Published in final edited form as:
Curr Neurol Neurosci Rep. 2014 March ; 14(3): 432. doi:10.1007/s11910-013-0432-6.

Epigenetics of Sleep and Chronobiology

Irfan A. Qureshi[1,2,3,6] and **Mark F. Mehler**[1,2,3,4,5,6,7,8,9,10,#]

[1]Roslyn and Leslie Goldstein Laboratory for Stem Cell Biology and Regenerative Medicine, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[2]Institute for Brain Disorders and Neural Regeneration, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[3]Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[4]Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[5]Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[6]Rose F. Kennedy Center for Research on Intellectual and Developmental Disabilities, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[7]Einstein Cancer Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[8]Ruth S. and David L. Gottesman Stem Cell Institute, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[9]Center for Epigenomics, Albert Einstein College of Medicine, Bronx, NY 10461, USA

[10]Institute for Aging Research, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Abstract

The circadian clock choreographs fundamental biological rhythms. This system is comprised of the master circadian pacemaker in the suprachiasmatic nucleus and associated pacemakers in other tissues that coordinate complex physiological processes and behaviors, such as sleep, feeding, and metabolism. The molecular circuitry that underlies these clocks and orchestrates circadian gene expression has been the focus of intensive investigation, and it is becoming clear that epigenetic factors are highly integrated into these networks. In this review, we draw attention to the fundamental roles played by epigenetic mechanisms in transcriptional and post-transcriptional regulation within the circadian clock system. We also highlight how alterations in epigenetic factors and mechanisms are being linked with sleep-wake disorders. These observations provide important insights into the pathogenesis and potential treatment of these disorders and implicate epigenetic deregulation in the significant but poorly understood interconnections now emerging between circadian processes and neurodegeneration, metabolic diseases, cancer, and aging.

[#]Corresponding author Mark F. Mehler, Albert Einstein College of Medicine, Rose F. Kennedy Center, 1410 Pelham Parkway South, Room 401, Bronx, NY 10461, USA, Telephone: (718) 430-3543, mark.mehler@einstein.yu.edu.
Irfan A. Qureshi, Albert Einstein College of Medicine, Rose F. Kennedy Center, 1410 Pelham Parkway South, Room 401, Bronx, NY 10461, USA, Telephone: (718) 430-4288, irfan@jhu.edu

Compliance with Ethics Guidelines

Conflict of Interest
Irfan A. Qureshi declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

Keywords

circadian; chromatin; DNA methylation; epigenetic; histone modification; long non-coding RNA; microRNA; non-coding RNA; RNA editing; sleep

Introduction

Epigenetics is a revolutionary new discipline. It provides, for the very first time, a robust framework for understanding how genomic programs are dynamically regulated and executed in each cell and tissue in response to complex intrinsic and environmental cues. The advent of epigenetics is, therefore, leading to profound insights in every field of biological science and to the development of innovative diagnostic and therapeutic strategies across all areas of medicine [1–6].

This review outlines the emerging role of epigenetics in sleep and chronobiology. We discuss the principal epigenetic mechanisms: DNA cytosine methylation, histone protein post-translational modifications and chromatin remodeling, non-coding RNA regulation, and RNA editing (Table 1). We present data demonstrating that the expression and function of associated epigenetic factors follow circadian patterns and are regulated by “core” members of the clock machinery and their well documented transcriptional and translational feedback loops. Furthermore, we describe how epigenetic mechanisms seem to be involved in mediating these transcriptional and translational processes—and the more recently identified post-transcriptional events—that comprise circadian clock circuitries in the suprachiasmatic nucleus (SCN) of the hypothalamus and in other neural and non-neural sites [7–9]. We note that epigenetic processes play critical roles in post-transcriptional mechanisms that have emerging roles in circadian clocks, such as transcriptional termination, alternative splicing, polyadenylation and mRNA metabolism; however, future studies are necessary to uncover more explicit mechanistic relationships.

We also highlight salient examples linking deregulation of epigenetic factors and mechanisms to the pathophysiology of sleep-wake disorders and chronobiological disturbances and to their comorbidities. These connections can either be causal, modulatory, or more correlative, and they can be described by the following emerging paradigms: (1) mutations in genes that encode factors involved in epigenetic pathways cause sleep-wake disorders, directly, (2) polymorphisms in these genes influence the fidelity of sleep-wake cycles and modify the susceptibility to sleep-wake disturbances, (3) epigenetic mechanisms deregulate—and/or they are deregulated by—the circadian clock and are associated with sleep-wake disturbances, indirectly. Our understanding of the impact of this spectrum of alterations in epigenetic factors and mechanisms on the risk, onset, and progression of sleep-wake disorders and chronobiological disturbances is still preliminary. Nevertheless, our growing recognition of the overall importance of epigenetics in the circadian clock, in physiology generally, and in myriad disease states has major implications, including the potential to help to define the molecular basis for the poorly understood relationships that exist between circadian processes and neural developmental and neurodegenerative disorders, metabolic diseases, cancer, and aging.

DNA methylation

Description

DNA methylation is involved in mediating complex genomic programs, such as X chromosome inactivation (XCI), genomic imprinting, DNA repair, and transcriptional regulation as well as in the maintenance of genomic stability. This process is remarkably

dynamic, not static as was initially believed. At a molecular level, DNA methylation refers to the covalent modification of cytosine residues by DNA methyltransferase (DNMT) enzymes, which leads to the formation of 5-methylcytosine (5mC) [1, 3]. Particular DNMT enzymes, such as DNMT3A/B, are responsible for the *de novo* generation of 5mC. Other enzymes, such as DNMT1, actively promote the maintenance of 5mC when this "mark" is already present. A compound related to folate and homocysteine, S-adenosyl methionine, serves as the methyl group donor for these methylation reactions. DNA hydroxymethylation refers to the oxidation of 5mC residues, which is catalyzed by Ten-Eleven Translocation enzymes and leads to the formation of 5-hydroxymethylcytosine (5hmC). The biological functions of 5mC are relatively well studied, whereas those of 5hmC and the additional oxidized derivatives, 5-formylcytosine and 5-carboxylcytosine, are still emerging. These marks are distributed throughout the genome in gene regulatory regions, inter- and intra-genic sequences, and repetitive elements in complex patterns. Various proteins differentially bind to methylated cytosine residues and serve as "readers" of methylation marks. These factors include methyl-CpG-binding domain proteins, Kaiso and Kaiso-like proteins, and SRA domain proteins. These readers recruit various combinations of accompanying proteins with regulatory and other activities to methylated genomic loci, where they exert their functions. Methylated DNA is also subject to active demethylation by Gadd45, DNA excision repair, and cytidine deaminase enzymes, further highlighting the dynamic and intricate nature of the DNA methylation process. Indeed, the expression of these sundry enzymes and binding proteins and associated DNA methylation profiles are highly tissue- and cell type-specific and even activity-dependent in the nervous system, where their functions are now being uncovered. These include roles in mediating neural development and aging, homeostasis, synaptic and neural network plasticity, and associated higher cognitive and behavioral functions (e.g., learning and memory), as well as disease pathogenesis [10, 11].

