HIGHLIGHTED TOPIC | The Role of Clock Genes in Cardiometabolic Disease

Molecular control of circadian metabolic rhythms

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Li S, Lin JD. Molecular control of circadian metabolic rhythms. J Appl Physiol 107: 1959–1964, 2009. First published July 2, 2009; doi:10.1152/japplphysiol.00467.2009.—Circadian metabolic rhythms are fundamental to the control of nutrient and energy homeostasis, as well as the pathogenesis of metabolic disease, such as obesity, lipid disorders, and type 2 diabetes. This temporal organization of tissue metabolism is coordinated through reciprocal cross talk between the biological timing system and the metabolic regulatory networks. In this review, we discuss the signaling mechanisms that serve to couple metabolic regulation to the circadian pacemaker, in particular the role of the peroxisome proliferator-activated receptor-γ coactivator-1 transcriptional coactivators in integrating clock and energy metabolism.

peroxisome proliferator-activated receptor-γ coactivator-1α; nuclear receptor

CIRCADIAN CLOCK AND ENERGY METABOLISM

THE TEMPORAL ORGANIZATION of tissue metabolism is emerging as a central feature of energy homeostasis in mammals. The concentrations of blood glucose, lipids, and circulating hormones display robust daily cycles (28, 70). This diurnal oscillation of nutrients and hormones is accompanied by rhythmic activation of diverse metabolic pathways. For example, hepatic gluconeogenesis, de novo lipogenesis, and xenobiotic detoxification are precisely timed and reach their respective peaks at distinct periods throughout the light-dark cycle (18, 26, 55). Recent transcriptome profiling has revealed extensive circadian regulation of mRNA levels of genes involved in glucose, lipid, amino acid, and mitochondrial metabolism, many of which encode rate-limiting enzymes (1, 50, 54, 74, 79, 90). These findings suggest that circadian signals likely play an important role in the control of metabolic fluxes. The restriction of behaviors are coordinated by biological clocks residing in the brain and also in the peripheral tissues (32, 49, 51, 62, 66, 84).

In mammals, the circadian rhythms of physiology and behaviors are coordinated by biological clocks residing in the brain and also in the peripheral tissues (32, 49, 51, 62, 66, 84). These molecular clocks consist of transcriptional activators and repressors assembled into autoregulatory loops that generate cyclic transcriptional activation with a period of ~24 h. In the center of this network are Bmal1 and Clock, basic helix-loop-helix transcription factors that heterodimerize and positively regulate the expression of Period 1 (Per1), Per2, and Per3, and Cryptochrome 1 (Cry1) and Cry2 (20, 27, 67). Per and Cry proteins subsequently form transcriptional repressor complexes that negatively regulate Bmal1/Clock activity, leading to the inhibition of their own transcription. The expression of Bmal1 is regulated by nuclear receptors retinoid-related orphan receptor (ROR)-α and Rev-erb-α, which stimulates and represses Bmal1 transcription, respectively (68, 79, 87). ROR-α physically interacts with transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1 transcriptional coactivators to recruit Bmal1 to the proximal Bmal1 promoter and activates Bmal1 transcription (46). In contrast, Rev-erb-α competes with ROR-α for the common regulatory elements and interacts with the nuclear corepressor/histone deacetylase repressor complex (87). This dichotomous recruitment of the ROR-α/PGC-1α activator complex and the Rev-erb-α/nuclear corepressor repressor complex drives rhythmic expression of Bmal1. Posttranslational mechanisms also play an important role in the regulation of clock function (48, 62, 63, 82). For example, casein kinase 1δ, casein kinase 1e, and glycogen synthase kinase 3 exert their effects on circadian pacemaker through modulating the stability and/or function of core clock components (19, 62). Recently, Fbxl3 was identified as a key regulator of Cry degradation through its association with the Skp1-Cullin 1 F-box ubiquitin ligase complex (5, 22, 71).

METABOLIC REGULATION BY THE PGC-1 COACTIVATORS

The transcriptional networks that regulate glucose and lipid metabolism are sensitive to nutritional status and respond to diverse physiological signals. These nearly constant adjustments of metabolic gene expression ensure energy and nutrient homeostasis at both cellular and organismal levels. Nuclear hormone receptors comprise a unique class of transcriptional regulators that are capable of sensing the concentrations of metabolites, including lipids, oxysterols, heme, and bile acids (4, 7, 16). Through switching between coactivator and corepressor recruitment, nuclear receptors serve to link metabolite sensing to transcriptional responses in diverse cell types (21,
The liver in response to starvation, whereas it is rapidly induced in skeletal muscle in response to physical activity (3, 23). In PGC-1α responsive to nutritional status and other physiological signals. In PGC-1α, gluconeogenesis and fatty acid metabolism, including mitochondrial biogenesis, fatty acid oxidation, hepatic gluconeogenesis, as well as heme biosynthesis through nuclear respiratory factors 1 and 2, estrogen receptor-related receptor-α, peroxisome proliferator-activated receptor-α, hepatic nuclear factor (HNF) 4-α, and estrogen receptor-related receptor-α, drives rhythmic metabolic gene expression and energy metabolism. SIRT1 engages cellular nutrient-sensing pathways and transduces these signals to both clock and metabolic regulators through its deacetylation of PGC-1α and Bmal1. Per, period; Cry, cryptochrome.

