review article

Transcriptional control of circadian metabolic rhythms in the liver

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Diurnal metabolic rhythms add an important temporal dimension to metabolic homeostasis in mammals. Although it remains a challenge to untangle the intricate networks of crosstalk among the body clock, nutrient signalling and tissue metabolism, there is little doubt that the rhythmic nature of nutrient and energy metabolism is a central aspect of metabolic physiology. Disruption of the synchrony between clock and metabolism has been causally linked to diverse pathophysiological states. As such, restoring the rhythmicity of body physiology and therapeutic targeting directed at specific time windows during the day may have important implications in human health and medicine. In this review, we summarize recent findings on the integration of hepatic glucose metabolism and the body clock through a regulatory network centred on the PPARγ coactivator 1 (PGC-1) transcriptional coactivators. In addition, we discuss the transcriptional mechanisms underlying circadian control of the autophagy gene programme and autophagy in the liver.
Keywords: autophagy, circadian rhythm, clock, glucose, lipid, liver, metabolism, PGC-1, transcription

Circadian Clock and Energy Metabolism

The body clock controls many aspects of mammalian physiology, including the sleep–wake cycle, locomotor activity, hormone release and metabolism. These biological rhythms are entrained by the light–dark (LD) and feeding cycles. In mammals, the master clock is located in the suprachiasmatic nucleus (SCN) of the ventral hypothalamus. Light resets the phase of the central pacemaker, which in turn synchronizes clocks in peripheral tissues. Remarkably, peripheral oscillators can be uncoupled from the central clock in response to restricted feeding [1–3], underscoring the importance of nutritional signals in circadian timing in the peripheral tissues. Clock oscillators regulate diverse organismal functions, but also operate in a cell-autonomous manner. Tissue explants can maintain robust rhythmic patterns of gene expression over a period of days to weeks in culture dishes [4]. Further, individual fibroblasts harbour intrinsic rhythms that undergo synchronization in response to a variety of stimuli, such as brief serum shock and exposure to glucocorticoids or forskolin [5,6].

Nutrient and energy metabolism is tightly integrated with the circadian timing system in mammals [7–9]. The concentrations of plasma metabolites such as amino acids and lipids, and circulating hormones, exhibit varying degrees of circadian rhythms. Recent metabolomic profiling of plasma in mice and humans revealed extensive diurnal oscillation [10–12], probably as a result of a combination of feeding cycles and endogenous metabolic rhythms. This daily ebb and flow of metabolites is accompanied by cyclic activation of major metabolic pathways in the liver, including gluconeogenesis, *de novo* lipogenesis, lipoprotein secretion, cholesterol

biosynthesis and xenobiotic detoxification. At the molecular level, transcriptional profiling using microarray and RNA sequencing has revealed extensive rhythmic expression of hepatic genes throughout the LD cycles [13–17]. A significant number of these cycling genes encode enzymes involved in glucose, lipid, amino acid and mitochondrial oxidative metabolism. As such, many metabolic processes are restricted not only to certain tissues in the body but also during specific time periods during the day.

> ❝The integration of body clock and metabolism is orchestrated by reciprocal signalling between the clock oscillator and metabolic pathways.❞

Temporal restriction of metabolic functions may provide advantages for organisms as they anticipate and synchronize their physiology to the environment. It is therefore not entirely surprising that disruptions of circadian timing have been associated with diseases such as sleep disorders, cardiovascular disease, metabolic syndrome and certain cancers [18–23]. In the context of metabolic homeostasis, circadian misalignments have been shown to elevate blood glucose and insulin concentrations in healthy individuals, accompanied by reduced circulating leptin levels and increased arterial blood pressure [24,25]. Whole body insulin sensitivity and glucose tolerance were significantly impaired following disruption of normal sleep cycles. In mice, high-fat feeding directly impacts the body clock while meal timing by restricted feeding profoundly alters the course of diet-induced obesity and its associated metabolic disorders [26]. The metabolic phenotype of several clock gene mutants has been characterized. Clock mutant mice develop obesity and display characteristics of metabolic syndrome [27], whereas mice lacking *Bmal1*, a heterodimer partner for *Clock*, have

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impaired glucose homeostasis and develop accelerated ageing [28,29]. Pancreas-specific inactivation of *Bmal1* markedly impaired glucose-stimulated insulin secretion and resulted in hyperglycaemia in mice [30,31]. Deficiency of both *Rev-erbα* and *Rev-erbβ* resulted in elevated blood glucose and plasma triglyceride levels and hepatic steatosis [32,33]. These observations show that different clock components exert distinct and tissue-specific effects on energy metabolism, underscoring the complex relationship between the molecular clock and metabolic pathways.

