Chapter 20
Circadian Clocks, Metabolism, and Food-Entrained Rhythms

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Abstract The circadian clock is one of the most conserved systems in mammals. It is an important regulator of many biological processes, such as the sleep-wake cycle, hormone secretion, and body temperature, which can influence both cellular and organ-level metabolic functioning. At the molecular level, the circadian system consists of autoregulatory feedback loop that dictates the timing of behavioral and physiological processes. This molecular clock is persistent in all of the central and peripheral tissues. Metabolism can also affect the circadian clock via feeding, or by metabolites which expression is controlled by food intake. Therefore, the current chapter emphasizes the cross-talk between the circadian system and metabolism at the molecular level, and its physiological outcome.

20.1 Introduction

The circadian clock is one of the most conserved systems in mammals. It is an important regulator of many biological processes, such as the sleep-wake cycle, hormone secretion, and body temperature, which can influence both cellular and organ-level metabolic functioning. At the molecular level, the circadian system consists of autoregulatory feedback loop that dictates the timing of behavioral and physiological processes. This molecular clock is persistent in all of the central and peripheral tissues. Metabolism can also affect the circadian clock via feeding, or by metabolites which expression is controlled by food intake. Therefore, the current chapter emphasizes the cross-talk between the circadian system and metabolism at the molecular level and its physiological outcome.
20.2 Organization of the Circadian Clock

The central clock is located in the hypothalamus, within a paired structure above the optic chiasm called the suprachiasmatic nuclei (SCN). The SCN play an important role for rhythmic secretion of hormones and the regulation of locomotor activity [1–3], and they hierarchically dictate the circadian timing system. They appear to be the central conductor that is orchestrating the other clocks and entrain the circadian system to the 24-h light/dark cycle. The SCN coordinate the synchronization of peripheral clocks, which is essential to ensure temporal organization of physiological processes [4].

The molecular links responsible for the circadian system is the core complex established by the transcription factors CLOCK and BMAL1 [5]. They heterodimerize and drive the transcription of many clock-controlled genes (CCGs) by binding to E-box sites (CACGTG) within their own promoters. CLOCK and BMAL1 also regulate the transcription of their own repressors, the members of period (per1/2/3) and cryptochrome (cry 1/2) families. The increased transcriptional level of per and cry genes causes the accumulation of the circadian repressors PER and CRY, which inhibit the transcription driven by CLOCK/BMAL1. Additional elements such as the orphan nuclear receptors ROR and REV-ERB are involved in the global circadian regulation as well.

20.3 Connection Between Clocks and Metabolism

20.3.1 Evidence from Studies in Mice

Studies on mice deficient for clock genes have shown that circadian rhythms play a key role in metabolism. Some evidence was found in clock mutant mouse studies. They revealed that mice having arrhythmic feeding and locomotor activity showed hyperphagia, hyperlipidemia, hyperglycemia, and hypoinsulinemia (6). Studies on mice lacking Bmal1 showed disrupted adipogenesis and carbohydrate metabolism in the liver [7–9]. Furthermore, asynchronous dietary cues may modify glucose homeostasis via their interactions with peripheral molecular clocks [8]. Mice with a liver-specific deletion of Bmal1 exhibited hypoglycemia restricted to the fasting phase of the daily feeding cycle, exaggerated glucose clearance, and loss of rhythmic expression of hepatic glucose regulatory genes [7]. Clock and Bmal1 mutants show impaired glucose tolerance, reduced insulin secretion, and defects in size and proliferation of pancreatic islets that worsen with age [10].

Metabolic alterations have also been found in deficient mice for target genes of BMAL1/CLOCK. For example, PER2 interacts with PPARγ, which leads to a time-dependent modulation of lipid metabolism. Accordingly, per2-deficient mice display altered lipid metabolism with drastic reduction of total triacylglycerol and nonesterified fatty acids [11]. Glucocorticoids and glucocorticoid receptor (GR) are
involved in regulation of glucose homeostasis. It is reported that the cry1/2 physically interacts with GR [12]. Through this interaction, the expression of the phosphoenolpyruvate carboxykinase 1 (Pck1), which is directly regulated by GR, is reduced, and subsequently cry-deficient mice show the increased Pck1 expression. The Rev-Erba, which is the major suppressor of Bmal1 transcription [13], is expressed in a variety of tissue types, including brown fat, skeletal muscle, and liver [14,15]. In addition to its action in the circadian clock mechanism, the biological function of Rev-Erba has been implicated in metabolism [16,17]. In the case of Rev-Erba knockout (KO) mice, although these animals showed altered expression of clock components, they did not show disrupted rhythms of behavior [13]. The study on Rev-Erba and β double KO mice also revealed the link with lipid metabolism [16,17]. These animals displayed altered locomotor activity and disrupted lipid metabolism. Feeding-induced obese mice treated with Rev-Erb agonists showed decreased fat mass, weight gain, triglyceride, and cholesterol levels [18]. Interestingly, overexpression of Rev-Erba in the liver caused disrupted lipid metabolism [19].

