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Review

Clocks, Metabolism, and the Epigenome

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Many behaviors and physiological activities in living organisms display circadian rhythms, allowing the organisms to anticipate and prepare for the diurnal changes in the living environment. In this way, metabolic processes are aligned with the periodic environmental changes and behavioral cycles, such as the sleep/wake and fasting/feeding cycles. Disturbances of this alignment significantly increase the risk of metabolic diseases. Meanwhile, the circadian clock receives signals from the environment and feedback from metabolic pathways, and adjusts its activity and function. Growing evidence connects the circadian clock with epigenomic regulators. Here we review the recent advances in understanding the crosstalk between the circadian clock and energy metabolism through epigenomic programming and transcriptional regulation.

Introduction

The approximately 24 hr cycling of light and dark drives the cyclic changes in the living environments for most organisms on Earth, from cyanobacteria to human beings. To adapt to changing environments, organisms anticipate the periodic changes and adjust their activities with the time of the day using an internal 24 hr clock system, known as the circadian clock. In human beings and other mammals, the clock governs many important behaviors and physiological processes, including sleep/wake, feeding, body temperature, hormone secretion, and metabolism.

Human beings are diurnal creatures. We conduct most of our activities during the day, including feeding, exercising, and working, and rest at night. Circadian clocks in our bodies provide time cues for activities and meanwhile synchronize the metabolic reactions with the anticipated activity cycles. The synchronization of behaviors and metabolism by the clock ensures the energy supply and maintains the internal homeostasis. However, this delicate system has been increasingly challenged in modern society. Modern life is characterized by increases in night activities, for instance, shift work, overtime work, night eating, sleep disruption, and deprivation. Misalignment of activities with the internal clock and metabolic rhythms could disrupt the clock and energy homeostasis. Evidence suggests that shift workers have a higher risk of metabolic diseases, including obesity, diabetes, metabolic syndromes, and cardiovascular diseases (Wang et al., 2011). Similar effects were also observed with sleep deprivation, sleep disruption, and night eating (reviewed in Huang et al., 2011).

In recognition of these concerns, much recent research focuses on the crosstalk between the circadian clock and metabolism. The core mammalian biological clock consists of interlocked activators and repressors of transcription that function via epigenomic mechanisms, which can be tuned with metabolic signals, including hormones and metabolites, and also have direct effects on metabolic events. In this review, we will summarize recent advances in understanding how circadian clocks crosstalk with metabolic pathways through epigenomic mechanisms.

Environment, the Epigenome, and Metabolism

In addition to the linear genomic DNA sequences, information affecting the expression of individual genes can be encoded in the chromatin using mechanisms such as DNA methylation, histone modification, and chromatin remodeling. This additional layer of gene regulation may be referred to as the epigenome. Epigenomic modification provides plasticity in gene expression and cellular functions in multicellular organisms, and allows reversible changes in response to changes in the their environment, including light, temperature, food availability, and dietary composition, which can affect many physiological processes, including development, aging, and metabolism (reviewed in Christensen and Marsit, 2011).

Metabolism is tightly regulated, and imbalance of energy intake and expenditure leads to accumulation of nutrients and metabolites and thus contributes to metabolic diseases, cardiovascular diseases, cancer, and other diseases. A common theme in metabolic control is transcriptional regulation of rate-limiting metabolic enzymes, usually involving epigenomic mechanisms. For instance, hepatic glucose production and secretion are regulated by phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), respectively. *Pepck* and *G6Pase* are activated by glucagon and fasting through cAMP-responsive binding element protein (CREB), repressed by insulin through the forkhead O box protein 1 (FOXO1), and stimulated by glucocorticoids through glucocorticoid receptor (GR) (reviewed in Jitrapakdee, 2012). Environmental factors such as nutrition, exercise, aging, and stress can signal through metabolic hormones, such as insulin and leptin, and metabolites, such as nicotinamide adenine dinucleotide (NAD^+), ADP, acetyl-CoA, and S-adenosylmethionine (SAM) (Christensen and Marsit, 2011). These signals regulate the epigenome by modulating the function of chromatin-modifying enzymes as well as transcription factors that are responsible for recruiting these enzymes.

Take calorie restriction (CR) as an example, in which amount of daily calorie intake is reduced by 30%–50% compared to ad libitum feeding. CR can increase life span and delay the onset of many aging-related diseases, including cancer and diabetes. CR is known to decrease blood insulin and thyroid hormone

levels in human (reviewed in Vaquero and Reinberg, 2009). Insulin signaling leads to phosphorylation and inhibition of FOXO1, whose targets include stress response genes and gluconeogenic genes (Dong et al., 2008).

