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Invited Review

INVITED REVIEW

Epigenetic mechanisms in diurnal cycles of metabolism and neurodevelopment

Weston T. Powell and Janine M. LaSalle*

Medical Microbiology and Immunology, Genome Center, MIND Institute, University of California, Davis, CA 95616, USA

*To whom correspondence should be addressed at: Medical Microbiology and Immunology, One Shields Ave., Davis, CA 95616, USA. Tel: +1 5307547598; Fax: +1 5307528692; Email: jmlasalle@ucdavis.edu

Abstract

The circadian cycle is a genetically encoded clock that drives cellular rhythms of transcription, translation and metabolism. The circadian clock interacts with the diurnal environment that also drives transcription and metabolism during light/dark, sleep/wake, hot/cold and feast/fast daily and seasonal cycles. Epigenetic regulation provides a mechanism for cells to integrate genetic programs with environmental signals in order produce an adaptive and consistent output. Recent studies have revealed that DNA methylation is one epigenetic mechanism that entrains the circadian clock to a diurnal environment. We also review recent circadian findings in the epigenetic neurodevelopmental disorders Prader–Willi, Angelman and Rett syndromes and hypothesize a link between optimal brain development and intact synchrony between circadian and diurnal rhythms.

Introduction

All organisms have evolved to exist in a rhythmically cycling environment driven by the 24-h day and seasonal variations. As a result, they have adapted their physiological rhythms to mirror their environment. Physiological rhythms are driven by both ongoing environmental influences (light/dark cycling, food availability, wake/sleep) and an intrinsic, genetically encoded clock mechanism.

In mammals, the genetically encoded clock resides in the suprachiasmatic nucleus (SCN) located within the hypothalamus to regulate cyclical metabolism in peripheral tissues. This mammalian clock is called the 'circadian' cycle because it continues to cycle in the absence of environmental inputs. The circadian cycle orchestrates a complex rhythm of transcription, post-transcription, translation and post-translational regulation with the circadian factors CLOCK, BMAL1, PERIOD and CRYPTOCHROME genes (reviewed in 1).

In contrast, the environmentally driven cycle is called the 'diurnal' cycle (Fig. 1). In most instances, the two cycles are in synchrony, with the diurnal cycle acting to entrain the circadian cycle. However, the two can become unsynchronized as is experienced with jet lag, sleep deprivation, starvation or constant darkness. In this review, we will focus on recent work highlighting the role of epigenetic mechanisms, in particular, DNA methylation, at the interface of circadian and diurnal cycles, and give examples of epigenetic neurodevelopmental disorders with circadian and diurnal rhythm disruptions.

Epigenetic mechanisms act to integrate complex genetic and environmental signals in order to regulate the output from systems, such as the circadian clock. Historically, epigenetics has referred to heritable traits not directly associated with genetic mutations; however, more recently, epigenetics refers to modifications of nucleotides or chromatin that do not change the underlying sequence but alter gene expression. An expanded definition of epigenetics has become important in understanding how the genomes of long-lived organisms such as humans adapt, develop and function within a changing environment.

Epigenetic layers above the DNA sequence include DNA methylation, histone modifications, chromatin looping, noncoding RNA and chromosomal organization (2). The role of epigenetics in integrating genetic signals with environmental influences has been most extensively investigated in analyses of

