chen et al., 2007). The periodic cycles of glycolysis and respiration are closely coupled to the replicative phase of the cell cycle. Intriguingly, disruption of the synchrony of these cycles resulted in an increased rate of spontaneous DNA mutation. Together, these studies suggest that rhythmic partitioning of metabolic processes can enhance survival by coupling the cell division cycle with cycles of energy storage and utilization. The availability of mutant mice with different circadian periods now provides the opportunity to formally test the concept that circadian "resonance" (i.e., synchrony between the external light-dark cycle and the internal period) is important in metabolic health and energy balance.

Physiological Roles of Clock Genes

Analysis of mammals with genetic lesions that disrupt circadian rhythms has recently provided insight into the role of several of the major circadian clock genes in physiology. Homozygous *Clock* mutant mice of the C57BL/6J genetic background have severe alterations in energy balance, with a phenotype with many characteristics of metabolic syndrome, including obesity, hyperlipidemia, hepatic steatosis, high circulating glucose, and low circulating insulin (Turek et al., 2005). The feeding rhythm in these mice is dampened, with increased food intake during the day, resulting in significantly increased overall food intake. It is likely that this phenotype results, at least in part, from altered rhythms of neuropeptides in the hypothalamus—ghrelin, CART, and orexin are all expressed at constitutively low levels in the *Clock* homozygous mutant mice (Turek et al., 2005). Whether loss of clock function in the peripheral tissues contributes to this metabolic phenotype still remains to be seen. Interestingly, a lean phenotype is observed in *Clock* mutant mice outbred onto an ICR genetic background. However, this phenotype is caused by impaired lipid absorption in animals with an ICR genetic background (Oishi et al., 2006).

Circadian Clock Function in the Periphery

An important metabolic role for circadian clock function in the periphery has also been observed in other genetic models. For example, alterations in lipid and glucose homeostasis occur with mutations in clock-related genes, such as the *Nocturnin* knockout mice. Although the *Nocturnin* gene is not itself part of the central clock mechanism, it is involved in posttranscriptional regulation of rhythmic gene expression (Baggs and Green, 2003; Garbarino-Pico et al., 2007). *Nocturnin*$^{-/-}$ mice have normal feeding behavior as well as normal food intake and activity levels. However, they remain lean on a high-fat diet. This is likely due to changes in lipid uptake or

utilization because these mice exhibit a loss of rhythmicity in genes important for these lipid pathways (Green et al., 2007). The *Nocturnin*$^{-/-}$ mice also have changes in glucose tolerance and improved whole-body insulin sensitivity.

Mice lacking the *Bmal1* gene also have altered gluconeogenesis and improved whole-body insulin sensitivity (Ramsey et al., 2007; Rudic et al., 2004), suggesting clock control of these pathways. Furthermore, loss of *Bmal1* in mouse embryonic fibroblasts results in the failure of these cells to differentiate into adipocytes. The overexpression of BMAL1 results in induction of several genes involved in lipogenesis and increased lipid synthesis, suggesting that the circadian clock may also be important in proper adipocyte function (Shimba et al., 2005). However, the pleiotropic functions of BMAL1 make assessment of the global knockout phenotype particularly difficult since these animals become cachectic with age and develop both arthritic and myopathic complications (Kondratov et al., 2006).

Mutation in another central clock gene, *Per2*, results in increased bone density in mice (Fu et al., 2005). Histomorphometric analyses showed that *Per2* null mice have an increased ratio of osteoblast to osteoclast activity, in addition to altered expression of *Cyclin D1* and *c-Myc*. These pathways are regulated by leptin, and it appears that *Per2* may play a role in mediating the leptin-dependent sympathetic regulation of bone formation. Since bone and adipose tissue share a common ontogeny, it is possible that these findings may also have implications for adipogenesis (Gimble et al., 2006). The *Per2* gene has also been implicated in cell cycle regulation in thymocytes (Fu et al., 2002). *Per2* null mice that have been irradiated with gamma radiation are more prone to tumor development, suggesting that *Per2* functions as a tumor suppressor in these cells.