CR response is also mediated by metabolites. Low energy intake in CR elevates the NAD^+/NADH ratio and AMP/ATP ratio, which can be sensed by NAD^+-dependent histone deacetylase (HDAC) sirtuins and AMP-activated protein kinase (AMPK), respectively. In mammals, sirtuin 1 (SIRT1), activated by NAD^+, deacetylates histone marks H4K16Ac and H3K9Ac and histone methyltransferase (HMT) SUV39H1, thus promotes formation of facultative heterochromatin, and represses transcription. SIRT1 also deacetylates and activates FOXO1 and PGC-1α, and promotes gluconeogenic gene expression (reviewed in Vaquero and Reinberg, 2009). This process might involve changes in the recruitment of histone acetyltransferases (HATs) by FOXO1 and PGC-1α. AMPK is activated by increase in AMP/ATP ratio, and directly phosphorylates PGC-1α, enabling SIRT1-mediated deacetylation and activation of PGC-1α. FOXOs might also be regulated similarly (reviewed in Cantó and Auwerx, 2011). Through these transcription factors and coregulators, AMPK can direct epigenomic remodeling and metabolic reprogramming. Sensing the nutritional/metabolic state through sirtuins and AMPK is a common theme, and also mediates responses to fasting and exercise (reviewed in Freyssenet, 2007; Cantó and Auwerx, 2011).

Environment and the Clock

Metabolism is also controlled by the internal circadian rhythm. The cell-autonomous clock machinery consists of several transcriptional-translational feedback loops, which allows autonomous oscillation with a period of approximately 24 hr (Figure 1). In mammals, the first feedback loop in the basic clock machinery contains a heterodimer of transcription activators the brain and muscle ARNTL-like protein 1 (BMAL1) and the circadian locomoter output cycles kaput (CLOCK). BMAL1/CLOCK activates transcription of Crytochromes (CRYs) and Periods (PERs). CRY and PER proteins negatively regulate their own expression by binding BMAL1/CLOCK and inhibiting their transcriptional activity (reviewed in Bass and Takahashi, 2010). Another critical feedback loop drives the cyclic transcription of BMAL1 using nuclear receptor (NR) REV-ERBα/REV-ERBβ. BMAL1 activates *Rev-erbα* transcription, which then suppresses *Bmal1* transcription (Preitner et al., 2002). This second loop, once considered auxiliary, was recently proved to be essential for the clock function (Bugge et al., 2012; Cho et al., 2012; Solt et al., 2012). The clock machinery is featured by the redundancy of its components: BMAL1/BMAL2, CLOCK/NPAS2 (neuronal PAS domain containing protein 2), CRY1/CRY2, PER1/PER2/PER3, and REV-ERBα/REV-ERBβ (Bugge et al., 2012; Cho et al., 2012; reviewed in Bass and Takahashi, 2010) (Figure 1). Other factors that are important for clock function include the retinoid-related orphan receptor RORs (RORα, RORβ, RORγ), which activate transcription of BMAL1 and REV-ERBα and are repressed by REV-ERBα (reviewed in Jetten, 2009). Levels of the clock proteins are also subject to posttranslational regulation by casein kinases (CKIε and CKIδ) and ubiquitin E3 ligases β-TrCP, FBXL3, and ARF-BP1/PAM (Yin et al., 2010; reviewed in Bass and Takahashi, 2010). Although the transcriptional-translational clocks are ubiquitous in mammalian cells, nontranscriptional mechanisms are also sufficient to sustain circadian oscillation. In picoeukaryotic alga *Ostreococcus tauri*, oxidation of peroxiredoxin proteins undergoes circadian cycles independent of the transcriptional circadian clock of the alga (O'Neill et al., 2011). Recently this mechanism was shown to be conserved in higher organisms, including human, at least in human red blood cells (O'Neill and Reddy, 2011). The widespread presence of transcription-independent redox oscillations suggests that crosstalk between metabolic cycles and circadian clocks has long played a significant role in both physiology and evolution.

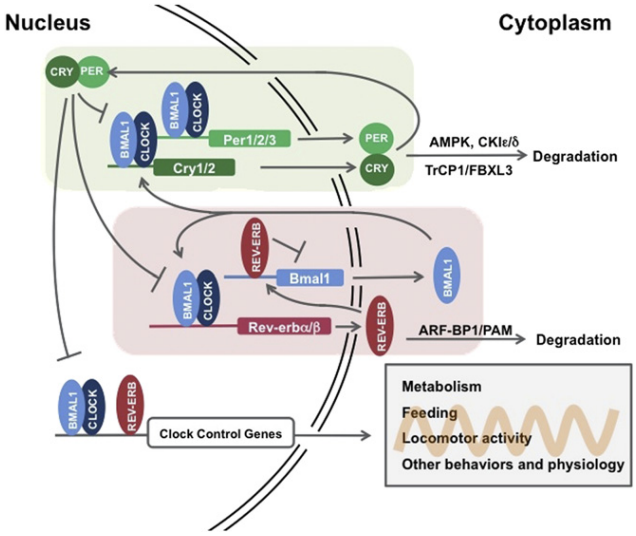

Figure 1. The Basic Clock Machinery Consists of Negative Transcriptional-Translational Feedback Loops

In the first loop, BMAL1/CLOCK drives *Per/Cry* transcription, while PER/CRY binds and inhibits transcriptional activity of BMAL1/CLOCK. In the second loop, BMAL1/CLOCK drives REV-ERB expression, which in turn represses *Bmal1* transcription. Both loops are essential for maintaining circadian rhythm. In addition, posttranslational modification, as shown for PER/CRY and REV-ERB, is also important in regulating clock activity. The core clock machinery can drive rhythmic behavioral and physiological activities, such as metabolism.

