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Chromatin landscape and circadian dynamics: Spatial and temporal organization of clock transcription

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Circadian rhythms drive the temporal organization of a wide variety of physiological and behavioral functions in ∼24-h cycles. This control is achieved through a complex program of gene expression. In mammals, the molecular clock machinery consists of interconnected transcriptional–translational feedback loops that ultimately ensure the proper oscillation of thousands of genes in a tissue-specific manner. To achieve circadian transcriptional control, chromatin remodelers serve the clock machinery by providing appropriate oscillations to the epigenome. Recent findings have revealed the presence of circadian interactomes, nuclear "hubs" of genome topology where coordinately expressed circadian genes physically interact in a spatial and temporal-specific manner. Thus, a circadian nuclear landscape seems to exist, whose interplay with metabolic pathways and clock regulators translates into specific transcriptional programs. Deciphering the molecular mechanisms that connect the circadian clock machinery with the nuclear landscape will reveal yet unexplored pathways that link cellular metabolism to epigenetic control.

circadian rhythms | chromatin | epigenetics | nuclear organization

The circadian clock is an endogenous timekeeper present in almost all life forms. This evolutionarily conserved timing system confers organisms with the ability to predict and anticipate daily fluctuations in the environment, thus allowing appropriate physiological adaptation. As a consequence, many aspects of metabolism, homeostatic balance, and behavior are under circadian control (1). Circadian rhythms are remarkably pervasive. For example, daily and sustained oscillations have been described for the oxidation state of peroxiredoxin proteins, which are strongly conserved (2). These circadian oscillations are present in organisms as disparate as mammals, insects, plants, fungi, cyanobacteria, and even in the archaeon *Halobacterium* (2). In higher organisms, circadian rhythms have evolved into a complex physiological and molecular system demonstrated by sleep–wake cycles, daily fluctuations in body temperature, blood pressure, cellular regeneration, and behavior such as food intake and alertness levels (3). Metabolism and body homeostasis are also under circadian control, displaying rhythms in the levels of circulating hormones and metabolites, as well as enzymes within the biochemical pathways participating in their biosynthesis (1, 4).

A fundamental characteristic of circadian rhythms is that they persist in the absence of environmental cues. Indeed, the clock system is not driven by external "zeitgebers" (a German word that means time-giver), but it is rather synchronized or entrained by zeitgebers every day to adjust to the 24-h period. In the absence of environmental zeitgebers, the clock "free runs" in periods close to 24 h long. The light–dark cycle is the most powerful circadian zeitgeber although other cues may influence the clock, such as external temperature or food availability (5). Thus, the circadian clock generates an internal biological rhythm that synchronizes and adapts to the changing environment.

Circadian clocks are intimately linked with cellular metabolism so that misregulation of circadian rhythms may lead to a number of pathologies such as obesity, metabolic syndrome, diabetes, cardiovascular diseases, inflammation, sleep disorders, and some tumorigenic processes (1). A number of studies have contributed to decipher the molecular mechanisms underlying clock function. The core clock molecular machinery consists of transcription factors and regulators, both activators and repressors, which act in concert to drive circadian expression of an important fraction of the genome. It is estimated that 3–30% of the transcripts are controlled by the clock, depending on the tissue or cell type (6–9). This wide program of gene expression is achieved through events of cyclic chromatin remodeling and epigenetic control. Here, we review these processes and present recent evidence on clock-controlled transitions in the nuclear landscape that contribute to the coordination of circadian gene expression through the physical association of genes in circadian interactomes.

The Clock Machinery: Driving Circadian Transcription

The circadian system is organized in a network of cellular oscillators present in virtually every cell of the organism. In mammals, the master clock resides in the suprachiasmatic nucleus (SCN) of the hypothalamus, which receives external light information from the retina through the retinohypothalamic tract. The electrical activity of the neurons in the SCN oscillates in synchrony, and the SCN is thought to synchronize all other oscillators, including those located in other areas in the brain and in peripheral tissues. A key feature of SCN neurons is a complete intercellular coupling of circadian oscillations, being highly resistant to phase perturbations (10). On the contrary, the phase of peripheral oscillators is entrained and synchronized by signaling from the SCN, consisting of neuronal and humoral messengers. Therefore, specific lesion of the SCN results in arrhythmic behavior in rodents (11, 12). The mechanisms involved in the communication between the SCN and the periphery remain poorly explored although they are thought to be complex and multilayered (10, 13).