Integration of Clock and Metabolism by the PGC–1 Coactivators

The PPARγ coactivator 1 (PGC-1) family of transcriptional coactivators includes PGC-1α, PGC-1β and PGC-related coactivator [34,35]. *PGC-1α* expression is highly inducible in brown fat by cold exposure [36] in the liver following starvation [37,38] and in skeletal muscle in response to physical exercise [39,40]. Hepatic *PGC-1β* expression is responsive to dietary intake of saturated fats [41]. In many cell types, PGC-1α and PGC-1β stimulate the expression of mitochondrial gene programmes encoded by both nuclear and mitochondrial genomes. PGC-1α deficiency leads to impaired mitochondrial oxidative metabolism in several tissues, including the brain, heart, skeletal muscle, brown fat and the liver [42]. Similar defects of mitochondrial gene expression and respiratory function were also observed in *PGC-1β null* mouse tissues [43], suggesting that they play non-redundant roles in maintaining mitochondrial oxidative metabolism. A remarkable feature of PGC-1 action is that they coordinate diverse aspects of metabolic adaptation in tissues in response to nutritional and hormonal cues. For example, mice lacking PGC-1α exhibited impaired cold-induced thermogenesis and exercise endurance and had aberrant induction of hepatic starvation response. Beyond mitochondrial metabolism, PGC-1 coactivators also regulate diverse biological processes in a cell type-specific manner. PGC-1α stimulates hepatic gluconeogenesis [38] and oxidative muscle fibre formation [44], whereas PGC-1β promotes lipoprotein secretion [41,45] and bone homeostasis [46].

Previous studies have shown that the expression of *PGC-1α* and *PGC-1β* exhibits strong diurnal rhythms in the liver and skeletal muscle [47,48]. PGC-1α induces the expression of several core clock genes, particularly *Bmal1*, *Clock*, *Per2* and *Rev-erbα*, in a cell-autonomous manner. The induction of clock gene expression is at least in part mediated through its coactivation of orphan nuclear receptor RORα (Figure 1). The PGC-1α/ROR and Rev-erbα/HDAC transcriptional complexes appear to play an antagonistic role in the transcriptional regulation of *Bmal1* expression [49,50]. The physiological role of PGC-1α in normal clock function was supported by significant impairments of diurnal rhythms of locomotor activity, body temperature and metabolic rate in *PGC-1α null* mice. Similarly, mice lacking PGC-1β also display abnormal diurnal locomotor activity patterns [51]. These results strongly implicate the PGC-1 coactivators as a nodal point in integrating energy metabolism and the body clock.
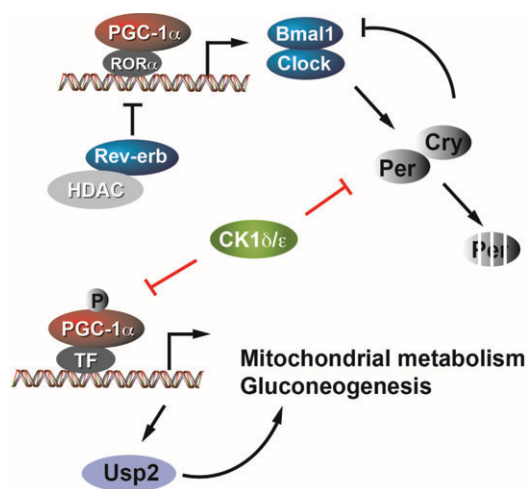
Figure 1. Integration of clock and hepatic metabolism by PGC-1α. PGC-1α coordinately regulates clock and metabolic gene expression through coactivation of its transcriptional partners. PGC-1α is reciprocally modulated by the biological clock through CK1δ-mediated phosphorylation. PGC-1, PPARγ coactivator 1; CK1δ, casein kinase 1δ.