In mammals, the basic clock machinery is present in most organs, assembled in a hierarchical system in which the central clock can entrain peripheral clocks (Figure 2). The central clock is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus, while peripheral clocks are present in most organs, including metabolic organs such as liver, adipose, heart, muscle, and kidney (reviewed in Dibner et al., 2010). Both the central clock and peripheral clocks can be reset by environmental cues, also known as Zeitgebers ("time giver"). The predominant Zeitgeber for the central clock is light, which is sensed by retina and signals directly to SCN. The central clock can entrain the peripheral clocks through neuronal and hormonal signals, as well as body temperature, aligning all clocks with the external light/dark cycle (Balsalobre et al., 2000; Brown et al., 2002; reviewed in Dibner et al., 2010). Peripheral clocks in metabolic tissues are also entrained by the central clock through feeding/fasting cycles (Figure 2). Fasting/feeding alters the levels of key

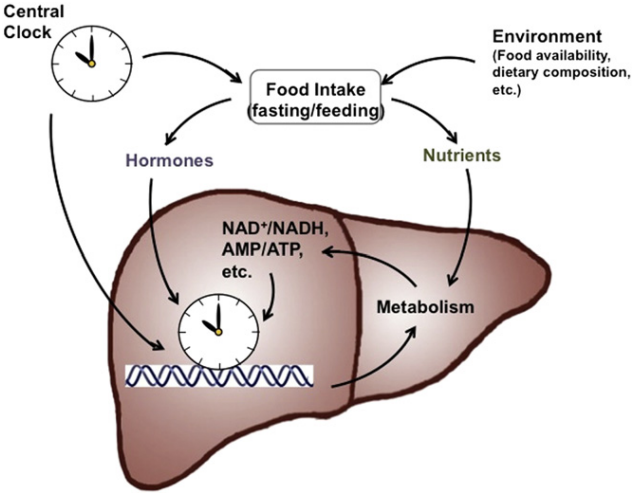

Figure 2. Overview of the Interplays between Environment, Circadian Clocks, and Metabolism

The circadian clock in the cells comprising metabolic organs, such as liver, functions as an epigenomic programmer and controls metabolic outputs. This autonomous apparatus is regulated by the central clock in the SCN and by food intake via hormones and nutrients/metabolites. Both the central clock and food availability contribute to the temporal regulation of food intake.

endocrine hormones and the intracellular metabolic state, which can modulate the function of peripheral clocks. When restricted food availability shifts the fasting/feeding cycles, peripheral clocks can be reset separately from the central clock, suggesting that food availability is the predominant Zeitgeber for these clocks (Damiola et al., 2000; Stokkan et al., 2001).

Rhythmic Regulation of Gene Expression: The Clock Mechanism

How does the basic clock machinery coordinate metabolic activities in different metabolic organs, as well as other behaviors and physiology? Growing evidence suggests that circadian clocks control physiology through transcription. Transcriptomic studies in different mouse tissues revealed that a large proportion of the whole transcriptomes, from 3% to 20%, undergo circadian oscillation (reviewed in Green et al., 2008). The circadian transcriptomes are tissue specific and tightly correlated with cellular functions. In SCN, it includes genes involved in protein-neuropeptide synthesis, secretion, and degradation and regulation of locomoter activity, while in liver, it includes genes involved in glucose, lipid, and xenobiotic metabolism (Akhtar et al., 2002; Panda et al., 2002). Circadian control of activities can also be mediated through translational and posttranslational regulation (Reddy et al., 2006). However, here we will focus on transcriptional regulation and epigenomic reprogramming.