Emerging roles in the molecular mechanisms underlying sleep and chronobiology

Data from human subjects and model systems reveals that DNA methylation is linked with circadian processes. Levels of DNA methylation, and those of associated enzymes and factors, exhibit rhythmic oscillations. For example, measuring plasma homocysteine and global DNA methylation in whole blood from healthy human subjects demonstrates diurnal variation, with homocysteine levels peaking in the evening and reaching a nadir overnight and global DNA methylation levels displaying an inverse pattern [12]. Consistent with these observations, an analysis of circadian gene expression across human brain regions found that the 5-methyltetrahydrofolate homocysteine methyltransferase gene exhibits one of the highest and most consistent degrees of rhythmicity, phasing, and amplitude [13]. Parallel studies in mice reveal not only that the expression of DNA methylation enzymes, such as Dnmt3b, (and associated levels of DNA methylation) exhibit circadian oscillations but also suggest that these factors are explicitly regulated by the circadian clock [14]. Furthermore, DNA methylation associated factors are subject to modulation by zeitgebers. For example, methyl-CpG-binding domain protein 2 (MECP2) is phosphorylated in response to light signaling in the master circadian pacemaker, the SCN [15]. Conversely, clock genes are targets of, and thus regulated by, DNA methylation across a range of species and tissues. For example, in *Neurospora*, a model system used for studying circadian processes, DNA methylation at the frequency gene locus is involved in setting the proper phase of the circadian clock [16]. In mice, the period 1 (*Per1*) gene is demethylated within the SCN during perinatal development, leading to *Per1* up regulation [17]. Also, studying clock gene methylation in stomach, kidney, striatum, and spleen in mice of differing ages reveals tissue- and age-specific patterns that include a significant decrease in *Per1* methylation in the stomach in older mice and a significant increased in cryptochrome 1 (*Cry1*), aryl hydrocarbon receptor nuclear translocator-like 2 (*Arntl2/Bmal2*), and neuronal PAS domain

protein 2 (*Npas2*) in the spleen in older mice [18]. These observations suggest roles for DNA methylation in both central and peripheral circadian clocks. In addition, clock genes exhibit selective DNA methylation profiles in tissues derived from human subjects, including differential patterns associated with a range of diseases. For example, examining the methylation status of clock genes (*Per1, Per2, Per3, Cry1, Cry2, Bmal1, and Clock*) in autopsy specimens demonstrates variation between patients and across tissue types in a single individual [19]. Clock gene DNA methylation profiles are also selectively deregulated in many disease processes including, most prominently, cancer as well as obesity and metabolic syndrome [20–24].

Clinical implications

Data from animal models links DNA methylation with circadian behaviors and sleep-wake associated pathological states. For example, Mecp2 deficient mice display alterations in daily rhythms (i.e., core body temperature and mobility) and electroencephalographic (EEG) cortical delta wave measurements [25]. Moreover, neonatal rats that are exposed to intermittent hypoxia exhibit DNA methylation changes associated with exaggerated responses to hypoxia, irregular breathing with apneas, and autonomic dysfunction as adults [26]. This neonatal programming can be mitigated by decitabine, a DNA methylation inhibitor.

Evidence from human subjects also reveals key roles of DNA methylation in circadian disorders. A range of mutations in the *MECP2* gene is responsible for causing the vast majority of cases of Rett syndrome, an X chromosome linked autism spectrum disorder associated with 1 in 10,000 female births that is characterized by the presence of significant sleep-wake disturbances and related patterns of breathing abnormalities [27]. Similarly, four distinct mutations in the *DNMT1* gene have been identified as causative for autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCA-DN) in six different kindreds [28, 29]. The clinical spectrum of this disorder includes narcolepsy without cataplexy, cerebellar ataxia, and sensorineural hearing loss as early features and, in later stages, narcolepsy with cataplexy, peripheral neuropathy, and dementia. Patients typically present with the full ADCA-DN syndrome at ages 30–50, though the initial manifestations of this neurodegenerative disorder can be present decades earlier. Pathogenic *DNMT1* mutations occur specifically in exon 21—p.Val606Phe, p.Ala570Val, p.Gly605Ala, and p.Cys596Arg—within its replication focus targeting sequence domain, which is implicated in controlling the subcellular localization, mediating interactions with molecular partners, and competitively inhibiting the catalytic function of the enzyme [30]. However, the precise roles of these mutations in the molecular pathogenesis of ADCA-DN remain unknown, as yet.

DNA methylation can be modulated by sleep-wake behaviors. Studies correlating DNA methylation levels measured in peripheral blood specimens derived from people with long term exposure to shiftwork have demonstrated these types of effects. These include significant alterations in methylation levels associated with (1) repetitive elements (i.e., *Alu* and *LINE-1*), a surrogate for global DNA methylation; (2) imprinted gene loci (i.e., distal-less homeobox 5, IGF2 antisense RNA, and tumor protein p73); (3) circadian gene loci; (4) immune system- and inflammation-related gene loci (i.e., interferon-γ and tumor necrosis factor); and (5) miRNA gene loci (i.e., *miR-219*) [31–34]. These findings suggest that DNA methylation changes are partly responsible for mediating the risk of developing of comorbid diseases associated with shiftwork. In turn, they imply that these profiles can potentially be used as clinically relevant markers of disruption in circadian processes and even as therapeutic targets for cancers and metabolic disorders linked with aberrant DNA methylation in clock genes [20–24].

Further studies demonstrate that DNA methylation levels associated with clock genes as well as other genes are deregulated in disorders that are associated with circadian abnormalities and sleep disturbance phenotypes. For example, examining blood samples derived from pediatric patients with obstructive sleep apnea (OSA) reveals that DNA methylation associated with the forkhead box P3 *(FOXP3)* gene—a master regulator of immune responses—correlates with levels of high sensitivity C reactive protein and the myeloid-related protein 8/14 complex along with the apnea-hypopnea index, suggesting a role for *FOXP3* methylation in mediating the OSA inflammatory phenotype and related endorgan comorbidities [35]. Similarly, orexin-A methylation and corresponding mRNA expression levels are deregulated in patients with major depressive disorder (MDD), implicating DNA methylation in mediating aberrant circadian rhythms and energy homeostasis and other manifestations associated with MDD [36]. Moreover, patients with Parkinson's disease exhibit DNA methylation changes associated with clock genes, including decreased methylation of the *NPAS2* gene [37]. Also, in patients with dementia, DNA methylation profiles associated with nine circadian genes (i.e., *PER1, PER2, PER3, CRY1, CRY2, CLOCK, BMAL1*, rho guanine nucleotide exchange factor 5, and casein kinase 1e) are selective for the subtype of dementia (i.e., dementia with Lewy bodies versus Alzheimer's disease) [38]