Clock Gene Effects on Metabolic Tissues

Several studies have also suggested a link between circadian clock genes and cell survival that may have an important effect on metabolic tissues. For example, there is increased sensitivity of *Clock* mutant mice to either cyclophosphamide or partial hepatectomy (Gorbacheva et al., 2005; Matsuo et al., 2003). Moreover, both *Per1* mutant and *Clock* mutant animals display reduced expression of *c-Myc*, *cyclin D1*, and *Wee-1*, three cell cycle genes that exhibit pronounced circadian patterns of expression. CLOCK/BMAL1 may also impact cell growth, in part through direct effects on chromatin remodeling, or by influencing the generation of reactive oxygen species (ROS) (Doi et al., 2006; Kondratov et al., 2007).

How might molecular clock gene function influence the production of ROS? Studies of the clock disruption in myocardium have begun to shed light on the mechanism of ROS generation and its contribution to cardiovascular dysfunction. Under normal growth conditions in myocardial cells, circadian clock expression influences the selection of fuel source and the balance between β-oxidation and glycolysis (Durgan et al., 2006). Increased β-oxidation, which occurs after clock gene disruption in myocardium, contributes to increased production of ROS. Since altered circadian clock function may also limit the availability of glutathione, clock function may be important in ROS inactivation (Kondratov et al., 2006). Interestingly, recent work in *Foxo* mutant flies has suggested a tie between the generation of ROS and the regulation of the master neural pacemaker. Specifically, flies deficient in the FOXO transcription factor display increased oxidative stress after exposure to paraquat and altered circadian locomotor activity patterns (Zheng et al., 2007). Since the mammalian ortholog of the fly FOXO (Foxo-1) is an essential regulator of insulin signaling in liver and other metabolic tissues (Matsumoto et al., 2006), it will be important to identify upstream factors linking circadian and metabolic cycles. Investigation into the convergence of insulin signaling pathways with circadian systems may ultimately provide insight into the role of clock in β-oxidation and insulin resistance (Boudina et al., 2007).

Emerging evidence indicates that clock gene function also plays a crucial role in many other metabolic tissues (Young, 2006). For example, clocks within skeletal muscle may affect alternation between glycolysis and β-oxidation. Clock expression may also be important in adipogenesis (a process that also affects insulin sensitivity), as well as impact both adrenal and pancreatic physiology. Moreover, clock gene function has been shown to influence catecholamines within the adrenal medulla; circadian function at the level of both brain and peripheral organs may influence the counter-regulatory response to hypoglycemia (Bartness et al., 2001; Oster et al., 2006). These wide-ranging phenotypes of clock gene disruption may reflect physiological problems at the tissue level, or it may be that clock dysfunction alters the phase relationship among multiple peripheral metabolic tissue clocks, thereby causing disruption of overall internal circadian resonance. These studies beg the question of what precisely causes the metabolic phenotypes that occur in many of the circadian cycle mutant mice. Are the phenotypes due to a loss of circadian function within specific metabolically relevant tissues, or are they the result of certain metabolic tissues being prone to circadian-misalignment-induced dysfunction? In addition, it will be important to compare the impact of multiple clock gene mutations, in particular metabolic phenotypes, to distinguish between gene-specific effects and the more general effects on specific processes of disrupting the clock network. Soon, studies exploiting both conditional rescue and ablation approaches will provide opportunities to dissect the cell-autonomous and multitissue role of the clock gene network in metabolic physiology (Hong et al., 2007; McDearmon et al., 2006). Analysis of biological oscillations produced by the clock network provides an ideal model for complex systems biology. A full understanding of the dynamic function of the circadian network will require integration of genetic, cellular, and physiological analyses.