Circadian Regulation of Hepatic Gluconeogenesis by PGC–1α

At the molecular level, the PGC-1 coactivators exert their effects through physical interaction with transcription factors and chromatin-remodelling complexes. Both PGC-1α and PGC-1β interact with nuclear respiratory factor 1 (NRF1), oestrogen receptor-related receptor α (ERRα) and ERRγ to regulate mitochondrial biogenesis [34,35]. These coactivators recruit histone acetyltransferase (HAT), the TRAP/DRIP/mediator complex, the SIRT1 histone deacetylase complex and the SWI/SNF chromatin-remodelling complexes to regulate the epigenetic state of local chromatin [52–55]. At least two non-mutually exclusive mechanisms are engaged by PGC-1α to regulate rhythmic expression of genes involved in hepatic gluconeogenesis: direct transcriptional regulation and indirect regulation through ubiquitin-specific protease 2 (Usp2). Previous studies have established hepatic nuclear factor 4α (HNF4α), glucocorticoid receptor (GR) and forkhead box O1 (FoxO1) as key mediators of gluconeogenic gene regulation by PGC-1α [38,56]. As such, it is probably that cyclic induction of PGC-1α leads to rhythmic activation of its transcriptional partners and target gene expression.

> **PGC-1 coactivators directly regulate the core clock and metabolic gene programmes through coactivating specific transcriptional partners.**

Alternatively, PGC-1α also regulates hepatic glucogenesis indirectly through its induction of Usp2 [57,58]. The deubiquinating enzymes are a family of cysteine proteases that catalyse the removal of ubiquitin from their target proteins. These proteases deubiquitinate a wide array of substrate proteins and regulate diverse biological processes, including

vesicular trafficking, signal transduction, gene transcription as well as metabolism. In primary hepatocytes, the expression of *Usp2* was strongly stimulated by PGC-1α. Further, *Usp2* expression exhibits strong diurnal rhythms in several tissues, including the liver and skeletal muscle. It is notable that Usp2 is the only ubiquitin-specific protease exhibiting significant circadian regulation. At the functional level, Usp2 is required for the induction of gluconeogenic and fatty acid β-oxidation genes by PGC-1α in hepatocytes. Adenoviral-mediated expression of *Usp2* in the liver increases hepatic glucose production and exacerbates glucose intolerance in high-fat diet-induced obese mice, whereas *in vivo* RNAi knockdown of *Usp2* improves systemic glycemic control. Importantly, hepatic rhythmic expression of *Usp2* is required for maintaining diurnal glucose regulation in response to restricted feeding. Together, these studies delineate a novel pathway that links hormonal and circadian signals to gluconeogenesis and glucose homeostasis.

Posttranslational mechanisms also play an important role in clock function [59–62]. In particular, casein kinase 1δ (CK1δ) and CK1ε regulate the length of circadian period through phosphorylating several clock proteins. Mutation of *CK1ε* is responsible for the *tau* mutant phenotype in Syrian hamsters, which is characterized by shortened circadian period [63,64]. Interestingly, mutation of *Doubletime*, a *Drosophila* ortholog of *CK1ε*, also results in altered period length [65–67]. A missense mutation (T44A) of *CK1δ* causes familial advanced sleep-phase syndrome, which is characterized by early sleep onset and early awakening in the morning [68]. Phosphorylation of clock proteins by CK1δ regulates their stability, subcellular localization and protein–protein interaction. In a search for proteins associated with PGC-1α, it was discovered that both CK1δ and CK1ε physically interact with PGC-1α [69]. PGC-1α serves as a substrate of CK1 with phosphorylation sites mapped to a domain near the C-terminus of PGC-1α. CK1-mediated phosphorylation reduced PGC-1α protein stability and transcriptional activity, resulting in impaired hormonal induction of gluconeogenic gene expression and glucose output. These findings illustrate that the clock oscillator reciprocally signals to *PGC-1α* and its downstream metabolic gene programmes through transcriptional and posttranslational mechanisms.

Circadian Regulation of Autophagy

Autophagy is a conserved cellular process responsible for turnover of long-lived proteins and organelles, and plays an essential role in organismal adaptation to starvation [70,71]. Nutrient limitation initiates a cascade of vesicle nucleation, autophagosome formation, docking and fusion with lysosome and subsequent lysosomal degradation. Autophagy is markedly induced in neonatal tissues and in adult tissues in response to starvation [72,73]. Defects in autophagy result in lower plasma glucose and amino acid concentrations during the early postnatal period, leading to impaired adaptation and survival. In addition, autophagy is required for removing protein aggregates, damaged organelles and certain pathogens [71]. Autophagy deficiency has been implicated in the pathogenesis

of several disease conditions, such as cancer, diabetes, hepatic steatosis, skeletal myopathy and neurodegeneration [74].

