Chromatin Dynamics of Circadian Transcription

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Abstract The molecular circadian clock orchestrates the daily cyclical expression of thousands of genes. Disruption of this transcriptional program leads to a variety of pathologies, including insomnia, depression, and metabolic disorders. Circadian rhythms in gene expression rely on specific chromatin transitions which are ultimately coordinated by the molecular clock. As a consequence, a highly plastic and dynamic circadian epigenome can be delineated across different tissues and cell types. Intriguingly, genome topology appears to coordinate cyclic transcription at circadian interactomes, in which circadian genes are in physical contact within the cell nucleus in a time-specific manner. Moreover, the clock machinery shows functional interplays with key metabolic regulators, thereby connecting the circadian epigenome to cellular metabolism. Unraveling the molecular aspects of such interplays is likely to reveal new therapeutic strategies towards the treatment of metabolic disorders.

Keywords Circadian rhythms · Chromatin · Epigenetics · Sirtuins · NAD⁺

Introduction

Life has evolved to adapt to daily changes in the environment provided by the Earth's rotation. As a consequence, 24-h period rhythms in physiology and behavior are observed in almost all living organisms [1]. Indeed, circadian rhythms are remarkably pervasive across evolution and can be traced back

to the ancient life form *Archaeobacteria* [2••]. In mammals, circadian rhythms are apparent in sleep-wake cycles, feeding behavior, circulating levels of hormones (e.g., melatonin, insulin, leptin, adrenocorticotrophic hormone, or cortisol), body temperature, bowel movements, heartbeat, muscle performance, etc., all of which fluctuate with a period of ~24 h [3]. These oscillations are sustained by the circadian clock, an endogenous timekeeper that provides the organisms with the ability to anticipate daily fluctuations in the environment and adapt accordingly. Thus, circadian rhythms persist in the absence of environmental cues to provide internal temporal organization and simultaneously, they can be entrained by certain external cues (zeitgebers) to ensure synchrony with the environment. Light is the most powerful zeitgeber [4].

The mammalian circadian system is organized as a hierarchy of oscillators, with the master pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus [5]. Circadian oscillators are present in almost all tissues of an organism, and the SCN orchestrates their coordinated function. In mammals, the SCN is composed of ~20,000 neurons whose activity is coupled and oscillate in synchrony. Coupling mechanisms involve precise network architecture and the release of specific neuropeptides and neurotransmitters, including neuropeptide Y (NPY), serotonin, vasoactive intestinal neuropeptide (VIP), and arginine vasopressin (AVP). The SCN directly receives photic information from the retina through the retinohypothalamic tract and feedback from certain target structures such as the pineal gland [6]. Through an intricate system of efferent projections and humoral signaling, the SCN entrains ancillary oscillators which are located in other areas in the brain and in peripheral tissues [5].

Circadian rhythms in behavior, physiology, and metabolism are essential to sustain adequate organism homeostasis. Indeed, misalignment of the circadian clock can lead to severe disorders such as obesity, type II diabetes, or metabolic syndrome [7]. The control that the circadian clock exerts on

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cellular metabolism is complex and multilayered, yet numerous underlying molecular mechanisms are being unraveled. Intriguingly, a number of rate-limiting enzymes controlling the pace of critical metabolic pathways are under circadian control of expression or activity [8]. This regulation is exerted by the circadian clock, a well-coordinated transcription-translation feedback system that orchestrates and integrates gene expression, protein stability, and metabolite production to keep correct time. The core components of the circadian clock are transcription factors which impose rhythmicity to a significant portion of the transcriptome in a tissue or cell-specific manner [9]. Transcriptional rhythms are accompanied by rhythmic chromatin transitions supported by a highly plastic and dynamic circadian epigenome [10]. Here, we summarize findings showing how the circadian machinery shapes the chromatin landscape and the active role of specific metabolites in regulating these events. The coordination of circadian gene expression in specific nuclear territories through the physical association of genes in circadian interactomes is also reviewed.