Mutations and the Circadian/Metabolic Systems

Mutations that affect the functions of the circadian system have a broad impact on metabolic function, but the reverse is also true. Mutations or challenges that cause metabolic problems also have been shown to affect the circadian system. Recent studies in high-fat-fed animals have shown that introduction of calorically dense chow leads to rapid changes in both the period of locomotor activity in constant darkness and to increased food intake during the normal rest period under

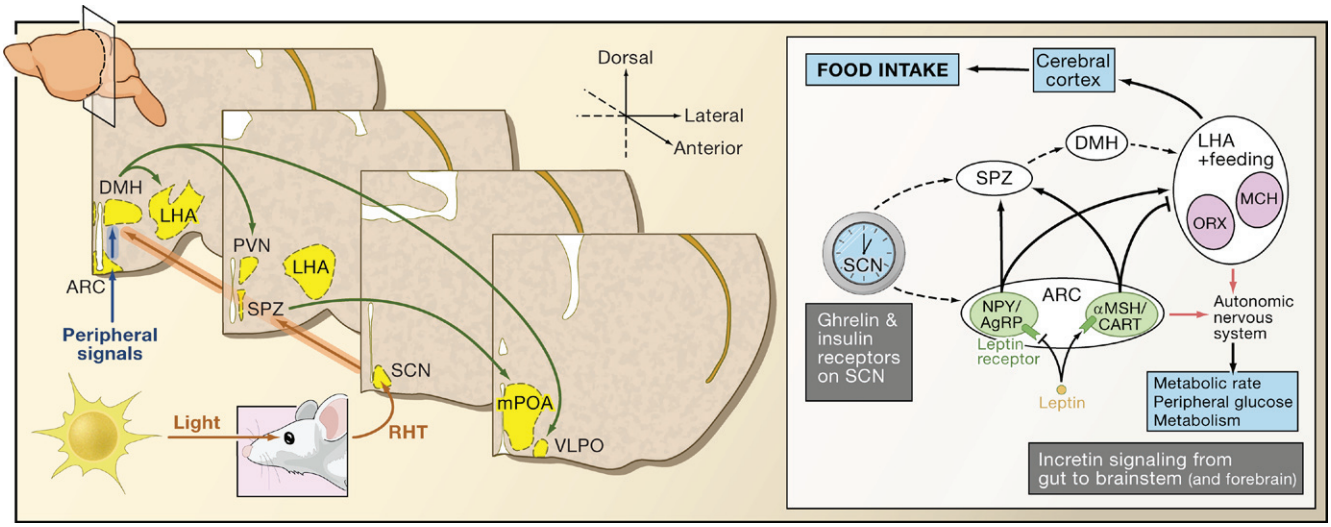

Figure 5. Neural Pathways Linking Circadian and Metabolic Systems

Neuroanatomical studies have implicated several nodal points that may connect circadian, sleep, and metabolic centers within the brain. External cues such as light are transmitted from the eyes via the retinohypothalamic tract (RHT) to the SCN. Projections from the suprachiasmatic nucleus (SCN) extend toward the subparaventricular zone (SPZ) and from the SPZ to the dorsomedial hypothalamus (DMH). Also shown are the medial preoptic area (mPOA) and the ventrolateral preoptic nucleus (VLPO), two relay regions of the hypothalamus that receive projections from the DMH and SPZ and may integrate circadian and wakefulness signals. *Clock* gene expression has been identified in DMH neurons, and the DMH has emerged as an important site in the activity response to food (the food-entrainable oscillator). The DMH has many outputs to other regions of the brain, including the lateral hypothalamus (LHA), which controls circadian regulation of the sleep/wakefulness and fasting/feeding cycles. Inset: The LHA also receives neuroendocrine input from the arcuate (ARC) neurons producing anorexigenic and orexigenic neuropeptides.The hormone leptin produced by adipose tissue activates the production of anorexigenic neuropeptides such as αMSH/CART, which in turn blocks production of the orexigenic peptides orexin (ORX) and melanin-concentrating hormone (MCH) in the LHA. In the absence of leptin, orexigenic neurons in the ARC produce the neuropeptide NPY/AgRP that stimulates hunger and decreased energy expenditure via signaling to the LHA. In addition to insulin, ghrelin, and other incretins may also influence circadian behavioral rhythms through direct effects on SCN or indirectly through actions within other regions of midbrain and brainstem. Arrows in inset indicate functional links.