The mammalian clock machinery is structured as an intricate network of transcriptional–translational feedback loops (14). The core clock proteins CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 (Brain and Muscle ARNT-Like 1) are basic HLH (helix-loop-helix)-PER-ARNT-SIM (bHLH-PAS) transcriptional activators that drive the expression of many clock-controlled genes (CCGs) (Fig. 1). CLOCK and BMAL1 heterodimerize through their PAS domains and bind to E-boxes in the promoters of CCGs to then activate their expression. Among the CCGs, there are other genes encoding other core clock proteins, specifically the repressors Period (PER1 to -3) and Cryptochromes (CRY1 to -2). PER and CRY proteins heterodimerize in the cytoplasm and translocate to the nucleus to interact and inhibit CLOCK:BMAL1. The stability of PER:

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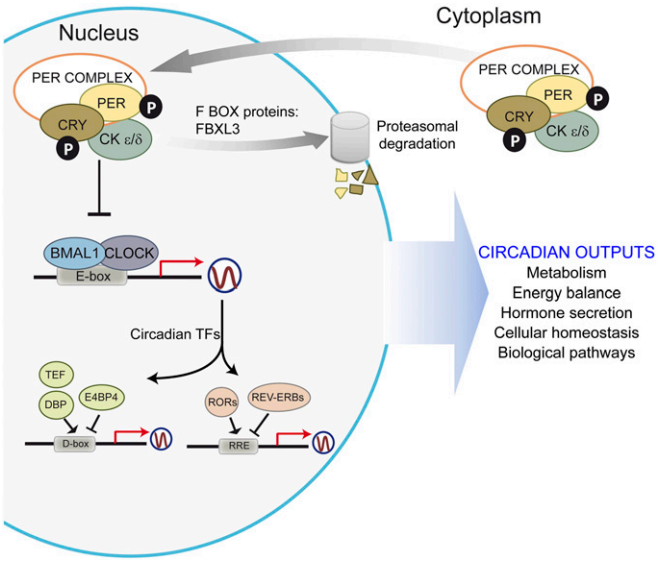

Fig. 1. Transcriptional–translational loops control circadian rhythms in mammals. The positive loop is driven by the transcription factors CLOCK:BMAL1, which activate the expression of clock-controlled genes through binding to E-box elements at their promoters. *Per* and *Cry* genes give rise to the components of the negative loop. Thus, PER and CRY proteins heterodimerize in the cytoplasm and are stabilized by phosphorylation events catalyzed by associated kinases (CKε/δ). The PER/CRY complex translocates into the nucleus and inhibits CLOCK:BMAL1 activity. F BOX proteins act in concert with the proteasome to degrade the PER/CRY complex with 24-h rhythmicity, yielding to a new round of transcription by CLOCK:BMAL1. Several transcription factors (TFs), including DBP/TEF/E4BP4 and RORs/REV-ERBs, are then acting to initiate additional oscillations in downstream genes, through rhythmic binding to D-box and ROR elements, respectively. These interconnected loops generate the circadian output, which is apparent in rhythms in metabolism, energy levels, hormone secretion, and many other biological pathways, depending on the tissue and environmental conditions.

CRY complexes is tightly regulated by posttranslational modifications involving a number of kinases (15) and ubiquitination events (16–19). These modifications lead to time-controlled clearance of repressors in the nucleus, priming for a new cycle of CLOCK:BMAL1-driven gene expression (Fig. 1). This timely regulated cycle dictates the 24-h rhythmic expression of several additional transcription factors, including D-site Binding Protein (DBP), Hepatic Leukemia Factor (HLF), Thyrotroph Embryonic Factor (TEF), and E4 Promoter–Binding Protein 4 (E4BP4), and the bHLH transcription factors Differentially Expressed in Chondrocytes 1 and 2 (DEC1 and DEC2), Retinoic Acid-Related Orphan Receptor (ROR) proteins, and Reverse Erithroblastosis Virus α and β (REV-ERBα/β). DBP, HLF, TEF, and E4BP4 bind to D-boxes in the genome whereas REV-ERBα/β and ROR proteins bind to the Rev-Erb/ROR-binding element (RRE) and subsequently impose rhythmicity to downstream genes (Fig. 1). These interconnected transcriptional feedback loops, in conjunction with other regulatory factors, provide remarkable plasticity to the circadian clock and generate multiple daily oscillations in the transcriptome (20).