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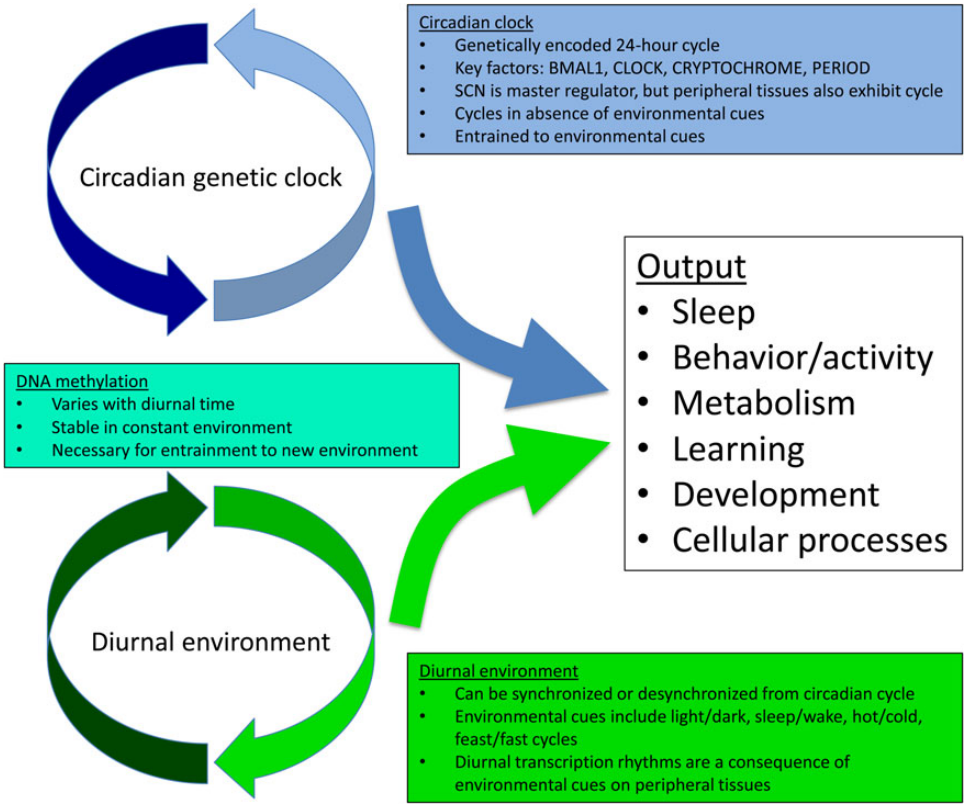

Figure 1. The mirrored cycles of the circadian genetic clock with the diurnal environment. Top (blue): Circadian factors expressed from the suprachiasmatic nucleus (SCN), including BMAL1, CLOCK, CYTOCHROME and PERIOD are genetically encoded to a 24 h rhythmicity independent of environmental factors. The circadian clock can be reset and entrained by the diurnal environment. Bottom (green): The diurnal cycle is regulated by environmental cues in peripheral (non-SCN) tissues, including light/dark, sleep/wake, hot/cold, feast/fast and synchronizes with the circadian cycle under optimal environmental conditions. Middle (teal): The epigenetic layer of DNA methylation is stable under conditions of constant darkness but varies with diurnal time in peripheral tissues and is necessary for entrainment of the circadian cycle to a new diurnal environment. DNA methylation therefore acts at the interface of genetic and environmental factors influencing outputs important for human health.

DNA methylation. DNA methylation over gene promoters reduces transcription, and methylation levels over gene bodies may serve as a marker of past transcription with inverse levels as the promoter.

While DNA methylation marks have historically been considered stable over a lifespan, evidence supporting a dynamic pattern of cyclical methylation for some environmentally responsive genes has been found (3). Therefore, the emerging evidence for diurnal cyclical DNA methylation in humans and animal models will be discussed. Furthermore, over the past few years, alterations in normal circadian and diurnal rhythms have been implicated in the phenotypes and pathogenesis of multiple diseases (4). In particular, alterations to normal cycling have become recognized as contributing to several epigenetically based neurodevelopmental disorders with comorbid sleep abnormalities.

Circadian Rhythms have far-reaching Metabolic and Transcriptional Impacts

Exposure of predictable patterns of light and dark set up the synchronization of the circadian and diurnal cycles with two distinct phases of cellular metabolism. The wake stage is characterized by catabolism, energy and protein usage resulting in low levels of protein synthesis and high AMP/ATP ratio. In contrast, the sleep stage is characterized by anabolism, a restorative time for ATP levels and increased protein synthesis (5). Such synchronization is important for proper cellular functioning, and current research has investigated the transcriptional rhythms under circadian (constant environment) and diurnal (cycling environment) conditions, as well as the regulatory mechanisms that control such activity.