20.3.2 Evidence from Studies in Humans

In humans, several genetic studies showed a connection between circadian clock and metabolism. Genetic variants of Clock and Bmal1 have been related with metabolic syndrome [20–24]. Per2 variants are also involved in metabolic syndrome such as high blood glucose levels and abdominal obesity [25,26]. Cry2 variants are also related to glucose homeostasis [27,28]. Thus, several genetic studies in humans revealed a close connection between clock disruption and metabolic syndrome.

20.3.3 Evidence from Genome-Wide Profiling Studies

Studies using genome-wide profiling have provided evidence for a close link between circadian clock and metabolism. A recent study showed that around 15% of the liver transcriptome is expressed in a circadian manner and manipulation of feeding schedule can regulate the expression of these genes [29]. In the liver, these include DBP, TEF, and HLF [30], E4BP4 [31], the Krüppel-like factors KLF10 [32] and KLF15 [33], and nuclear receptors [34]. These transcription factors are reported to regulate genes that are involved in the metabolism.
20.4 Metabolic Feedback onto Clock Genes

SIRT1 plays an important role in nutrient availability, liver gluconeogenesis, lipolysis, and insulin secretion [35]. SIRT1 deacetylates histones and several transcription factors [36]. SIRT1 physically interacts with CLOCK/BMAL1 heterodimers, resulting in a rhythmic deacetylation of BMAL1 [37] and degradation of PER2 [38]. Since activity of SIRT1 depends on the nicotinamide adenine dinucleotide (NAD$^+$), it seems that SIRT1 can connect metabolism to the circadian network. Furthermore, SIRT1 also influence the activity of PPARα [39] and PGC-1α [40]. PGC-1α seems to be connected to the circadian clock because its expression is rhythmic and it can be a co-activator of ROR [41], which is known as an activator of transcription of Bmal1 (Fig. 20.1). Since SIRT1 activity is regulated by availability of the NAD$^+$, studies were performed for understanding if this cofactor could be a circadian target as well. The rate-limiting enzyme in NAD$^+$ biosynthesis is nicotinamide phosphoribosyltransferase (NAMPT). Increased NAMPT results in increased synthesis of NAD$^+$, which increases the activity of SIRT1, also known as NAD$^+$-dependent deacetylase. The ratio of NAD$^+$/NADH modulates CLOCK/BMAL1 DNA-binding activity [42]. It is also reported that in the liver the activity of poly(ADP-ribose) polymerase 1 (PARP-1), a NAD$^+$-dependent ADP-ribosyltransferase, oscillates in a daily manner and is regulated by feeding [43]. PARP-1 is known to bind and poly-ADP-ribosylate CLOCK at the light phase. Thus, loss of PARP-1 increases the activity of CLOCK/BMAL1 and influences the circadian rhythmicity of Per and Cry. As a consequence, parp-1-deficient mice show altered expression of genes regulated by CLOCK/BMAL1.

Fig. 20.1 Metabolic regulators and circadian clock components. A schematic representation of the interactions between metabolic regulators with clock components in the liver is shown. Krüppel-like factor 10 (KLF10) and PPARs regulate Bmal1 expression by binding to the corresponding recognition sequences in the Bmal1 promoter. Levels of NAD+ influence SIRT1 activity, which deacetylates BMAL1 and PER2 proteins. AMPK, which is a major sensor for the AMP/ATP ratio, can directly phosphorylate CRY1, leading to destabilization and degradation of CRY1.
Glucose is also involved in regulation of circadian clock. Glucose appears to upregulate KLF10 [44]. It is known to bind to GC-rich elements in the Bmal1 promoter and subsequently represses Bmal1 transcription. Another important factor for metabolic state is the ratio between AMP and ATP. A major sensor for the ratio between AMP and ATP is adenosine monophosphate-dependent protein kinase (AMPK). It can directly phosphorylate and destabilize CRY1 [45]. Food components can also affect circadian clocks. When mice are fed with ketogenic diet, circadian rhythmicity of wheel-running activity under constant darkness is altered [46]. High-fat diet also causes a significant reformation of circadian oscillation of transcripts and metabolites in the liver [47]. These can be explained both by interfering with recruitment of CLOCK/BMAL1 to chromatin and by inducing PPARγ-driven oscillation on noncyclic genes.