Electron microscopy studies performed in the 1970s showed that the number of autophagic vacuoles varies throughout the day in several cell types, including the inner segment of retina rod cells, cardiomyocytes, hepatocytes, pancreatic acinar cells and proximal tubules of kidney in rats [75,76]. Using a combination of autophagy markers and flux measurements, recent studies indicated that autophagy activity exhibits a robust diurnal rhythm in the liver, heart and skeletal muscle [77,78]. Autophagy flux can be estimated by measuring the degradation rate of microtubule-associated protein1 light chain 3-II (LC3-II), a marker for autophagy induction. This measurement revealed that autophagy flux in the liver reached a peak in the afternoon and decreased to lower levels in the dark phase. Importantly, the cyclic activation of autophagy flux in the liver is associated with rhythmic expression of autophagy genes [78]. The physiological significance of autophagy cycles remains unknown. It is possible that close coupling of autophagic protein degradation to the body clock may provide unique advantages for mammals to maintain systemic nutrient and energy homeostasis. Further, time-dependent remodelling of cellular proteomes and organelles may be fundamentally important for metabolic homeostasis along the temporal axis. The latter potentially serves an important function in restricting cellular metabolic functions to specific time periods during LD cycles.

Transcriptional regulation of autophagy genes has emerged as an important mechanism in autophagy. Several transcription factors have been identified that regulate various aspects of the autophagy gene programme, including Forkhead transcription factor O3 (FoxO3) and transcription factor EB (TFEB). FoxO3 was identified as a regulator of autophagy in skeletal myocytes by stimulating the expression of autophagy genes, including *LC3B*, *Gabarapl1*, *Bnip3* and *Bnip3l* [79]. TFEB induces the expression of a large number of genes involved in lysosome biogenesis, autophagy and lysosomal exocytosis [80,81]. TFEB is colocalized with mTORC1 under normal growth conditions and undergoes nuclear translocation in response to starvation and lysosomal stress [82,83]. In the context of circadian autophagy, CCAAT/enhancer binding protein β (C/EBPβ) is emerging as a key regulator of rhythmic autophagy gene expression in the liver [78]. C/EBPβ is a basic leucine zipper transcription factor that regulates diverse biological processes. The expression of C/EBPβ itself is highly rhythmic and is dependent on the liver clock. Gain-of-function studies using adenoviral-mediated gene expression indicated that C/EBPβ promotes the programme of autophagy gene expression and induces autophagic protein degradation in cultured hepatocytes. C/EBPβ directly binds to the promoters of autophagy genes and activates their transcription [78]. *C/EBPβ* itself is a target of core clock and is required for the regulation of rhythmic autophagy in the liver.

> **❝**Circadian control of autophagy rhythm is mediated by transcriptional control of autophagy gene expression by C/EBPβ and PGC-1β.**❞**

PGC–1β and Rhythmic Autophagy Regulation

The physiological role of PGC-1 coactivators in transcriptional regulation of autophagy genes has not been established. In co-immunoprecipitation (co-IP) studies, PGC-1β was found to be physically associated with C/EBPβ in transiently transfected 293T cells (Figure 2A), suggesting that it may function as a transcriptional coactivator for C/EBPβ. Importantly, adenoviral-mediated overexpression of *PGC-1β* stimulated the expression of many C/EBPβ-regulated autophagy genes in primary hepatocytes, including *FIP200, Ulk1, LC3B, Atg2a, Gabarapl1, Bnip3, Atg16l1* and *Atp6v0a2*, a subunit of vacuolar proton pump (Figure 2B). PGC-1β also stimulates the expression of *C/EBPβ*, suggesting that these two factors may form a feed-forward regulatory loop. Recent studies have shown that the mammalian Ulk1-FIP200-Atg13 kinase complex, which is equivalent to the Atg1 complex in yeast, integrates diverse nutrient signals and controls the initiation of autophagy in the cell [84,85]. Liver-specific inactivation of *FIP200* severely impaired autophagy in hepatocytes and resulted in liver injury [86]. As such, FIP200 and Ulk1 may provide an important link between PGC-1β and the autophagy pathway.