In the core clock machinery, BMAL1/CLOCK, REV-ERBs, PERs, CRYs, and RORs are all transcription regulators. Both BMAL1/CLOCK and REV-ERBs display rhythmic genome binding and can drive the rhythmic expression of their targets (Ripperger and Schibler, 2006; Feng et al., 2011; Rey et al., 2011; Bugge et al., 2012). For instance, REV-ERBα directly regulates gluconeogenic enzymes *G6Pase* and *Pepck*, and many lipid biosynthetic genes (Yin et al., 2007; Feng et al., 2011). More importantly, the core clock machinery can drive circadian expression of many transcription factors, thus extending and enhancing its regulatory function. Among these factors are NRs, such as retinoic acid receptors (RARs), TRs, peroxisome proliferator-activated receptors (PPARs), GR, and short heterodimer partner (SHP) (Yang et al., 2006). Other transcription factors include the three members of the proline- and acidic amino acid-rich (PAR) basic leucine zipper proteins: albumin D site binding protein (DBP), thyrotroph embryonic factor (TEF), and hepatocyte leukemia factor (HLF), and the related protein E4BP4. All four of these proteins bind to D boxes, but while DBP, TEF, and HLF are activators, E4BP4 is a repressor. Many of these factors are direct targets of the clock. BMAL1 controls *Pparα* and *Dbp*, and REV-ERBα controls *Shp* and *E4bp4* (Canaple et al., 2006; Duez et al., 2008). The circadian clock also regulates stability and activity of these factors. PER2 binding promotes ERα degradation, regulates PPARγ DNA binding, and coactivates PPARα-mediated transcription (Gery et al., 2007; Grimaldi et al., 2010; Schmutz et al., 2010), while CRY binding represses GR (Lamia et al., 2011). The clock can also regulate these factors indirectly, for instance, through their ligands, such as glucocorticoid for GR (Reddy et al., 2007). The clock signal can also be mediated by transcription coactivators PGC-1α and BAF60a, as well as microRNAs (Wu et al., 2009; Tao et al., 2011; Gatfield et al., 2009).

Clock Regulation of the Epigenome

Transcription factors control gene transcription by facilitating recruitment and activation of the transcription machinery, by altering the epigenome to recreate a more favorable environment for transcription, or most of the time by both (reviewed in Farnham, 2009). Accumulating evidence, summarized in this section, implicates the core clock genes as partners of chromatin-modifying enzymes (Table 1).

Histone Acetylation

Histone acetylation undergoes cyclic oscillation with the clock, as evident in mouse liver, not only at the promoters of the clock genes but also in a genome-wide scale (Etchegaray et al., 2003; Curtis et al., 2004; Naruse et al., 2004; Ripperger and Schibler, 2006; Feng et al., 2011). Histone acetylation is controlled by a battle between HATs and HDACs, both of which participate in clock-regulated rhythmic gene transcription.

The BMAL1/CLOCK or BMAL1/NPAS2 heterodimer recruits both HATs and HDACs. BMAL1 binds transcription coactivator p300 and possibly the CREB-binding protein (CBP), while CLOCK and NPAS2 bind p300 and the CBP-associated factor (PCAF) (Takahata et al., 2000; Etchegaray et al., 2003; Curtis et al., 2004). All three coactivators have intrinsic HAT activity. In liver and heart, p300 exhibits a circadian association with CLOCK or NPAS2, correlating with increase in *Per1* mRNA and histone H3 acetylation on the *Per1* promoter (Etchegaray et al., 2003; Curtis et al., 2004). BMAL1/CLOCK also directly interacts with SIRT1, recruits SIRT1 to the *Dbp* gene in a timely manner, and drives rhythmic histone acetylation and gene transcription (Asher et al., 2008; Nakahata et al., 2008) (Figure 3). Moreover, CLOCK itself is a HAT, and its HAT activity is essential for the rhythmic acetylation at the *Per1* promoter and the circadian expression of *Per1* and *Dbp* (Doi et al., 2006).

Table 1. Clock-Associated Histone-Modifying Activity

Clock Component	Associated Chromatin Modifier	Targets	References
BMAL1/CLOCK	CLOCK	H3K9, H3K14 Bmal1(K537), GR	Doi et al., 2006; Nader et al., 2009; Charmandari et al., 2011
	p300	H3	Takahata et al., 2000; Etchegaray et al., 2003
	PCAF		Curtis et al., 2004
	CBP		Takahata et al., 2000; Curtis et al., 2004
	SIRT	H3K9, H3K14, Bmal1(K537), Per2	Asher et al., 2008; Nakahata et al., 2008
	MLL1	H3K4	Katada and Sassone-Corsi, 2010
	EZH2	H3K27	Etchegaray et al., 2006
	JARID1a		DiTacchio et al., 2011
BMAL1/NPAS2	p300	H3, H4	Curtis et al., 2004
	PCAF	H3, H4	Curtis et al., 2004
	ACTR		Curtis et al., 2004
PER	PSF-SIN3A-HDAC1	H3, H3K9, H4K5	Duong et al., 2011
	WDR5	H3K4	Brown et al., 2005
CRY	SIN3B-HDAC1/2	Histone H3, H4	Naruse et al., 2004
REV-ERBα,β	NCoR-HDAC3	H3, H4, H3K9	Ishizuka and Lazar, 2003; Yin and Lazar, 2005; Feng et al., 2011; Bugge et al., 2012

PER and CRY direct the negative feedback signal to BMAL1/CLOCK probably through attenuating its affinity for DNA (Figure 1) (Ripperger and Schibler, 2006). In addition, through interaction with BMAL1/CLOCK, CRY1 can recruit SIN3B corepressor and HDAC1/2 to the *Per1* promoter and repress *Per1* transcription through histone deacetylation (Naruse et al., 2004). Similarly, PER2 recruits SIN3A corepressor and HDAC1 through the polypyrimidine tract-binding protein-associated splicing factor (PSF) to the *Per1* promoter (Duong et al., 2011).