20.5 Entrainment of Peripheral Clocks by Feeding Cues

At the systemic level, the circadian clock mechanism is related to metabolism. One of the most important external inputs to the metabolic systems are feeding signals. Through the temporal feeding restriction, the link between the central clock and the liver clock can be disrupted [48]. A recent study also revealed that time of feeding can regulate circadian rhythmicity in hepatic gene expression [29]. They used distinct feeding and fasting paradigms on wild-type (WT) and circadian clock-deficient mice. Accordingly, both food availability and the temporal pattern of feeding determined the phase and amplitude of the circadian transcriptome in WT liver.

20.6 Food-Anticipatory Activity and Food-Entrainable Oscillator

Food availability is a strong external cue that modulates locomotor activity in rodents. Under ad libitum feeding condition, nocturnal animals (such as mice and rats) favor food intake during the active phase (i.e., subjective nighttime). Under ad libitum feeding condition, the mealtime and its associated behavior are coordinated by the SCN [49]. When food access is restricted to daytime, mice display preprandial locomotor activity, preprandial rise in the body temperature, and the release of corticosterone. Such daily arousal and prefeeding activity around 2–3 h prior to the mealtime is designated as “food-anticipatory activity (FAA)” (Fig. 20.2). Under the normal light/dark cycle (LD) and daytime RF condition, mice display FAA in the daytime with subsequent reduction in nighttime activity. In addition, FAA is also observed in animals subjected to constant dark (DD) or constant light (LL) condition. When mice were shifted from restricted feeding to ad libitum
feeding condition, FAA appeared at the expected time for several days [50]. This demonstrates the presence of another internal circadian timing system, which allows mice to adapt and memorize their own mealtime. It means that FAA is not a momentary phenomenon generated by acute fasting. This internal clock predicting the feeding time is called the “food-entrainable oscillator (FEO),” and FAA is a behavioral manifestation of the FEO.

20.6.1 FEO Is Independent of the SCN

The SCN has been identified as the prime site of the light-entrainable oscillator [3], and the underlying molecular mechanism depends on the interlocking autoregulatory feedback system of clock genes [5]. On the other hand, the FEO
location and underlying molecular mechanism remain to be elusive. Since the SCN is the circadian pacemaker, it was postulated that this region could be one of the components of the FEO, but specific experiment did not confirm this hypothesis. Indeed SCN ablation in rats led to arrhythmicity in their day-night locomotor activity, but FAA and feeding-induced corticosterone rhythms still persisted. When SCN-damaged rats were shifted from RF to total food deprivation, FAA was still observed for several days. Furthermore, FAA is readily observable under feeding a regimen with a period within circadian range, which highlights the limits of entrainment [49,51]. Considering the strong evidence showing that FAA remains after the SCN lesion, the FEO may be present in locus or loci outside of the SCN.

20.6.2 Location of the FEO

Following the trail of light into the brain led to the discovery of the SCN as a site of a master circadian clock; a similar approach was applied to the FEO search. The strategy consisted of manipulating or destroying the communication pathways, which may transmit food-related cues to the central nervous system. Some studies aimed to detect those properties of food, which can convey mealtime information to drive FAA. These properties include gustatory, olfactory, food digestion-induced stimuli, and nutrients in the bloodstream. However, nasal epithelium disruption [52], olfactory bulb ablation [53], alteration of hepatic function [54], and subdiaphragmatic vagotomy [55] did not affect food-anticipatory behavior in rats.

The SCN lesion study provided an important methodological approach to quest of FEO location. Ablation of specific brain area was performed, and mice were subjected to a temporal restricted feeding condition. Because of the involvement of the hypothalamus in the regulation of the circadian clock, food intake, and satiety signal processing, it was one of the structures likely to harbor the FEO. However, lesions of the paraventricular hypothalamus (PVN) [50], the lateral hypothalamic (LH) [56], the arcuate nucleus (AN) [57], the ventromedial hypothalamus (VMH) [58], the nucleus accumbens (NAc) [59], the hippocampus, and the amygdala [60] showed weak or no influence on FAA expression. The hindbrain possesses various nuclei, which are involved in control of food intake and hunger. The lesions of caudal brainstem nuclei such as the nucleus of the solitary tract (NTS) [61], the area postrema (AP) [62], and the parabrachial nucleus (PBN) [63] did not abolished FAA.