Activation of autophagy enhances the degradation of sequestosome 1 (p62), an LC3-interacting protein that delivers cargos for lysosomal degradation. Consistently, we found that PGC-1β significantly reduces steady-state p62 levels in transduced hepatocytes (Figure 2C). Similar effects on p62 levels were also observed in the livers from mice transduced with *PGC-1β* adenovirus (Figure 2D). Together, these studies show

that PGC-1β may serve as a coactivator for C/EBPβ to stimulate autophagy gene expression and enhance autophagy activity. As the expression of *PGC-1β* exhibits diurnal rhythms in the liver, it is likely that the rhythmic induction of *C/EBPβ* and *PGC-1β* may mediate circadian signalling to drive rhythmic activation of autophagy.

Perspectives

Circadian metabolic rhythms are a core feature of mammalian nutrient and energy homeostasis and have important implications in health and disease. The mechanisms that integrate metabolic functions with circadian timing in tissue are complex and probably involve reciprocal crosstalk between the metabolic regulatory networks and the biological clock. The molecular clock directly regulates the expression of downstream metabolic genes and participates in driving diurnal metabolic cycles. In parallel, the clock oscillator also responds to nutritional and hormonal cues by adjusting the phase and/or amplitude of rhythmic gene expression. Consequently, while the regulatory circuits are somewhat hardwired between the clockwork and the metabolic gene programmes, remarkable flexibility exists to allow fine-tuning of metabolic output from tissues. The ebb-and-flow of tissue metabolism is emerging to represent unique opportunities for therapeutic intervention by restoring the rhythmicity of the body clock and/or targeting therapies to certain phases of the metabolic cycle.
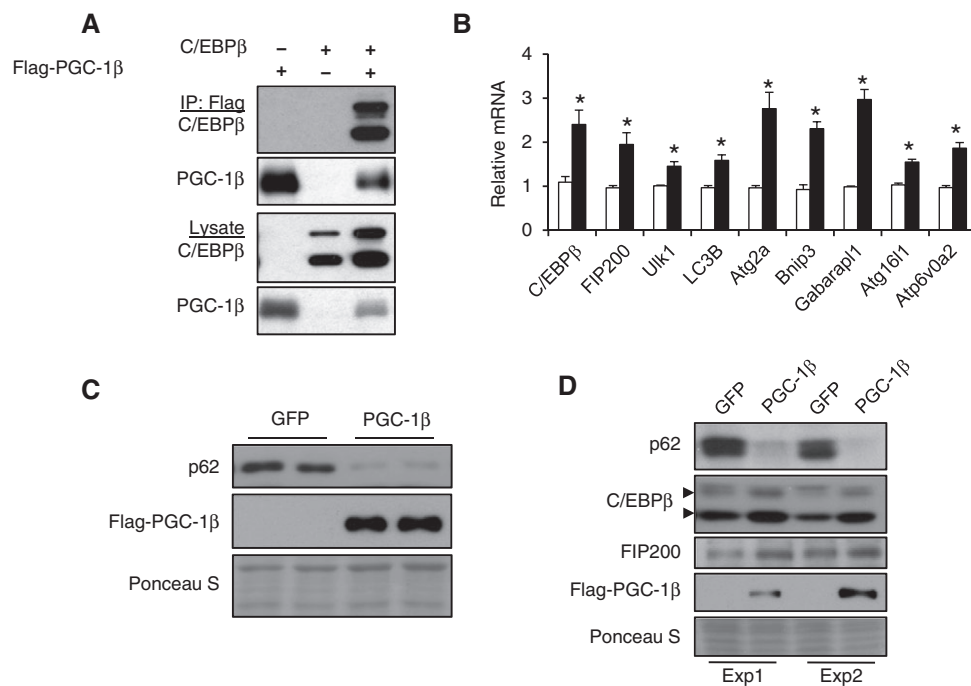
Figure 2. Induction of autophagy by the C/EBPβ/PGC-1β transcriptional complex. (A) Physical interaction between PGC-1β and C/EBPβ. (B) qPCR analysis of hepatocytes transduced with green fluorescence protein (*GFP*) (open) or *PGC-1β* (filled) adenovirus. Shown is mean ± standard deviation of one representative experiment in triplicates, *p < 0.01. (C) Immunoblots of total lysates from hepatocytes transduced with *GFP* or *PGC-1β* adenovirus. (D) Immunoblots of pooled lysates from transduced livers obtained in two independent experiments. C/EBPβ, CCAAT/enhancer binding protein β; PGC-1, PPARγ coactivator 1; qPCR, quantitative polymerase-chain-reaction.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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