Interactions between the circadian clock and metabolism: there are good times and bad times

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An endogenous circadian (∼24 h) clock regulates rhythmic processes of physiology, metabolism and behavior in most living organisms. While able to free-run under constant conditions, the circadian clock is coupled to day:night cycles to increase its amplitude and align the phase of circadian rhythms to the right time of the day. Disruptions of the circadian clock are correlated with brain dysfunctions, cardiovascular diseases and metabolic disorders. In this review, we focus on the interactions between the circadian clock and metabolism. We discuss recent findings on circadian clock regulation of feeding behavior and rhythmic expression of metabolic genes, and present evidence of metabolic input to the circadian clock. We emphasize how misalignment of circadian clocks within the body and with environmental cycles or daily schedules leads to the increasing prevalence of metabolic syndromes in modern society.

Keywords circadian clock; feeding; metabolism; metabolic disorder

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Introduction

Endogenous circadian clocks are found in most organisms. Widely believed to be an adaptation to the day:night cycles on earth, such a circadian timing system ensures rhythms of metabolic reactions and physiological processes to occur at the right time of the day, and thus offers adaptive values to organisms living in the cyclic environment. Indeed, direct competition studies using plants [1] and bacteria [2] demonstrated that a functional circadian clock benefits growth and survival in day:night cycles. Although not a focus of this review, it is important to note that molecular circadian clocks are not only entrained by the environmental cues (the most prominent being light:dark cycles), they are also self-sustaining in constant conditions without environmental signals, which is a hallmark feature of circadian timing systems. Within each clock cell, the molecular clock drives rhythmic expressions of genes important for metabolism and physiology. Recent genome-wide transcriptional profiling studies have identified a large number of clock controlled genes (ccgs) [3–7]. About 2%–35% of the genome display rhythmic expressions in eukaryotes, depending on the detection methods and species used, whereas in the photosynthetic cyanobacteria the circadian clock regulates global gene transcription [8]. The physiological importance of circadian clocks is further demonstrated by recent findings that expressions of ccgs within various pathways are orchestrated to coordinate sequential biochemical reactions and metabolic activities [9–11]. Importantly, some components of the circadian clock are directly involved in metabolic pathways. On the other hand, metabolic reactions impinge on the circadian clock, thereby couples metabolic cycles to the circadian system. Such reciprocal interactions strengthen the circadian clock and enable it to adapt to changes in the cellular environment. Here we first describe the basic molecular framework of the circadian clock and illustrate the organization of the circadian timing system in animals, and then discuss molecular mechanisms underlying circadian regulation of metabolism and present evidence of metabolic input to the circadian clock.

Organization of the Circadian Clock

The basic molecular framework of the circadian clock is highly conserved

The first circadian clock gene, period, was identified in the fruit fly Drosophila melanogaster in 1971 [12]. Cloning of the period gene in the early 1980s [13–15] ushered in a new era of molecular studies of circadian biology. Over the past decades, a number of core clock genes have been identified and characterized. Studies of these bona fide clock genes in multiple model organisms have revealed a
remarkably conserved molecular framework for circadian timing. In principle, the molecular circadian oscillator consists of a transcriptional–translational feedback loop: the circadian activator promotes transcription of the repressor genes, and accumulated repressor proteins feedback to inhibit their own transcription. A series of post-transcriptional and post-translational regulations generate a time-lag to form a circadian cycle \[16\]. Non-transcription-based circadian oscillators have been reported \[17\], but they are beyond the scope of this review.

In *Drosophila*, the circadian activator is the heterodimeric complex of Clock (CLK) \[18\] and Cycle (CYC) proteins \[19\] (Fig. 1). During the daytime, CLK–CYC promotes mRNA transcription of repressor genes *period* (*per*) and *timeless* (*tim*) \[20–23\]. Newly synthesized PER protein is phosphorylated by Doubletime (DBT) (*Drosophila* homolog of casein kinase 1) \[24,25\], followed by the E3 ligase-mediated proteasomal degradation \[26,27\]. TIM is degraded by light-dependent and independent mechanisms \[27–30\]. At night, accumulated TIM and PER proteins form heterodimers and translocate into the nucleus to repress the transcriptional activity of CLK–CYC \[23,31,32\]. Degradation of TIM and subsequently PER at daybreak allows CLK–CYC to start a new cycle of transcription. Importantly, expression of *Clk* itself is regulated by another feedback loop, where CLK–CYC activates mRNA transcription of the negative regulator *vrille* (*vri*) and the positive regulator *Pdp1e* (*PAR domain protein 1 e*), which in turn feeds back to inhibit and activate *Clk* transcription, respectively \[33\]. Since total CLK protein levels do not seem to cycle, the significance of *Clk* mRNA oscillation is not clear. However, the phase of *Clk* mRNA cycling parallels that of CLK phosphorylation (which promotes CLK degradation) \[34,35\], thus oscillating levels of *Clk* mRNA appear to maintain total CLK levels. Such a regulation is important for the maintenance of stable circadian rhythms: since the amount of CLK protein is limiting \[36\], a reduction of CLK or CYC levels renders a long circadian period \[18,19\]. Notably, loss of *Pdp1e* results in a decreased level of CLK proteins in the central clock neurons and mutant flies are behaviorally arrhythmic \[37\].