Circadian Rhythms in the Chromatin Fiber

As circadian transcription takes place in the chromatin fiber, specific mechanisms allowing cyclic chromatin transitions occur in a genome-wide scale (21). Several chromatin remodelers have been found to display circadian activity on chromatin, thus directing a number of cyclic events in the cell nucleus. In fact, the master circadian regulator CLOCK functions as an acetyltransferase on histone H3 at K9 and K14 (22), both marks associated to a chromatin state permissive for transcription. Most likely, CLOCK acts in concert with other histone acetyltransferases (HATs) to sustain cycles in the acetylation state of histones at promoters of CCGs (23). Indeed, the CLOCK:BMAL1 heterodimer has been shown to interact with CREB binding protein (CBP), p300, and with the CBP-associated factor PCAF (23–26). Acetylation by these various HATs is counterbalanced by a number of histone deacetylases (HDACs). For example, the circadian repressor PER recruits SIN3A-HDAC1, a mechanism that is thought to rhythmically deliver histone deacetylases to the *Per1* promoter, thus contributing to transcriptional repression (27). Similarly, the complex SIN3B-HDAC1/2 interacts with the circadian repressor CRY1 (28). In mice, the hepatic NCoR–HDAC3 complex is rhythmically recruited to chromatin via REV-ERBα, an event that regulates lipid metabolism in the liver by controlling the circadian epigenome at specific gene promoters (29, 30). Thus, although more information is needed, it would seem that a variety of circadian repressive complexes exist and that their different functions could be specific for given autoregulatory loops. Among the HDACs involved in circadian function, the nicotinamide adenine dinucleotide (NAD^+)-dependent, class III of enzymes deserves special mention. The founding member of this family of mammalian deacetylases, SIRT1, derives its name from the yeast homolog *Sir2* (silent mating type information regulation) and is involved in metabolism, inflammation, and aging. These enzymes, collectively referred to as sirtuins, have varied intracellular localization, being nuclear, cytoplasmic, or mitochondrial. Because they represent a direct molecular link between NAD^+-dependent metabolism and deacetylase activity, the finding that both SIRT1 and SIRT6 contribute to circadian control has provided the first molecular link between metabolism, aging, and the circadian clock (31, 32).

In addition to histone acetylation, other posttranslational modifications have been linked to clock function. Indeed, the very first evidence that chromatin modifications could play a role in circadian responses was the light-inducible phosphorylation at H3-S10 in SCN neurons (33). Histone methylation is a critical modification that has been linked to clock control. Specifically, H3K4me3 seems to be essential to permit circadian chromatin transitions and control CCG expression (34). The histone methyltransferase (HMT) MLL1 was demonstrated to be responsible for CLOCK:BMAL1 recruitment to chromatin and for the cyclic trimethylation at H3K4. Another methyltransferase of the same family, MLL3, was also shown to play a role (35, 36). Similarly, the repressive epigenetic mark H3K27me3 is clock-controlled at the *Per1* promoter through a mechanism involving the methyltransferase EZH2 (37). Moreover, the histone demethylase JARID1a inhibits histone deacetylase 1 (HDAC1) and enhances CLOCK:BMAL1-mediated transcription (38), and so does the flavin adenine dinucleotide (FAD)-dependent demethylase LSD1 in a mechanism dependent on its circadian phosphorylation by the protein kinase PKCα (39).

A Dynamic Epigenome: Rhythms in the Nuclear Landscape

Because various epigenetic processes associated to circadian gene expression are being revealed, the linear view of chromatin remodeling should be challenged. Although it is assumed that CLOCK:BMAL1 binding to promoters contributes to cyclic chromatin reorganization in combination with chromatin remodelers (21, 40), these studies do not provide explanation for the diversity of circadian outputs among different tissues. Indeed, the application of genome-wide tools to explore global transitions in the circadian epigenome has revealed an unexpected degree of complexity. For example, RNA-seq experiments in the mouse liver have uncovered that only ∼25% of circadian genes show both intron- and exon-derived mRNA oscillations, indicating that de novo transcription is happening at a limited number of circadian

transcripts whereas oscillations in others might be driven by rhythmic posttranscriptional processes (41). Genome-wide sequencing of nascent RNA (Nascent-seq) (42) and high-throughput chromatin immunoprecipitation (ChIP-seq) analyses for temporal DNA occupancy profiles by RNA polymerase II (Pol II) (41, 43, 44) from mouse livers confirm this observation. These effects could be explained at least in part by circadian-controlled splicing events and mRNA stability (45). For example, the oscillation in the cold-inducible RNA-binding protein (CIRP) is driven by circadian rhythms in temperature (46). Intriguingly, CIRP binds *Clock* mRNA among others, thereby stabilizing it (46). Cyclic alternative splicing occurs in the mouse liver, impacting the mRNA levels of certain circadian genes such as *Npas2, Hlf*, or *Usp2* (47), and light-inducible alternative spicing events at the *U2af26* gene in the mouse brain generates a protein variant that regulates the levels of PER1, leading to broad effects on circadian gene expression (48). Moreover, several mRNAs exhibit rhythmic poly(A) tail lengths necessary for circadian protein translation (49). Furthermore, alterations in the m(6)A-RNA methylation machinery affect the circadian transcriptome and period length, with this machinery dictating the pre-mRNA/mature RNA distribution of the clock genes *Per2* and *Arntl* (50). Remarkably, RNA methylation processes are sensitive to the availability of S-adenosylmethionine (SAM), the universal methyl donor, and to the relative levels of its by-product, S-adenosylhomocysteine (SAH), which inhibits transmethylation reactions (51), thus connecting circadian mRNA stability with cellular metabolism.