Histone post-translational modifications and chromatin remodeling

Description

Along with DNA methylation, histone protein post-translational modifications (PTMs) and higher-order chromatin remodeling coordinate a broad range of genomic processes that include XCI, genomic imprinting, transcriptional regulation, DNA replication and repair, and the maintenance of genomic integrity. Chromatin is composed of histone proteins and other factors responsible for packaging genomic DNA into a compact structure within the cell nucleus [1, 3]. A nucleosome is the most basic element of chromatin, and it refers to DNA that is folded around octamers of core histone proteins (i.e., H2A, H2B, H3, H4). Nucleosomes are connected to each other by linker DNA and histones (i.e., H1), forming the typical "beads-on-a-string" arrangement. These fibers are assembled into progressively higher-order chromatin structures that can have varying degrees of condensation. Euchromatic regions are those with loosely packaged and thus accessible DNA, whereas heterochromatic regions have more densely packaged DNA. Importantly, these chromatin states are dynamic and subject to modification at multiple levels, from histone protein PTMs to nucleosome repositioning and remodeling of hierarchical structures. In turn, actively occurring changes in chromatin states are responsible for controlling the accessibility and interactions of DNA sequences with other factors in the nucleus. These can include other DNA sequences, RNAs, DNA binding proteins, transcription factors, transcriptional co-regulators, and additional nuclear machineries, such as those that mediate transcription and DNA replication and repair. These functions are mediated by specialized epigenetic factors, which act as readers, "writers", and "erasers" of chromatin "codes". Specifically, histone-modifying enzymes are responsible for catalyzing site-specific histone protein PTMs (e.g., lysine [K] acetylation and mono-, di- and tri-methylation; arginine methylation; and serine phosphorylation). These complementary families of enzymes include histone acetyltransferases and deacetylases (HATs and HDACs) and histone methyltransferases and demethylases (HMTs and HDMs). Nucleosomes can be repositioned and remodeled by ATP-dependent (e.g., SWI/SNF) enzymes. Often, complementary epigenetic writer and eraser enzymes form macromolecular complexes with each other and with other factors such as those that read, or recognize, particular chromatin states. These include, for example, specific bromodomain, chromodomain and Tudor domain, plant homeodomain finger, and malignant brain tumor domain proteins. Key examples of these types of chromatin

remodeling complexes are the Polycomb, Trithorax, REST, and CoREST complexes. The expression of these reader, writer, and eraser proteins and associated profiles of epigenetic marks are, like those linked to DNA methylation, highly tissue- and cell type-specific and activity-dependent in the nervous system, where they also underpin nervous system development, homeostasis, plasticity responses (e.g., learning and memory), and disease pathogenesis.

Emerging roles in the molecular mechanisms underlying sleep and chronobiology

Chromatin states are intimately linked with the molecular machinery of the circadian clock. In fact, one of the principal regulators of circadian gene expression, the CLOCK protein, acts both as a transcription factor and also as an acetyltransferase that targets the core histone proteins, H3 and H4, illustrating the high degree of integration that exists between these systems [39]. Indeed, circadian clocks control the expression and deployment of histone-modifying enzymes and corresponding levels of histone marks; and conversely, histone-modifying enzymes play a role in regulating circadian gene expression and the core clock genes. For example, H3 histone acetylation of the *Per1*, *Per2* and *Cry1* genes exhibits circadian rhythms that parallel those in present in transcribed mRNA [40]. The abundance and genomic binding distribution of the HMT enzyme, mixed lineage leukemia 3 (MLL3), and related genome-wide profiles of histone H3 lysine lysine 4 trimethylation (H3K4me3), a mark of transcriptional activation, follow circadian patterns [41]. Correspondingly, catalytic inactivation of MLL3 leads to a loss of H3K4me3 at thousands of genomic loci, widespread perturbations in circadian gene expression, and disruption of the oscillation of core clock genes, including *Bmal1*, *Cry1*, *Per2*, and *Rev-erbα*. Another HMT, mixed lineage leukemia 1 (MLL1), also mediates circadian gene expression and H3K4me3 levels; and, the mechanism of action of MLL1 includes forming a macromolecular complex with the circadian regulators, CLOCK and BMAL1 [42]. In fact, there are many examples of epigenetic factors interacting with core clock proteins and, thereby, participating in circadian gene regulation. The lysine specific demethylase 5A (KDM5A/JARID1A) also forms a complex with CLOCK and BMAL1, which results in transcriptional activation of the Period genes and maintenance of circadian oscillations [43]. In addition, there is a spectrum of other epigenetic factors that play key roles in mediating circadian gene expression, including those with HAT, HMT, and HDM histone-modifying and chromatin remodeling enzyme activities [44]. Some of these factors have roles in coupling the molecular clock machinery with key processes, such as metabolism [7, 8]. For example, sirtuin 1 (SIRT1) acts as a metabolic sensor and effector and oscillates in a circadian manner. SIRT1 is a deacetylase that targets histone H3 and other proteins such as BMAL1 and PER2; and, SIRT1 enzyme activity requires nicotinamide adenine dinucleotide, a coenzyme linked to metabolic state and regulated by the circadian clock [45–48].

Clinical implications

Data from model systems links chromatin regulation with circadian behaviors and sleep-wake associated pathological states. For example, loss of elongator complex protein 3, a HAT enzyme, in *Drosophila* during neural development leads to sleep loss and a hyperactive phenotype in adult flies that is associated with an increase in synaptic bouton number and in axonal length and branching [49]. Similarly, loss of HAT activity of Tip60 also disrupts sleep-wake cycle regulation in *Drosophila* by influencing the small ventrolateral circadian pacemaker neurons [50].

In addition, data from human subjects reveals key roles for chromatin regulatory factors in circadian behaviors and disorders. For example, a genome wide association study performed as a part of the Sleep Heart Health Study with participants drawn from the Framingham Offspring Study found a significant association between sleepiness (defined as Epworth

Sleepiness Scale score) and a single nucleotide polymorphism (*rs2218488*) in the eyes absent homolog 1 (*EYA1*) gene, which acts as a histone phosphatase [51]. Further, haploinsufficiency of the histone-lysine N-methyltransferase gene, and deletions of chromosome 9q34.3 in which this gene is embedded, causes Kleefstra syndrome [52, 53]. The clinical spectrum of Kleefstra syndrome encompasses frequent nocturnal awakenings, parasomnias, and excessive daytime sleepiness [54]. Similarly, haploinsufficiency of the *HDAC4* gene causes the chromosome 2q37 deletion syndrome, also known as brachydactyly-mental retardation syndrome, which is associated with cognitive and behavioral abnormalities including sleep disturbances such as multiple arousals throughout the night in childhood, and prolonged bouts of uninterrupted sleep (~18 hrs) with absence of REM sleep in adulthood [55, 56]. These patients exhibit reduced expression of retinoic acid induced 1 (RAI1), which regulates the *CLOCK* gene and circadian rhythms and is linked to Smith-Magenis syndrome, implying that HDAC4 modulates RAI1 [57]. Also, a range of transcriptional and epigenetic factors including the cohesin-loading protein, nipped-B homolog; the core cohesin components, structural maintenance of chromosomes 1A and 3; and the histone-modifying enzyme, HDAC8 are implicated in causing Cornelia de Lange syndrome [58]. The clinical features of this disorder include sleep disordered breathing, insomnia, and sleepiness [59, 60].