PGC-1α AS A CLOCK REGULATOR

PGC-1α regulates the transcription of target genes through physical interaction and coactivation of its transcriptional partners. In the case of clock genes, PGC-1α coactivates ROR-α and is recruited to the ROR binding sites (ROR response element) present on the proximal Bmal1 promoter (46). This results in enhanced association of histone acetyltransferases, TRAP/DRIP/mediator complex, sirtuin (SIRT) 1 histone deacetylase, and SWI/SNF complex, and regulate gene transcription through its modulation of local chromatin structure (56, 64, 83).

Recent analysis of the PGC-1α transcriptional complex, as well as the genomewide coactivation, has led to the identification of a comprehensive list of transcriptional partners for this factor (39, 40). PGC-1α physically interacts with several chromatin-remodeling complexes, such as histone acetyltransferase, TRAP/DRIP/mediator complex, sirtuin (SIRT) 1 histone deacetylase, and SWI/SNF complex, and regulate gene transcription through its modulation of local chromatin structure (56, 64, 83).

PGC-1α has been demonstrated to interact with DNA-binding transcription factors, as well as chromatin-remodeling complexes. Several transcription factors, including hepatic nuclear factor-α, glucocorticoid receptor, and FOXO1, have been implicated in mediating the induction of gluconeogenic gene expression (57, 89). On the other hand, PGC-1α regulates mitochondrial biogenesis through nuclear respiratory factors 1 and 2, estrogen receptor-related receptor-α, PPAR-α, and PPAR-δ (31, 42). This family of transcriptional coactivators, such as cAMP response element binding protein-1 (PGC-1), play an important, sometimes even dominant, role in the control of glucose, lipid, and mitochondrial oxidative metabolism (15, 34, 42, 58). This family of transcriptional coactivators consists of PGC-1α, PGC-1β, and PGC-related coactivator, all of which are highly conserved among vertebrates. PGC-1α regulates several major aspects of energy metabolism, including mitochondrial biogenesis, fatty acid β-oxidation, hepatic gluconeogenesis, as well as heme biosynthesis. Adenoviral-mediated and transgenic expression of PGC-1α results in increased mitochondrial biogenesis and cellular respiration (85). In the liver, PGC-1α induces hepatic gluconeogenesis and fatty acid β-oxidation and plays an essential role in the induction of hepatic starvation response (89).

Consistently, metabolic adaptation in response to stresses is severely impaired in PGC-1α null mice (38, 44). In contrast, PGC-1β stimulates triglyceride synthesis and lipoprotein secretion in the liver in response to dietary fats (45). Deficiency in PGC-1α or PGC-1β leads to impaired mitochondrial oxidative metabolism in several tissues, including the brain, heart, skeletal muscle, brown fat, and the liver (38, 41, 44, 72, 81).

A key feature that distinguishes PGC-1 from other transcriptional coactivators, such as cAMP response element binding protein-binding protein and p300, is that their expression is highly responsive to nutritional status and other physiological signals. PGC-1α was first discovered as a cold-inducible coactivator for peroxisome proliferator-activated receptor (PPAR)-γ in the brown adipose tissue (59). The mRNA levels of PGC-1α are elevated in the liver in response to starvation, whereas it is rapidly induced in skeletal muscle in response to physical activity (3, 23). In contrast, PGC-1β expression is regulated by fatty acids, as well as cytokines, and its expression rises significantly during adipocyte differentiation (43, 45, 80). In addition to nutritional regulation of PGC-1α and PGC-1β, these two coactivators are also controlled by circadian signals in the liver and skeletal muscle (46). Interestingly, the phase of these two factors appears to be different in the liver, with PGC-1β peaking ~4 h before PGC-1α. To date, the exact molecular signals that mediate the circadian regulation of PGC-1α and PGC-1β remain unknown. Nevertheless, these observations raise the possibility that the PGC-1 coactivators function to orchestrate the temporal organization of energy metabolism through cross talk with circadian pacemakers.