Temporal and genome-wide transitions in histone modifications and clock protein DNA occupancy profiles have been delineated for the mouse liver (41, 43, 52–54). These studies confirm a striking feature of the clock: DNA occupancy of the CLOCK:BMAL1 complex is specific and temporally restricted to the light phase, between ZT4 and ZT8, whereas the circadian repressor PER and CRY proteins appear in an antiphasic manner, during the dark period (41, 52, 53). However, histone modifications follow a more complex pattern. Although H3K4me3, H3K9ac, and H3K27ac closely follow the transcriptional oscillation of circadian genes (41, 54), there is widespread heterogeneity in temporal occupancy across the genome of H3K4me3, suggesting that many noncircadian genes also exhibit rhythms in epigenetic marks (41). Overall, these notions illustrate the complexity of the circadian epigenome as time-specific nuclear events are coordinated to generate cyclic outputs.

An additional regulatory layer is achieved through noncoding RNAs. Genetic disruption of the microRNA (miRNA) pathway by mutating *Dicer* either in the mouse or in cell lines affects the circadian transcriptome (55, 56). Indeed, hepatic miR-122 controls the phase and amplitude of several metabolic genes (57). In mouse SCN, miR-139 and miR-219 modulate the clock entrainment and its period, respectively (58), and, in *Drosophila*, manipulations of miR-279 levels impact rest–activity rhythms (59). In addition, strand-specific and small RNA sequencing in mouse liver has revealed numerous oscillating antisense RNAs and miRNA transcripts, including the antisense mRNA from the *Per2* gene (54). Further investigations are necessary to decipher the functional characteristics of these oscillatory noncoding RNAs and how they impact circadian transcription.

Oscillations in 3D: Revealing the Circadian Interactome

To efficiently accomplish DNA functions, the genome is highly organized in the cell nucleus. During interphase, individual chromosomes occupy distinct nuclear territories, and specific regions intermingle in interchromosomal contacts (60). The recent development of high-throughput imaging techniques and chromosome conformation capture (3C)-based technologies has provided remarkable insights into nuclear architecture, spatial chromosome folding, and genome functions (61). Mammalian genomes seem to be folding in topological domains with pervasive features across different cell types, tissues, or even species (62, 63). Functional territories include domains with active transcription toward the center of the nucleus and repressive domains localized at the nuclear periphery (64). Similarly, spatial segregation of epigenetic marks has been reported (65–67).

Because circadian transcription occurs in the context of the 3D nucleus, it is thereby plausible that the principles governing gene positioning directly impinge on the circadian output. Interestingly, in the Cyanobacterium *Synechococcus elongatus*, manipulations of the topological state of its chromosome impact circadian gene expression in a predictable manner, indicating that genome folding defines its circadian output (68, 69). In mammalian cells, fluorescence in situ hybridization (FISH) experiments show that the circadian gene *Dbp* undergoes rhythmic changes in chromatin condensation (70), suggestive of dynamic topological fluctuations along the circadian cycle.

The first demonstration that circadian genes are organized in functional nuclear territories was obtained by chromosome conformation capture on chip (4C) technology (71). This comprehensive study was carried out in mouse embryonic fibroblasts (MEFs) and demonstrated cyclic chromosomal arrangements using the circadian gene *Dbp* as bait. This gene is located in a highly gene-dense genomic environment, and its interchromosomal contacts are largely stable during the circadian cycle (71). Remarkably, a number of specific, cyclic variations in *Dbp* genomic interactions occur, thereby defining a circadian interactome (Fig. 2*A*). These genomic interactions depend on a functional clock because they are drastically less dynamic in *Bmal1*$^{-/-}$ MEFs (71). It is thought that coregulated genes cluster in dedicated nuclear locations that favor the formation of transcriptionally active sites, also known as specialized "transcription factories." In this scenario, genes physically associated are coregulated because they share factors and regulators that reside within a transcription factory (72–74). Interestingly, specific genes that cluster within the *Dbp* interactome are also circadian, and they do so at their peak of expression. It is thereby reasonable to assume that they are coregulated through the sharing of a common transcription factory (71). Among the genes dynamically coupled within the *Dbp* interactome and coregulated with *Dbp* is *Ash1l*, which encodes an H3K36 methyltransferase. This finding suggests a link between circadian chromatin remodeling and chromosomal organization.

Spatial congregation of transcription factor binding sites into preestablished architectural domains is a recurrent characteristic of various transcriptional networks (75–77). Circadian E-box elements appear 2.5-fold enriched at the *Dbp* contact sites, illustrating that the circadian program of gene expression in MEFs happens in established nuclear domains (71). Indeed, other transcription factor binding sites are highly represented. Thus, it is tempting to speculate that the spatial architecture of the genome, through combinatorial associations between multiple DNA elements at specialized transcription sites, provides groundwork for cell type-specific circadian transcriptional outputs, as occurs at certain cell-specific transcriptional networks (78–80) (Fig. 2*B*). Intriguingly, functional annotation of genes concurrent with *Dbp* in the nuclear space shows that these genes are involved in biological pathways known to be circadian, such as xenobiotic detoxification and metabolic control (71), supporting the notion that additional and specific transcription factors act in concert with chromosomal organization to maintain homeostasis and circadian rhythms.