Table 1 summarizes the recent genomic investigations of circadian and diurnal rhythms at the epigenetic, transcriptional and proteomic levels that have demonstrated a large portion of the genome (up to 43% of protein-coding genes) interacting at some level with circadian and diurnal rhythms (6–9). Furthermore, multiple peripheral tissues, including liver, brain, adipose and heart all show evidence for diurnal rhythmicity of a subset of transcripts. Transcriptional activity, particularly over long non-coding RNAs (lncRNA), is a means by which the epigenetic signature of a locus or chromosomal domain can be re-written and modified, leaving behind a memory of recent or past transcriptional events (2,11,16). A recent finding of ~1000 lncRNA with circadian rhythms suggests that such widespread cyclical transcription in multiple peripheral tissues may generate epigenetic plasticity from interactions with genetic and environmental inputs (9).

Interestingly, mice lacking the core clock gene *Bmal1* specifically in the SCN maintained transcriptional rhythmicity under diurnal light/dark cycles and more rapidly adapted to a shifted diurnal cycle than wild-type controls (17). Under the environmental conditions of constant darkness, however, the SCN clock-ablated mice showed a gradual desynchronization, suggesting that a SCN-independent pathway synchronizes peripheral tissue transcriptional cycles with external time. Similarly,

Table 1. Genome-wide studies of diurnal and circadian transcripts

Tissue	Species	Time points	Circadian or Diurnal?	Methods	# Oscillating transcripts	Epigenetic changes?	Effects of genotype or environment	Reference
Liver	Mouse	q4 h over 48 h	Circadian (Constant dark)	RNA-seq, ChIP-seq	1371 intron and 2037 exon RNA	RNAPII-8WG16, RNAPII-Ser5P, H3K4me3, H3K9ac and H3K27ac at TSS; H3K36me3 and H3K79me2 in gene body	n/a	(6)
Brain dorsolateral prefontal cortex	Human	n/a	Diurnal, time determined by time of death	RNA-seq, Illumna 450 k bead chip methylation,	Not given	DNA methylation nadir near TSS ~05:30 outside TSS ~20:30	n/a	(7)
Liver	Mouse	q3 h over 24 h	Circadian (constant dark)	RNA-seq, ChIP-seq, MethylC-seq	1262	No DNA methylation changes, H3K4me3, H3K9ac, H3K4me1, H3K27ac and H3K36me3,	n/a	(8)
Aorta, adrenal gland, brainstem, brown fat (anterior dorsum adipose), cerebellum, heart, hypothalamus, kidney, liver, lung, skeletal muscle (gastrocnemius) and white fat (epididymal adipose)	Mouse	q2 h over 48 h	Circadian (constant dark)	RNA-seq, microarray	8508 (across all tissues), range: 3186 (liver) to 642 (hypothalamus)	n/a	n/a	(9)
Liver	Mouse	q4 h over 24 h	Diurnal	5-methylcytosine quantification	n/a	Global DNA methylation nadir at ZT1 (end of dark)	n/a	(10)
Brain cortex	Mouse	Zt6, Zt16	Diurnal	RNA-seq	n/a	n/a	*Snord116* ± had 6467 genes altered when compared with WT	(11)
SCN	Mouse	CT4	Circadian (constant dark)	RNA-seq, MeDIP	n/a	1294 DMRs when entrained to 22 h day	22 h day had 292 loci with >2-fold transcript level changes at CT4	(12)
Forebrain	Mouse	6 h after sleep deprivation	Diurnal	Microarray expression, MeDIP promoter array	n/a	227 DMRs with sleep deprivation	1298 altered transcript levels with 6H sleep deprivation	(13)
Blood	Human	n/a	Diurnal	Microarray expression	1502 with normal sleep cycle, 237 with desynchrony	n/a	1502 with normal sleep cycle, 237 with desynchrony	(14)
Blood	Human	n/a	Diurnal	Microarray expression	1855 with normal sleep, 1481 with sleep deprivation	n/a	1855 with normal sleep, 1481 with sleep deprivation	(15)

transcript oscillations were observed from peripheral tissues under experimental conditions of either diurnal light/dark cycles or a period of constant darkness following light/dark entrainment (Table 1) (6,9,14,15). Together, these results suggest that peripheral transcriptional oscillations are influenced more by the memory of past environmental and metabolic clock-related cycles than by the SCN-encoded clock machinery.