All PGC-1 members share characteristic transcription activation domain at the NH2-terminus, several LXXLL motifs that serve as docking sites for nuclear hormone receptors, and RNA recognition motifs at the COOH-terminus. PGC-1α has demonstrated to interact with DNA-binding transcription factors, as well as chromatin-remodeling complexes. Several transcription factors, including hepatic nuclear factor-α, gluocorticoid receptor, and FOXO1, have been implicated in mediating the induction of gluconeogenic gene expression (57, 89). On the other hand, PGC-1α regulates mitochondrial biogenesis through nuclear respiratory factors 1 and 2, estrogen receptor-related receptor-α, PPAR-α, and PPAR-δ (31, 42). This family of transcriptional coactivators, such as cAMP response element binding protein-1 (PGC-1), play an important, sometimes even dominant, role in the control of glucose, lipid, and mitochondrial oxidative metabolism (15, 34, 42, 58). This family of transcriptional coactivators consists of PGC-1α, PGC-1β, and PGC-related coactivator, all of which are highly conserved among vertebrates. PGC-1α regulates several major aspects of energy metabolism, including mitochondrial biogenesis, fatty acid β-oxidation, hepatic gluconeogenesis, as well as heme biosynthesis. Adenoviral-mediated and transgenic expression of PGC-1α results in increased mitochondrial biogenesis and cellular respiration (85). In the liver, PGC-1α induces hepatic gluconeogenesis and fatty acid β-oxidation and plays an essential role in the induction of hepatic starvation response (89). Consistently, metabolic adaptation in response to stresses is severely impaired in PGC-1α null mice (38, 44). In contrast, PGC-1β stimulates triglyceride synthesis and lipoprotein secretion in the liver in response to dietary fats (45). Deficiency in PGC-1α or PGC-1β leads to impaired mitochondrial oxidative metabolism in several tissues, including the brain, heart, skeletal muscle, brown fat, and the liver (38, 41, 44, 72, 81).

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induce the expression of Bmal1 and Rev-erb-α (Lin JD, unpublished observations). Furthermore, PGC-1β null mice have altered circadian rhythm of locomotor activity that is characterized by reduced activity levels during early dark phase (72). In contrast, PGC-1α null mice are hyperactive (44). While the effects of PGC-1β deficiency on the free-running period remain to be determined, these results suggest that both PGC-1α and PGC-1β may participate in the regulation of clock function. It is possible that altered locomotor activity may feed back to the central clock. Because PGC-1 coactivators are expressed in several brain areas, including the suprachiasmatic nucleus, it is likely that they may directly modulate central pacemaker function (46, 75). Future studies are needed to clarify the diurnal expression patterns of PGC-1 in the suprachiasmatic nucleus and their role in the regulation of the central clock.

Since PGC-1α expression is highly responsive to nutritional and circadian signals, it is likely that this factor may transduce extracellular signals to the circadian pacemaker (Fig. 1). In this case, PGC-1α responds to metabolic signals, as well as timing cues, and directly regulates Bmal1 gene expression through its coactivation of ROR-α. PGC-1α also induces the expression of Rev-erb-α, a repressor of Bmal1 transcription. Whether PGC-1α stimulates Rev-erb-α expression through coactivation of ROR-α or indirectly through Bmal1 remains unknown. The fact that PGC-1α modulates both positive and negative arms of the clock transcriptional circuitry suggests that this factor may have a broader impact on the properties of clock oscillator.

**METABOLIC SIGNALING TO THE CIRCADIAN PACEMAKER**

Nutrient intake provides an important timing cue for the metabolic activity and clock phase in peripheral tissues. Meal timing in rodents, as well as in humans, resets the phase of hepatic lipogenesis and plasma leptin concentrations (6, 14, 69). The phase of clock gene expression in peripheral tissues, but not in the suprachiasmatic nucleus, undergoes drastic phase resetting in response to restricted feeding (12). Thus the nutritional and hormonal signals elicited by feeding serve to synchronize peripheral clock to the underlying metabolic oscillators. While meal timing exerts profound effects on peripheral clocks, the types of nutrients also influence both central and peripheral clocks. Feeding mice with a high-fat diet leads to behavioral perturbations, as well as altered clock gene expression, in hypothalamus and peripheral tissues (35). It is likely that dietary fats may engage similar nutrient-responsive pathways to transduce metabolic signals to the circadian pacemaker. In this context, nuclear hormone receptors and their cofactors are potential candidates that link dietary nutrients to the clock pathway. Remarkably, mRNA expression of many nuclear receptors exhibits robust daily cycles in a wide range of tissues, including the liver, skeletal muscle, and brown and white fats (86). The dynamic temporal profile of nuclear receptor gene expression supports the crucial role of these transcriptional regulators in orchestrating metabolic cycles, as well as core clock functions.