REV-ERBs and RORs are members of the NR family, a family known to employ multiple coactivator and corepressor complexes for chromatin modification. REV-ERBα recruits nuclear receptor corepressor (NCoR) and HDAC3 to deacetylate histones and repress transcription (Yin and Lazar, 2005). RORs interact with both corepressors and coactivators (reviewed in Jetten, 2009), and the rhythmic binding of RORs to the *Npas2* promoter is positively correlated with DNA accessibility and histone acetylation (Takeda et al., 2011).

Histone Methylation

Whereas histone acetylation is usually associated with transcriptional activation, methylation has a mixed effect on transcription, depending on the modification sites. CLOCK interacts

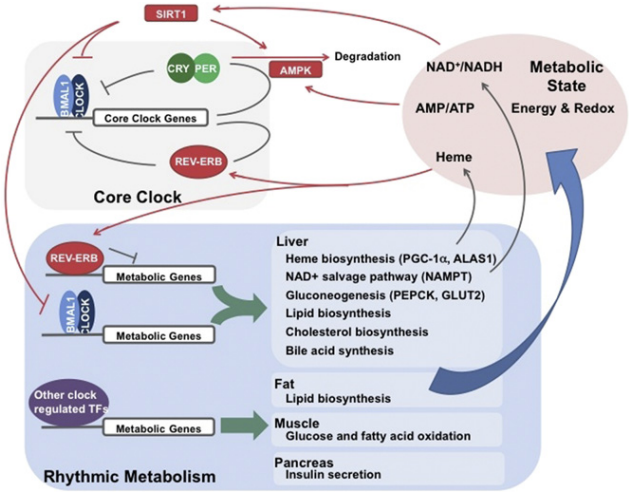

Figure 3. The Core Clock Machinery Drives Rhythmic Metabolic Activities and Receives Feedback from Intracellular Metabolites

BMAL1/CLOCK and REV-ERB drive rhythmic metabolic outputs, including NAD^+ and heme biosynthesis, while intracellular NAD^+ and heme feedback on the clock through their sensors, SIRT1 and REV-ERBs, respectively. Intracellular AMP levels regulate the circadian clock through activation of AMPK and degradation of PER and CRY. The core clock also drives many metabolic pathways in different tissues, which also contributes to the intracellular metabolite pool and metabolic state.

with and recruits the mixed lineage leukemia 1 (MLL1), a HMT that specifically promotes H3K4me3, an activation mark. MLL1 is required for circadian H3K4 methylation and H3 acetylation and for circadian gene expression (Katada and Sassone-Corsi, 2010). PER1 associates with WD repeat-containing protein 5 (WDR5), and loss of WDR5 abolishes the circadian H3K4 and H3K9 methylation at the *Rev-erbα* promoter (Brown et al., 2005). Interestingly, MLL1 and WDR5 can be present in the same methyltransferase complex. BMAL1/CLOCK binds polycomb protein EZH2 and methylates H3K27 at the *Per1* and *Per2* promoters, which is essential for CRY-mediated transcriptional repression (Etchegaray et al., 2006). BMAL1/CLOCK also recruits JumonjiC and ARID domain-containing histone lysine demethylase 1a (JARID1a) to the *Per2* promoter, though it appears that the circadian functions of JARID1a may be independent of its histone-modifying activity (DiTacchio et al., 2011).

Chromatin Remodeling

Chromatin remodeling often accompanies histone modification and transcriptional regulation (reviewed in Strahl and Allis, 2000). One study showed that RORs recruit the SWItch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex subunit BAF60a to drive rhythmic *Bmal1* and *G6Pase* expression (Tao et al., 2011). However, this aspect of clock function is understudied at the present time.

Rhythmic chromatin modification is not limited to the promoter of clock genes that have been studied, as ChIP-seq revealed that BMAL1 and REV-ERBs bind thousands of sites throughout the genome (Dufour et al., 2011; Feng et al., 2011; Rey et al., 2011; Bugge et al., 2012). In liver, REV-ERBs drive genome-wide circadian recruitment of HDAC3. As a

consequence, although HDAC3 expression is constant, it cyclically deacetylates histone and represses transcription at regions targeted by REV-ERBα and REV-ERBβ (Feng et al., 2011; Bugge et al., 2012). In addition, the clock can drive rhythmic epigenomic programming indirectly through clock-regulated transcription factors, such as NRs, which then recruit HATs and HDACs.

