Cell Metabolism
Review

Crosstalk between Components of Circadian and Metabolic Cycles in Mammals

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In mammals, most metabolic processes are influenced by biological clocks and feeding rhythms. The mechanisms that couple metabolism to circadian oscillators are just emerging. NAD-dependent enzymes (e.g., Sirtuins and poly[ADP-ribose] polymerases), redox- and/or temperature-dependent transcription factors (e.g., CLOCK, NPAS2, and HSF1), nutrient-sensing transcriptional regulatory proteins (e.g., CREB-CBP-CRCT2, FOXO-p300, nuclear receptors, PGC-1, and SP1 family members) and protein kinases (e.g., AMPK), are plausible candidates for conveying a cell's metabolic state to the core clock circuitry. The intertwining between these acute regulators and circadian clock components is so tight that the discrimination between metabolic and circadian oscillations may be somewhat arbitrary.

Introduction

It is likely that biological clocks have originally evolved in single-celled organisms to orchestrate cellular metabolism and adapt it to environmental conditions. The growth and proliferation of such organisms requires a huge number of chemical reactions, and some of these interfere with each other. In both unicellular and multicellular organisms, there are two ways to separate chemically incompatible pathways: their spatial sequestration to different subcellular compartments and their temporal distribution to different time windows. The latter process could, in principle, be handled solely by acute regulatory mechanisms, that is, mechanisms responding directly to changes in substrate concentrations. Mammalian glucose homeostasis—and in particular glycogen anabolism and catabolism in the liver—is a nice example for such substrate-driven processes (Lam et al., 2009). The maintenance of nearly constant plasma glucose levels is important, since insufficient glucose levels would compromise the functions of neurons while, at least in humans, excessive glucose levels disrupt the balance of body fluids and electrolytes (Tzamaloukas et al., 2008). It is then not surprising that glucose homeostasis involves a puzzling array of local and central glucose sensing mechanisms and a large number of hormones that influence uptake, storage, synthesis, and catabolism of carbohydrate fuels. It would be desirable to stockpile carbon fuels—like glucose—in the form of inert polymers—like glycogen—when they are plentiful and to disassemble these polymers when energy resources are on short supply. All this needs is a glucose-sensing mechanism that modulates the antagonistic activities of the stockpiling and storage consumption machineries in a concentration-dependent manner (Agius, 2008). When food-derived glucose enters liver cells or when glucose is produced in large amounts by hepatic gluconeogenesis, it causes the release of glucokinase (GK) from an inert nuclear complex containing the GK regulatory protein (GKRP). The derepressed GK then phosphorylates glucose to glucose-6-phosphate (G6P), and G6P acts as an alosteric inhibitor of glycogen phosporylase (GP) by promoting the dephosphorylation of this enzyme. Phosphorylated (active) GP is a potent inhibitor of glycogen synthase phosphatase (GSP), an enzyme that removes inhibitory phosphate groups from glycogen synthase (GS). Hence, the G6P-induced dephosphorylation of GP relieves the inhibition of GS and thereby turns the switch toward glycogen synthesis. When glucose is needed by cells incapable of gluconeogenesis, the glycogen stores are disassembled by phosphorolysis, a process initiated by the reduction of G6P levels. Acute signaling pathways of a similar complexity also govern the catabolism and anabolism of fatty acids, cholesterol, and bile acids, as well as the clearance of toxic xenobiotics (Jump et al., 2005; Kalaany and Mangelsdorf, 2006; Modica et al., 2009).

At the same time, all of these metabolic pathways also receive inputs from local and central circadian clocks. Thus, glycogen metabolism and gluconeogenesis are influenced by various clock-dependent mechanisms in addition to—or rather in cooperation with—nutrient sensing acute regulatory pathways. For example, mice with a liver-specific knockout of *Bmal1* display hypoglycemia during the postabsorptive phase, supposedly because acute signaling by glucose-sensing hormones (e.g., glucagon) is insufficient to compensate for the lack of food-derived glucose during this time window (Lamia et al., 2008). Conversely, the simultaneous inactivation of *Cry1* and *Cry2*, whose products attenuate CLOCK-BMAL1 transcriptional activity, results in hyperglycemia (Zhang et al., 2010).

In contrast to the reactive nature of acute regulatory mechanisms, the circadian timing system allows their possessors to anticipate metabolic reactions during the course of the day in a proactive manner. Xenobiotic detoxification nicely illustrates this case (Claudel et al., 2007). Food frequently contains noxious chemicals in addition to valuable nutrients, and these toxic xenobiotics must be inactivated and excreted by a detoxification system operative in a variety of tissues, including liver, kidney, and intestine. The detoxification system is composed of toxin-sensing transcription factors (e.g., the nuclear receptors PXR, CAR, and PPARα and the aryl hydrocarbon receptor AHR), monooxidases of the cytochrome P450 family, conjugating enzymes, ATP transporters, heme-synthesizing enzymes like

aminolevulinic acid synthase 1 (heme is the prosthetic group of cytochrome P450 enzymes), and cytochrome P450 oxidoreductase (required for the regeneration of cytochrome P450 enzymes) (Claudel et al., 2007). The expression of many of these regulators and enzymes oscillates in a circadian manner, and, as a consequence, the tolerability toward numerous noxious constituents varies dramatically during the day (Gachon et al., 2006). Hence, although the detoxification system is induced by toxic chemicals and thus contains an acute regulatory component, the circadian time keeping system greatly enhances its efficacy (Claudel et al., 2007).

Several excellent and comprehensive reviews have recently been published on the circadian orchestration of metabolism and the possible consequences of its disruption for health and disease (Green et al., 2008; Maury et al., 2010; Reddy and O'Neill, 2010; Takahashi et al., 2008). In this article, we will discuss recently emerging evidence suggesting that the temporal coordination of metabolism is accomplished by a tight crosstalk between components of acute signaling pathways and the core clock circuitry.

Cellular and Molecular Architecture of the Circadian Timing System

In mammals, the circadian timing system has a hierarchical architecture, composed of a central clock in the brain and subsidiary oscillators in virtually all cells of the body (Dibner et al., 2010). The brain's master pacemaker resides in the suprachiasmatic nuclei (SCN) and is synchronized to daily light-dark cycles by direct photic inputs received from the retina through the retinohypothalamic tract. The SCN then sets the phase of cell-autonomous, self-sustained cellular oscillators in peripheral tissues through a variety of signaling pathways, some of which will be discussed below.

Circadian oscillators have virtually the same molecular makeup in SCN neurons and peripheral cell types. However, while they are coupled in the SCN via synaptic and paracrine signals (Liu et al., 2007a; Maywood et al., 2007; Welsh et al., 2010), they do not appear to communicate with each other in peripheral tissues or cells grown in tissue culture. Therefore, as a result of intercellular differences in period length, phase coherence between cells is gradually lost in tissues of SCN-lesioned animals (Guo et al., 2006), tissue explants (Yamazaki et al., 2000; Yoo et al., 2004), and transiently synchronized cultured fibroblasts (Balsalobre et al., 1998; Nagoshi et al., 2004). The coupling of molecular oscillators in SCN neurons also renders them more resilient to genetic perturbations. For example, while in vitro-cultured dissociated SCN neurons and fibroblasts require the clock gene isoforms *Cryptochrome 1* (*Cry1*) and *Period 1* (*Per1*) to keep their clocks ticking, cellular SCN oscillators in organotypic brain slices from *Cry1* or *Per1* knockout mice are perfectly functional (Liu et al., 2007a).