12 hr light/12 hr dark conditions (Kohsaka et al., 2007). These changes in behavioral rhythmicity corresponded with altered clock gene expression within peripheral metabolic tissues, and as well as with altered cycling of the nuclear hormone receptors involved in sterol, lipid, and carbohydrate metabolism in both liver and visceral white adipose tissue. One implication of these findings is that nutritional status directly affects the phase of the SCN clock, as activity in darkness was lengthened. The findings also show that peripheral *clock* gene expression is sensitive to exposure to an obesity-inducing environment—a condition that is anticipated to reflect more common causes of clock dysfunction in humans than primary missense mutations in the function of the core *clock* genes.

In another example, locomotor activity rhythms were affected by disruption of the brain-specific Homeobox Factor *Bsx*, a gene encoding a recently identified key regulator of hypothalamic Npy/AgRP neuron development (Sakkou et al., 2007). *Bsx* mutant mice are not only resistant to obesity when crossed with leptin-deficient obese (*ob*) mice; they also display attenuated onset and dampened amplitude of nocturnal locomotor activity. Determination of the period length of locomotor activity under constant conditions in metabolic mutant mice will yield greater insight into the role of these signaling pathways in the SCN. They will also shed light on extra-SCN clock gene expression and function. Furthermore, understanding the effect of these important mediators of behavior and energy balance on both locomotor activity and sleep will broaden our knowledge of the links between circadian and metabolic systems.

Brain-Peripheral Tissue Crosstalk and Energy Balance

Identification of the interactions between transcriptional networks that control circadian, cell, and metabolic cycles may provide a framework to better understand the impact of circadian control not only within individual tissues, but also between brain and peripheral tissues that participate in energy storage and utilization.

Anatomical and molecular studies have colocalized several brain regions in which neural networks involved in circadian and metabolic systems overlap (Figure 5). Importantly, clock genes have been shown to cycle not only within the SCN, but also in several other brain regions. These regions include the forebrain in nuclei surrounding the third ventricle, where either orexigenic (NPY/AgRP) or anorexigenic (POMC/CART) neuropeptides are expressed, and the dorsomedial nucleus, a relay site to brainstem regions involved in wakefulness and sleep (Elmquist et al., 1998; Gooley et al., 2006; Mieda et al., 2006). Hypothalamic neuropeptides, particularly AgRP and POMC, are also expressed according to a pronounced diurnal rhythm, although the extent to which these oscillations are entrained by feeding, light, or nutrient signaling remains uncertain (reviewed in Kalra et al., 1999). Infusion of intralipid (an aqueous lipid emulsion), which induces elevation of circulating free fatty acids, has been shown to acutely modulate expression of orexin. The activity of orexin neurons has also been tied to glucose metabolism and leptin (Chang et al., 2004; Date et al., 1999; Yamanaka et al., 2003). Interestingly, recent studies in dogs fed high-fat diets suggest that nocturnal elevation (dur-

ing sleep) of free fatty acids may be an early hallmark of the insulin-resistant state (Kim et al., 2007). Taken together, both nutrients and the hormonal signals tied to nutrient balance are likely to impact regions of the hypothalamus important in control of circadian systems, wakefulness, and sleep.

Curiously, projections from the master pacemaker neurons of the SCN also synapse on the subparaventricular zone, a region that in turn projects to the lateral hypothalamic area (LHA) neurons that express hypocretin/orexin, indicating presence of a "hardwired" circuit joining centers involved in rhythmicity, wakefulness, and feeding (Horvath and Gao, 2005). Projections from forebrain regions also reach both the midbrain and the hindbrain to nuclei implicated in dopaminergic-reward aspects of feeding and in cholinergic- and incretin-mediated satiety signaling (Abizaid et al., 2006; Drucker, 2006; Farooqi et al., 2007; Fulton et al., 2006; Hagan et al., 2000; Harris et al., 2005; Williams et al., 2006). In *Clock* mutant mice, altered expression of hypothalamic neuropeptides within leptin-responsive neurons of the arcuate nucleus correlates with the development of hyperphagia and obesity. Both the temporal pattern and absolute expression levels of orexin and hypothalamic ghrelin are diminished in these animals (Turek et al., 2005). Moreover, *Clock* mutant animals display alterations in both hedonic drive (cocaine response) and cholinergic signaling (autonomic tone), suggesting additional neural effects of the clock network that may impact body weight and fuel utilization (Curtis et al., 2007; McClung et al., 2005; Reilly et al., 2007). A major goal now will be to unravel the role of clock gene expression within forebrain, midbrain, and hindbrain. It will be crucial to test the effect of clock gene function within neuronal subpopulations that may be important in the spectrum of metabolic and behavioral abnormalities that arise in mice harboring disrupted circadian cycle genes.