This basic framework of negative and positive feedback regulation is highly conserved from bacteria, fungi, plants, insects to rodents and humans \[16,38–42\], suggesting a convergent evolution of circadian timing mechanisms. In mammals, Clock forms the heterodimeric complex with the CYC homolog BMAL1 (Brain and muscle arnt-like protein 1) to promote the expression of repressor genes *Period* and *Cry*, and PER–CRY complex in turn negatively regulates their own gene transcription. However, more complexities are built into the feedback loop in mammals. There are three *Per* genes (*Per1*, *Per2*, and *Per3*) and two *Cry* genes (*Cry1* and *Cry2*) \[40\]. In addition, BMAL1 has a functionally redundant paralog BMAL2 \[43\], whereas the Clock paralog NPAS2 (Neuronal PAS domain protein 2) partners with BMAL1 to maintain the circadian clock function \[44,45\] in the absence of Clock protein \[46\]. The positive feedback loop is also more complex in mammals than that in flies: Clock–BMAL1 activates rhythmic mRNA expression of nuclear hormone

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**Figure 1** Basic molecular framework of the circadian clock is conserved from flies to mammals. In the fruit fly *D. melanogaster*, the CLK–CYC heterodimer binds to the promoters of repressor genes *per* and *tim* to promote their mRNA transcription during the day. Accumulated PER and TIM proteins form heterodimers to repress CLK–CYC activity at night. Post-translational modifications, mainly phosphorylation (p), regulate stability and nuclear localization of PER and TIM proteins. Degradation of TIM, and subsequently PER, by light-dependent and independent mechanisms allows a new cycle to begin at the daybreak. In another feedback loop, CLK–CYC activates transcription of *vri* and *Pdp1e*, and their proteins feedback to repress and activate *Clk* mRNA transcription, respectively. Similar negative and positive feedback loops also operate in the mammalian system, such that Clock–BMAL1 complex activates transcription of repressor genes *Per* and *Cry*, which feedback to repress the transcriptional activity of Clock–BMAL1; in another feedback loop, Clock–BMAL1 activates mRNA expression of nuclear hormone receptor genes *Rev-erb* and *Ror*, and their proteins feedback to negatively and positively regulate *Clock* and *Bmal1* transcriptions. Several circadian clock proteins are regulated by post-translational modifications such as phosphorylation (p), acetylation and sumoylation, which are important for their stability, localization or activity.
receptor genes Rev-erb (α and β) and Ror (α, β and γ); REV-ERB and ROR in turn drive rhythmic expression of Bmal1 [47–52], Clock [53], and Npas2 [54,55]. It should be noted that functions of these paralog genes are not fully redundant and they also have different spatial and temporal expression patterns [40,56]. For example, differential expression of NPAS2 may explain the distinct effect of Clock-deficiency on the molecular circadian clock in different tissues [57]. Furthermore, mRNA expression of core clock genes is regulated as a network [58,59] and in fact, these feedback loops are interlocked in mammals and flies [60,61].

Hierarchies of circadian clocks

In mammals, clock cells are present in most tissues. These clock cells harbor cell-autonomous molecular circadian oscillators as described above. However, they are also synchronized by systemic cues from the central pacemaker in the hypothalamus, the suprachiasmatic nuclei (SCN), which consists of a network of ~10,000 heterogeneous clock neurons that control a myriad of hormones and neurotransmitters to regulate circadian physiology and behavior [40]. In day:night cycles, light signal is transmitted through the retinohypothalamic tract (RHT) to entrain the SCN clocks [62], such that circadian clocks in the whole body is synchronized to daily light:dark cycles (Fig. 2). One of the SCN output that synchronizes peripheral clocks is the vasoactive intestinal polypeptide (VIP). Loss of VIP has been reported to alter the phase relationship between the SCN and some peripheral organs [63]. However, the preeminent role of VIP is to couple the network of circadian oscillators within the SCN [64].