The major purpose of the circadian clockwork circuitry is to produce rhythms in behavior and physiology. With regard to circadian metabolism, this can be accomplished by the rhythmic expression of output genes encoding regulators and enzymes of various metabolic pathways. A single circadian transcription factor with an inflexible activity phase would hardly fit the organism's needs, since different clock output pathways must have different phases. This is particularly important for clock-controlled mechanisms designed to sequester chemically incompatible pathways to different time windows. The currently held model for the molecular clockwork circuitry (Dibner et al., 2010), although still sketchy, offers plausible mechanisms for how vastly different phases in output gene regulation can be accomplished (Figure 1). The two period genes *Per1* and *Per2* and the two cryptochrome genes *Cry1* and *Cry2* lie at the heart of the circadian oscillator. The transcription of these genes is under the positive control of the PAS domain helix-loop-helix proteins CLOCK and BMAL1 and under the negative control of their own products, the PER and CRY proteins. CLOCK-BMAL1 heterodimers bind to E or G boxes within *cis*-acting promoter and enhancer regions of *Per1/2* and *Cry1/2* genes

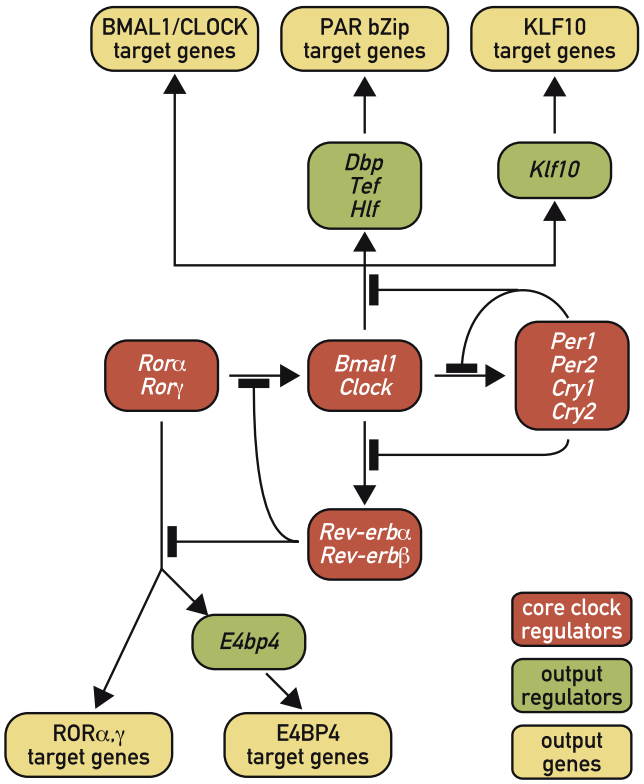

Figure 1. The Generation of Different Phases by the Molecular Oscillator

The molecular oscillator in peripheral organs consists of two interlocked feedback loops. The major and essential loop involves the transcriptional activation of period (*Per1*, *Per2*) and cryptochrome (*Cry1*, *Cry2*) genes by the PAS domain helix-loop-helix transcription factors CLOCK and BMAL1, and the autorepression of *Per* and *Cry* genes by their own protein products (see text). An accessory feedback loop is established through negatively and positively acting nuclear orphan receptors: the activators RORα and RORγ compete with the repressors REV-ERBα and REV-ERBβ for the occupancy of RORE elements within promoter and enhancer regions of *Bmal1* and possibly *Clock*. The circadian transcription of REV-ERBα (and probably REV-ERBβ) is governed by the major PER/CRY feedback loop, and the activity of RORE-driven transcription is thus nearly antiphasic to of E box-driven genes. Further phases are generated through additional clock-controlled transcription factors such as PAR bZip proteins and KLF10, whose expression is E box dependent, and E4BP4, whose expression is RORE dependent, (output regulators). The major and accessory feedback loops can therefore regulate widely different phases of genes encoding metabolic enzymes and modulators (output genes) involved in rhythmic metabolism. For the sake of simplicity, the cartoon does not include a large number of posttranscriptional regulatory events that contribute to circadian oscillator function and output.

and thereby stimulate the transcription of these genes. As a consequence, PER and CRY proteins accumulate and form heterotypic complexes with additional polypeptides, including the casein kinase 1 isoforms CK1δ and CK1ε (Lee et al., 2009), the putative splicing factor NONO, and the WD repeat protein 5 (WDR5) (Brown et al., 2005). These multisubunit complexes associate with CLOCK-BMAL1 heterodimers in the nucleus and annul their transcription activation potential. Chromatin immunoprecipitation studies and in vitro binding assays demonstrate that the DNA-binding activity of CLOCK and BMAL1 fluctuates with a robust daily amplitude, suggesting that the negatively acting PER-CRY complexes drive cyclic CLOCK-BMAL1 activity by attenuating the affinity of these transcription factors for DNA. The autorepression of *Per1/2* and *Cry1/2* genes by PER and CRY leads to the decrease of these proteins, and once their activity falls below a critical threshold level, a new cycle of *Per1/2* and *Cry1/2* transcription can ensue. The cyclic transactivation activity of the CLOCK-BMAL1 heterodimer also governs the robustly circadian expression of *Rev-erbα* and *Rev-erbβ*, encoding the heme-binding nuclear orphan receptors REV-ERBα (Nr1d1) and REV-ERBβ (Nr1d2). The repressors REV-ERBα and REV-ERBβ bind to RORE elements within the *Bmal1* promoter and thereby inhibit the circadian transcription of *Bmal1* and, to a lesser extent, of *Clock*. RORE elements are also bound by the three members of the RAR-related orphan receptors RORα, RORβ, and RORγ, which in contrast to REV-ERBα and REV-ERBβ act as transcriptional activators. While RORα and RORγ accumulate in most cell types, the expression of RORβ is neuron specific. Circadian *Bmal1* transcription is thus accomplished by the competition of ROR activators and REV-ERB repressors. Given that *Rev-erbα* and *Rev-erbβ* are target genes of BMAL1-CLOCK heterodimers, REV-ERB repressors and ROR activators couple the REV-ERB feedback loop driving cyclic *Bmal1* transcription directly to the central PER-CRY feedback loop. Although the REV-ERB feedback loop renders the circadian oscillator more stable, it is dispensable for circadian rhythm generation (Preitner et al., 2002). This suggests that circadian *Bmal1* mRNA accumulation is not a sine qua non for keeping the PER-CRY feedback loop—and hence circadian rhythm generation—operative. Rather, the major purpose of the accessory REV-ERB/ROR feedback loop may be the generation of a phase for output gene expression that is almost antiphasic to that governed by the canonical PER-CRY/CLOCK-BMAL1 feedback loop. Hence, the clockwork circuitry can directly regulate the expression of clock-controlled genes at two widely different phases (Ukai and Ueda, 2010). For example, in mice, the expression of E box- and RORE-driven genes reaches acrophases during the late afternoon and late night, respectively (Preitner et al., 2002; Ueda et al., 2002). Each of the two coupled feedback loops also regulates the expression of genes encoding circadian output transcription factors. For example, the cyclic transcription of the PAR bZip protein genes *Dbp*, *Hlf*, and *Tef* is accomplished through the interaction of CLOCK-BMAL1 with E boxes (Ripperger and Schibler, 2006) ,while that of the *Nfil3/E4bp4* gene involves the interplay of REV-ERBs with RORs on ROREs (Le Martelot et al., 2009). Since the accumulation of PAR bZip and *E4bp4* messenger RNAs (mRNAs) and proteins requires some time, the phases of these circadian output regulators are delayed by a few hours with regard to the phases of the core clock regulators that drive their rhythmic transcription (Ukai-Tadenuma et al., 2008).