The Circadian Molecular Clock in Mammals

Molecular clocks are present in almost all cells in the organism, and they share a molecular architecture with the ability to generate and sustain circadian rhythms on transcription of clock-controlled genes (CCG) [11]. Two transcriptional activators (CLOCK and BMAL1) and two repressors (CRY and PER proteins) compose the core clock machinery [12–16]. CLOCK and BMAL1 proteins heterodimerize and are rhythmically recruited to E-box elements located in the promoter of CCGs to subsequently activate their expression. The circadian repressors *per1-3* and *cry1-2* are among the CCGs. PER and CRY proteins accumulate and heterodimerize in the cytoplasm to subsequently translocate into the nucleus. This process is tightly regulated by posttranslational modification events, mostly involving phosphorylation of PER and CRY proteins by a number of kinases such as CKI ϵ or CKI δ [17]. In the nucleus, PER:CRY complexes repress CLOCK:BMAL1-mediated transcriptional activity on CCGs, thereby generating a negative feedback loop. The stability of the PER:CRY complex is regulated by specific F-Box proteins including FBXL3, which target them for degradation by the proteasome [18, 19, 20•, 21•]. These events result into time-controlled clearance of repressors from the nucleus, priming for a new cycle of CLOCK:BMAL1-driven gene expression. This time-ly regulated molecular mechanism is key to the rhythmic expression of CCGs.

Among the CCGs, there are transcription factors that further extend the clock regulatory system by imposing rhythmicity to downstream genes. Among these factors there are several nuclear receptors, such as REV-ERB α /REV-ERB β

and the retinoid orphan receptors RORs (ROR α , - β , - γ), which bind to ROR elements in the promoters and controls rhythmic expression on a subset of genes including *bmal1* [22, 23]. Additional circadian transcription factors are exemplified by DBP, TEF, HLF, and E4BP4, whose transcriptional activities impose rhythmicity to genes involved in xenobiotic detoxification pathways in the liver [24].

Dynamic Transitions on Chromatin

Transcription factors exert their function by recruiting activators of the transcription machinery, some of which can modulate the epigenome by contributing to an open chromatin environment permissive for transcription. Conversely, transcriptional repressors promote chromatin condensation, therefore limiting accessibility and activity of the transcription machinery [25, 26]. It is thereby required that specific chromatin modifiers in the cell nucleus cooperate with the clock to direct cyclic events which sustain circadian rhythms in transcription (Fig. 1). To this end, the circadian heterodimer CLOCK:BMAL1 interacts with histone acetyltransferases (HATs) and deacetylases (HDACs) to coordinate timed chromatin transitions in histone acetylation at CCGs promoters. Specifically, CLOCK:BMAL1 has been shown to interact with the HATs CBP (CREB binding protein), p300, and with the CBP-associated factor PCAF [27–30]. These molecular associations lead to cyclic oscillations in histone acetylation at CCG promoters, which in turn result in rhythmic chromatin transitions [11]. Moreover, the CLOCK protein itself is a HAT that rhythmically acetylates K9/K14 residues of histone H3 [31].

Histone acetylation by these various HATs is counterbalanced by specific HDACs. Notably, the transcriptional repressor complex SIN3-HDAC cooperates with PER proteins to elicit deacetylation of histones at *per1* gene promoter [32•]. Similarly, genomic recruitment of HDAC3 by REV-ERB α in mouse liver directs a circadian rhythm of histone deacetylation on a number of genes required for hepatic lipid homeostasis [33•]. Other HDACs important for circadian function are the one included in the class III of enzymes known as sirtuins. These enzymes are of particular interest, as their deacetylase activity depends on the metabolite nicotinic adenine dinucleotide (NAD⁺) [34]. There are seven mammalian sirtuins (SIRT1–7), among which SIRT1, SIRT3, and SIRT6 are involved in circadian control of gene expression and metabolism [35, 36•, 37, 38•]. Their characterization has provided the first molecular link between metabolism, epigenetics, and the circadian clock.

Specific histone methyltransferases (HMTs) have been related to clock function. For example, the *mixed-lineage leukemia* (MLL) family member MLL1 is critical to recruit the CLOCK:BMAL1 complex to chromatin and elicits rhythmic H3K4 tri-methylation at CCGs promoters, a mark that is

Fig. 1 Different regulatory layers delineate the circadian transcriptome. The circadian clock operates in the cell through interconnected mechanisms to regulate circadian gene expression. The tridimensional organization of circadian genes in circadian interactomes inside of the nucleus provides a framework for transcriptional regulation. Moreover, the clock coordinates with specific chromatin remodelers to generate

chromatin transitions which support circadian transcription. The core clock proteins CLOCK and BMAL1 rhythmically bind to promoters of clock-controlled genes and drive circadian gene expression. Finally, the circadian transcriptional output imposes rhythmicity to metabolic pathways and specific signaling events to maintain cellular homeostasis

associated with transcriptional activation. Another member of the same family, MLL3, has also been shown to contribute to circadian control [39•, 40•]. Additionally, the circadian component PER2 interacts with the H3K9 methyltransferase Suv39h1 to mediate repression of the *per1* gene [41]. Moreover, the repressive mark H3K27me3 appears rhythmic at the level of *per1* and *per2* promoters, an event dependent on the HMT EZH2 [42].