While in principle the SCN is the master circadian pacemaker that synchronizes peripheral clocks [40], cell-autonomous circadian clocks in peripheral tissues can sustain rhythmic cellular functions independent of the SCN. For example, a tissue-autonomous clock is sufficient to maintain the rhythm of olfactory sensitivity [65]. In humans, a number of metabolic pathways are directly controlled by the circadian clock [66]. Consistently, genome-wide transcriptional profiling demonstrated that the cell-autonomous hepatocyte clock can drive rhythmic expression of a significant amount of genes [67].

In Drosophila, most clock cells are directly entrained to day:night cycles due to the presence of the photoreceptor CRY, which directly transmits light signals to the molecular circadian clock [68]. Nonetheless, the brain clock regulates feeding behavior [69], and a network of about 150 clock neurons in the central brain orchestrates overt rhythms, such as sleep:wake cycles [70]. Within this network, a cluster of cells named small ventro-lateral neurons (s-LN vs) is the central pacemaker that drives rhythmic behavioral rhythms in constant conditions [71]. The s-LN vs release a neuropeptide called pigment dispersing factor (PDF) to carry out similar functions as the mammalian VIP: it synchronizes circadian clocks in the central brain [72,73]. Notably, s-LN vs are important for the normal clock function in the prothoracic gland [74], suggesting that hierarchical organization of circadian clocks also exists in Drosophila. However, like that in mammals, peripheral

**Figure 2 Interactions between the circadian clock and metabolism**

In mammals, light:dark cycles signal through the RHT, which directly innervates the SCN to entrain the central pacemaker. The brain clock not only regulates rest:activity cycles and feeding rhythms, it also directly sends signals to synchronize circadian clocks in peripheral tissues, such that peripheral clocks are coupled to the central clock-driven feeding:fasting cycles. Within metabolically active tissues such as the liver, circadian clocks and metabolic processes are tightly intertwined. Several central regulators of metabolism are critical components of the molecular circadian clock. Note that feeding signals can bypass or replace the SCN signals to entrain circadian clocks in some peripheral tissues. On the other hand, metabolic signals also feed into brain areas that are potentially regulated by the central pacemaker. Similar interactions may exist in Drosophila. However, most clock cells in peripheral tissues of Drosophila express the circadian photoreceptor CRY, thus they are directly entrained by light:dark cycles. Both the brain and peripheral clocks regulate feeding rhythms, and less is known about the effect of metabolites on circadian clocks in Drosophila.
clocks are sufficient to drive olfactory rhythms, independent of the brain clock [75].

**Circadian Regulation of Metabolism**

In mammals, the SCN orchestrates rhythmic metabolic processes through systemic regulation of hormones and transmitters to coordinate rhythmic feeding behaviors, appetite and metabolic rate [76–78]. For example, feeding rhythm is attenuated in some circadian clock mutants [76,79]. In addition, the phase of feeding rhythm is advanced in mice lacking either VIP or its receptor, and the overall metabolic rate is greatly reduced [78]. Furthermore, the SCN signals to peripheral clocks to coordinate their functions. It has been shown that brain-specific rescue of Clock in the Clock mutant mice not only restored the SCN-driven locomotor activity rhythm but also increased numbers of cycling transcripts in the liver, including some core circadian clock genes, even though the expression of most clock-controlled genes (ccgs) did not cycle in the brain-Clock-rescued animals [3]. It is possible that rhythmic expression of these clock genes is directly driven by rhythmic cues from the Clock-rescued-SCN, or indirectly by rhythmic behaviors, such as sleep : wake cycles and feeding rhythms (Fig. 2). In line with this argument, conditional disruption of BMAL1 function in the liver demonstrated that mRNA expression of Per2 continues to cycle, indicating that SCN-derived circadian cues directly drive some clock gene expression in the liver [80]. Similar results were obtained when CLK activity was disrupted in the Drosophila fat body (equivalent to the mammalian liver and adipose tissue) [81]. Interestingly, certain perturbations of the s-LN,s in the central brain altered lipid storage in the fat body [82]. The exact mechanism underlying such a regulation is unknown. Since rhythmic feeding behavior is regulated by the circadian clock [69,83] and both the brain clock and peripheral clocks contribute to the maintenance of feeding rhythms and energy metabolism [69], it will be interesting to determine if rhythmic feeding behavior is altered in the aforementioned flies.