In addition to the transcriptional mechanisms described above, many posttranscriptional events—and in particular post-translational protein modifications and protein-protein interactions—contribute to the molecular rhythm generation. Indeed, it cannot yet be firmly excluded that posttranscriptional regulatory mechanisms are actually the primarily driving force in circadian rhythm generation, similar to what has been described for the KaiABC phosphorylation clock in cyanobacteria (Nakajima et al., 2005). In the mammalian system, all known core clock components can be phosphorylated in a daytime-dependent fashion (Vanselow and Kramer, 2007). Moreover, some clock proteins can be acetylated (BMAL1, PER2) (Asher et al., 2008; Hirayama et al., 2007), sumoylated (BMAL1) (Cardone et al., 2005; Lee et al., 2008), ubiquitinated (probably most clock proteins) (Mehra et al., 2009), and poly(ADP-ribosylated) (Asher et al., 2010). Several excellent review articles on post-translational clock protein modifications have recently been published (Gallego and Virshup, 2007; Mehra et al., 2009), and in this article we will only refer to protein modifications possibly related to the interaction between metabolic and circadian cycles.

Systemic versus Local Circadian Regulation

The first indications pointing toward a tight connection between metabolism and circadian clocks came from genome-wide transcriptome profiling studies (Akhtar et al., 2002; Duffield et al., 2002; Hughes et al., 2009; Kornmann et al., 2007; Panda et al., 2002; Storch et al., 2002). For example, depending on the stringency of the algorithms and the sampling frequency used, between 2% and 15% percent of all expressed genes appear to follow daily accumulation cycles, some of which with vastly different phases. Among these transcripts, a large fraction encodes enzymes and regulators of carbohydrate, cholesterol, lipid metabolism, and endo- and xenobiotic detoxification. Circadian transcriptomes also encompass mRNAs specifying transcription regulatory proteins, which supposedly serve as output regulators of circadian clocks. In the liver, these include the three PAR bZip activator proteins DBP, TEF, and HLF (Gachon, 2007), the PAR bZip related repressor NFIL3/E4BP4 (Mitsui et al., 2001), the Kruppel-like factor KLF10/mGIF/TIEG1 (Hirota et al., 2010), and 20 nuclear hormone and orphan receptors, all known to be transcriptional regulators of metabolism (Yang et al., 2006). For several of these transcriptional regulators, knockout mice have been established and their liver transcriptomes have been compared with those of wild-type mice. Many target genes identified for these circadian transcription factors specify primarily enzymes involved in nutrient processing and metabolism (e.g., Gachon, 2007; Guillaumond et al., 2010; Le Martelot et al., 2009; Leuenberger et al., 2009).

The circadian system can influence the timing of metabolism through systemic cues, emanating from the SCN master clock, and through local oscillators present in peripheral tissues (Figure 2). For the mouse liver, the discrimination between these two routes has been accomplished by a circadian transcriptome analysis in mice with or without functional hepatocyte clocks (Kornmann et al., 2007). These experiments unveiled that about 50 out of a total 350 rhythmic mRNAs continued to display daily fluctuations with little changes in phase, amplitude, and

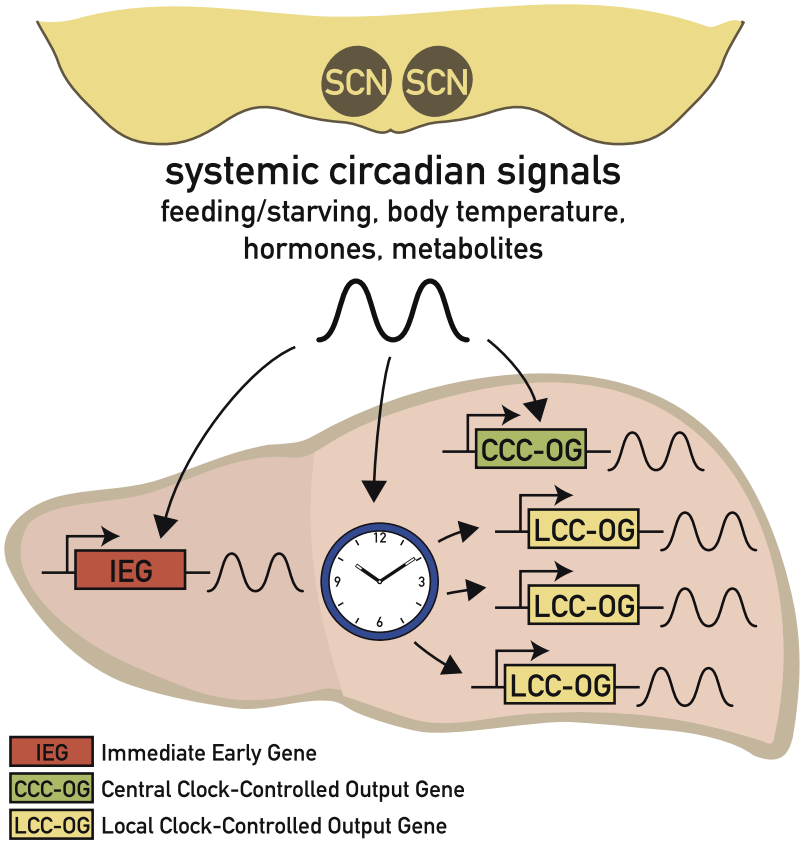

Figure 2. Circadian Gene Expression in Peripheral Tissues Can Be Regulated by Cyclic Systemic Cues and via Local Oscillators

The mammalian circadian timing system consists of an hierarchically organized network of self-sustained, cell-autonomous oscillators. The master pacemaker in the SCN, in the brain synchronizes subsidiary clocks in most body cells by controlling a variety of rhythmic signals, including cyclic hormones, body temperature oscillations, and—by imposing feeding-fasting rhythms. These signals are interpreted by immediate early genes (IEGs) conveying rhythmic systemic information to core clock genes or clock output genes. Hence, daytime-dependent gene expression in peripheral tissues, such as the liver shown in the cartoon, can be governed by synchronized local clocks (LCC-OG, local clock-controlled output genes) or directly via systemic signals emanating from the SCN master clock (CCC-OG, central clock-controlled output genes).

magnitude in liver cells with arrested oscillators. Hence, in liver the fractions of cyclic transcripts depending on systemic signals and local oscillators amount to approximately 14% and 86%, respectively. The systemically regulated liver genes would be expected to include (1) immediate early genes, which convey rhythmic signals to core clock genes of hepatocyte oscillators and thus are involved in the synchronization of liver clocks, and (2) genes directly participating in rhythmic liver physiology and metabolism. Although the discrimination of these two gene categories can be ambiguous in certain cases, it is likely that representatives of both classes were indeed identified among system-driven loci. The immediate early gene class contains several heat shock protein genes, known to be regulated by heat shock transcription factor 1 (HSF1), and target genes of serum response factor 1 (SRF1). These two immediate early transcription factors are thus likely players in the synchronization of circadian clocks.

The tight connection between circadian and metabolic cycles is supported by the impact of feeding rhythms on the phase of clocks in many peripheral tissues, including liver, pancreas, heart, skeletal muscle, and kidney (Damiola et al., 2000). In fact, when feeding rhythms are inverted from night to day feeding, the phase of peripheral circadian clocks gradually adapts to the new feeding regimen, reaching an inverted steady state phase after 10 to 14 days. Irrespective of whether the animals are kept under light-dark cycles (LD) or constant darkness (DD), the feeding inversion has little impact on the phase of the SCN. Therefore, feeding-fasting cycles are very strong Zeitgebers for peripheral clocks, even dominating the synchronization cues emitted by the master clock in the SCN. Obviously, under normal conditions the food- and SCN-dependent timing cues are not in conflict. Rather, the SCN imposes feeding rhythms by driving rest-activity cycles and thereby "uses" food-dependent Zeitgebers in addition to other cyclic cues (like body temperature or blood-borne signals) to set the phase in the periphery (Figure 3).