Food Ingestion and Circadian/Metabolic Systems

The prevailing model for understanding long-term body weight constancy holds that the tonic or baseline function of neurons in the hypothalamic orexigenic and anorexigenic centers is dynamically regulated by changes in the levels of peripheral hormones (principally leptin and insulin) that are produced in relationship to nutrient stores and food availability. The discovery of leptin was a watershed in yielding molecular insight into the pathways linking the peripheral and neural systems involved in feeding and energy expenditure. Although the major effect of leptin involves maintenance of body mass constancy during the challenge of famine, leptin itself is expressed in mice according to a diurnal rhythm (with relatively higher levels during the dark period) (Ahima et al., 1998). The absence of leptin during periods of food scarcity results in decreased energy expenditure and quiescent neuroendocrine systems, including the thyroidal and reproductive axes (Ahima and Flier, 2000). More recently, studies on hypothalamic nutrient sensing have uncovered an important role of hypothalamic leptin and nutrient signaling in the regulation of peripheral gluconeogenesis, a process that is particularly important during sleep, when the liver serves as the major source of glucose (Parton et al., 2007; Pocai et al., 2005; Sandoval et al., 2007).

Interestingly, although both leptin and melanocortinergic signaling mediate hypothalamic regulation of feeding, energy utilization, and glucose metabolism, the specific hypothalamic neurons involved in the control of gluconeogenesis appear to be distinct from those involved in energy expenditure. This suggests a sort of specialization within the neural energy circuit itself (Claret et al., 2007; Parton et al., 2007). With regard to hypothalamic control of the liver, it has also been demonstrated that mammalian glucose metabolism is subjected to pronounced diurnal variation across the light-dark cycle, with alternating cycles of gluconeogenesis and glycogen synthesis that are coordinated with the rest-activity cycle (reviewed in Ramsey et al., 2007). Temporal control of hypothalamic autonomic efferents to liver, fat, and possibly pancreas may play a role in human glucose constancy. Interestingly, in fibroblasts, activation of 5′-AMP-activated kinase (AMPK), an important nutrient sensor in the liver, led to a phase advance in clock protein expression. Activation of AMPK also correlated with phase advances in mice after treatment with metformin, an antihyperglycemic biguanide that is thought to target AMPK (Um et al., 2007). Clearly, it will be important to further examine the effects of the AMPK pathway on both peripheral interactions between circadian and metabolic pathways. AMPK signaling should also be assessed at the level of hypothalamic neurons, where AMPK has been implicated in feeding regulation (Martin et al., 2006).

In addition to evidence for interactions between circadian and metabolic systems at the level of adiposity hormones, the discovery of gut-derived polypeptides involved in control of meal size, meal timing, gastric emptying, and endocrine pancreas function provides an additional window into links between circadian and metabolic systems (Drucker, 2006; Murphy and Bloom, 2006; Nyholm et al., 1999). As mentioned above, studies with ghrelin have indicated effects on both SCN oscillators and locomotor activity patterns in mice, though the effects were dependent upon the nutritional state of the animal (Tang-Christensen et al., 2004; Yannielli et al., 2007). In addition, studies of gastrin-releasing peptide, a mediator of both feeding and locomotor activity, indicate an effect on circadian phase that opposes effects of NPY (discussed above) (Gamble et al., 2007; Ladenheim et al., 2002). Peptide tyrosine-tyrosine (PYY_{3-36}), a gut-derived member of the pancreatic polypeptide family, has also been shown to not only influence feeding but to also correlate with alterations to wakefulness and sleep architecture (Akanmu et al., 2006). A link between circadian physiology and responsiveness of endocrine pancreas to the incretin hormone glucagon-like peptide-1 has also been suggested in studies showing direct effects of melatonin on pancreatic β cells (Kemp et al., 2002). Studies of gastrointestinal clock gene expression in rodents have established expression of clock components within both stomach and colon, although clock function within the small intestine (specifically within cells that express anorectic and insulinotropic polypeptides) remains to be examined (Hoogerwerf et al., 2007). Nonetheless, the observation of clock gene expression within the colon is significant with regards to circadian control of incretin production, as there is abundant expression of PYY_{3-36} and related neuroendocrine polypeptides in the distal colon.