The extent of the influence that chromatin topological organization has on the circadian transcriptome needs to be determined. It will be of interest to apply recently developed higher-resolution technologies (such as Hi-C) to capture the conformation of genomes that provide a genome-wide view for all intra- and interchromosomal associations at 5- to 10-kb resolution (77, 81). Also, a key feature of circadian gene expression is the succeeding cycles of transcription with groups of genes

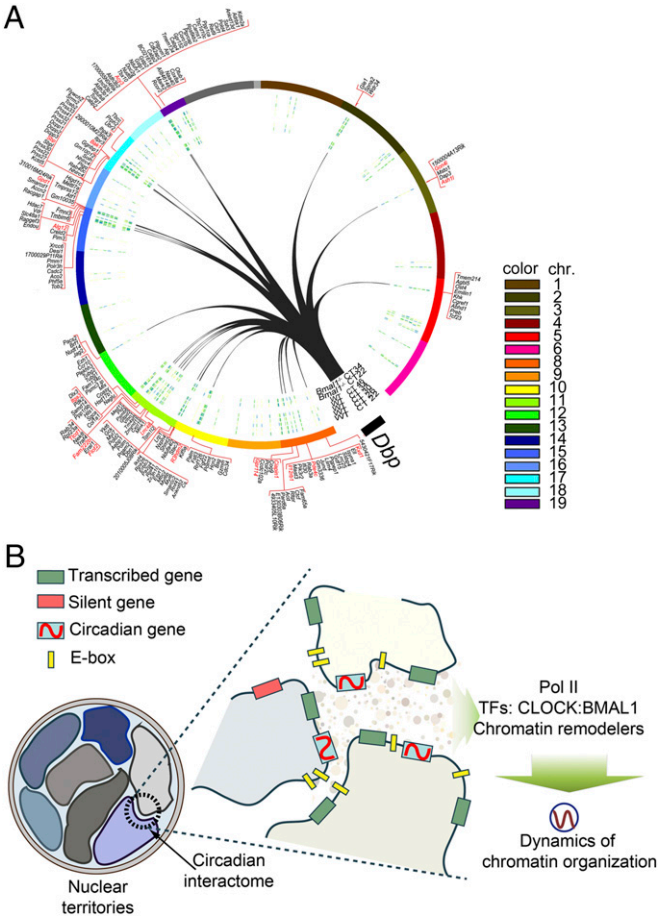

Fig. 2. The nuclear landscape and circadian interactome. (*A*) Circos plot representing the genome-wide view of *dbp* circadian interactions (black lines) with the corresponding chromosomes *in trans,* named *Dbp* circadian interactome. The gene content for each *Dbp* contact region is indicated in the outer layer of the plot. In red color are the names of circadian genes in MEFs. Each chromosome is represented as a color code, which is indicated on the right. The inner layers represent frequencies of interaction for WT or *Bmal1*$^{-/-}$ MEFs. Reprinted with permission from ref. 71. (*B*) Chromosome positioning in the nucleus is not random, and each chromosome occupies its own territory. Chromosomes intermingle in hubs and delineate the framework for chromatin functions. Circadian genes are positioned in transcriptionally active and gene-rich environments, delineating circadian interactomes. E-box elements cluster together in the nucleus, generating nuclear compartments supporting circadian transcription. Chromatin dynamics coordinate circadian cycles in clustering of certain circadian genes, possibly colocalizing at shared transcription factories in the nucleus. These nuclear regions might be highly enriched in regulatory proteins, including RNA polymerase II (Pol II), chromatin remodelers, and transcription factors (TFs). Thereby, CLOCK:BMAL1 specialized transcription factories might exist.

peaking at subsequent times. Thus, the presence of multiple circadian interactomes, in which one may lead to the organization of the following one, is plausible. In this respect, exploring other baits besides the *Dbp* gene is likely to deliver more information into the circadian topology of the genome. Finally, the integration of these topological data with profiling of known chromatin-associated epigenetic marks, Pol II occupancy, and recruitment of transcription factors to chromatin across the circadian cycle would be valuable.

Cellular Metabolism and Circadian Clock Intersect

Emerging evidence demonstrates reciprocal regulation between circadian rhythms and metabolism. A large number of human clinical and genetic studies and animal models illustrate the intimate link that the circadian clock has with cellular and organismal homeostasis (1, 82–86). Present knowledge favors a scenario in which the clock regulates metabolism by controlling the expression of key metabolic genes through epigenetic mechanisms. In addition, the oscillator itself seems to sense the energy state of the cell and modulate its function to adapt accordingly.