The characterization of signaling pathways participating in the phase entrainment of peripheral clocks is still in its infancy. At least in part, the difficulty in identify such pathways lies in their redundancy. Thus, the genetic abrogation of any particular pathway may have little impact on the steady state phase, since many parallel routes are employed to synchronize subsidiary oscillators in peripheral organs. The redundancy problem can be circumvented by recording the kinetics of feeding induced phase adaptation (Figure 3). As outlined above, the SCN and feeding rhythms transmit conflicting Zeitgeber cues to peripheral organs when feeding rhythms are reversed. Under such conditions, the elimination of direct SCN-dependent signaling pathways should accelerate feeding-induced phase inversion, while the absence of nutrient-dependent signaling pathways should delay this process. Experiments designed on the basis of this hypothesis have suggested that the glucocorticoid hormone receptor and poly (ADP-ribose) polymerase 1 (PARP-1, see below) participate in the phase resetting of liver clocks, the first as an SCN-dependent regulator and the latter as a feeding-dependent regulator (Asher et al., 2010; Le Minh et al., 2001).

The global impact of feeding on rhythmic liver gene expression was recently revealed by a circadian liver transcriptome profiling study by Panda and coworkers in wild-type and behaviorally arrhythmic *Cry1/Cry2* double-knockout mice (Vollmers et al., 2009). As expected, temporal changes in feeding regimens led to a phase adaptation of cyclic mRNAs in wild-type mice. More importantly, feeding rhythms could phase-entrain the accumulation of over 600 mRNAs in the liver of clock-less *Cry1/Cry2* double-knockout mice. These transcripts were issued primarily

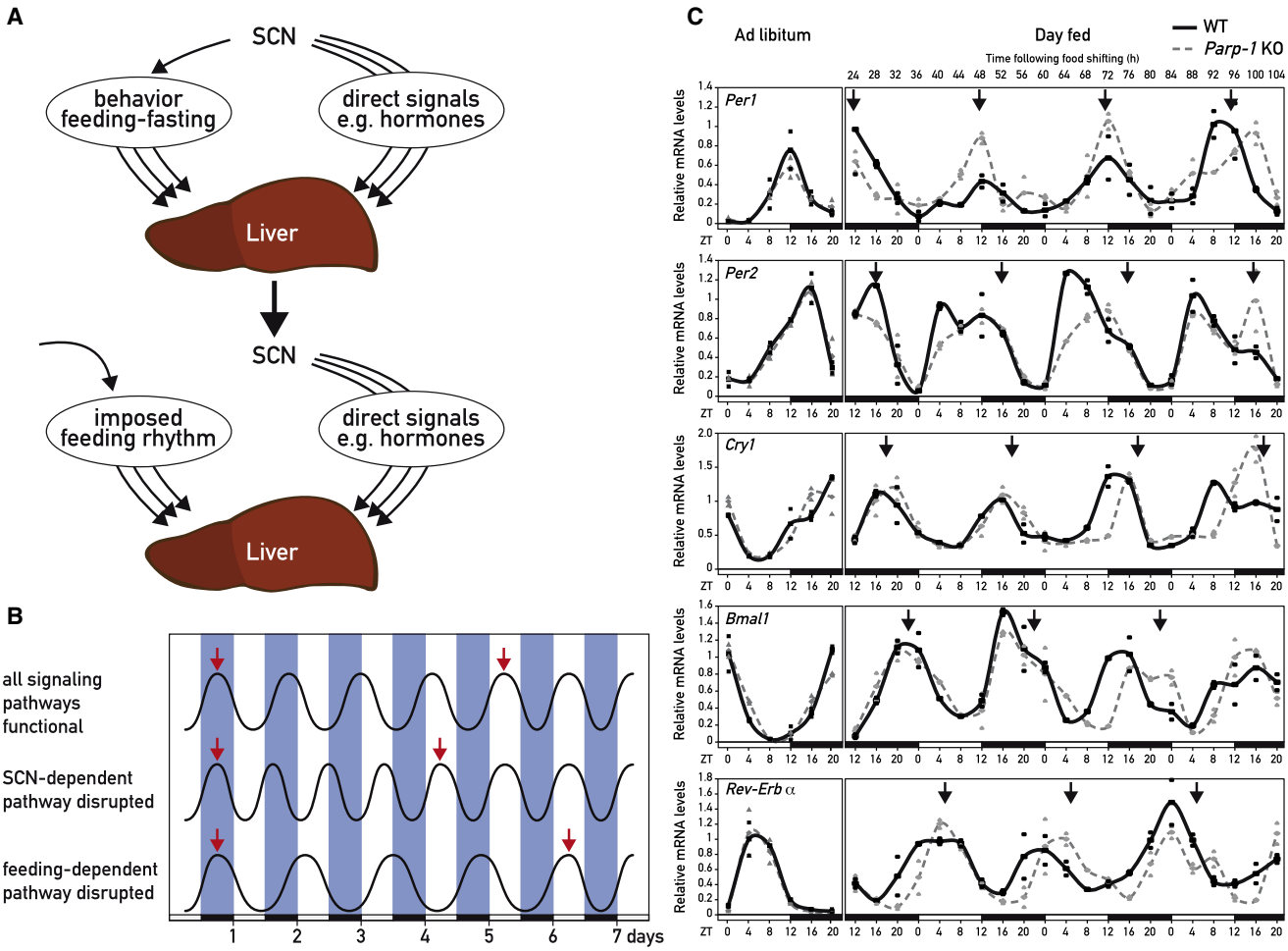

Figure 3. Kinetics of Circadian Gene Expression upon Inverting the Feeding Regimen

(A) The SCN master clock phase-entrain peripheral clocks through direct signaling pathways, such as cyclic hormones, (e.g., glucocorticoids) and indirect pathways depending on rest activity cycles, (e.g., feeding rhythms). In the cartoon, it is arbitrarily assumed that both direct cues and feeding rhythms synchronize peripheral oscillators (like those operative in the liver) through three different molecular signaling pathways. Under normal conditions, all of these pathways cooperate and act in synchrony. Feeding rhythms are dominant over direct SCN synchronization cues, and when antiphasic feeding rhythms are imposed upon animals (daytime-feeding for nocturnal mice and rats), they will eventually invert the phase of peripheral clocks and thereby uncouple it from the phase of the SCN pacemaker.

(B) In mice the food-driven phase inversion requires several days. The disruption of nutrient-dependent and SCN-dependent signaling pathways will slow down and accelerate, respectively, the kinetics of phase-resetting of peripheral circadian gene expression. The left and right red arrows in each tracing indicate, respectively, the phase (time of maximal gene expression) before day feeding and the new steady-state phase reached after several days of daytime feeding.

(C) Phase adaptation of circadian gene expression in the liver of wild-type and *Parp-1* knockout mice after switching from ad libitum feeding (under which condition the mice ingest food primarily during the night) to daytime feeding. Note that the accumulation of mRNAs issued from different clock genes displays different kinetics of phase resetting. The black arrows indicate the phase (maximal expression during ad libitum feeding). This illustration is adapted from Figure 6 in Asher et al. (2010), with permission from Cell Press.

from target genes of transcription factors known to serve as nutrient and stress sensors, such as CREB, SREBP1/2, FOXO1, HSF1, and ATF6. As the molecular circadian oscillators of all cells are inactivated in *Cry1*/*Cry2* double-mutant mice, this study could not assess the effect of feeding rhythms on circadian pathways downstream of core clock transcription factors and clock-controlled output regulators, such as the PAR bZip proteins DBP, HLF, and TEF. It would therefore be interesting to conduct similar studies with mice harboring functional circadian clocks in the periphery, but incapacitated oscillators in the brain. This could be accomplished by performing circadian liver transcriptome profiling studies with SCN-lesioned mice or mice with neuron-specific clock gene disruptions.