Temporal and genome wide analyses of histone modifications and clock proteins DNA occupancy using high throughput chromatin immunoprecipitation (ChIP-seq) technologies have revealed many aspects of the circadian epigenome in the mouse liver [11, 43•, 44, 45•]. These studies confirm that CLOCK:BMAL1 complex recruitment to chromatin is temporally restricted to the light phase, between ZT4 and ZT8. Conversely, the circadian repressors CRY and PER appear antiphasic, during the dark period. The genome-wide pattern of circadian histone modifications is complex, and globally these are not restricted to a specific time of the day, but rather oscillate in order to sustain the distinct phase of each CCG [43•, 45•]. Overall, these events reveal the coordination of multiple epigenetic regulatory components to generate the adequate circadian output (Fig. 1).

Intriguingly, RNA-seq experiments in the mouse liver in combination with ChIP-seq analyses for temporal DNA occupancy profiles by RNA polymerase II indicate that not every

circadian transcript is subjected to rhythmic “de novo” transcription [43•, 44, 46•]. Instead, a significant portion of the circadian transcriptome is driven by clock-controlled posttranscriptional mechanisms such as rhythmic splicing events, mRNA stability and translation, or regulation by non-coding RNAs (ncRNAs) and microRNAs (miRNAs) [47, 48]. For example, cyclic alternative splicing occurs in the mouse liver, controlling the mRNA levels of circadian genes such as *npas2* [49]. Additionally, alterations in the m(6)A-RNA methylation machinery affects circadian period length and disturbs the pre-mRNA/mature RNA subcellular distribution of the clock genes *per2* and *bmal1* [50•]. Moreover, a wide variety of ncRNAs are rhythmically expressed in the mouse liver including an antisense transcript on *per2*, which might have a regulatory function [45•, 51•]. Finally, genetic disruption of the microRNA pathway by mutating *dicer* affects the circadian transcriptome [52, 53•]. Indeed, specific miRNAs including miR-122, miR-139, and miR-219 appear to modulate the circadian clock in a tissue-specific manner [54, 55].

A Genomic Interactome Driven by the Clock

Specific posttranslational histone modifications have been associated to various levels of transcriptional control [25]. Studies on circadian chromatin remodeling provide time-

specific linear maps for the localization of epigenetic marks to the DNA sequence and their implication with rhythmic transcription. However, understanding of genome function requires considering the spatial organization of interphase chromosomes inside of the nucleus [56]. Indeed, mammalian genomes are highly organized in topological domains with pervasive features across different cell types, tissues, or even species [57, 58]. Functional territories include domains with active transcription towards the center of the nucleus and repressive domains mostly localized at the nuclear periphery and spatial segregation of epigenetic marks [59].

A recent study using chromosome conformation capture on chip (4C) technology demonstrates that mammalian circadian genes are organized in functional nuclear territories [60•]. In this study, mouse embryonic fibroblasts were used to decipher the genomic environment of the clock-controlled gene *dbp* along the circadian cycle. The location of *dbp* gene is consistently restricted to high gene dense nuclear domains, and specific interchromosomal contacts appear remarkably pervasive across the circadian cycle. A striking feature of the *dbp* interactome is the spatial congregation of clock-related DNA-elements, including a significant enrichment in E-box elements [60•]. These data support a scenario where the various phases of the circadian program of gene expression occur in specific nuclear domains.

Interestingly, some specific *dbp* genomic contacts are dynamic, and their interaction mirrors *dbp* expression, revealing a circadian interactome [60•]. These interactions do not appear in *bmal1*^{−/−} cells, indicating that the clock machinery is implicated in the *dbp* genomic environment, possibly favoring adequate circadian transcriptional regulation. Notably, a subgroup of genes located within the *dbp* circadian interactome is coordinately transcribed in a circadian fashion. This includes the H3K36 histone methyltransferase *ash1l* and the metabolic gene *acy3*. It is thereby conceivable that these genes are coregulated by sharing the transcription machinery within dedicated areas in the nucleus, possibly contributing to the assembly of specific circadian transcription factories [60•, 61–63]. Overall, these observations provide the first evidence of interplay between chromosome organization and the clock to regulate the circadian output in mammals. Additionally, the nuclear membrane has been demonstrated to regulate circadian rhythms, possibly by a direct interaction of the nuclear envelope component MAN1 with the promoter of the clock gene *bmal1*, thereby activating its expression [64•]. Thus, circadian gene positioning inside of the nucleus appears as an important regulatory layer by which cells can organize the cyclic expression of a large number of genes (Fig. 1).