Metabolism and chromatin remodeling intersect at the level of specific metabolic cofactors that enzymes need to catalyze reactions (21, 87, 88). For example, histone acetylation depends on metabolic pathways supplying acetyl-CoA in the nuclear compartment. In mammals, acetyl-CoA is produced from glucose, the main carbon source, by the enzyme adenosine triphosphate (ATP)-citrate lyase (ACLY). Interestingly, the murine hepatic levels of ACLY protein are oscillatory (89), and so are the levels of both glucose and acetyl-CoA metabolites (83, 90). ACLY activity has been shown to control global histone acetylation depending on glucose availability (91). Taken together, these findings support the notion that circadian changes in histone acetylation are tightly controlled not only by specific HATs, but also by interconnected metabolic pathways and enzymes supplying nuclear acetyl-CoA. This scenario extends to SAM, the metabolite used by methyltransferases to deliver methyl groups. The levels of SAM directly influence H3K4me3 levels in mouse pluripotent stem cells (92) and, importantly, treatment with 3-deazaadenosine (DAA), an inhibitor of SAH hydrolysis that hinders transmethylation, elongates the circadian period in cultured cells and mice (50). Although the effects of DAA are partially generated through inhibition of the RNA methylation machinery, additional influence by DNA or histone methyltransferases cannot be ruled out. Further research is necessary to decipher the impact of one carbon metabolism in the circadian transcriptome.

A key metabolite that influences the circadian epigenome is nicotinamide adenine dinucleotide (NAD^+), which shows robust diurnal rhythms in synchronized cells and mice (83, 85, 93–95). NAD^+ is the cofactor for class III of HDACs, the sirtuins. The mechanism of NAD^+ oscillation and the role played by these deacetylases in clock function are discussed in the next section.

Changing metabolic states may influence circadian rhythms by a direct impact on the activity of the core machinery. An in vitro biochemical approach has indicated that the DNA-binding activities of NPAS2:BMAL1 and CLOCK:BMAL1 heterodimers to E-boxes can be influenced by the redox states of NAD(H) or NADP(H) (96), implying that CLOCK:BMAL1 transcriptional activity could be sensitive to the cellular redox. Moreover, circadian oscillations in intracellular redox potentials are evolutionarily conserved (2). Although the ability of NPAS2 or CLOCK proteins to sense the intracellular redox state in vivo remains to be proven, crystallographic analyses on the mammalian repressor complex CRY1–PER2 suggest an intriguing scenario. Indeed, a disulfide bond between two cysteine residues in CRY1 weakens its interaction with PER2 whereas a reduced state of CRY1 stabilizes the complex and facilitates transcriptional repression (97). This atomic interplay could act as a sensor of the metabolic status of the cell. Remarkably, mammalian CRY2 retains specific flavin adenine dinucleotide (FAD)-binding activity, and FAD competes for the CRY2-binding pocket with the ubiquitin ligase complex SCF^{FBXL3}, which is known to regulate period length by destabilizing CRYs (16, 18, 98). Thus, pharmacological modulation of this interaction switch with small molecules resembling FAD could provide means to adjust circadian period length (98, 99).

Several posttranslational modifications of the core clock proteins transduce signals from cellular metabolism to the circadian transcription factors, thereby modifying their transcriptional output. In this respect, the circadian components CLOCK, BMAL1, and PER2 can be O-linked *N*-acetylglucosamine (GlcNAc)-modified by the enzyme O-GlcNAc transferase (OGT), thereby modulating

their activities (100, 101). Liver-specific ablation of OGT in the mouse leads to dampened oscillation of *Bmal1* and gluconeogenic genes. GlcNAc arises from the hexosamine biosynthetic pathway (HBP), a minor branch of glycolytic metabolism (102). Thus, glucose levels dictate the availability of GlcNAc, with OGT being a signal transducer between cellular metabolism and circadian components. Along the same lines, phosphorylation of CRY1 by the nutrient sensor AMP-activated protein kinase (AMPK) connects cellular energy levels with the clock by exposing it to the AMP/ATP ratio and intracellular signaling (103, 104).

Finally, circadian rhythms in the metabolism of human red blood cells are illustrated by oscillations in peroxiredoxin oxidation–reduction, hemoglobin tetramer–dimer transitions and NADH/NADPH (105, 106). Because red blood cells are anucleated and therefore cannot perform transcription, these findings reveal the interdependency between nontranscriptional oscillations in metabolism and transcription-driven circadian rhythms. Similarly, the persistence of redox rhythms in the absence of transcription was also demonstrated (106, 107).