Crosstalk between Nutrient-Sensing Regulators and Circadian Clock Components

As aforementioned, many signaling pathways may transmit food-dependent cues to core components of the circadian clock. These include nutrient-sensing hormones, protein kinases, and nuclear receptors, as well as redox-sensing enzymes and transcription factors. Below, we discuss a few regulators that are plausible candidates for conveying nutrient-dependent metabolic states to cellular circadian oscillators. We are aware of the somewhat arbitrary subdivision of these players into different classes. In reality, the mechanisms elaborated below are not working in isolation, but are part of a highly interactive metabolic network.

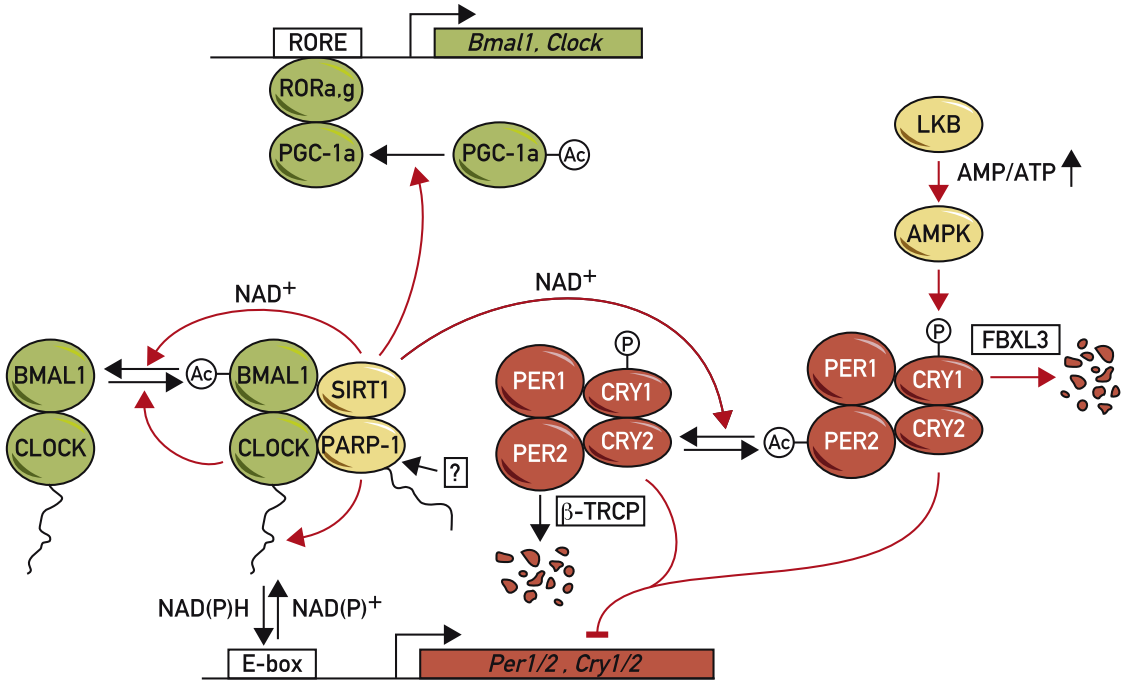

Figure 4. Interaction of Metabolic Regulators with Clock Components

The cartoon schematically represents interactions of metabolic regulators (yellow) with core clock components of the negative limb (red) and the positive limb (green) of the coupled feedback loops. FBXL3 and β-TRCP are F box proteins of ubiquitin ligase complexes that mark CRY and PER proteins, respectively, for degradation. LKB phosphorylates AMPK when the AMP/ATP ratio increases. AMPK then phosphorylates CRY1 and thereby targets it for degradation. SIRT1 deacetylates BMAL1 (which can be acetylated by CLOCK), PER2 (acetyl-transferase yet unknown), and the coactivator PGC-1α (which can be acetylated by the general control nonrepressed protein 5 [GCN5] and p300) (Dominy et al., 2010). The SIRT1-mediated deacetylation decreases the half-life of PER2 and enhances PGC-1α activity, which together with RORα,γ activates *Bmal1* transcription. The NAD^+ levels oscillate in a circadian fashion. In addition to modulating the activity of transcription factors by deacetylation, SIRT1 may also affect circadian transcription by the deacetylation of histone H3 tails, as suggested by (Nakahata et al., 2008). The box with a question mark indicates an unknown regulator of circadian PARP-1 activity. Red arrows indicate interactions involving metabolic regulators, and black arrows represent interactions between clock components.

NAD-Dependent Enzymes: SIRT1 and PARP-1

Nicotinamide adenine dinucleotides $NAD(P)^+$ and NAD(P)H serves as a readout for the cellular redox and metabolic states. The first clues for the implication of $NAD(P)^+$ and NAD(P)H in the modulation of circadian clock components came from a biochemical study by Rutter and colleges (Rutter et al., 2001). At least in the test tube, the binding of CLOCK-BMAL1 and NPAS2-BMAL1 heterodimers to their E box cognate sequence is exquisitely sensitive to the $NAD(P)^+$/NAD(P)H ratio. While reduced NADH and NADPH stimulate this process, their oxidized equivalents NAD^+ and $NADP^+$ strongly inhibit it. Obviously, this mechanism would only be relevant if NAD levels were subject to daily oscillations. In liver, this was found to be the case, (Nakahata et al., 2009; Ramsey et al., 2009) in part because of the rhythmic expression of nicotinamide phosphoribosyltransferase NAMPT (the enzyme driving the NAD^+ salvage pathway) produces oscillations in cytosolic—and probably also nuclear—accumulation of NAD^+. It should be emphasized, however, that cytosolic and, supposedly, nuclear NAD^+ levels can be modulated by many additional mechanisms, for example by the reduction of pyruvate to lactase in glycolysis, by NADPH consumption during fatty acid synthesis, and by the DNA damage-induced activation of PARP-1, which in cultured cells can dramatically reduce NAD^+ levels (Berger, 1985). In addition to the CLOCK/NPAS2-BMAL1 heterodimers, the NAD^+-dependent enzymes SIRT1 and PARP-1 are plausible candidates as NAD^+ sensors. SIRT1 is a protein deacetylase that deacetylates not only histones but also several transcription factors (Blander and Guarente, 2004). Recently, the levels (Asher et al., 2008) and/or activity (Nakahata et al., 2008) of SIRT1 were found to be daytime dependent. Furthermore, SIRT1 physically interacts with CLOCK-BMAL1 heterodimers, and this leads to the rhythmic deacetylation of BMAL1, histone H3 (Nakahata et al., 2008), and PER2 (Asher et al., 2008). In the case of PER2, the SIRT1-mediated deacetylation leads to a decrease in stability, and the increased accumulation of PER2 protein in SIRT1-deficient mouse embryonic fibroblasts (MEFs) severely compromises circadian gene expression in these cells. SIRT1 may also influence circadian gene expression and metabolism in the liver by modulating the activity of the transcription factors PPARα (Purushotham et al., 2009) and FOXO3 (Brunet et al., 2004) and the coactivators PGC-1α (Rodgers et al., 2005) and CRTC2 (also known as TORC2) (Liu et al., 2008), which are known SIRT1 targets. PGC-1α has recently been shown to be tightly linked to core clock function, both in fibroblasts and in the liver. It is expressed in a circadian fashion, activated by SIRT1-mediated deacetylation, and serves as a coactivator of ROR orphan nuclear receptors, which are pivotal transcriptional activators of *Bmal1* transcription (Figures 1 and 4) (Liu et al., 2007b). The FOXO1-CBP/p300 and CREB-CBP/p300-CRTC2