Non-coding RNA regulation

Description

The human genome encodes not only proteins but also a vast and increasing number of non-coding RNAs (ncRNAs) [1–3]. ncRNAs are found in cell type- and developmental stage-specific and activity-dependent profiles and underlie nervous system development and function [2]. Indeed, ncRNA expression is highest in the brain, highlighting the importance of these factors in neural processes. These ncRNAs are generally classified by their structural features and by their regulatory and functional activities. One of the best-characterized classes is referred to as microRNAs (miRNAs). miRNA molecules are ~20–23 nucleotides (nt) in length, they bind to regulatory regions in mRNA transcripts through sequence-selective interactions, and they are involved in post-transcriptional regulation of these mRNAs. The microprocessor complex and DICER1 ribonuclease are responsible for miRNA biogenesis. miRNA function is mediated by Argonaute family RNA binding proteins and formation of the RNA-induced silencing complex (RISC). This process, referred to as RNA interference (RNAi), represents an extraordinarily powerful paradigm for regulating large networks. A single miRNA can target hundreds of different mRNAs that contain a complementary binding sequence, and conversely, an individual mRNA can harbor binding motifs for several different miRNAs. Other classes of so-called “short” ncRNAs include, endogenous short-interfering RNAs, PIWI-interacting RNAs, and small nucleolar RNAs (snoRNAs) that are each associated with their own unique biogenesis and effector pathways. For example, snoRNAs serve as guides for RNA modifying enzymes that are responsible for pseudouridinylation and methylation of rRNAs and alternative splicing of particular mRNAs. In contrast to these short classes of ncRNAs, long ncRNAs (lncRNAs) are defined as transcripts that are greater than 200 nt in length and they can be much larger (i.e., hundreds of kilobases). Indeed, lncRNAs are quite diverse in terms of their sizes, genomic contexts from which they are transcribed, how they are regulated, secondary and tertiary structures that they adopt, post-transcriptional life cycles, molecular interacting partners, mechanisms of action, and overall functional repertoires. lncRNAs can be derived from genomic loci encompassing protein-coding genes in configurations that are sense or antisense (e.g., natural antisense transcripts, overlap partially or completely, and share regulatory regions). These genomic contexts are important in determining lncRNA activity, with lncRNAs often regulating the histone modifications, transcription, post-transcriptional

processing, stability, transport, and functioning of the corresponding protein-coding gene. Furthermore, lncRNAs are also implicated in mediating genome-wide histone modifications and chromatin remodeling, nuclear subdomain formation, transcriptional regulation, post-transcriptional RNA processing, nuclear-cytoplasmic shuttling, translational control at the synapse, and can serve as "hosts" for short ncRNAs, such as snoRNAs, released by cleavage of the lncRNA [61].

Emerging roles in the molecular mechanisms underlying sleep and chronobiology

Different classes of short and long ncRNAs are intimately linked with the molecular machinery of the circadian clock. Particular ncRNAs are expressed in oscillating patterns that are modulated by circadian clocks, sleep-wake behaviors, and zeitgebers, such as light and feeding; and conversely, ncRNAs regulate core clock genes, circadian rhythms, and sleep-wake cycles.

An increasing number of studies performed utilizing central and peripheral tissues derived from model systems reveal rhythmic profiles of miRNA expression [62–71]. For example, four miRNAs, *miR-171*, *miR-398*, *miR-168* and *miR-167* display diurnal expression with increased levels during the light and decreased levels in the dark, in *Arabidopsis* [64]. Two miRNAs, *miR-263a* and *miR-263b*, exhibit robust daily changes with peak expression at night in adult wild-type *Drosophila* head regions, and these oscillations are absent in flies with *clock* gene mutations that are arrhythmic [65]. Six miRNAs from the *Drosophila miR-959-964* genomic cluster are expressed in a circadian manner in the adult head fat body, are responsive to food intake, and are implicated in modulating innate immunity, metabolism, and feeding behavior [66]. Connections between miRNAs and sleep-wake behaviors and zeitgebers are highlighted by data showing that sleep deprivation modulates miRNA expression in the rat brain and also that certain miRNAs—*miR-138*, *let-7b*, and *miR-125a-5p*—are differentially expressed in the transitions between light and dark in the cortex, hippocampus, and hypothalamus [67, 68]. A number of studies are beginning to reveal how miRNAs are embedded within the circadian clock and involved in modulating rhythmic behaviors and sleep-wake cycles. For example, miRNAs expressed in fly circadian tissues target core clock genes (i.e., clock, vrille, and clockworkorange) and are associated with RISC; and modulating one of these miRNAs, *bantam*, increases the circadian period by 3 hours (h) [72]. In mammals, the period gene family is regulated by the *miR-192/194* cluster of miRNAs, which reduce the length of the circadian period when overexpressed [73]. The *Bmal1* gene is also targeted by miRNAs, such as *miR-494*. Intriguingly, this miRNA is contained within microvesicles secreted by cells in the brain and other organ systems into the peripheral circulation, suggesting that circulating miRNAs have roles in signaling between central and peripheral clocks [74]. Further, *miR-219* and *miR-132* are expressed in a circadian pattern in adult mouse SCN, and they serve as targets of the CLOCK/BMAL1 complex and of cAMP responsive element binding protein 1, respectively [69]. *miR-219* modulates the length of the circadian period, whereas *miR-132* affects the ability of light to reset, or entrain, the clock [75]. Moreover, miRNA biogenesis and effector pathways play important roles in regulating circadian processes. For example, in *Drosophila*, the gawky protein (i.e., GW182 homologue) is involved in translational repression, deadenylation, and decay of miRNA targets, and it also modulates pigment-dispersing factor neuropeptide signaling, which is responsible for the synchronization of circadian neurons and mediates responses of the circadian neural network to light [76]. In mice, down regulation of Drosha, Dicer, or Argonaute 2 leads to a release in *miR-185*-mediated control of the rhythmicity of *Cry1* mRNA translation [77]. Similarly, Dicer-deficient mice exhibit circadian rhythms with a period that is shortened by 2 h, and this effect is caused by loss of three miRNAs (*miR-24*, *miR-29a*, and *miR-30a*) that regulate the *Per1* and *Per2* mRNAs [78].

In addition, lncRNAs are also implicated in circadian processes. For example, profiling circadian and diurnal transcription in *Drosophila* brain revealed that approximately 10% of rhythmically expressed genes represent ncRNAs, primarily lncRNAs that serve as snoRNA host genes [79]. Analyzing rat pineal gland demonstrated that lncRNAs exhibit circadian patterns of expression, and further, that these are reversible with light administration, maintained in darkness but abrogated by disrupting SCN-pineal pathways, and mediated by norepinephrine and associated cAMP signaling, like corresponding circadian expression profiles for protein-coding genes [80]. Measuring nascent RNA levels in mouse liver uncovered that lncRNAs are similarly transcribed in a circadian manner [81].

Clinical implications

Data from model systems links both miRNAs and lncRNAs with sleep-wake behaviors and associated pathological states. For example, administering a *miR-132* mimetic into rat brain modulates a range of sleep-related parameters, including the duration of non-rapid-eye-movement sleep (NREMS) and rapid eye movement sleep during the light phase, and EEG slow-wave activity during NREMS, measures of the integrity of sleep [82]. Similarly, specific inhibitors of *miR-138* and *let-7b* reduce the amount of sleep and associated NREMS EEG delta power [68]. In terms of lncRNAs, mutations in the *yar* lncRNA gene disrupt sleep homeostasis in *Drosophila* by decreasing the length of night time bouts of sleep and also due to an inhibition of daytime sleep that occurs as a response to sleep deprivation [83].