The dorsomedial hypothalamic nucleus (DMH) is one of the important components in various neural and humoral pathways regulating feeding behavior and body weight. It is connected to other brain regions, which are involved in the regulation of the sleep-awake cycle and body temperature [64]. The cell-specific lesion in DMH had abolished the preprandial rise in body temperature, locomotor activity, and wakefulness. In addition, neuronal activity and period gene expression in the DMH entrained to mealtime under scheduled feeding conditions [65,66]. In another report, rats with complete and fractional DMH ablation displayed normal FAA
relative to intact ones. Hence, the DMH seems not exclusively and explicitly responsible for FAA [67]. However, earlier studies suggested that the DMH actively inhibits the SCN output and facilitates functioning of the FEO located elsewhere in the brain. Thus, the DMH can play a modulatory role in FAA expression [68]. Taken together, the role of the DMH in FEO is still highly contentious. Further functional rescue experiments will be needed to explain the role of the DMH in FAA.

Numerous contradictory observations from single central substrate lesion studies suggested that FAA expression might be an integrated outcome of a network of brain structures. Under RF conditions, \textit{c-Fos} showed temporal expression profile in the PVN, LH, and DMH [69]. In addition, brain stem nuclei, particularly the NTS and PBN, showed neuronal activation in phase with feeding time [70]. This indicates that brain stem nuclei may deliver necessary neuronal entraining information to hypothalamic nuclei for FAA. Increased \textit{c-Fos} expression at expected mealtime was observed in some other structures involved in motivational process such as the prefrontal cortex, lateral septum, stria terminalis, paraventricular thalamic nucleus, and NAc, except in the hippocampus. This suggests a relevant role of corticolimbic structure in the FEO [71]. Another strategy involves the assessment of local cerebral metabolic rate for glucose in RF during FAA expression. It was hypothesized that structures showing significant alteration in glucose utilization under RF would be part of a system responsible for FAA appearance. The intergeniculate leaflets and the paraventricular hypothalamic and the arcuate nuclei showed decreased glucose metabolic rate during food anticipation [72].

All these findings indicate and support the concept of a multi-structural organization of the FEO, which appears to be composed of several hypothalamic, extrahypothalamic, and peripheral tissues.

### 20.6.3 Clock Gene Mutation and FAA

The discovery of clock genes has steered the insights into the molecular and genetic working of the circadian clock. The plausible question was raised, i.e., whether the same clock complex regulates the FEO. In order to address this question, various clock gene mutant mice were subjected to temporal RF conditions. It was hypothesized that the clock gene mutation that is critical for FEO functioning should show impaired FAA. \textit{Clock} mutant mice exhibited FAA, when subjected to temporal restricted food access in LD and DD conditions. This indicated that the \textit{Clock} gene is not necessary for FAA expression [73]. \textit{Npas2}-deficient mice did not show any major changes in their circadian behavior relative to WT [74]. Under RF conditions \textit{Npas2} knockout mice showed delayed FAA expression. It implies that NPAS2 is responsible for adaptability to food restriction [75]. In a report published in 2005, \textit{Cry1/Cry2} double mutant mice developed less stable food-anticipatory rhythms with delayed onset compared to their littermate controls. It suggests alteration, but
not complete loss of FAA in Cry1/Cry2 double mutant mice [76]. It appears that the cry genes are not essential, but necessary for the stability of FAA expression.

It has been demonstrated that Per1 and Per2 genes play important roles in light-induced synchronization of the SCN [77]. Therefore, Per genes may be responsive to other external synchronizer such as food. Per1 mutant and WT mice did not show significant difference in FAA, when challenged with temporal RF condition. Surprisingly, Per2 mutant mice failed to anticipate the mealtime [78]. These results highlighted a role of Per2 in molecular regulation of the FEO. Pendergast et al. (2009) has reported that Bmal1-deficient mice exhibited FAA during RF in LD condition and robust FAA in DD condition [79]. Though FAA is unstable and imprecise in a 24-h feeding regimen, Per1/Per2/Per3 triple mutant mice were able to anticipate mealtime [80].

Some clock gene mutant mice showed unchanged or reduced FAA amplitude. But, lack of food-anticipatory rhythms in Per2-deficient mice suggested the involvement of one circadian clock component in the FEO mechanism. Studying FAA in tissue-specific clock gene knockout mice might provide critical inputs in FEO search.