transcription factor-coactivator complexes appear to orchestrate gluconeogenesis in a time-specific fashion after the onset of fasting. In the fed state, FOXO1 and CRTC2 reside as inert phosphoproteins in the cytosol. During early fasting, these proteins become dephosphorylated, migrate into the nucleus, and become acetylated by the CBP/p300. This acetylation activates CRTC2, but inactivates FOXO1. During a more prolonged fasting (>6 hr), NAD^+ levels and SIRT1 activity increase, and this leads to the deacetylation of CRTC2 and FOXO1. Whereas the NAD^+-dependent, SIRT1-mediated deacetylation stimulates the transactivation potential of FOXO1, it dampens that of CRTC2 (Liu et al., 2008). Conceivably, the acetylation state of FOXO1 and CRTC2 could also convey nutrition states to the circadian clock and clock-controlled genes. Remarkably, cAMP signaling via CREB and related factors has been shown to be an integral part of the molecular circadian oscillator, both in SCN neurons (O'Neill et al., 2008) and peripheral cell types (Wang and Zhou, 2010). This underscores again how tightly acute signaling pathways are intertwined with core clock mechanisms. In fact, these processes are probably inseparable in many instances, similar to what is described above for SIRT1- and cAMP-dependent signaling of metabolic states to the circadian clockwork circuitry.

Mice with a hepatocyte-specific disruption of *Sirt1* display liver steatosis and an upregulation of SREBP-1a/c and SREBP-2 target genes encoding enzymes involved in fatty acid and cholesterol synthesis (Ponugoti et al., 2010). Recently, a plausible mechanism underlying this phenotype has been uncovered. SREBP isoforms have been known to be acetylated by CBP/p300 at lysine residues in their DNA-binding domains. Walker et al. now demonstrated that SIRT1-mediated deacetylation reduces the stability of SREBP proteins (Walker et al., 2010). Thus, the inactivation of SIRT1 elicits a higher than normal accumulation of SREBPs, and this leads to increased cholesterol and fatty acid synthesis. Sterols and free fatty acids serve as ligands and modulators for several nuclear receptors, such as LXR, the three PPAR family members (Hong and Tontonoz, 2008), and ROR isoforms (Wang et al., 2010a). In liver, PPARα and RORs have been shown to conduct partially redundant function in activating *Bmal1* transcription (Canaple et al., 2006), and it can be speculated that their relative contribution to regulating circadian *Bmal1* transcription is influenced through their interaction with fatty acids and sterols, respectively. Indeed, fatty acids serve as activating ligands for PPARα by promoting their interaction with coactivators (Viswakarma et al., 2010), whereas oxysterols reduce the transactivation potential of RORs by compromising the recruitment of coactivators (Wang et al., 2010a; Wang et al., 2010b). In addition, several nuclear receptors, including PPARα and RORs, have recently been shown to modulate PER2 activity by direct physical interactions (see below) (Schmutz et al., 2010).

It should be emphasized that the nutrient-related SIRT1 regulatory mechanisms outlined above have been performed on fed and starved mice. Hence, it is not yet clear to what extent imposed restricted feeding regimens reflect the physiology relevant during normal feeding-fasting cycles of animals fed ad libitum. During the postabsorptive resting phase, laboratory rodents still ingest about 15% to 20% of the total daily food rations (Kohsaka et al., 2007), and some of the molecular responses observed in artificially fasted animals may not be elicited during the postabsorptive phase of natural feeding rhythms in animals having free access to food. In future experiments, it will therefore be important to compare the phase shifting kinetics of circadian gene expression in wild-type mice and mice with a hepatocyte-specific disruption of *Sirt1*, in order to examine whether the rhythmic SIRT1 activity indeed participates in the food-driven synchronization of circadian gene expression in vivo. Such experiments have recently been performed for PARP-1, another potential NAD^+ sensor (Asher et al., 2010). In mice subjected to daytime feeding, the phase inversion of clock gene expression was significantly delayed in the liver of *Parp-1* knockout mice when compared to wild-type mice, suggesting that PARP-1 activity is indeed implicated in the phase entrainment of peripheral oscillators. The precise molecular mechanisms involved in this process are still elusive, but biochemical studies revealed a clear connection between PARP-1 activity and circadian gene expression. Thus, PARP-1-mediated poly (ADP-ribosylation) activity is daytime dependent, reaching zenith and nadir values at ZT04–ZT08 and ZT16, respectively. As cyclic PARP-1 activity persists in liver with disabled hepatocyte oscillators and is phase-inverted in day-fed animals, it is probably driven by feeding rhythms rather than local circadian clocks. For several reasons, however, NAD^+ oscillations engendered by circadian NAMPT expression are unlikely to account for rhythmic poly(ADP-ribosylation) activity: (1) the phase of PARP-1 activity does not match that of hepatic NAMPT expression and NAD^+ accumulation, (2) unlike NAMPT expression, oscillating PARP-1 activity does not require the CLOCK-BMAL1 heterodimer, (3) cyclic PARP-1-dependent poly(ADP-ribosylation) can be reconstituted in a cell-free system with temporally staged liver nuclear extracts in the presence of a huge excess of NAD^+, and (4) PARP-1 requires the BRCT protein interaction domain to display daytime-dependent activity. PARP-1 poly(ADP-ribosylates) not only itself, but also histones and a number of transcription factors, including CLOCK (Asher et al., 2010) and SP1 (Zaniolo et al., 2007). Interestingly, both of these transcription factors display circadian DNA-binding activity, which in both cases appears to be blunted by PARP-1-mediated poly (ADP-ribosylation). At least in vitro, the binding of SP1 complexes to a GC-rich recognition sequence within the *Per2* promoter is dramatically increased in *Parp-1* knockout mice. PARP-1 also binds to FOXO1 and thereby attenuates its transactivation potential. Although FOXO1 does carry poly(ADP-ribose) chains, it appears that the physical interaction of this protein with PARP-1, rather than poly(ADP-ribosylation), modulates the activity of FOXO1 (Sakamaki et al., 2009).

The interactions between the metabolic sensors SIRT1, PARP-1, and AMPK (see below) and core clock components are schematically outlined in Figure 4.

Nuclear Receptor-Dependent Mechanisms

Of the 49 nuclear receptor genes of the mouse genome, 20 are expressed in a circadian manner in liver, at least on the mRNA level (Yang et al., 2006). These include all three members of the PPAR (PPARα,β/δ,γ) and ERR families (ERRα,β,γ), both REV-ERB isoforms (REV-ERBα,β), two ROR isoforms (RORα,γ) FXRβ, SHP, a small heterodimerization partner and antagonist of several nuclear receptors, thyroid receptor α (TRα), and orphan receptors serving as immediate early transcription

factors in signaling pathways. Virtually all of these nuclear receptors are involved in the regulation of catabolic and anabolic metabolic processes. In addition, some of them may also modulate the activity of circadian clock function, either as direct players of the core clock circuitry (RORs, REV-ERBs; see above), by directly interacting with them (see next paragraph), or by stimulating or repressing pathways producing putative small molecular ligands for core clock components. For example, LXR stimulates whereas FXR and SHP repress the expression of *Cyp7a1*, encoding the rate-limiting enzyme in the conversion of cholesterol to bile acids (Inagaki et al., 2005; Peet et al., 1998). These receptors may thus have an impact on the intracellular levels of various sterol compounds, which in turn may suppress the transactivation activities of the core clock transcription factors RORα and RORγ in the liver.