In humans, in addition to causing disease directly, variation in genes encoding miRNAs can modify the risk of developing sleep disturbances. For example, patients with MDD harboring the T allele of the *rs76481776* polymorphism in the *miR-182* gene are susceptible to chronobiological subphenotypes (i.e., late insomnia) [84]. This *miR-182* gene polymorphism is associated with increased expression of *miR-182* and repression of its targets—adenylate cyclase 6, CLOCK, and delta sleep-inducing peptide—which have roles in mediating circadian rhythms and sleep. In terms of lncRNAs, Prader-Willi syndrome, which is associated with feeding and sleep and other circadian disturbances, is caused by abnormalities on the paternal chromosome 15q11-q13 region. This locus encodes an lncRNA, *116HG*, which is a snoRNA host gene, implying that it plays roles in post-transcriptional RNA modifications. It also acts as a subnuclear scaffold that is particularly active in neurons during sleep and seems to be involved in regulating metabolic and core circadian genes—*Clock*, *Cry1*, and *Per2*—suggesting it also has roles in mediating diurnal energy expenditure in the brain [85].

RNA editing

RNA editing refers to a process for post-transcriptional RNA modification that results in altering nucleotides within RNA molecules, from adenosine-to-inosine or cytidine-to-uridine [3]. These forms of editing are catalyzed by the adenosine deaminase that act on RNA (ADAR) family of enzymes and the activation-induced deaminase/apolipoprotein B editing catalytic subunit (AID/APOBEC) family of enzymes, respectively. ADAR enzyme targets include mRNAs, particularly those involved in synaptic transmission, as well as ncRNAs. RNA editing influences its targets by altering the amino acid coding capacity of mRNAs, patterns of alternative splicing, regulatory sequences, subcellular localization and transport, and assorted interactions. AID/APOBECs have similar functions, and they can also act on DNA molecules—referred to as DNA editing—and are involved in genomic processes such as DNA demethylation. Both of these editing events occur prominently in the brain and are regulated in complex spatiotemporal patterns during development, adult life, and aging and in response to stress and environmental stimuli. Intriguingly, there is a bidirectional connection between circadian rhythms and RNA editing. A transcriptomic analysis in *Drosophila* brain found significant deregulation in profiles of RNA editing, including site-

specific hyper-and hypoediting, in period-null mutants [79]. In addition, *Drosophila* with decreased levels of Adar expression (< 80%) exhibit changes in circadian motor patterns [86].

Perspectives

The evidence we have highlighted provides valuable insight into the emerging roles played by DNA methylation, histone PTMs and chromatin remodeling, ncRNAs, and RNA editing in sleep and chronobiology. These epigenetic mechanisms are clearly regulated by the circadian clock. In addition, they are also involved in orchestrating oscillatory gene expression, circadian rhythms and sleep-wake behaviors, and the function of the circadian clock, itself. Therefore, not surprisingly, epigenetic deregulation is responsible for the emergence of circadian rhythm and sleep-wake disorders, and measuring epigenetic profiles represents a novel approach for identifying genomic loci and assessing pathophysiological processes linked to these chronobiological disorders. Further studies focusing on the broader spectrum of epigenetic regulatory mechanisms that are now being recognized and associated higher-order epigenetic processes, such as genomic imprinting, will likely reveal even more critical functions within the clock machinery, analogous to those of histone-modifying enzymes and chromatin remodeling factors, which are relatively well known. Indeed, transcriptional regulation and kinetics (e.g., transcription termination) and post-transcriptional processes (e.g., alternative splicing, polyadenylation, and mRNA metabolism) are emerging as critical nodes in the molecular mechanisms underlying the circadian system [9]; and, epigenetic factors are, by definition, central players in these mechanisms. For example, a very intriguing study recently revealed that methylation of the N^6 position of adenosine (m^6A) is a major post-transcriptional mRNA modification that targets many core clock gene transcripts and, further, that silencing the m^6A methylase (methyltransferase like 3) in mouse liver leads to deregulation of these genes and to circadian period elongation [87]. Notably, because epigenetic mechanisms are responsible for coordinating the cell- and tissue-specific deployment of genomic programs, they are likely therefore to underpin specific features of pacemakers residing in the SCN, in other brain regions, and in other tissues. Moreover, extracellular epigenetic signaling seems to be involved in mediating communication between central and peripheral circadian clocks. Systems level analyses are necessary to better define the relationships between the whole organism circadian system, associated physiological processes, and related comorbidities [88]. The rapidly evolving field of epigenetics provides a robust framework for doing so across central and peripheral tissues and also promises to identify pharmacological and other treatment strategies that are novel and based on integrative molecular mechanisms.

We regret that space constraints have prevented the citation of many relevant and important references.

Acknowledgments

Mark F. Mehler is supported by grants from the National Institutes of Health (NS071571, HD071593, MH66290), as well as by the F.M. Kirby, Alpern Family, Harold and Isabel Feld and Roslyn and Leslie Goldstein Foundations.

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Mehler MF. Epigenetic principles and mechanisms underlying nervous system functions in health and disease. Prog Neurobiol. 2008; 86:305–341. [PubMed: 18940229]
2. Qureshi IA, Mehler MF. Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease. Nat Rev Neurosci. 2012; 13:528–541. [PubMed: 22814587]
3. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol [Review]. 2010; 28:1057–1068.
4. Qureshi IA, Mehler MF. Understanding neurological disease mechanisms in the era of epigenetics. JAMA neurology [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2013; 70:703–710.
5. Qureshi IA, Mehler MF. Developing epigenetic diagnostics and therapeutics for brain disorders. Trends Mol Med. 2013
6. Qureshi IA, Mehler MF. Long Non-coding RNAs: Novel Targets for Nervous System Disease Diagnosis and Therapy. Neurotherapeutics: the journal of the American Society for Experimental NeuroTherapeutics. 2013; 10:632–646. [PubMed: 23817781]
7. Masri S, Sassone-Corsi P. The circadian clock: a framework linking metabolism, epigenetics and neuronal function. Nat Rev Neurosci. 2012; 14:69–75. [PubMed: 23187814]
8. Albrecht U. Timing to perfection: the biology of central and peripheral circadian clocks. Neuron [Research Support, Non-U.S. Gov't Review]. 2012; 74:246–260.
9. Lim C, Allada R. Emerging roles for post-transcriptional regulation in circadian clocks. Nat Neurosci. 2013; 16:1544–1550. [PubMed: 24165681]
10. Mellen M, Ayata P, Dewell S, et al. MeCP2 Binds to 5hmC Enriched within Active Genes and Accessible Chromatin in the Nervous System. Cell. 2012; 151:1417–1430. [PubMed: 23260135]
11. Jin SG, Wu X, Li AX, Pfeifer GP. Genomic mapping of 5-hydroxymethylcytosine in the human brain. Nucleic Acids Res [Research Support N.I.H. Extramural]. 2011; 39:5015–5024.
12. Bonsch D, Hothorn T, Krieglstein C, et al. Daily variations of homocysteine concentration may influence methylation of DNA in normal healthy individuals. Chronobiol Int. 2007; 24:315–326. [PubMed: 17453850]
13. Li JZ, Bunney BG, Meng F, et al. Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. Proc Natl Acad Sci U S A [Research Support N.I.H. Extramural Research Support U.S. Gov't, Non-P.H.S.]. 2013; 110:9950–9955.
14. Maekawa F, Shimba S, Takumi S, et al. Diurnal expression of Dnmt3b mRNA in mouse liver is regulated by feeding and hepatic clockwork. Epigenetics [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2012; 7:1046–1056.
15. Zhou Z, Hong EJ, Cohen S, et al. Brain-specific phosphorylation of MeCP2 regulates activitydependent Bdnf transcription, dendritic growth, and spine maturation. Neuron [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2006; 52:255–269.
16. Belden WJ, Lewis ZA, Selker EU, et al. CHD1 remodels chromatin and influences transient DNA methylation at the clock gene frequency. PLoS Genet [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2011; 7:e1002166.
17. Ji Y, Qin Y, Shu H, Li X. Methylation analyses on promoters of mPer1, mPer2, and mCry1 during perinatal development. Biochem Biophys Res Commun [Research Support, Non-U.S. Gov't]. 2010; 391:1742–1747.
18. Zhang L, Lin QL, Lu L, et al. Tissue-specific modification of clock methylation in aging mice. Eur Rev Med Pharmacol Sci [Research Support, Non-U.S. Gov't]. 2013; 17:1874–1880.
19. Nakatome M, Orii M, Hamajima M, et al. Methylation analysis of circadian clock gene promoters in forensic autopsy specimens. Leg Med (Tokyo). 2011; 13:205–209. [PubMed: 21596611]
20. Neumann O, Kesselmeier M, Geffers R, et al. Methylome analysis and integrative profiling of human HCCs identify novel protumorigenic factors. Hepatology [Research Support, Non-U.S. Gov't]. 2012; 56:1817–1827.
21. Hanoun M, Eisele L, Suzuki M, et al. Epigenetic silencing of the circadian clock gene CRY1 is associated with an indolent clinical course in chronic lymphocytic leukemia. PLoS ONE. 2012; 7:e34347. [PubMed: 22470559]