Metabolically active tissues such as the liver, pancreas and gastrointestinal organs all possess circadian clocks that regulate tissue-specific functions [84]. Notably, disruption of the circadian clock in the islet leads to hypoinsulinemia and diabetes due to defective β-cell functions [85]. Liver-specific deletion of Bmal1 disrupts the rhythm of glucose metabolism and results in low fasting glucose levels [86]. Consistently, hepatic overexpression of Cry1, the negative regulator of BMAL1 activity, inhibits gluconeogenesis and reduces blood glucose levels [87]. An important question, however, is whether these metabolic defects are due to lack of oscillating clock gene activities, or due to altered transcriptional activities of these genes per se. In support of the latter, it has been reported that mRNA expression of a significant number of genes that normally do not cycle (thus they are not defined as ccgs) are also reduced in Clock mutants [5]. However, cyclic expression of most ccgs in the liver is regulated by the cell-autonomous clock. These ccgs are directly or indirectly regulated by a number of core clock genes [10,88,89]. A large number of ccgs are involved in metabolism of carbohydrate, lipid, protein and detoxification [3–7]. Importantly, a significant portion of these genes encodes central regulators of metabolic processes [90–94]. Furthermore, clock-controlled metabolic reactions in turn regulate the transcriptional activity of other metabolic genes [95], and a large number of circadian regulated pathways related to metabolism are coordinated in a way that the product of one pathway feeds into another pathway [9]. Such complex regulation of metabolism by the circadian clock explains why metabolic syndromes develop when the circadian clock is disrupted [48,76,79,96–100].

Similarly in Drosophila, genome-wide transcriptional profiling of the fat body demonstrated that a large number of genes involved in metabolism are rhythmically expressed, and fat body-specific disruption of CLK was sufficient to alter feeding rhythms and abolish cyclic expression of most of these ccgs [81], again suggesting that most ccgs in the fat body are regulated by the cell-autonomous circadian clock (Fig. 2).

**Metabolic Processes Feedback to the Circadian Clock**

While the central pacemaker directly signals to peripheral clocks in mammals, systemic cues generated by the central pacemaker plays a critical role in entraining circadian clocks in metabolically active tissues (Fig. 2). As discussed above, a functional SCN clock can regenerate the rhythmic expression of some core clock genes in the liver that has a defective clock [3,80], likely due to rhythmic feeding behavior driven by the SCN. Direct evidence for this effect can be demonstrated by scheduled feeding : fasting cycles. In fact, time-restricted feeding (RF) was able to restart rhythmic expression of some clock genes in a clock-defective background, in addition to restoring rhythmic expression of a large number of ccgs in the liver [101]. Furthermore, RF in abnormal timeframe can uncouple peripheral clocks from the SCN clock [102,103].

Such strong effects of feeding on circadian clocks are seemingly in conflict with the prominent role of the SCN in regulating peripheral clocks. On the other hand, these findings support the notion that feeding : fasting cycle is one major output from the SCN to synchronize the liver clock. In fact, the circadian clock and rhythmic metabolic processes are interconnected, such that systemic regulation of
feeding and metabolic processes by the SCN results in cyclic changes of cellular environment, which in turn feedback to modulate the circadian clock [104]. Notably, the liver circadian clock drives rhythmic expression of the nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme of NAD⁺ biosynthesis [105,106]. On the other hand, in vitro experiments demonstrated that the DNA-binding activity of Clock-BMAL1 is sensitive to NAD⁺/NADH ratio [107]. In vivo, it has been shown that the NAD⁺-dependent poly(ADP-ribose) polymerase 1 modulates the DNA-binding activity of Clock–BMAL1 [108]. Furthermore, cyclic levels of NAD⁺ modulate SIRT1 activity, which in turn regulates the acetylation of BMAL1, PER2, and PGC-1, all of which subsequently affects Clock–BMAL1 activities [109–111]. In addition, the AMP-activated protein kinase acts on CRY through phosphorylation and subsequent degradation [112]. Since fasting results in elevated levels of NAD⁺ through the upregulation of NAMPT [113], and expression levels of AMP [114] and SIRT1 are also increased during fasting [115], the circadian clock and metabolic cycles are effectively interlocked in metabolically active tissues (Fig. 2).