Several nuclear receptors expressed in hepatocytes have recently been shown to physically interact with PER2 (Schmutz et al., 2010). These comprise PPARα, HNF4, TRα, NURR1, REV-ERBα, and, to a lesser extent, RORs. The surface of PER2 necessary for these interactions encompasses an LXXLL motif, which in coactivators mediates the binding to their cognate nuclear receptors. It is not yet clear whether and in what cases the association of PER2 with nuclear receptors leads to a stimulation or repression of target gene expression. However, in cotransfection experiments, PER2 potentiated the activation of a *Bmal1*-luciferase reporter gene, conceivably by competing with the corepressor N-CoR1 binding to REV-ERB repressors (Schmutz et al., 2010). The PER2-REV-ERBα interaction also participates in the orchestration of glucose homeostasis, in particular by modulating glycogen metabolism and gluconeogenesis. Thus, in mice deficient for both PER2 and REV-ERBα, glycogen levels are nearly invariable throughout the day. This glycogen phenotype is accompanied by the constant expression of G6P and the temporally deregulated expression of the transcripts encoding glucokinase, glycogen synthase, glycogen phosphorylase, phosphofructokinase, fructose-1, 6-bisphosphatase, the glucose transporter Glut2, and phosphoenolpyruvate carboxykinase (PEPCK), (Schmutz et al., 2010).

Glucose Sensing by the Circadian Repressor TIEG1/mGIF/KLF10

Circadian gene expression in cultured fibroblasts can be transiently synchronized by the activation of a puzzling variety of signaling pathways, and most of these trigger the acute induction of period gene expression (*Per1* and/or *Per2*) to higher than circadian zenith levels (Balsalobre et al., 2000; Balsalobre et al., 1998). PER protein overexpression rapidly represses the transcription of *Per* and *Cry* genes to nadir values and thus synchronizes all cells to the same phase within a few hours. High glucose concentrations can also synchronize fibroblast clocks, but they do so by an opposite mechanisms, namely by acutely repressing the transcription of *Per1*, *Per2*, and *Bmal1* genes (Hirota et al., 2002). DNA microarray studies identified the mRNA encoding TIEG1 (also known as mGIF and KLF10; see above), a negatively acting Zn^{2+} finger transcription factor of the Sp1 family, to be strongly upregulated after glucose treatment (Hirota et al., 2002). The same group has now deciphered a molecular pathway that may account for the glucose-dependent downregulation of *Bmal1* transcription (Hirota et al., 2010). TIEG1/MGIF/KLF10 binds to two GC-rich elements within the *Bmal1* promoter and, at least in cotransfection experiments, dampens *Bmal1* transcription. More importantly, *Tieg1/mGif/Klf10* mRNA accumulation follows a robust diurnal rhythm in mouse liver that, at least in part (see below) may be driven by oscillating intracellular glucose concentrations. Work by Teboul and coworkers with *Tieg1/mGif/Klf10* knockout mice adds another facet to this story (Guillaumond et al., 2010). These mutant mice display a postprandial and fasting hyperglycemia, and transcriptome profiling studies revealed 158 TIEG1/MGIF/KLF10 target genes involved in glucose and lipid metabolism. Hepatic glucose overproduction by an abnormally high expression PEPCK (the rate-limiting enzyme of gluconeogenesis) probably accounts for the daytime-dependent hyperglycemia in TIEG1/MGIF/KLF10-deficient mice. The same authors also established that robust circadian *Tieg1/mGif/Klf10* transcription requires *Bmal1*, as the mRNA specified by this gene accumulated to low, nearly constant levels in *Bmal1* knockout mice. Hence, TIEG1/MGIF/KLF10 appears to be imbedded into a feedback loop circuitry involving core clock transcription factors and glucose homeostasis. Circadian CLOCK-BMAL1 activity and glucose absorbed with the food or generated by gluconeogenesis stimulate *Tieg1/mGif/Klf10* expression. This leads to the repression of genes encoding enzymes involved in gluconeogenesis and glucose export. Curiously, this feedback loop seems to affect glucose homeostasis only in males, as female mice are normoglycemic. In females, TIEG1/MGIF/KLF10 is however implicated in circadian lipid and cholesterol homeostasis (Guillaumond et al., 2010).

Body Temperature, Feeding, and Heat Shock Transcription Factor 1

Systemically regulated genes identified in the liver of mice with disabled hepatocyte clocks include several heat shock protein/chaperone genes (*Hsp1a*, *Hspca*, *Hspa4*, *Hspa41*, *Hsp8*, *Hsp110*, and *Sip1*) (Kornmann et al., 2007). Moreover, HSF1, the major regulator of temperature-dependent gene expression of these genes, has been identified in a screen dubbed Differential Display of DNA-binding proteins (DDDP) as a rhythmically active transcription factor in liver (Reinke et al., 2008). As insinuated by its name, HSF1 has initially been thought to require elevated temperatures, such as those caused by high fever, for its activation. In nonstressed cells, HSF1 forms an inert complex with chaperones (mostly HSP90) that rapidly shuttles between the cytoplasm and the nucleus. Elevated temperature and stress-inducing chemicals like reactive oxygen species lead to the denaturation of proteins, and the denatured polypeptides compete with HSF1 for the binding to HSP90, thereby liberating HSF1 from the complex (Whitesell and Lindquist, 2009). Free HSF1 then associates into homotrimers that bind heat shock element (HSE) in promoter regions of HSF1 target genes. In addition, the activity of HSF1 depends on the phosphorylation by several protein kinases. For example, phosphorylation by the kinases polo-like kinase 1 (PLK1) and calcium/calmodulin-dependent protein kinase II (CaMKII) increase the HSF1 transactivation potential, while phosphorylation on certain HSF1 residues by glycogen synthase kinase 3 beta (GSK-3β), PKC isoforms, and extracellular signal-regulated kinase (ERK1) attenuate it (Whitesell and Lindquist, 2009).

Although the expression of HSF1 target genes is indeed highest at the maximal temperature tolerated by cells, shallow

temperature oscillations imposed on cultured fibroblasts are sufficient to drive robustly rhythmic transcription of a reporter gene carrying multiple HSF1 recognition sequences (HSEs) (Reinke et al., 2008) and to synchronize circadian gene expression (Brown et al., 2002; Buhr et al., 2010). Hence, body temperature rhythms are likely to contribute to the synchronization of peripheral oscillators in the intact animal via the modulation of HSF1 activity. This conjecture is further supported by the observation that the accumulation of the mRNA encoding CaMKII, an HSF1-activating kinase, follows a system-driven daily rhythm (Kornmann et al., 2007).

In the liver, HSF1 activity can also be induced by feeding, and it was proposed that the purpose of nutrient-induced heat shock protein accumulation may reduce the oxidative stress caused by feeding (Katsuki et al., 2004). Indeed, in cultured cells, HSP27 overexpression led to an increase of glutathione production. As feeding rhythms are the dominate Zeitgebers for clocks in many peripheral tissues, HSF1 may be one of the many players conveying nutrient signals to the circadian clockwork circuitry. Intriguingly, HSF1 is also a substrate of SIRT1 (Westerheide et al., 2009), a well established NAD^+ sensor. HSF1 is acetylated on nine lysine, of which one (K80) is located within the DNA-binding domain. In the nonacetylated state, K80 increases the affinity to DNA by establishing a salt bridge with a phosphate of the DNA backbone. Acetylation of K80 dramatically lowers the affinity of HSF1 to its cognate DNA sequences (Westerheide et al., 2009); thus, SIRT1 may augment HSF1 DNA-binding activity by the deacetylation of K80.