22. Hoffman AE, Zheng T, Yi CH, et al. The core circadian gene Cryptochrome 2 influences breast cancer risk, possibly by mediating hormone signaling. Cancer Prev Res (Phila) [Research Support N.I.H. Extramural]. 2010; 3:539–548.

23. Taniguchi H, Fernandez AF, Setien F, et al. Epigenetic inactivation of the circadian clock gene BMAL1 in hematologic malignancies. Cancer Res [Research Support, Non-U.S. Gov't]. 2009; 69:8447–8454.

24. Milagro FI, Gomez-Abellan P, Campion J, et al. CLOCK, PER2 and BMAL1 DNA methylation: association with obesity and metabolic syndrome characteristics and monounsaturated fat intake. Chronobiol Int [Research Support, Non-U.S. Gov't]. 2012; 29:1180–1194.

25. Wither RG, Colic S, Wu C, et al. Daily rhythmic behaviors and thermoregulatory patterns are disrupted in adult female MeCP2-deficient mice. PLoS ONE [Research Support, Non-U.S. Gov't]. 2012; 7:e35396.

26. Nanduri J, Makarenko V, Reddy VD, et al. Epigenetic regulation of hypoxic sensing disrupts cardiorespiratory homeostasis. Proc Natl Acad Sci U S A [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2012; 109:2515–2520. This paper demonstrated that DNA methylation plays a role in the neonatal programming of hypoxic sensitivity and subsequent autonomic deregulation during adulthood.

27. Young D, Nagarajan L, de Klerk N, et al. Sleep problems in Rett syndrome. Brain Dev [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2007; 29:609–616.

28. Winkelmann J, Lin L, Schormair B, et al. Mutations in DNMT1 cause autosomal dominant cerebellar ataxia, deafness and narcolepsy. Hum Mol Genet [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2012; 21:2205–2210.

29. Pedroso JL, Povoas Barsottini OG, Lin L, et al. A novel de novo exon 21 DNMT1 mutation causes cerebellar ataxia, deafness, and narcolepsy in a Brazilian patient. Sleep. 2013; 36:1257–1259. 1259A. [PubMed: 23904686]

30. Syeda F, Fagan RL, Wean M, et al. The replication focus targeting sequence (RFTS) domain is a DNA-competitive inhibitor of Dnmt1. J Biol Chem [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't Research Support U.S. Gov't, Non-P.H.S.]. 2011; 286:15344–15351.

31. Bollati V, Baccarelli A, Sartori S, et al. Epigenetic effects of shiftwork on blood DNA methylation. Chronobiol Int. 2010; 27:1093–1104. [PubMed: 20636218]

32. Zhu Y, Stevens RG, Hoffman AE, et al. Epigenetic impact of long-term shiftwork: pilot evidence from circadian genes and whole-genome methylation analysis. Chronobiol Int [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2011; 28:852–861.

33. Shi F, Chen X, Fu A, et al. Aberrant DNA methylation of miR-219 promoter in long-term night shiftworkers. Environ Mol Mutagen [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2013; 54:406–413.

34. Jacobs DI, Hansen J, Fu A, et al. Methylation alterations at imprinted genes detected among long-term shiftworkers. Environ Mol Mutagen [Randomized Controlled Trial Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2013; 54:141–146.

35. Kim J, Bhattacharjee R, Khalyfa A, et al. DNA methylation in inflammatory genes among children with obstructive sleep apnea. Am J Respir Crit Care Med [Research Support N.I.H. Extramural]. 2012; 185:330–338.

36. Rotter A, Asemann R, Decker A, et al. Orexin expression and promoter-methylation in peripheral blood of patients suffering from major depressive disorder. J Affect Disord [Research Support, Non-U.S. Gov't]. 2011; 131:186–192.

37. Lin Q, Ding H, Zheng Z, et al. Promoter methylation analysis of seven clock genes in Parkinson's disease. Neurosci Lett [Research Support, Non-U.S. Gov't]. 2012; 507:147–150.

38. Liu HC, Hu CJ, Tang YC, Chang JG. A pilot study for circadian gene disturbance in dementia patients. Neurosci Lett [Research Support, Non-U.S. Gov't]. 2008; 435:229–233.

39. Hirayama J, Sahar S, Grimaldi B, et al. CLOCK-mediated acetylation of BMAL1 controls circadian function. Nature [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2007; 450:1086–1090. This study revealed that the CLOCK protein serves as a histone acetyltransferase enzyme and that this activity plays a role in mediating circadian gene expression.