Not only is the cycling of metabolites important for the circadian clock, primary metabolic signaling pathways, such as the insulin and target of rapamycin (TOR) pathways, also regulate circadian clock in mammals and flies [116,117]. In fact, some key regulators of metabolic pathways, such as PGC-1 [118] and nuclear hormone receptors REV-ERBs and RORs, are essential components of the circadian clock [47,48,52,90]. A dual role of these proteins in metabolism and the circadian clock further blurs the distinct separation of these two fundamental processes.

**Deleterious Effect of Circadian Disruptions on Health**

In modern 24/7 human societies, the circadian clock is frequently disrupted by reduced exposure to sun light and excessive night-time illumination and activities. While the modes of disruptions differ, all circadian disruptions result in a misalignment of circadian clocks, either between tissues, or between the body and environmental cycles. One prominent example of circadian disruption is jetlag. Explant monitoring of Per1-luciferase activities has shown that while the SCN clock adjust quickly to the day : night cycle in the new time zone, the rest of the body tissues are much slower to adapt [119]. More recently, it was found that there is a strong heterogeneity of entrainment kinetics not only among different tissues, but also among core clock genes within each tissue, including the SCN [120]. Interestingly, scheduled feeding improved the resetting of the SCN-driven rhythms of behavior and core body temperature in a rat model of jetlag [121].

The adverse effect of circadian misalignment on metabolism has only begun to be elucidated [122–125]. As discussed above, sleep : wake and feeding : fasting cycles are intrinsically hardwired. Under normal conditions, feeding during the active time ensures an optimal coordination of circadian entrainment driven by the light : dark cycle and feeding : fasting cycle. Feeding during the sleep phase shifts circadian clocks in metabolically active tissues, thus resulting in a desynchrony within the body [102,103,126]. Furthermore, altered feeding schedule elicits different responses from different organs [102,127,128], similar to the effect of jetlag. Indeed, timing, duration, and frequency of meals all affect circadian clocks in different ways [129–132]. In addition, frequent ingestion of food causes deregulations in the brain. For example, rhythmic expression of ghrelin is regulated by the circadian clock in the stomach, but also directly influenced by feeding [133], thus feeding at the wrong time of the day sends conflicting signals to the brain and other peripheral tissues (Fig. 2). Altered feeding may also underlie metabolic dysfunctions observed in the shiftwork schedule [134,135]. Notably, while *ad libitum* feeding of high-fat diet disrupted the circadian clock in the liver, time-restricted feeding of high-fat diet during the normal active time (night-time for mice) reversed its adverse effect on the circadian clock and metabolism [136–138].

In fruit flies, restricted feeding at a time when they are normally resting also disrupted energy metabolism and decreased reproductive fitness [81]. Importantly, disruption of the fat body clock and brain clock resulted in opposite effects on energy metabolism, indicating that a proper coordination between the brain clock and peripheral clocks is essential for energy homeostasis [69,86].

**Perspective**

Like many other biological reactions, functional separation of the circadian timing system and metabolic cycles is achieved by spatial separation: the central pacemaker in the brain is largely insulated from daily fluctuations of metabolic reactions. In animals, the central pacemaker couples peripheral clocks to sleep : wake and feeding : fasting cycles, such that overt rhythms of behavior, physiology, and metabolic functions are aligned to environmental light : dark cycles. This hierarchical organization ensures optimal evolutionary fitness in the cyclic environment. In line with the notion that the SCN-driven feeding : fasting cycle couples peripheral clocks, circadian clocks and metabolic processes are tightly intertwined in metabolically active tissues, such that circadian and metabolic cycles reinforce each other to sustain robust rhythms. However, such alignments are increasingly being disrupted in the industrialized world by irregular light exposure, shiftwork schedule, and...
unlimited food access. Meanwhile, circadian disruptions impair metabolic functions. Thus mutual disruption of the circadian clock and metabolism supports a vicious cycle that may contribute to the epidemics of metabolic syndromes [124,139—141]. A better mechanistic understanding of the communication between the central pacemaker and peripheral clocks, and interactions between the circadian clock and metabolic cycles will eventually lead to strategies aimed to prevent or even treat circadian abnormalities and metabolic disorders.

Concluding Remarks

In summary, several key points are discussed in this review: (i) central and peripheral clocks coordinate metabolism through feeding: fasting cycles and direct regulation of metabolic reactions; (ii) metabolic processes feedback to regulate circadian clocks through changes of levels of metabolites and activities of metabolic regulators; (iii) circadian disruptions result in metabolic disorders; and (iv) interconnected circadian clock and metabolic cycle strengthen the function of both processes, but also forms a vicious cycle to impact health when either one is disrupted.

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