Metabolite profiling in yeast indicates that extensive oscillation of intracellular metabolite levels is correlated with robust metabolic rhythms (76, 77). While it is unlikely that individual mammalian tissues have the capacity to generate self-sustained metabolic cycles, the diurnal fluctuation of metabolites is expected to exert a major effect on rhythmic expression of clock and metabolic genes. An important intracellular nutrient sensor is SIRT1, an NAD+ -dependent histone deacetylase that has been implicated in several aspects of energy homeostasis (25). Two recent studies demonstrate that SIRT1 also regulates circadian clock function (2, 52). SIRT1 associates with the Bmal1/Clock transcriptional complex and modulates the acetylation status of Bmal1, which, itself, is a substrate for Clock-mediated acetylation. SIRT1 deacetylates Bmal1 and is required for diurnal rhythms of histone modification at Bmal1 target loci. Interestingly, SIRT1 also appears to exert its influence on the clockwork through deacetylation and degradation of Per2. The enzymatic activity of SIRT1 requires NAD+, which is generated through de novo biosynthesis from tryptophan and a salvage pathway that is controlled by nicotinamide phosphoribosyltransferase. Circadian expression of nicotinamide phosphoribosyltransferase itself is under the control of Bmal1/Clock complex and is further modulated by SIRT1 (53, 61). A major target of SIRT1 is PGC-1α, which is activated by SIRT1-mediated deacetylation (36, 64). This should, in principle, enable PGC-1α to integrate multiple metabolic signals, including circulating hormones and nutrient-sensing and energy-sensing pathways, and transduce these signals to peripheral clocks.

**INTEGRATION OF BIOLOGICAL CLOCK AND ENERGY METABOLISM**

The observations that PGC-1α also directly impacts the clock oscillator highlight the complex cross talk between the clockwork and metabolic regulatory networks. It is increasingly apparent that many transcriptional regulators serve the dual purpose of modulating both clock and metabolic pathways. A clear example is the ROR and Rev-erb families of nuclear receptors, which activate and repress the expression of target genes, respectively. ROR-α is an essential component of the clock oscillator that positively regulates Bmal1 and Rev-erb-α expression. In contrast, Rev-erb-α is a transcriptional repressor that functions to antagonize ROR-α, thereby constituting a negative feedback loop in the clock transcriptional network. While the biological function of ROR-α and Rev-erb-α in circadian control is well established, accumulating evidence indicates that these two factors also play an important role in the regulation of glucose and lipid metabolism (13, 29). ROR-α has been demonstrated to regulate lipoprotein metabolism through its regulation of apolipoprotein gene expression in the liver. This factor also appears to play a role in the regulation of genes involved in fatty acid oxidation in muscle cells. More recently, Rev-erb-α was found to be a heme-binding protein that represses gluconeogenic gene expression in the liver and modulates hepatic glucose production in response to heme (60, 87, 88). On the contrary, ROR-α stimulates the expression of glucose-6-phosphatase and regulates glycogen metabolism in the liver (8). Circadian clock regulates diverse physiological processes, collectively referred to as the output pathways. In this hierarchical model, the molecular clock serves as a master regulator of downstream pathways through direct or indirect mechanisms. While this model is conceptually satisfying, it is becoming apparent that the distinction between clock and metabolic regulators is not always, if at all, straightforward. Key
components of the molecular clock are also regulators of glucose and lipid metabolism, whereas major metabolic regulators, in particular the PGC-1 transcriptional coactivators, also directly control clock gene expression. This genetically ‘hard-wired’ cross talk between biological timing and metabolic homeostasis may serve an ancient purpose to rapidly synchronize tissue metabolism with environmental cues.

CIRCADIAN RHYTHMS AND METABOLIC DISEASE

Abnormal circadian rhythms have been associated with sleep disorder, cardiovascular disease, metabolic syndrome, cancer, and rheumatoid arthritis (9–11, 17, 30, 33). In addition, there is increased risk for metabolic disorders in shift workers, suggesting that disruption of normal biological clock adversely impacts energy homeostasis. Remarkably, acute disruption of sleep rhythms leads to decreased insulin sensitivity in humans (73). These studies illustrate a potentially causal role of sleep disruption in the pathogenesis of insulin resistance and glucose intolerance. Although the mechanisms involved are far from clear, these observations underscore the crucial role of circadian timing signals in metabolic regulation and energy homeostasis. The significance of this relationship in the ongoing epidemic of metabolic syndrome awaits further investigation.

Interestingly, Clock mutant mice develop obesity and display characteristics of metabolic syndrome (78). In addition, mice lacking Bmal1, a heterodimer partner for Clock, have impaired glucose homeostasis, likely as a result of reduced hepatic gluconeogenesis (37, 65). While there is little doubt that diurnal cycles of hormones and nutrients have profound effects on glucose and lipid metabolism, direct cross talk between the circadian pacemaker and metabolic regulators remains poorly understood. Novel therapeutic targets are expected to emerge from the signaling pathways that transmit timing cues to tissue energy metabolism.

REFERENCES