40. Etchegaray JP, Lee C, Wade PA, Reppert SM. Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. Nature [Research Support, U.S. Gov't, Non-P.H.S. Research Support U.S. Gov't PHS.]. 2003; 421:177–182.
41. Valekunja UK, Edgar RS, Oklejewicz M, et al. Histone methyltransferase MLL3 contributes to genome-scale circadian transcription. Proc Natl Acad Sci U S A [Research Support, Non-U.S. Gov't]. 2013; 110:1554–1559. This analysis showed that the expression and function of the mixed lineage leukemia 3, a histonemodifying enzyme, is controlled by the circadian clock and is involved in regulating rhythmic gene expression on a genome-wide scale.
42. Katada S, Sassone-Corsi P. The histone methyltransferase MLL1 permits the oscillation of circadian gene expression. Nat Struct Mol Biol [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2010; 17:1414–1421.
43. DiTacchio L, Le HD, Vollmers C, et al. Histone lysine demethylase JARID1a activates CLOCK-BMAL1 and influences the circadian clock. Science [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2011; 333:1881–1885.
44. Cha J, Zhou M, Liu Y. CATP is a critical component of the Neurospora circadian clock by regulating the nucleosome occupancy rhythm at the frequency locus. EMBO Rep [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2013; 14:923–930.
45. Nakahata Y, Kaluzova M, Grimaldi B, et al. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2008; 134:329–340. This paper reported that NAD^+-dependent sirtuin 1 is a histone deacetylase, which is involved in regulating circadian gene expression, and whose enzymatic activity is both, dependent on cellular metabolic state and subject to circadian regulation.
46. Asher G, Gatfield D, Stratmann M, et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell [Research Support, Non-U.S. Gov't]. 2008; 134:317–328.
47. Nakahata Y, Sahar S, Astarita G, et al. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2009; 324:654–657.
48. Ramsey KM, Yoshino J, Brace CS, et al. Circadian clock feedback cycle through NAMPTmediated NAD+ biosynthesis. Science [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2009; 324:651–654.
49. Singh N, Lorbeck MT, Zervos A, et al. The histone acetyltransferase Elp3 plays in active role in the control of synaptic bouton expansion and sleep in Drosophila. J Neurochem [Research Support N.I.H. Extramural]. 2010; 115:493–504.
50. Pirooznia SK, Chiu K, Chan MT, et al. Epigenetic regulation of axonal growth of Drosophila pacemaker cells by histone acetyltransferase tip60 controls sleep. Genetics. 2012; 192:1327–1345. [PubMed: 22982579]
51. Gottlieb DJ, O'Connor GT, Wilk JB. Genome-wide association of sleep and circadian phenotypes. BMC medical genetics [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2007; 8(Suppl 1):S9.
52. Kleefstra T, Smidt M, Banning MJ, et al. Disruption of the gene Euchromatin Histone Methyl Transferase1 (Eu-HMTase1) is associated with the 9q34 subtelomeric deletion syndrome. J Med Genet [Evaluation Studies Research Support, Non-U.S. Gov't]. 2005; 42:299–306.
53. Kleefstra T, Brunner HG, Amiel J, et al. Loss-of-function mutations in euchromatin histone methyl transferase 1 (EHMT1) cause the 9q34 subtelomeric deletion syndrome. Am J Hum Genet [Research Support, Non-U.S. Gov't]. 2006; 79:370–377.
54. Verhoeven WM, Kleefstra T, Egger JI. Behavioral phenotype in the 9q subtelomeric deletion syndrome: a report about two adult patients. Am J Med Genet B Neuropsychiatr Genet [Case Reports]. 2010; 153B:536–541.
55. Williams SR, Aldred MA, Der Kaloustian VM, et al. Haploinsufficiency of HDAC4 causes brachydactyly mental retardation syndrome, with brachydactyly type E, developmental delays, and behavioral problems. Am J Hum Genet [Case Reports Research Support, Non-U.S. Gov't]. 2010; 87:219–228.

56. Villavicencio-Lorini P, Klopocki E, Trimborn M, et al. Phenotypic variant of Brachydactyly-mental retardation syndrome in a family with an inherited interstitial 2q37.3 microdeletion including HDAC4. Eur J Hum Genet. 2013; 21:743–748. [PubMed: 23188045]
57. Williams SR, Zies D, Mullegama SV, et al. Smith-Magenis syndrome results in disruption of CLOCK gene transcription and reveals an integral role for RAI1 in the maintenance of circadian rhythmicity. Am J Hum Genet [Research Support, Non-U.S. Gov't]. 2012; 90:941–949.
58. Deardorff MA, Bando M, Nakato R, et al. HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle. Nature [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2012; 489:313–317. This investigation identified loss-of-function mutations in the histone deacetylase 8 gene that deregulate the cohesin complex and are responsible for causing Cornelia de Lange syndrome.
59. Rajan R, Benke JR, Kline AD, et al. Insomnia in Cornelia de Lange syndrome. Int J Pediatr Otorhinolaryngol. 2012; 76:972–975. [PubMed: 22503448]
60. Stavinoha RC, Kline AD, Levy HP, et al. Characterization of sleep disturbance in Cornelia de Lange Syndrome. Int J Pediatr Otorhinolaryngol [Comparative Study]. 2011; 75:215–218.
61. Askarian-Amiri ME, Crawford J, French JD, et al. SNORD-host RNA Zfas1 is a regulator of mammary development and a potential marker for breast cancer. RNA [Research Support, Non-U.S. Gov't]. 2011; 17:878–891.
62. Clokie SJ, Lau P, Kim HH, et al. MicroRNAs in the pineal gland: miR-483 regulates melatonin synthesis by targeting arylalkylamine N-acetyltransferase. J Biol Chem [Research Support N.I.H., Intramural]. 2012; 287:25312–25324.
63. Xu S, Witmer PD, Lumayag S, et al. MicroRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. J Biol Chem [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2007; 282:25053–25066.
64. Sire C, Moreno AB, Garcia-Chapa M, et al. Diurnal oscillation in the accumulation of Arabidopsis microRNAs, miR167, miR168, miR171 and miR398. FEBS Lett [Research Support, Non-U.S. Gov't]. 2009; 583:1039–1044.
65. Yang M, Lee JE, Padgett RW, Edery I. Circadian regulation of a limited set of conserved microRNAs in Drosophila. BMC Genomics [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2008; 9:83.
66. Vodala S, Pescatore S, Rodriguez J, et al. The oscillating miRNA 959-964 cluster impacts Drosophila feeding time and other circadian outputs. Cell Metab [Research Support N.I.H. Extramural]. 2012; 16:601–612.
67. Davis CJ, Bohnet SG, Meyerson JM, Krueger JM. Sleep loss changes microRNA levels in the brain: a possible mechanism for state-dependent translational regulation. Neurosci Lett [Research Support N.I.H. Extramural]. 2007; 422:68–73.
68. Davis CJ, Clinton JM, Krueger JM. MicroRNA 138, let-7b, and 125a inhibitors differentially alter sleep and EEG delta-wave activity in rats. J Appl Physiol. 2012; 113:1756–1762. [PubMed: 23104698]
69. Cheng HY, Papp JW, Varlamova O, et al. microRNA modulation of circadian-clock period and entrainment. Neuron. 2007; 54:813–829. [PubMed: 17553428] This paper provided the first, robust mechanistic evidence that environmentally responsive microRNA activity is involved in the circadian clock within the suprachiasmatic nucleus.
70. Gatfield D, Le Martelot G, Vejnar CE, et al. Integration of microRNA miR-122 in hepatic circadian gene expression. Genes Dev [Research Support, Non-U.S. Gov't]. 2009; 23:1313–1326.
71. Na YJ, Sung JH, Lee SC, et al. Comprehensive analysis of microRNA-mRNA co-expression in circadian rhythm. Exp Mol Med [Comparative Study Research Support, Non-U.S. Gov't]. 2009; 41:638–647.
72. Kadener S, Menet JS, Sugino K, et al. A role for microRNAs in the Drosophila circadian clock. Genes Dev [Research Support N.I.H. Extramural Research Support, Non-U.S. Gov't]. 2009; 23:2179–2191.
73. Nagel R, Clijsters L, Agami R. The miRNA-192/194 cluster regulates the Period gene family and the circadian clock. FEBS J [Research Support, Non-U.S. Gov't]. 2009; 276:5447–5455.