One hypothesis to explain how genes and tissues not directly linked to the circadian machinery are transcriptionally influenced by the circadian clock is that genes residing within a locus epigenetically impacted by the circadian cycling may gain increased sensitivity to environmental signals at different times of day. Since most of the genes from the atlas of circadian transcripts (including the lncRNA genes) exhibited circadian oscillation in a tissue-specific manner, such epigenetic plasticity would also be expected to be tissue-specific. Such interplay between tissue-specific gene networks and the core circadian machinery leads to each tissue having its own set of cyclical transcripts, and makes each tissue uniquely responsive to perturbations in the diurnal environment (9,18).

Circadian and Diurnal Rhythms in DNA Methylation

Under physiological diurnal cycles, evidence for cyclical DNA methylation patterns has been observed. An analysis of DNA methylation changes that related to time of death in 738 post-mortem human dorsolateral prefrontal cortex tissues found an enrichment of high-amplitude CpG methylation sites within 1 kb of transcription start sites (TSS), with the nadir of methylation at these sites occurring in the early morning preceding the peak of the closest transcript by 1–3 h (7). The proximity to transcription start sites suggests that the dynamic nature of the DNA methylation changes may be occurring at promoter sites. In mouse livers, diurnal oscillation in global DNA methylation levels, as measured by total percentage of 5-methylcytosine and methylation of LINE-1 CpG sites, was lost in *Per1/Per2* double knock-out mice, demonstrating a genome-wide impact of circadian clock machinery on epigenetic changes in the context of a normal cycling environment (10). In humans and mice, global DNA methylation outside of TSS has a nadir near the end of the wake period, but in humans this corresponds to the end of the light period, whereas in mice this is the end of the dark period (7,10).

In contrast to diurnal methylation variation, studies using whole genome analysis (MethylC-seq) on liver DNA or methylated DNA immunoprecipitation (MeDIP) investigation of brain DNA from mice in constant darkness did not reveal any circadian changes in DNA methylation at promoters or other regions (8,12). Such a discrepancy could point to a key difference between intrinsic clock circadian cycling, and the transcriptional cycling that occurs in the presence of a diurnal environment, with DNA methylation acting as mediator between the environment and intrinsic transcriptional clock cycling.

Supporting the hypothesis that DNA methylation acts as a key mediator between the environment and an intrinsic clock circuit, the entrainment of mice to a 22-h cycle depends on DNA methylation (12). Similar to previous work, no diurnal variation in DNA methylation was found for mice housed in constant darkness. However, once mice were entrained to a 22-h cycle, 1294 differentially methylated regions were identified in the SCN compared with mice entrained to a 24-h cycle. When DNA methyltransferase activity was inhibited by zebularine, the mice did not adjust to the new 22-h cycle as robustly as control mice, indicating that DNA methylation was necessary to change the intrinsic period of the circadian clock machinery. The DNA methylation differences correlated with altered transcription of nearby genes, and were enriched near genes important for synaptogenesis, axonal guidance and neurohormonal signaling, suggesting that neurodevelopment is influenced by the synchronization of diurnal and circadian cycles (12). DNA methylation changes were observed in the *Dio3* gene locus following photoperiod changes in hamster hypothalamus, also supporting the idea that methylation changes are in response to environmental perturbations to diurnal cycles (19). There may also be brain region-specific variation in the role of DNA methylation during diurnal cycles, as a recent study interrogating the *Bdnf* promoter found diurnal variation in methylation at the *Bdnf* promoter in cortex, but not in basal forebrain (20).

Environmental Disruption and Epigenetic Changes

The evidence that epigenetic regulation may be a factor in integrating the diurnal environment and the genetic circadian clock also comes from experiments in which the environment is manipulated. In a study of the effect of sleep loss on human blood transcriptome by using extended light period, resetting the 24-h period was seen to result in reduced amplitude of transcript level changes (15). In a follow-up study, the impact of forced desynchronization with the intrinsic clock was investigated by putting individuals in a 28-h light/dark cycle (14). Desynchronization led to a marked reduction in the number of transcript levels demonstrating circadian rhythmicity, more than had been seen with simple sleep deprivation in the setting of a 24 h cycle (237 transcripts cycled with desynchrony versus 1485 with sleep deprivation) (14,21). The mechanism by which sleep deprivation or desynchronization causes such widespread transcriptional changes is not well understood. However, one intriguing hypothesis is that DNA methylation could mediate interactions between the diurnal and the intrinsic circadian gene network. In support of this hypothesis, sleep deprivation led to altered promoter usage in a diurnal fashion that correlated with loss of diurnal DNA methylation variation at *Bdnf* (20).