Nonhistone Targets of Clock-Associated Histone Modifiers

Histone-modifying enzymes also target transcription factors, cofactors, and enzymes. CLOCK acetylates its partner BMAL1 and facilitates CRY1 binding and CRY1-mediated transcriptional repression (Hirayama et al., 2007). CLOCK also acetylates GR, attenuates its DNA binding, and regulates glucocorticoid response (Nader et al., 2009; Charmandari et al., 2011). SIRT1 counteracts CLOCK-mediated BMAL1 acetylation, and deacetylates and degrades PER2, thus derepressing BMAL1/CLOCK (Nakahata et al., 2008; Asher et al., 2008) (Figure 3). Activation or inhibition of transcription activity will also alter the epigenomic state of the target genes. Also, clock-associated histone modifiers can be recruited by other transcription factors and function independent of the clock machinery.

Circadian Regulation of Metabolism

Metabolic activities undergo diurnal changes, as reflected by the oscillating hormone levels in blood and rhythmic activities of metabolic pathways. Hormones mediate the crosstalk between the central nervous system and major metabolic organs, and are essential to metabolic homeostasis. Some of them are secreted in a circadian manner, including insulin and glucagon from pancreas, adiponectin and leptin from adipose, and ghrelin from stomach (reviewed in Froy, 2007). Many metabolic pathways display rhythmic activities, consistent with levels and activities of the rate-limiting enzymes. For instance, gluconeogenesis peaks in the daytime with PEPCK activity (Kida et al., 1980). Rhythmic activities were also observed in xenobiotic metabolism, glycogen metabolism, and amino acid metabolism, and in other metabolic tissues, such as fat (reviewed in Davidson et al., 2004; Gimble and Floyd, 2009).

The role of circadian clocks in metabolic regulation is well supported by genetic evidence that mutation in clock genes disturbs rhythmic expression of key metabolic genes and causes metabolic disorders. Key metabolic pathways under clock control will be discussed in this section. In addition, other metabolic or related pathways are also regulated by the circadian clock, including cardiovascular function and inflammation (reviewed in Bass and Takahashi, 2010).

Glucose Homeostasis

Loss of BMAL1 attenuates the diurnal variation in glucose and triglyceride levels, impairs gluconeogenesis, and causes glucose intolerance, while the Clock mutant mice (ClockΔ19) develop hyperglycemia and hypoinsulinemia (Rudic et al., 2004; Lamia et al., 2008; Turek et al., 2005). Both genetic models suggest that BMAL1/CLOCK plays a critical role in glucose homeostasis. The study was followed by tissue-specific deletion of *Bmal1* in liver and pancreatic β cells, both of which support the critical role of peripheral clocks in metabolic tissues. Loss of BMAL1 in liver abolishes rhythmic expression of glucose metabolic genes, e.g., *Pepck* and *Glut2*, and leads to hypoglycemia only in the fasting phase of the day, while loss of BMAL1 in pancreatic β cells impairs insulin secretion (Figure 3) (Lamia et al., 2008; Marcheva et al., 2010).

Other clock components also play a role. CRYs inhibit gluconeogenic gene expression, probably through regulating CREB activity. Therefore, hepatic depletion of CRY1/2 increases circulating glucose, while CRY1 overexpression reduces fasting blood glucose and improves whole-body insulin sensitivity in *db/db* mice (Zhang et al., 2010). CRYs also transrepress glucocorticoid-induced *Pepck* transcription, and loss of CRYs results in glucose intolerance (Lamia et al., 2011). Similarly, depletion of both REV-ERBs increases fasting blood glucose (Cho et al., 2012), and RORα is known to activate *G6Pase* through SRC-2 and BAF60a (Chopra et al., 2008; Tao et al., 2011).

Lipid Metabolism

Clock mutation and *Bmal1* deficiency also impair lipid metabolism, as shown by hyperleptinemia, hyperlipidemia, and hepatic steatosis (Turek et al., 2005; Shimba et al., 2011). The mechanism behind this remains unclear. Ablation of REV-ERBs also causes hepatic steatosis, in part via derepression of lipogenesis (Feng et al., 2011; Bugge et al., 2012). Indeed, evidence suggests that REV-ERBs might be responsible for circadian lipid biosynthesis in liver, which has been recognized for more than three decades (Hems et al., 1975; Alenghat et al., 2008; Feng et al., 2011). Moreover, by inhibiting the lipid biosynthesis and driving lipid storage, it reroutes gluconeogenic metabolites and indirectly promotes gluconeogenesis (Sun et al., 2012). REV-ERB agonists inhibit lipid and cholesterol synthesis in liver and fat, and promote fatty acid and glucose oxidation in muscle, resulting in increased energy expenditure (Figure 3). These agonists significantly improve dyslipidemia and hyperglycaemia in a diet-induced obesity model (Solt et al., 2012). In addition, BMAL1 and REV-ERBα both regulate adipocyte differentiation (Laitinen et al., 2005; Shimba et al., 2005).