Sirtuins: Metabolism and Epigenetics Converge

The coordination and integration of metabolic pathways within the circadian epigenome appear intricate. Although some metabolites are thought to freely diffuse between different cell compartments, others require specific transporters and even energy consumption to move across compartments. Thus, the availability of a metabolite in close proximity of its protein sensor may provide an additional, critical regulatory level (88). In this context, the circadian metabolite NAD^+ represents a revealing paradigm. A rate-limiting step within the NAD^+ biosynthetic salvage pathway controls the conversion of nicotinamide (NAM) to β-nicotinamide mononucleotide (NMN); this step is catalyzed by the enzyme nicotinamide phosphoribosyltransferase (NAMPT, also known as visfatin). The *Nampt* gene is clock-controlled, through direct binding of CLOCK:BMAL1 to E-boxes located in the promoter (94, 95). NMN is converted to NAD^+ by the enzymes nicotinamide mononucleotide adenylyltransferase 1–3 (NMNAT1 to -3) (Fig. 3). Thereby, NAD^+ biosynthesis and availability seem circadian through a transcriptional-enzymatic feedback loop, possibly imposing circadian rhythmicity to the activities of various NAD^+-dependent enzymes. Also, NAD^+ levels and NAD^+-dependent enzymes are differentially regulated in various cell compartments (108). The circadian clock seems to have an intimate interplay with the NAD^+ pathways and NAD^+-dependent enzymes such as sirtuins. There are seven mammalian sirtuins (SIRT1 to -7), three of which (SIRT1, SIRT3, and SIRT6) have been functionally linked to the oscillator and modulate circadian outputs in response to metabolic cues (Fig. 3) (109).

Mitochondrial SIRT3 displays robust changes in its deacetylase activity in response to NAD^+ levels (110, 111). SIRT3 is a key regulator of mitochondrial function, including fatty acid oxidation and intermediary metabolism (112). Some of the SIRT3 targets include rate-limiting enzymes for mitochondrial biochemical processes, and their acetylation levels impact their activity. These enzymes can be modulated by NAD^+ availability through SIRT3, providing a possible explanation for the circadian rhythmicity observed in mitochondrial fatty acid oxidation and protein acetylation (111, 113) (Fig. 3). The oxidative ability of mitochondria isolated from *Bmal1*$^{-/-}$ mice appears reduced, correlating with decreased mitochondrial NAD^+ levels in these mutants (111). Interestingly, recent reports show that a crosstalk between nucleus and mitochondria mediates a similar effect during aging (108), suggesting that nuclear genes intervening in mitochondrial oxidative phosphorylation become repressed during aging in a mechanism involving the nuclear SIRT1. This deleterious mechanism can be partially reversed by exogenous NAD^+ administration (108). In this scenario, it is plausible that

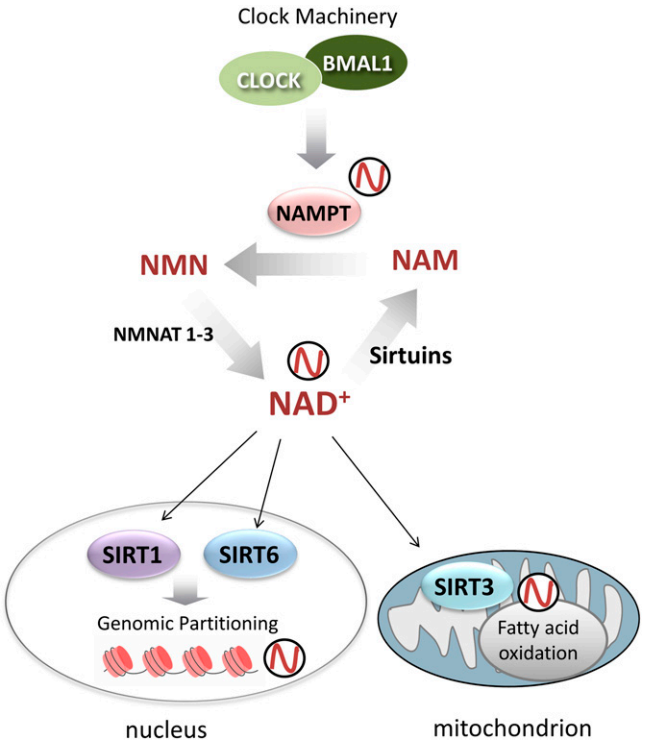

Fig. 3. NAD^+ and sirtuins interconnect cellular compartments and control metabolism during the circadian cycle. NAD^+ levels are circadian, as the rate-limiting enzyme NAMPT is encoded by a clock-controlled gene (94, 95). Rhythms on the availability of NAD^+ impose rhythmicity to the NAD^+-dependent deacetylases known as sirtuins. SIRT1 and SIRT6 regulate circadian rhythms in the nucleus, and SIRT3 in the mitochondria (32, 111). In the cell nucleus, SIRT1 and SIRT6 exert different control mechanisms on CLOCK:BMAL1 and specific transcription factors such as SREBP1, resulting in a SIRT6- and SIRT1-specific partitioning of the circadian genome in genomic subdomains, paralleled by differential metabolic phenotypes (32). Similarly, rhythms in SIRT3 activity elicit cycles of deacetylation at mitochondrial proteins, including enzymes involved in fatty-acid oxidation. Acetylation of these proteins controls their functionality, and, thereby, rhythms in fatty-acid oxidation are observed.

circadian control is involved, given the strict relationship between SIRT1, NAD^+, and circadian rhythms (114).