The mechanism by which DNA methylation is altered in a diurnal manner is not yet established. One hypothesis is that changes in DNA methyltransferase levels diurnally may lead to DNA methylation changes, as some studies have shown this correlation (13). However, an alternative explanation is that diurnal transcriptional changes may alter methylation patterns, especially at CpG island promoters, where transcription can lead to formation of an RNA:DNA hybrid (R-loop) that protects against methylation, and at gene bodies in which higher methylation correlates with higher transcription (22–24).

The adaptation of the circadian rhythm to a changing diurnal environment is also important in early development. Daily rhythms in fetuses are regulated by maternal rhythms, but then must adjust to the *ex utero* environment and establish an autonomous rhythm soon after birth (25). From infancy to adolescence, the mammalian brain undergoes rapid development, synaptogenesis and maturation of neurons with widespread epigenetic and transcriptional changes in neurons. Interestingly, DNA methylation appears to play a unique role in the proper development of neuronal networks, memory, learning and brain development during this time frame (23,26). It is likely that alterations due to circadian, diurnal or epigenetic mechanisms in establishing the epigenetic patterns of diurnal rhythms are likely to have profound impacts on neurodevelopment.

Neurodevelopmental Disorders and Diurnal/ Circadian Disruption

Over the past few years, multiple studies have begun to elucidate a link between genetic disorders affecting epigenetic pathways and circadian rhythms. In researching the pathogenesis of neurodevelopmental disorders involving epigenetic pathways, a common thread of sleep abnormalities and circadian disruption has emerged. In particular, molecular links to circadian rhythms have been described for the parentally imprinted disorders Prader–Willi (PWS) and Angelman syndromes (AS), as well as the X-linked Rett syndrome caused by mutations in *MECP2*, encoding an epigenetic reader of DNA methylation.

Prader–Willi syndrome

PWS is characterized by hypotonia and failure to thrive in the newborn period with progression to hyperphagia, metabolic disturbances and obesity in adulthood (27). Children with PWS also suffer from pubertal insufficiency, developmental delay, behavioral problems and sleep disturbances. While sleep problems in PWS have historically be credited to the obesity and apnea, actigraphic measurements have recently shown that sleep abnormalities are a central and reproducible feature of PWS, characterized by frequent night waking and daytime sleepiness (28). The genetic cause of PWS is most commonly large paternal deletion of chromosome 15q11.2–q13.3; however, small microdeletion cases involving the *SNORD116* locus or the imprinting control center (PWS-ICR) are also observed (Fig. 2). Uniparental disomy, in which two intact chromosome 15s are present, but both are maternal in origin also contributes to approximately one-third of PWS cases.

Expression across the PWS locus is under the control of the PWS-ICR with methylation of the PWS-ICR occurring on the maternal allele and leading to silencing of *SNRPN* and other paternally expressed transcripts (Fig. 2). The unmethylated, paternal PWS-ICR is the promoter for the protein-coding gene *SNRPN* and a long, non-coding transcript that is processed to give rise to the *SNORD116*, *SNORD115*, *116HG*, *115HG* and *UBE3A-AS* RNAs. Together, the genetic evidence for PWS implicates the loss of the paternally expressed *SNORD116* transcripts in the central disorder, although other paternally expressed transcripts *NDN* and *MAGEL2* may contribute to the phenotype in large deletion and maternal disomy cases.

Two PWS mouse models lacking the syntenic *Snord116* locus have been described to recapitulate some PWS-like phenotypes (29,30). However, in contrast to humans with PWS who exhibit failure to thrive followed by obesity, mice lacking the syntenic *Snord116* locus remain smaller and leaner through adulthood (11,29,30). While transcriptional differences were not identified in the hypothalamus of *Snord116del* mice, our recent genomic analysis of cortex identified over 6000 transcripts with 94% of these higher in *Snord116del* compared with WT cortex (11). Since many of the known circadian genes were upregulated, including *Clock*, *Cry1*, *Cry2*, *Per1* and *Per2*, we further explored diurnal phenotypes in this PWS mouse model. *Snord116del* mice exhibited increased metabolic rates with increased fat oxidation during light (sleep) hours. The missing *Snord116* region encodes a lncRNA (*116HG*) and small, nucleolar RNAs (snoRNAs) only expressed in neurons. Consistent with a diurnal regulatory role for the lncRNA, the size of the *116HG* nuclear cloud was significantly larger during sleep than wake and the transcriptional increases in *Snord116del* mice were specific to the light phase. The