The extent of the influence that chromatin topological organization has on the mammalian circadian transcriptome deserves to be further studied. Notably, in the in the Cyanobacterium *Synechococcus elongatus*, the circadian clock regulates a chromosome compaction rhythm, and

experimental manipulations of the chromosome topological state impact circadian gene expression in a predictable manner, indicating that genome folding defines its circadian output [65•, 66].

The NAD⁺-Sirtuins Pathway Connects Metabolism to Epigenetics

Accumulating evidence demonstrates that the circadian epigenome and metabolism are intimately connected [3]. Studies from genetically modified mice lacking circadian components indicate that the clock controls a large portion of metabolic homeostasis and reveals important connections between disruption of circadian rhythms and disease [7]. For example, clock mutant mice are hyperphagic and obese and develop a severe metabolic syndrome [67]. Moreover, selective ablation of the circadian gene *bmal1* within the pancreas leads to profound hyperglycemia and β -cell failure, leading to diabetes mellitus [68•]. Additionally, mice with a liver-specific deletion of *bmal1* are unable to maintain glucose homeostasis and exhibit hypoglycemia and defective expression of hepatic glucose regulatory genes [69]. It is thereby essential to decipher molecular connections between circadian rhythms and metabolism, as these could help design of pharmacological strategies towards the treatment of a number of metabolic diseases.

In this context, the NAD⁺-dependent class III family of deacetylases, named sirtuins, represents a revealing paradigm. There are seven mammalian sirtuins (SIRT1–7), of which SIRT1, SIRT3, and SIRT6 have been linked to the circadian clock [70]. SIRT1, the founding member of this family of enzymes, specifically deacetylates the circadian components BMAL1 and PER2 [35, 37], thereby impacting their activities. Also, SIRT1 modulates acetylation on H3K9/K14 at CCGs promoters [37, 71•]. As a consequence, circadian gene expression is altered in the liver from mice with a hepatic-specific deletion of *sirt1* [36•, 37]. Moreover, SIRT1 controls the expression of the *bmal1* gene in the SCN, and influences circadian amplitude and period length [72•]. Notably, the activity of SIRT1 is circadian, a function that has been linked to the cyclic availability of its cofactor NAD⁺ [73•, 74•]. Interestingly, the rate-limiting step within the NAD⁺ biosynthetic pathway is catalyzed by the enzyme NAMPT (nicotinamide phosphoribosyltransferase), and the gene *nampt* is clock controlled, through rhythmic binding of CLOCK:BMAL1 to E-boxes located on its promoter [73•, 74•]. Thereby, NAD⁺ biosynthesis appears circadian through the coupling of two transcriptional and enzymatic feedback loops. Rhythmic fluctuations in NAD⁺ levels have been observed both in synchronized cells and tissues [73•, 74•].

Recent work reveals that the nuclear sirtuins SIRT1 and SIRT6 control circadian gene expression of differential groups of genes, defining distinctly partitioned classes of circadian

genes [36•, 70]. Thereby, SIRT1 and SIRT6 operate through distinct mechanisms to delineate the circadian output. Although both of them interact with CLOCK:BMAL1, they differentially regulate the function of the heterodimer. Interestingly, SIRT6 controls CLOCK:BMAL1 transactivation on promoters of genes involved in fatty acid biosynthesis, possibly by coordinating its function with specific transcription factors such as the sterol regulatory element-binding protein 1 (SREBP1) [36•]. Moreover, the genomic partitioning by SIRT1 and SIRT6 results in parallel segregation of cellular metabolism. Specifically, SIRT6 dictates circadian metabolism of fatty acids, including triglycerides, membrane and signaling lipids, and medium- and long-chain fatty acids [36•].

Additionally, the mitochondrial sirtuin SIRT3 shows cyclic deacetylase activity driven by circadian rhythms in NAD⁺ availability [38•]. SIRT3 coordinates the mitochondrial acetylome in response to nutrient status to keep homeostasis. Particularly, SIRT3 regulates acetylation of proteins involved in several metabolic pathways such as fatty acid oxidation and tricarboxylic acid cycle [75, 76]. Many of these acetylation events impact protein activity and appear circadian, suggesting that SIRT3 dictates rhythms in mitochondrial function [38•, 77]. Notably, rhythms in fatty acid oxidation are disrupted in *Bmal1*^{-/-} mice and can be restored by exogenous administration of NAD⁺, demonstrating that the clock contributes to the control of mitochondrial oxidative function [38•].