SIRT1 is both cytoplasmic and nuclear. When in the nucleus, it is mostly nucleoplasmic although it can be recruited to chromatin on demand. SIRT6, on the other hand, is exclusively nuclear and mostly chromatin-bound, localized at transcriptionally active genomic loci. Recent findings reveal that SIRT1 and SIRT6 operate through distinct mechanisms to differentially coordinate the clock machinery and consequently delineate the circadian transcriptional output (31, 32, 115). In the mouse liver, these proteins coordinate circadian transcription of separate groups of genes (32). Whereas SIRT1 targets include histones and nonhistone proteins, SIRT6 exerts its function by coordinating CLOCK:BMAL1 recruitment to specific chromatin sites (31, 32, 115). Indeed, SIRT1 also deacetylates clock proteins, such as BMAL1 and PER2 (31, 94, 115, 116), whereas SIRT6 is not able to do so. Another intriguing example of SIRT1-mediated deacetylation that is controlled by the clock relates to the enzyme acetyl-CoA Synthetase 1 (AceCS1) (90). This acetylation switch controls AceCS1 activity and imposes rhythmicity on the synthesis of acetyl-CoA levels (90). In contrast, SIRT6 deacetylase activity seems to be markedly slow on proteins whereas it might be more efficient in removing long-chain fatty acids from lysine residues (117, 118). Interestingly, the activity of SIRT6 seems to depend not only on NAD^+, but

also on fatty acids (119). This specific aspect of SIRT6 places this sirtuin at the crossroad between fatty acid metabolism and the clock, as CLOCK:BMAL1 transactivation on promoters of genes involved in fatty acid biosynthesis is modulated by SIRT6 (32).

A comprehensive analysis of the circadian transcriptome and metabolome in mice with liver-specific ablation of either SIRT1 or SIRT6 reveals a specific role for SIRT6 in dictating the circadian synthesis and breakdown of fatty-acid pathways, as well as their storage into triglycerides. This control is exerted at least in part through the chromatin recruitment of the sterol regulatory element-binding protein 1 (SREBP1) (32). The high-throughput profiling of the SIRT6- and SIRT1-dependent transcriptome revealed that these two deacetylases control partitioned classes of circadian genes, which results in parallel segregation of cellular metabolism (32) (Fig. 3).

Considering the role of genome topology in circadian control (71), it is tempting to speculate that the sirtuin-driven partitioning of the circadian epigenome may be contributing to the assembling of cyclic interactomes. Also, the case of SIRT1 and SIRT6 may be only the first example of a more general concept in which chromatin remodelers may control specific transcriptional networks through changes in the nuclear landscape. Moreover, the intranuclear localization of NAD^+ could provide an additional regulatory layer, by restricting NAD^+ distribution to "niches" of activity (88). This notion seems to be validated by the regulation of the NAD^+-dependent nuclear enzyme poly (ADP ribose) polymerase-1 (PARP1), which recruits to its proximity the enzyme NMNAT1 involved in the NAD^+ salvage pathway, thereby allowing for local NAD^+ supply to support its activity on DNA (120). Interestingly, a role for PARP1 in enhancing the binding of CLOCK:BMAL1 to chromatin has been reported (121). To which extent genome topology senses circadian metabolism remains to be explored.

Concluding Remarks

A series of fascinating discoveries in the field of circadian rhythms have revealed the direct implication of the clock in the maintenance of cellular homeostasis. The ability of the clock machinery for sensing the metabolic state of the cell and its remarkable plasticity place it in a strategic position. Thus, the clock can integrate environmental and metabolic signals and directly modify gene expression to favor the adaptation of the organism to specific conditions. Modulation of the circadian epigenome by environmental cues impinges on the circadian output through the specific coordination with key chromatin remodelers and transcription factors. Many of these proteins can sense the intracellular metabolic state and communicate this information to the clock machinery, coordinating its transcriptional activity accordingly. However, the circadian transcriptional landscape seems highly complex as it implicates dynamic changes in nuclear organization (88). Understanding how the nuclear landscape integrates metabolic cues and shapes the transcriptional output will be of great importance. Indeed, metabolic-related diseases and their intimate link with the disruption of the circadian clock have attracted widespread interest from researchers and pharmaceutical enterprises. Unravelling the mechanisms leading to metabolic syndromes is critical because it may expose key molecular players in the circadian control of glucose or lipid homeostasis. These insights could provide new strategies toward the development of therapeutic targets for the treatment of common metabolic-related pathologies.

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