74. Shende VR, Goldrick MM, Ramani S, Earnest DJ. Expression and rhythmic modulation of circulating microRNAs targeting the clock gene Bmal1 in mice. PLoS ONE. 2011; 6:e22586. [PubMed: 21799909]
75. Alvarez-Saavedra M, Antoun G, Yanagiya A, et al. miRNA-132 orchestrates chromatin remodeling and translational control of the circadian clock. Hum Mol Genet. 2011; 20:731–751. [PubMed: 21118894]
76. Zhang Y, Emery P. GW182 controls Drosophila circadian behavior and PDF-receptor signaling. Neuron [Research Support N.I.H. Extramural]. 2013; 78:152–165.
77. Lee KH, Kim SH, Lee HR, et al. MicroRNA-185 oscillation controls circadian amplitude of mouse Cryptochrome 1 via translational regulation. Mol Biol Cell [Research Support, Non-U.S. Gov't]. 2013; 24:2248–2255.
78. Chen R, D'Alessandro M, Lee C. miRNAs Are Required for Generating a Time Delay Critical for the Circadian Oscillator. Curr Biol. 2013; 23:1959–1968. [PubMed: 24094851]
79. Hughes ME, Grant GR, Paquin C, et al. Deep sequencing the circadian and diurnal transcriptome of Drosophila brain. Genome Res [Research Support N.I.H. Extramural]. 2012; 22:1266–1281. This study performed RNA sequencing of Drosophila brain and identified hundreds of transcripts with circadian expression profiles, including many non-coding RNAs and those that were targets of RNA editing.
80. Coon SL, Munson PJ, Cherukuri PF, et al. Circadian changes in long noncoding RNAs in the pineal gland. Proc Natl Acad Sci U S A [Research Support N.I.H., Intramural Research Support, Non-U.S. Gov't]. 2012; 109:13319–13324. This investigation of long non-coding RNA profiles in the rat pineal gland found diurnal expression patterns for 112 transcripts that were responsive to light exposure and regulated by norepinephrine signaling from the suprachiasmatic nucleus.
81. Menet JS, Rodriguez J, Abruzzi KC, Rosbash M. Nascent-Seq reveals novel features of mouse circadian transcriptional regulation. eLife. 2012; 1:e00011. [PubMed: 23150795]
82. Davis CJ, Clinton JM, Taishi P, et al. MicroRNA 132 alters sleep and varies with time in brain. J Appl Physiol. 2011; 111:665–672. [PubMed: 21719725]
83. Soshnev AA, Ishimoto H, McAllister BF, et al. A conserved long noncoding RNA affects sleep behavior in Drosophila. Genetics [Research Support N.I.H. Extramural]. 2011; 189:455–468.
84. Saus E, Soria V, Escaramis G, et al. Genetic variants and abnormal processing of pre-miR-182, a circadian clock modulator, in major depression patients with late insomnia. Hum Mol Genet. 2010; 19:4017–4025. [PubMed: 20656788]
85. Powell WT, Coulson RL, Crary FK, et al. A Prader-Willi locus lncRNA cloud modulates diurnal genes and energy expenditure. Hum Mol Genet. 2013; 22:4318–4328. [PubMed: 23771028] This analysis of the 116HG long non-coding RNA encoded by the imprinted Prader-Willi locus suggested that this factor is involved in transcriptional regulation of key metabolic and clock genes, in post-natal neurons and during sleep, via the formation of a novel subnuclear domain.
86. Jepson JE, Savva YA, Yokose C, et al. Engineered alterations in RNA editing modulate complex behavior in Drosophila: regulatory diversity of adenosine deaminase acting on RNA (ADAR) targets. J Biol Chem [Research Support, Non-U.S. Gov't]. 2011; 286:8325–8337.
87. Fustin JM, Doi M, Yamaguchi Y, et al. RNA-Methylation-Dependent RNA Processing Controls the Speed of the Circadian Clock. Cell. 2013; 155:793–806. [PubMed: 24209618] This paper demonstrated that post-transcriptional RNA methylation is a novel component of the circadian clock responsible for targeting core clock transcripts and thereby regulating the length of the circadian period.
88. Qureshi IA, Mehler MF. Towards a 'systems'-level understanding of the nervous system and its disorders. Trends Neurosci. 2013; 36:674–684. [PubMed: 23988221]

Table 1

The principal epigenetic mechanisms and examples of their emerging roles in chronobiological disorders.

Epigenetic mechanism	Description	Disorder	Abnormality
DNA methylation	Covalent modifications of cytosine residues in DNA molecules that are responsible for mediating complex genomic programs, such as X chromosome inactivation, genomic imprinting, DNA repair, and transcriptional regulation as well as the maintenance of genomic stability.	Autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCA-DN)	DNA methyltransferase 1 gene mutations
		Rett syndrome	Methyl-CpG-binding domain protein gene 2 mutations
		Dementia with Lewy bodies	Aberrant DNA methylation profiles at circadian gene loci
		Shiftwork	Aberrant DNA methylation profiles at repetitive elements, imprinted gene loci, circadian gene loci, immune system- and inflammation-related gene loci, and microRNA loci
		Pediatric obstructive sleep apnea	Aberrant DNA methylation profiles at forkhead box P3
		Parkinson's Disease	Aberrant DNA methylation profiles at circadian gene loci (e.g., *NPAS2* gene)
		Major depressive disorder	Aberrant DNA methylation profiles at orexin-A
Histone post-translational modifications and chromatin remodeling	Hierarchical modifications of histone proteins, nucleosomes, and related factors that package DNA molecules within the cell and also control the accessibility of DNA to other molecules, including RNAs, DNA binding proteins, transcriptional co-regulators, transcription factors, and additional nuclear machineries, such as those involved in transcription and DNA replication and repair.	Chromosome 2q37 deletion syndrome (Brachydactylymental retardation syndrome)	Clinical symptoms are caused by deletion of histone deacetylase 4
		Cornelia de Lange syndrome	Cohesin-loading protein, nipped-B homolog; the core cohesin components, structural maintenance of chromosomes 1A and 3; and histone deacetylase 8 mutations
		Kleefstra syndrome	Euchromatic histone-lysine N-methyltransferase 1 haploinsufficiency
Non-coding RNA (ncRNA) regulation	RNA molecules that regulate histone modifications and chromatin remodeling and transcription, post-transcriptional processing, stability, transport, and functioning of other factors via sequence-specific and conformational interactions.	Major depressive disorder	T allele of the *rs76481776* single nucleotide polymorphism in *pre-miR-182*, is associated with chronobiological subphenotypes (i.e., insomnia)
		Prader-Willi syndromes	Caused by defects on chromosome 15q11-13, which encodes the long non-coding RNA, 116HG