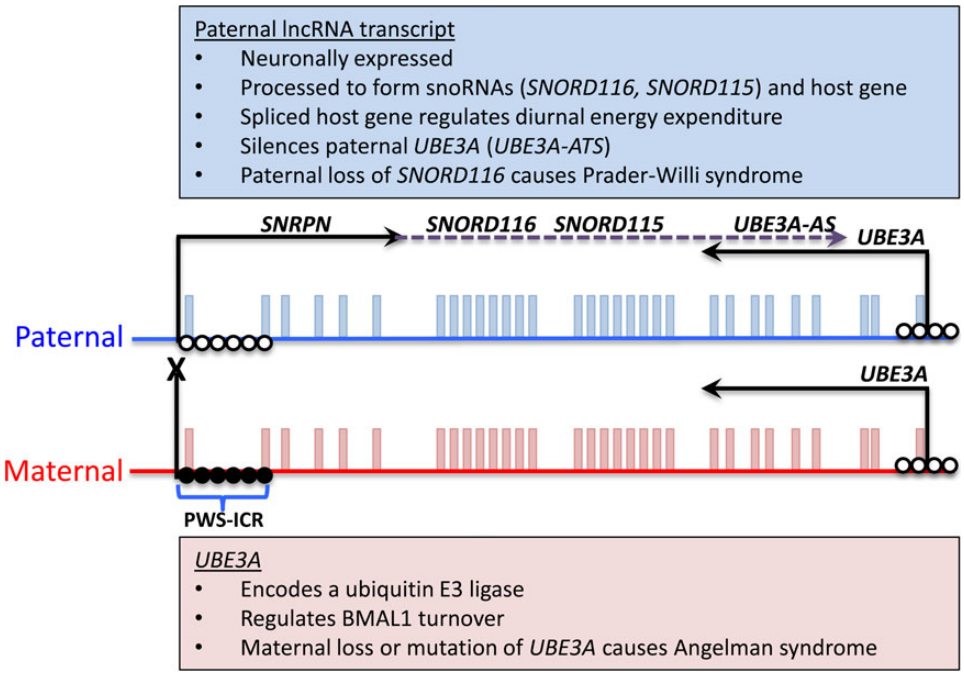

Figure 2. Circadian and diurnal factors regulated by parental imprinting in the Prader–Willi/Angelman syndrome locus. The Prader–Willi (PWS) and Angelman (AS) critical region on human chromosome 15q11.2–q13.3 is shown, depicting transcripts specifically expressed from the paternal (blue) or maternal (red) alleles. DNA methylation (closed circles) on the maternal allele of the PWS imprinting control region (PWS-ICR) silences the expression of *SNRPN* (solid arrow) and the long non-coding transcript expressed in neurons (dotted arrow) that encompasses repeated snoRNA clusters (including *SNORD116* and *SNORD115*) and the antisense transcript to *UBE3A* (*UBE3A-AS*). The SNORD loci are further processed to snoRNAs, which localize to the nucleolus, and spliced host gene (*116HG*, *115HG*) transcripts that localize in a nuclear cloud to the site of transcription and regulate diurnal energy and transcript levels in cortex during sleep. *UBE3A* encodes an E3 ubiquitin ligase protein that regulates protein turnover of the circadian factor BMAL1. Since the paternal *UBE3A* allele is silenced by expression of the *UBE3A-AS* in neurons, deletion or mutation of the maternal copy of *UBE3A* causes AS.

importance of circadian and diurnal rhythms of metabolism in the pathogenesis of PWS was consistent with earlier work showing that mice with paternal deficiency of *Magel2* show dysregulation circadian cycles (31,32). Further understanding of the link between diurnal cycles and the complex molecular mechanisms of the imprinted genes with the PWS locus is expected to be important for improving treatments for PWS.