The observations described above suggest that HSF1 may integrate many signals of various cellular pathways related to stress and metabolism. Genome-wide transcriptome profiling and ChIP-seq experiments may unveil whether HSF1 is a direct transcriptional regulator of circadian core clock genes.

The Nutrient-Sensing Protein Kinase AMPK

The AMP/ATP ratio depends on cellular metabolism, and once this ratio increases, cells attenuate ATP-consuming pathways and accelerate ATP-generating pathways. AMP-dependent protein kinase (AMPK) is a major sensor of the AMP/ATP ratio, and when AMP becomes abundant it binds to the γ subunit of AMPK and elicits a structural change that is transmitted to the catalytic α subunit. This favors the phosphorylation of a threonine in the activating T loop of the α subunit by liver kinase B1 (LKB1), probably by rendering it a better LKB1 substrate and by inhibiting dephosphorylation at this threonine by unknown phosphatases (McBride and Hardie, 2009).

Recently, the activation of AMPK has been shown to have an impact on circadian clock function through various mechanisms. Thus, AMPK can directly phosphorylate CRY and thereby shortens the half-life of this core clock protein (Lamia et al., 2009). In keeping with a function of AMPK in the circadian regulation of CRY1 stability, its accumulation in the nucleus oscillates during the day with a cycle that is antiphasic to that of CRY1 accumulation. The induction of AMPK activity by the antidiabetes drug Metformin has also been demonstrated to promote the degradation of PER2, albeit by an indirect mechanism. Thus, it appears that AMPK phosphorylates S389 of casein kinase 1ε (CK1ε) and thereby enhances the CK1ε-mediated phosphorylation of PER2, which accelerates the degradation of this protein. Accordingly, PER2 accumulates to higher levels in organs of *Ampkα2* knockout mice, lacking the catalytic subunit α-2 of AMPK (Um et al., 2007).

Phosphorylation by AMPK may not only modulate the activity of transcriptional regulatory proteins involved in metabolism and circadian clock function, but also may act directly on chromatin encompassing genes reacting to nutrient deprivation. In a recent report, AMPK was found to phosphorylate histone H2B at serine 36 in p53 target genes that are implicated in survival after stress such as glucose starvation (e.g., *Reprimo*, *CyclinG*, and *Cpt1c*), and that AMPK-mediated phosphorylation of H2B enhances the resilience of mouse embryonic fibroblasts to glucose deprivation (Bungard et al., 2010).

All in all, the observations made on the impact of AMPK on clock gene expression render AMPK a plausible regulator for coupling circadian clocks to metabolic cycles.

Are Circadian Clocks Essential for the Control of Metabolism?

In spite of the apparent benefits of circadian clocks in assisting the temporal orchestration of metabolism, clock-less rodents are perfectly viable in the laboratory, irrespective of whether they have been rendered arrhythmic by the surgical ablation of the SCN or by the genetic disruption of core clock genes. So are circadian clocks merely a capricious byproduct of evolution—an epiphenomenon, so to speak? Unlikely so! In fact, circadian clocks have evolved many times independently and are omnipresent in light-sensitive organisms encompassing cyanobacteria, fungi, algae, protozoans, plants, and metazoans (Schibler, 2006). Moreover, insects and mammals have conserved most of their core clock genes, and these must therefore have been under positive selection during more than 500 million years (Rosbash, 2009). Yet, as revealed by sequence comparisons of genomes from closely related species, genes that become superfluous—due to adaptive changes in lifestyle—do not remain functional for such lengthy time periods. For example, the genomes of humans and chimpanzees contain pseudogenes for many olfactory receptors genes whose counterparts are still functional alleles in the other species (Go and Niimura, 2008). These genes must thus have been under positive selection for less than ten million years in a species-specific manner.

The interconnection between circadian clocks and metabolism is also underscored by work on other model organisms. For example in *Drosophila*, FOXO and GSK3b/Shaggy, well established regulators of metabolism and energy homeostasis, are required for robust circadian rhythm generation (Martinek et al., 2001; Zheng et al., 2007).

The disruption of various mammalian core clock or clock output genes have been associated with increased morbidities and mortalities, ranging from disturbances in metabolism (Green et al., 2008), xenobiotic detoxification (Gachon et al., 2006), and bone homeostasis (Fu et al., 2005) to epileptic seizures (Gachon et al., 2004). Unfortunately, however these findings only demonstrate the importance of the respective genes for the phenotypes under study; they do not address the question of whether the health problems were caused by a perturbation of circadian clocks. Indeed, it is likely that many processes controlled by clock-related transcription factors just depend upon these regulators, but not upon their cyclic accumulation and/or activity.

One way to discriminate between phenotypes caused by clock dysfunction and phenotypes elicited by clock gene disruption would be to perform genetic rescue experiments with transgenes producing constant intermediate levels of a given clock or clock output regulator in mice homozygous null for the corresponding gene. Due to extensive regulation at the posttranslational level, such experiments are very difficult to accomplish, at least for core clock components. For example, the accumulation of CRY2 protein oscillates with much higher amplitude than that of its mRNA (Preitner et al., 2002). Apparently, the association of CRY2 with other rhythmic clock proteins such as CRY1, PER1, and PER2 and/or cyclic posttranslational modifications is sufficient to engender robust daily CRY2 fluctuations via the regulation of its stability.

So how can we examine the importance of circadian oscillators in the laboratory? The best approaches to tackle this endeavor are so-called resonance experiments. If circadian oscillators had evolved to anticipate an organism's physiological needs during the daily light-dark cycle, their period length—tau (τ), in chronobiology jargon—should be roughly in resonance with the period of the light-dark cycle —"T cycle" in chronobiology parlance. For example, an organism with a τ of approximately 24 hr should perform better at a resonating T cycle of 24 hr than at discordant T cycles of, say, 20 or 30 hr. Conversely, mutant organisms with oscillators generating τs of 20 and 30 hr should thrive best at T cycles of 20 and 30 hr, respectively. If the resonance and nonresonance of τ with T cycles respectively increases and decreases an organism's fitness, then it must be the interaction of the clock with environmental rhythms rather than the mutation of the clock gene itself that is responsible for the better or worse performance. Beautiful resonance experiments with unambiguous outcomes have been conducted with cyanobacteria strains harboring *kaiC* wild-type alleles as well as short-period, long-period, and arrhythmic *kaiC* mutant alleles (KaiC is a central component of the cyanobacteria oscillator) (Ouyang et al., 1998; Woelfle et al., 2004). The following conclusions emerged from these approaches: in mixed cultures, strains with a resonating circadian clock outgrew strains with nonresonating or arrested oscillators after only ten generations and arrhythmic mutant strains grew slightly better than rhythmic strains when kept under constant light conditions. A clear benefit of resonating clocks could also be demonstrated for Arabidophis thaliana strains (Dodd et al., 2005). Such plants grew faster, displayed higher photosynthesis rates, assimilated more nitrogen, and were more resilient to environmental stress than plants with discordant circadian clocks. Again, theses differences were most pronounced when the plants were grown in mixed, dense cultures, in which plants with concordant and discordant oscillators had to compete.

The time is now ripe for conducting similar resonance studies with mammalian organisms to examine the possible virtues of circadian oscillators in the temporal orchestration of metabolism. For both hamsters (Ralph and Menaker, 1988) and mice (Meng et al., 2008), mutant strains with vastly different free-running periods (τs) are now available. Moreover, drug screens have revealed chemicals which significantly affect circadian period length, at least in cells (Hirota and Kay, 2009). It will be exciting to examine whether the harmony between τs and T cycles ameliorates energy homeostasis and reduces phenotypes associated with its dysfunction.

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