Bile Acid Metabolism

REV-ERBα is an important regulator of bile acid synthesis. Genetic ablation of REV-ERBα lowers bile acid synthesis and decreases bile acid accumulation in the liver, which is correlated with altered phase and total decrease of *Cyp7a1* expression (Duez et al., 2008; Le Martelot et al., 2009). REV-ERBα may indirectly activate *Cyp7a1* via E4BP4 and SHP, or via LXR (Duez et al., 2008; Le Martelot et al., 2009). REV-ERBα also regulates SREBP function through its regulator INSIG2 and thus the SREBP targets involved in cholesterol and lipid metabolism (Le Martelot et al., 2009). Loss of BMAL1 increases circulating cholesterol, and loss of PER1/2 upregulates bile acid synthesis and results in hepatic cholestasis (Ma et al., 2009; Shimba et al., 2011).

Rhythmic Regulation of Metabolism by Fasting/Feeding

A key unanswered question is whether the rhythmic feature of metabolism is a passive response to the fasting/feeding cycles in metabolic tissues or an active anticipatory mechanism directly governed by the circadian clock. Recent transcriptomic studies in mouse liver provide some insight. Liver-specific overexpression of REV-ERBα abolishes oscillation of almost all the rhythmic transcripts found in wild-type mice, suggesting that they are controlled by the liver clock (Kornmann et al., 2007). Meanwhile, only a small subset of these transcripts, including the clock

genes, maintain their oscillation under fasting, suggesting most of the rhythmic transcripts are also regulated by fasting/feeding (Vollmers et al., 2009). Interestingly, in clock-deficient mice, restricted feeding can resume oscillation of about 60% of the rhythmic transcripts and even increase their amplitude, along with many other nonrhythmic genes (Vollmers et al., 2009). These observations suggest that under normal conditions, peripheral clocks and fasting/feeding cycles work together to drive rhythmic gene expression and metabolic activities.

Fasting/feeding activities are usually aligned with sleep/wake cycles and are timed by the central clock. Therefore, normally both the central clock and peripheral clocks synergize in rhythmic metabolic regulation. In addition to feeding/fasting, the central clock can also drive metabolic activities through hormones and body temperature (Brown et al., 2002; Reddy et al., 2007). For instance, blood glucocorticoid levels exhibit circadian oscillation (Oster et al., 2006). In the absence of the central clock, daily glucocorticoid injection can restore about 60% of the liver circadian transcriptome, probably through hepatocyte nuclear factor 4α (HNF4α) (Maywood et al., 2007; Reddy et al., 2007). Therefore, under normal conditions, the central clock synchronizes peripheral clocks, fasting/feeding cycles, hormone secretion, and body temperature changes, and all of these contribute to the rhythmic metabolic activities throughout the body (Figure 2).

On the other hand, fasting/feeding is also subject to food availability in the environment, and changes in metabolic profiles as induced by fasting/feeding can impact and even reset the peripheral clocks in metabolic tissues. Thus, in addition to the clock regulating metabolism, metabolism can regulate the clock.

Metabolic Regulation of the Clock

The circadian clock is regulated by metabolic signals such as fasting/feeding and dietary factors. Fasting and refeeding regulate the clock gene expression in peripheral tissues, particularly liver (Kawamoto et al., 2006; Tahara et al., 2011). High-fat diet (HFD) lengthens the circadian period and attenuates clock oscillation (Kohsaka et al., 2007). Clock-associated histone modifiers such as p300 and SIRT1 are also regulated. Fasting decreases p300 phosphorylation and activates p300, and induces SIRT1 expression, while HFD inhibits p300 phosphorylation, lengthens the circadian period, and attenuates clock oscillation (Rodgers et al., 2005; Liu et al., 2008). The impact of metabolism might be mediated through hormones, such as glucagon and insulin (Liu et al., 2008; Tahara et al., 2011), as well as metabolites. The circadian clock can sense the intracellular metabolite levels and adjust its own rhythm and functions, and allows a fine and precise temporal regulation of metabolic pathways within individual cells. We will focus on the key metabolites such as NAD^+, AMP, and heme, though other metabolites may also play a role.

NAD^+/NADH

NAD^+/NADH redox equilibrium indicates the metabolic state of the cell. NADH and NADPH directly bind CLOCK or NPAS2 and enhance BMAL1/CLOCK (or NPAS2) DNA binding, while the reduced form NAD^+ binds and inhibits (Rutter et al., 2001). NAD^+/NADH ratio also regulates SIRT1, which deacetylates PER2, BMAL1, and histones (Asher et al., 2008; Nakahata et al., 2008) (Figure 3). Intracelluar NAD^+ levels are circadian and are critical for circadian clock functions (Sahar et al., 2011).