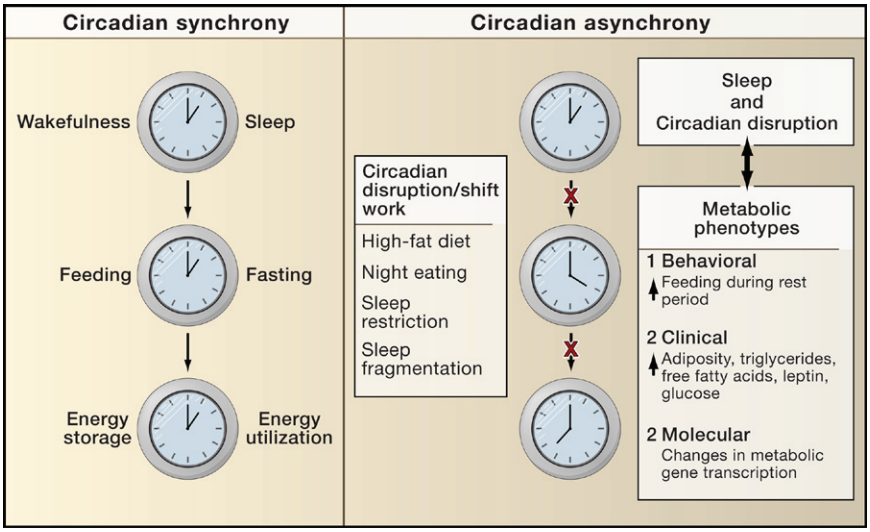

Figure 6. Circadian Synchrony and Metabolic Disease

Many aspects of metabolic physiology are known to occur at specific times each day. Gene expression patterns corresponding to periods of energy storage and energy utilization have been tied to the function of the peripheral clock in the liver and are shown as outputs of the clock (the cycle of energy storage and utilization). Many disorders such as myocardial infarction peak at certain times during a 24 hr day, suggesting a potential link between disruption of circadian rhythms and disease pathology. Emerging evidence suggests that disruption of synchrony between periods of rest/activity with feeding/fasting and energy storage/utilization may be tied to dysregulation of not only body weight but also diverse metabolic processes such as glucose metabolism, vascular reactivity, thrombosis, and lipid homeostasis.

Circadian Disruption and Metabolic Disease

At the clinical and epidemiologic level, several lines of evidence suggest that circadian disruption is associated with cardiovascular and metabolic complications across large segments of the human population (reviewed in Laposky et al., 2007) (Figure 6). Cross-sectional studies have uncovered an increased prevalence of metabolic syndrome, high body mass index (BMI), and cardiovascular events in shift workers (Ellingsen et al., 2007; Karlsson et al., 2001). These observations raise the possibility that chronic misalignment between the cycles of rest and activity, and those of fasting and feeding, may contribute to the initiation and progression of obesity and metabolic syndrome. With regard to feeding, intriguing behavioral studies in humans suggest that nocturnal feeding patterns ("night-eating syndrome") may represent an independent risk for metabolic disease (Allison et al., 2007). These findings are reminiscent of recent experimental rodent studies showing that excess energy intake during diet-induced obesity leads to increased energy intake only during the rest period and not during the active period when the animal normally eats (Kohsaka et al., 2007). Thus, it appears that the capacity to defend long-term energy constancy is related both to the time of day when food is consumed and to the relationship between meal (or snack) time and the sleep-wake cycle.