Angelman syndrome

In contrast to PWS, which results from loss of paternally expressed non-coding genes, AS results from maternal loss or mutation of the protein-coding gene *UBE3A* (33,34). Children with AS have developmental delay, speech impairments, intellectual disability, seizures, ataxic movements, hyperactivity and abnormal sleep patterns (35).

UBE3A lies within the 15q11–13 imprinted region and is imprinted specifically in neurons (36). As shown in Figure 2, silencing of the paternal *UBE3A* allele occurs due to progression of paternal transcription through the *Snord116* and *Snord115* locus to an antisense transcript of *UBE3A* (*UBE3A-AS*) (37–41).

The role of UBE3A in regulating dendritic growth and neuronal function has been well described; however, a novel role for *UBE3A* has been described in two recent studies that link *UBE3A* function to circadian rhythms (42–44). The first study from Gossan *et al.* (45) found that *Ube3a* activation in mouse embryonic fibroblasts led to increased ubiquitination and degradation of Bmal1 with a concomitant dampening of robust transcriptional cycling. A second group showed circadian defects in two different AS mouse models due to the loss of Bmal1 protein turnover in neurons (46). The mice exhibited prolonged free-running circadian cycles and adjusted more rapidly to light/dark environmental signals. Since *Ube3a* is only imprinted in neurons, these data suggest that the behavioral defect observed is due to dysregulation of the central circadian clock, supported by evidence of disrupted *Per2* cycling in the SCN. However, the phenotype may also be due to altered cycling in behavior centers of the cortex as well since maternal *Ube3a* is absent in all neuronal tissues of AS mouse models.

Importantly, a circadian defect of increased period of Per2 rhythmicity in *Ube3a* deficient mice was reversed by de-repressing the silenced paternal *Ube3a* in neuronal tissues using the topoisomerase inhibitor topotecan (46). Topotecan acts to inhibit antisense transcriptional progression through *Ube3a-AS* on the paternal allele, and targeting the *Ube3a-AS* transcript has been proposed as potential therapy for AS (37,40,41). Interestingly, Shi *et al.* (46) found that topotecan treatment led to a shortening (albeit not statistically significant) of the circadian period in WT mice, suggesting that re-expression of the paternal allele may have subtle dosage impacts on circadian machinery in the SCN. Together, these studies implicate UBE3A in regulating the circadian clock transcriptional regulator Bmal and suggest a possible cross-talk with diurnal regulation of the paternal PWS transcripts (Fig. 3).

Rett syndrome

Rett syndrome is a neurodevelopmental disorder characterized by loss of early spoken language, developmental regression, abnormal hand movements, breathing abnormalities and disrupted sleep patterns (47). Rett syndrome is most commonly caused by mutations in the X-linked gene *MECP2*, encoding methyl-CpG-binding protein 2 (48). Due to the function of MeCP2 as a DNA-methylation binding protein and the role of X inactivation