Through modulation of sirtuins, the cofactor NAD⁺ is emerging as key regulator of metabolism and circadian rhythms. Interestingly, sirtuins control longevity in different species and are intimately related with aging process [34]. Also NAD⁺ levels decay with aging [78, 79], and these changes are paralleled by mis-regulation in amplitude and phase of clock genes [80, 81•]. In the central pacemaker, some of these age-dependent circadian defects are controlled by SIRT1 [72•]. Thereby, the NAD⁺-sirtuin pathway provides a link between metabolism, circadian rhythms, and longevity [82].

Metabolites Talk to the Clock

Oscillating metabolites are important for the maintenance of cellular rhythmicity, and NAD⁺ is a revealing example. Present knowledge favors a scenario in which the clock appears to sense the cellular energy state and modify its transcriptional function to adapt. Indeed, certain metabolites have been shown to directly interact with components of the clock machinery. For example, it was proposed that CLOCK:BMAL1 DNA-binding affinities to E-boxes are affected by the redox states of NAD(H) or NADP(H), although “in vivo” evidence for these observations has been lacking [83]. Intriguingly, recent crystallographic studies suggest that the repressor complex CRY1:PER2 is destabilized by a

disulfide bond between two cysteine residues in CRY1 [84•], which could act as a redox sensor modulating the complex. Moreover, the mammalian CRY2 protein presents a conserved FAD (flavin adenine dinucleotide) binding pocket, which is also targeted by the ubiquitin ligase protein FBXL3 [18, 19, 85•]. This interplay regulates CRY2 stability and impacts circadian period length [85•, 86].

Additionally, posttranslational modifications of the clock machinery provide means to connect cellular metabolism and the oscillator. For example, the metabolite *O*-linked β -*N*-acetylglucosamine (O-GlcNAc) can be linked to the circadian proteins CLOCK, BMAL1, and PER2 by the enzyme OGT (O-GlcNAc transferase), thereby modifying the turnover of the proteins [87•, 88•]. Interestingly, O-GlcNAc is becoming increasingly recognized as a key signal transducer from glucose metabolism [89]. Furthermore, the nutrient sensor protein AMPK (AMP-activated protein kinase) was shown to directly phosphorylate the clock component CRY1, thereby destabilizing it. Notably, this particular posttranslational modification is sensitive to cellular glucose levels; thereby, AMPK functions as a signal transducer between glucose metabolism and the oscillator [90•].

The extent of metabolic influence on clock function needs further investigation. The use of metabolomic approaches reveals that diet composition and nutritional cues have unforeseen effects on the circadian clock. For example, mice fed a high-fat diet become obese and diabetic, and remarkably they show a specific reorganization of metabolic pathways in the liver, leading to profound reprogramming of the hepatic clock [91•]. These changes are illustrated by advanced recruitment of CLOCK:BMAL1 heterodimer to promoters of CCGs and specific epigenetic events leading to deep alterations in the circadian transcriptome [91•]. Interestingly, these alterations are reversible by switching to normal diet, and also can be alleviated by time-restricted feeding [91•, 92•], highlighting the remarkable plasticity of the oscillator.

Conclusion

Accumulating data reveal the role of the circadian clock in maintaining cellular homeostasis. It is becoming increasingly evident that the oscillator senses and integrates information from metabolic pathways and modifies its function to adapt accordingly. Remarkably, the molecular clock, through the coordination of chromatin remodelers, modulates a vast epigenomic landscape, leading to plastic and dynamic chromatin transitions which delineate the specific circadian transcriptome. The integration of cellular signaling by the clock in the nucleus extends to regulation of circadian genes positioning, a mechanisms which might be critical to define the circadian output. The decoding of the language that links nutrients and metabolites to the circadian clock will be important to

decipher how mis-regulation of circadian rhythms leads to chronic diseases such as obesity and diabetes.

Compliance with Ethics Guidelines

Conflict of Interest Lorena Aguilar-Amal and Paolo Sassone-Corsi declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All animal studies performed by any of the authors were in accordance to the Institutional Animal Care and Use Committee (IACUC) guidelines at the University of California, Irvine.

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