How might circadian misalignment impact the metabolic comorbidities of obesity, diabetes, and cardiovascular disease? Several lines of evidence suggest that circadian dysregulation may exert a broad impact not only on glucose control but also on inflammation, fibrinolysis, fluid balance, and vascular reactivity. Indeed, circadian control of glucose metabolism in humans is a well-recognized aspect of clinical diabetes management, and an alteration in the normal cyclic pattern of glucose tolerance is a hallmark of type 2 diabetes (Holterhus et al., 2007). This phenomenon of circadian control of glucose metabolism is perhaps most familiar to individuals with type 1 diabetes mellitus. These patients must adjust their daily insulin requirements around the light-dark cycle according to fluctuations in the basal requirement for insulin, as well as in response to changes in glycemic excursion after meals in the morning and at night (Van Cauter et al., 1997). Consideration of the physiological and molecular basis for changes in the circadian pattern of glucose tolerance, and for variation in the counterregulatory response to hypoglycemia, may ultimately lead to better strategies for diabetes management.

In addition to control of glucose metabolism, circadian systems may also participate in additional components of the metabolic syndrome. A central node linking metabolic and circadian pathways involves the nuclear receptor superfamily, as reviewed above. The pathways regulated include those downstream of Rev-ERBα and the RORs that modulate the core clock and diverse metabolic processes ranging from adipogenesis to inflammation and thrombosis (reviewed in Duez and Staels, 2008). Indeed, PAI-1, an important component of the fibrinolytic cascade that signals the breakdown of coagulation products, is subjected to circadian control and is a downstream target of Rev-erbα and RORα. Further studies are needed to address whether the strong clustering of acute cardiovascular catastrophes within restricted times of the day may be related to disruptions in normal circadian regulation of these pathways within liver, adipose tissue, or perhaps the vasculature.

In experimental animal models, repeated misalignment of internal phase with the light cycle, a so-called "jet lag" paradigm, has also been associated with accelerated cardiomyopathy and premature mortality (Davidson et al., 2006; Penev et al., 1998). Both epidemiologic and clinical studies have begun to probe whether restricted sleep, or altered cycling of sleep-wakefulness states, may contribute to hyperphagia and metabolic dysregulation. Reduced sleep time is associated with increased BMI, and forced sleep restriction in humans is tied to alterations of neuroendocrine control of appetite and glucose tolerance (Spiegel et al., 2004; Taheri et al., 2004; Tasali et al., 2008).

Although previous work has focused on the impact of sleep loss on cognitive and mood disorders, further investigation is needed to examine the link between altered circadian synchrony and human health. To achieve this goal, it will be necessary to establish standard methodologies for the analysis of circadian parameters in studies of feeding and glucose metab-

olism in human subjects. Furthermore, experimental genetic strategies need to be applied in animal models to pinpoint the underlying pathways important in coordinating these systems. The availability of temporal and metabolic phenotype data in humans will ultimately enable association studies to evaluate the contribution of circadian gene variation to human diabetes and obesity. Biological oscillations represent an area of metabolic physiology that, although familiar to most of us, is often overlooked in both clinical trial design and in experimental genetic studies. It seems opportune to consider timing and the clock system as we search for new mechanism-based treatments for metabolic disease.

Future Perspectives

The past decade has witnessed major strides in our understanding of the neurobehavioral basis of both feeding and circadian timing. Interestingly, both circadian and metabolic systems involve extensive crosstalk between CNS and peripheral tissues through humoral, nutrient, and direct neural wiring. At the organismal level, physiologic observations suggest that the circadian clock may be important in a wide range of pathologies that cluster at specific times of day, including myocardial infarction, arrhythmogenicity, congestive heart failure, thrombosis, and carbohydrate and lipid turnover. Yet, we still have little knowledge of the identities of the internal environmental sensors that couple circadian systems with processes ranging from nutrient homeostasis to coagulation, cytokine production, respiration, and myocardial contractility. In addition, circadian clock proteins, such as CLOCK and BMAL1, may also have functions independent of their roles as components of the circadian oscillator. Thus, both inter- and intraorgan asynchrony may also contribute to chronic pathologies such as diabetes, obesity, and cardiovascular disease. Current gaps in our understanding of the circadian system include the contributions of CNS oscillators versus peripheral oscillators for regulating circadian homeostasis and the detailed mechanisms by which peripheral organ clocks modulate tissue physiology. Studies of the circadian clock have opened a window on the molecular control of behavior, and now open a unique opportunity to probe the molecular links between behavior and metabolism.

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