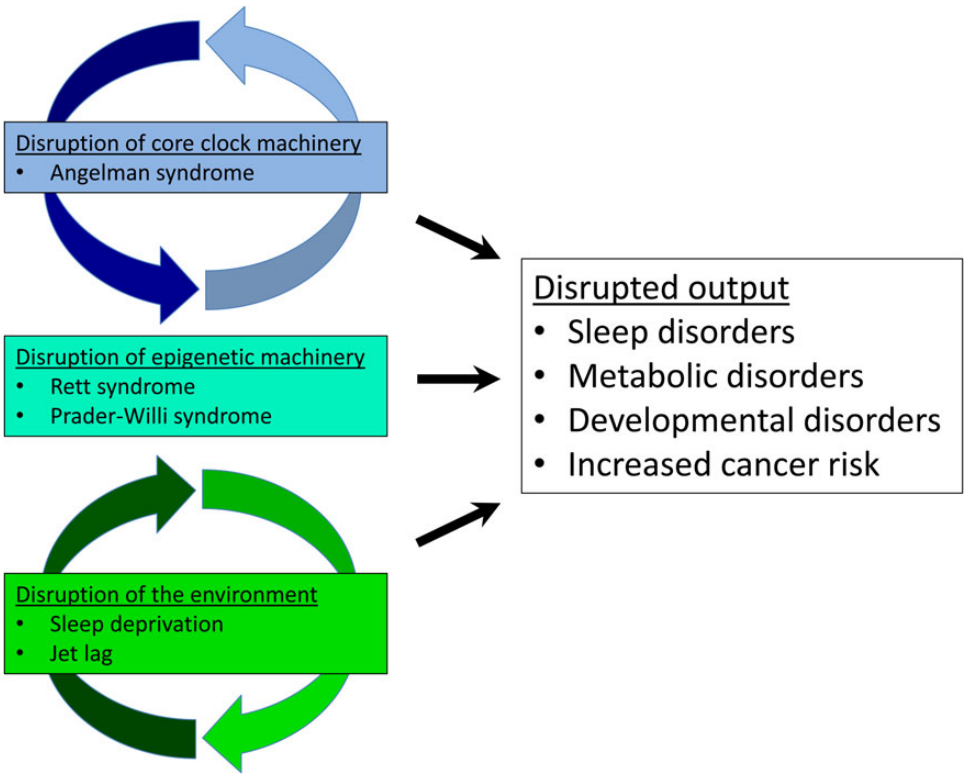

Figure 3. Genetic and environmental disruptions to the synchrony of circadian and diurnal cycles. Top (blue): Maternal loss of *UBE3A* in Angelman syndrome leads to alterations in turnover in BMAL1, a core component of the circadian clock. Middle (teal): Mutations in *MECP2* in Rett syndrome may act at the interface of reading the diurnal DNA methylation marks. Paternal loss of *116HG* in Prader–Willi syndrome may act at by regulating diurnal transcriptional cycling in the cortex. Bottom (green): Disruptions to the diurnal environment, such as in mouse models of jet lag or sleep deprivation also lead to epigenetic and transcriptional alterations.

in the disorder in females, Rett syndrome serves as prototypical 'epigenetic' disorder. MeCP2 is expressed highly in the hypothalamus and specifically the SCN around the time of birth and becomes phosphorylated in response to light exposure (49–51). In a recent study, investigating diurnal variations in MeCP2 levels in mouse forebrain, both *Mecp2* RNA and protein levels oscillated with light/dark cycles as RNA and protein levels were highest during light (sleep) hours and lowest during dark (wake) hours (52). Importantly, the oscillation in MeCP2 levels was correlated with chromatin accessibility at promoter regions of the genes *Bdnf, Dlk1* and *Sst*, which had corresponding oscillations in transcript levels. The authors hypothesize that the chromatin accessibility changes were due to changes in MeCP2 protein levels, and that the altered accessibility contributed to the changes in transcript levels (52). In a different study, sleep was found to be fragmented and delayed and structural abnormalities were observed in the number of VIP+ neurons in the SCN of *Mecp2* null compared with WT mice (53). Interestingly, subjecting the mice to a chronic 'jet lag' model of progressive weekly light phase adjustments resulted in significantly earlier lethality of *Mecp2* null compared with WT male mice (53).

Taken together with the findings of DNA methylation oscillations during light/dark cycles in human brains (7), an important avenue for future investigation would be to integrate MeCP2 levels with diurnal DNA methylation levels in brain. Interestingly, the nadir of methylation at most TSS occurred towards the end of the sleep (dark) period (~5:30 a.m.) in humans and mice, whereas the peak level of MeCP2 protein levels was during the corresponding sleep period in mice suggesting a possible interaction (7,10,52). The link between diurnal/circadian disruption has also been seen with haploinsufficiency of another DNA methylation binding protein, MBD5 (54).

Conclusion

The recent work into the genetic and epigenetic control between diurnal and circadian rhythms, as well as links between neurodevelopmental diseases to diurnal transcriptional alterations, highlights a promising area for further research. In particular, the epigenetic layer of DNA methylation appears to act as a critical mediator of the complex interactions between genetic, environmental and developmental systems in mammals. However, the role of epigenetic marks in regulating the mirrored cycles of circadian rhythm and diurnal rhythm is likely to only be part of the story, as research into the circadian machinery has revealed multiple layers of regulation beyond transcription. Post-transcriptional regulation including splicing, mRNA turnover, translation regulation and post-translational modification have also been shown to regulate circadian cycle output (6,55).

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