NAD^+ levels are determined both by the fasting/feeding cycles and by circadian rhythms. Fasting induces NAD^+ levels in liver, as well as SIRT1 protein levels (Rodgers et al., 2005). Circadian clocks regulate NAD^+ mainly through the NAD^+ salvage pathway, in which NAD^+ is synthesized from nicotinamide (NAM), the byproduct of sirtuins, through the rate-limiting enzyme nicotinamide phosphoribosyltransferase (NAMPT). BMAL1/CLOCK drives the circadian expression of NAMPT and contributes to the rhythmic NAD^+ levels. NAD^+ activates while NAM represses SIRT1, which interacts with BMAL1/CLOCK and inhibits NAMPT transcription. Therefore, NAD^+ drives a negative feedback on its own synthesis through SIRT1 and the circadian clock, and through NAD^+, the intracellular metabolic state can regulate the clock (Figure 3) (Nakahata et al., 2009; Ramsey et al., 2009).

NAD^+ is also a substrate of poly(ADP-ribose) polymerase 1 (PARP-1), an NAD^+-dependent ADP-ribosyltransferase. PARP-1 activity oscillates in a daily manner and is regulated by feeding. PARP-1 binds and poly(ADP-ribosyl)ates CLOCK at the onset of the light phase, regulates BMAL1/CLOCK interaction with PER and CRY and BMAL1/CLOCK DNA binding, and mediates food entrainment of the liver clock. NAD^+ might play a role in regulating PARP-1 activity by feeding, but other mechanisms are also involved (Asher et al., 2010).

AMP/ADP

AMP and ADP bind AMPK, inhibit AMPK dephosphorylation, and activate AMPK kinase activity. Therefore, AMPK functions as a sensor of energy state in the cell, which is reflected by AMP and ADP levels (Cantó and Auwerx, 2011). Several studies place AMPK as a circadian clock regulator (Um et al., 2007, 2011; Vieira et al., 2008; Lamia et al., 2009) with two different mechanisms: AMPK activates CKIε and promotes PER2 degradation (Um et al., 2007), and AMPK phosphorylates CRY1 and destabilizes it (Lamia et al., 2009) (Figure 3). AMPK also regulates the NAD^+/NADH ratio, through which it crosstalks with SIRT1 (Cantó et al., 2009).

Heme

Heme is a ligand of REV-ERBα and REV-ERBβ and enhances corepressor recruitment and transcriptional repression (Raghuram et al., 2007; Yin et al., 2007; Pardee et al., 2009; Phelan et al., 2010) (Figure 3). Through heme, REV-ERBs can sense the redox state, and gases such as O_2, NO, and CO, as they regulate REV-ERB structure and corepressor recruitment (Pardee et al., 2009). Also, heme binding can be regulated by the redox state of the REV-ERBβ protein (Gupta and Ragsdale, 2011). Heme is also a component of NPAS2, whose heterodimeric DNA binding with BMAL1 is inhibited by CO (Dioum et al., 2002; Gilles-Gonzalez and Gonzalez, 2004).

Like NAD^+, heme can also negatively regulate its own synthesis through the circadian clock. The control is exerted on the expression of the rate-limiting enzyme in heme biosynthesis, δ-aminolevulinate synthase (ALAS1), whose expression is circadian. Heme inhibits BMAL1/NPAS2-activated ALAS1 expression and heme biosynthesis (Kaasik and Lee, 2004). In parallel, through REV-ERBα, heme represses PGC-1α transcription, a potent inducer of heme biosynthesis (Wu et al., 2009)

(Figure 3). Both NAD^+ and heme are critical metabolites and indicators/sensors of the metabolic state in the cells. The negative metabolite feedback loops connect metabolism with the circadian clock and are critical for metabolic homeostasis and fine-tuning of the clock function.

Conclusions and Perspective

In this review, we have discussed the functions of the circadian clock as a rhythmic epigenomic programmer and transcriptional regulator of gene expression and metabolism, and the interplays between the circadian clock and metabolism on epigenome. Crosstalk between the circadian rhythm and metabolism is essential for maintaining metabolic homeostasis and preventing metabolic disorders. In addition, the environment, particularly food availability and energy intake, participates in the crosstalk, regulating the circadian clock as well as metabolic pathways. However, there are still gaps in our understanding. First, while much of our current knowledge focuses on the gene expression of clock components as well as metabolic factors, their effects on cellular and organismal phenotype depend upon protein levels and activity, which are highly regulated at the translational and posttranslational levels, respectively. Moreover, metabolic pathways are interdependent with multiple control points, such that the concept of a single rate-limiting step is likely simplistic. In addition, it remains to be determined whether transcriptional regulation can be predicted from knowledge of the modification of the epigenome, or if this is an epiphenomenon of other cellular determinants. Last, but not least, it remains to be further elucidated to what extent rhythmic metabolism is a passive response to fasting/feeding or an anticipatory effect of the clock. Future studies will advance our understanding, and it is hoped that this knowledge will lead to alleviation of the adverse effects of circadian misalignment and metabolic diseases in modern society.

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