20.7 Food Entrainment and Peripheral Oscillators

Feeding-related cues could entrain clock gene expression in peripheral tissues. The temporal restricted food access modulates clock gene expression in the liver, kidney, heart, and pancreas without affecting the phase of clock gene expression in the SCN [48]. The feeding-induced phase resetting occurred faster in the liver compared to other peripheral organs, shifting rhythms by 10 h in 2 days [81]. In SCN-lesioned mice, temporal feeding can entrain and reorganize the circadian profile of liver gene expression [82]. It indicates that metabolic changes caused by feeding cycles can lead to dissociation of peripheral oscillators from the SCN. In ad libitum food access, feeding behavior is associated with the activity phase, and the metabolic signals cycle in harmony with the SCN. Thus, the SCN can set the phase of clock genes in peripheral tissues. However, under RF conditions, many metabolic and hormonal signals are shifted according to the mealtime. These signals include glucose, free fatty acids, glucocorticoids, ketone bodies, and some hormones [83]. Different blood-borne factors alone or in combination with other signals can reset the clock in the peripheral tissues [84]. These metabolic and hormonal cues may play a role in FEO functioning.

Glucocorticoids can alter the circadian clock gene expression in many peripheral organs [85], implying a role of glucocorticoids in phase resetting of peripheral tissues. Under RF, corticosterone (CORT) level peaked prior to the mealtime. The correlative rise of CORT and increased prefeeding activity have led to the hypothesis that CORT may act as signal for FEO-related behavioral output [86]. However, the adrenalectomized rats showed no noticeable difference in the magnitude of FAA compared to intact ones [87]. This finding indicates that CORT is not able to
express scheduled feeding-induced behavior. Yamamoto et al. (2005) showed that CORT-induced Per1 expression does not cause a phase shift in clock gene expression [88]. Furthermore, adrenalectomized rats entrained rapidly to RF compared to intact animals [89]. Taken together, the role of glucocorticoids as an entraining signal for peripheral clocks is still controversial.

Acyl ghrelin and des-acyl ghrelin are gastrointestinal peptide hormones synthesized by gastric oxyntic cells and in medial-lateral hypothalamic nuclei. Ghrelin peptides can stimulate feeding in rats and mice. Food-restricted rats and mice revealed preprandial rise in plasma ghrelin. Temporal RF condition can entrain daily rhythms of circadian clock gene expression in oxyntic cells of the stomach [90]. The FAA examination in ghrelin ligand and ghrelin receptor knockout mice indicated that ghrelin is not necessary for FAA [91,92]. In contrast, Davis et al. (2011) showed that ghrelin receptor signaling was necessary for adaptation to the scheduled feeding-induced anticipatory response [93]. In addition to ghrelin, several other metabolic hormones such as leptin, insulin, glucagon, and glucagon-like peptide were assessed for their role in food-entrainable rhythms. However, these metabolic hormones are not necessary for FAA expression, but can act as cues to modulate FAA [94].

The peripheral oscillators have the ability to be entrained rapidly to feeding schedule and metabolic cues. Although they are not necessary for FAA expression, they can play a role in the modulation of the anticipatory activity. Coordination of central and peripheral oscillator systems need to be studied under RF conditions to elucidate the mechanism behind the FEO.

### 20.8 Clinical Relevance

Because circadian clocks and the metabolism are strongly coupled and clock gene mutant mice exhibit altered lipid and glucose homeostasis, the circadian clock mechanism seems to be related with a number of metabolic diseases such as obesity, dyslipidemia, and diabetes. Hepatic overexpression of Cry1 lowered blood glucose concentrations and improved insulin sensitivity in insulin-resistant db/db mice [95]. In primary hepatocytes, the synthetic CRY1/2 agonists inhibited glucagon-induced gluconeogenesis [96]. These studies suggest that compounds that enhance cryptochrome activity may provide therapeutic benefit to individuals with type 2 diabetes. The use of a PPARα agonist causes a phase advance of locomotor activity and feeding rhythm. Given that disrupted circadian rhythms lead to obesity, activation of PPARα can serve as a clinical target for the modulation of both circadian rhythms and metabolism [97]. As highlighted in this chapter, food entrainment can affect the circadian clock mechanism, suggesting that lifestyle can affect human health through the circadian clock mechanism. Given that meal timing plays an important role in synchronizing behavioral and physiological rhythms, it is possible that defects in food entrainment could impair circadian organization of physiology and metabolic function. These studies reveal the
potential of operating on circadian clocks as strategy toward therapeutics for metabolic diseases.

20.9 Summary

- Studies on clock gene-deficient animal models have revealed that circadian rhythms have a key regulatory function in metabolism.
- The clock machinery controls the expression of genes essential for numerous metabolic pathways at the molecular level.
- Circadian oscillators exist not only in the SCN but also in most peripheral tissues, and alterations in feeding rhythm can affect the circadian system.
- Scheduled feeding can organize and entrain peripheral circadian clocks.
- Numerous studies highlighted distributed location and integrated mechanism among central and peripheral clocks behind FEO.

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