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Time for Bed: Genetic Mechanisms Mediating the Circadian Regulation of Sleep

Ian D. Blum[1], **Benjamin Bell**[2], and **Mark N. Wu**[1,2,3,*]

[1]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21287

[2]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287

[3]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21287

Abstract

Sleep is an evolutionarily conserved behavior that is increasingly recognized as important for human health. While its precise function remains controversial, sleep has been suggested to play a key role in a variety of biological phenomena, ranging from synaptic plasticity to metabolic clearance. Although it is clear that sleep is regulated by the circadian clock, how this occurs remains enigmatic. Here, we examine the genetic mechanisms by which the circadian clock regulates sleep, drawing upon recent work in fruit flies, zebrafish, mice, and humans. These studies reveal that central and local clocks utilize diverse mechanisms to regulate different aspects of sleep, and a better understanding of this multilayered regulation may lead to a better understanding of the function(s) of sleep.

Keywords

Sleep; Circadian; Genes

From clockwork gears to sleep behavior

The biological clocks ticking within essentially all animals coordinate diverse and widespread physiological processes and behaviors across the 24 hr day. Starting with the seminal identification of the *period* mutant by Benzer and Konopka in 1971 and culminating with the recent awarding of the 2017 Nobel Prize in Physiology or Medicine to Drs. Hall, Rosbash, and Young [1], there has been intense focus on unraveling the molecular genetic basis of the core circadian oscillator. As our understanding of the mechanisms that mediate intrinsic rhythmicity and phase resetting of the core oscillator has matured, there has been increasing attention to how these **circadian clock** oscillations regulate various physiological

*Correspondence: marknwu@jhmi.edu.

functions and behaviors, such as sleep. In this review, after providing a general introduction to sleep, we will focus on recent advances in our understanding of the genetic mechanisms by which it is regulated by the circadian clock. These studies suggest that circadian orchestration of multiple genetic pathways enables multilayered control of different aspects of sleep, and a deeper understanding of these processes may ultimately yield insights into the fundamental functions of sleep.

Sleep is an ancient, conserved behavior with multiple proposed functions

Sleep is a vital, phylogenetically conserved phenomenon that has been identified in animals ranging from worms and jellyfish to birds and mammals [2–7]. Despite its near ubiquity, sleep remains a poorly understood process. While its function(s) remain an intensely debated topic, sleep has been proposed to be important for energetic and metabolic processes [8, 9], immune system function [10], clearance of neuronal waste products and maintaining ionic balance [11–13], synaptic homeostasis and neural plasticity [14–17], and learning and memory [18–20]. How a single behavioral entity plays a key role in such diverse processes is unclear, but may relate to the notion that sleep is not a unitary phenomenon, but rather comprises multiple distinct sub-states [21]. In more primitive organisms, such as jellyfish, it may be that sleep serves a single primordial function, and that various other functions for sleep have been added during the evolution of more complex animals. Sleep in humans, for example, can be measured by **electroencephalography** (EEG) and be broadly categorized into **rapid-eye movement** (REM) sleep, and “light” **non-REM** (NREM) vs “deep” NREM sleep (slow wave sleep), based largely upon waveform frequency/amplitude patterns. These distinct sleep sub-states preferentially occur during different times during the night, are differentially regulated, and have been proposed to be important for distinct processes [22]. Beyond these canonical sleep sub-states, there is growing recognition that these basic categories do not adequately describe the full spectrum of potential sleep sub-states [23]. Distinct sleep sub-states associated with different electrophysiological signatures, arousal thresholds, and/or behaviors have also been described in invertebrates, suggesting that the diversity of sleep states is an evolutionarily conserved phenomenon [24–27].

Studies of the regulation and function of sleep have been greatly aided by the use of model organisms. In this review, in addition to research in humans, we focus on circadian/sleep work performed in fruit flies, zebrafish, and mice. How is sleep measured in these model organisms? Sleep can be identified using electrophysiological measures such as EEG, or by behavioral criteria. The latter includes sustained periods of quiescence occurring in a circadian pattern, reduced sensitivity to stimuli (increased arousal threshold), and homeostatic rebound following prolonged wakefulness [28]. Thus, characterization of sleep phenotypes in model organisms utilizes electrophysiological or behavioral (consolidated immobility associated with an increased arousal threshold) measures. Sleep is regulated by a homeostatic process (which reflects sleep need) and a circadian process (which reflects endogenous circadian time) [29, 30]. Here, we will mainly discuss the latter, but will first discuss the molecular and circuit basis of the core circadian clock.

Tick-Tock… Canonical Clocks Coordinating Rhythms

Forward genetic screens in animal models have identified canonical circadian clock genes which form a **transcriptional-translational feedback loop** (TTFL), turning over at a rate which approximates the solar day (~24hrs) [for review see 31]. The molecular basis of the core circadian oscillator and its basic functions are largely conserved from flies to humans (Fig. 1). In mammals, the basic TTFL comprises transcriptional activators (CLOCK/NPAS2 and BMAL1) that drive the expression of transcriptional repressors (PERIOD and CRYPTOCHROME), which then feedback to inhibit CLOCK/BMAL activity and suppress their own expression [32]. The oscillatory activity of these transcription factors and their regulators (clock genes, CGs) lead to the rhythmic expression of a large network of genes (clock-controlled genes, CCGs), which has been estimated to comprise up to 43% of the transcriptome across the entire body [33]. In this way, the molecular clocks found in almost every single cell in our bodies can locally coordinate oscillatory gene expression [34].

These cell-autonomous clocks throughout the brain and body are generally synchronized with each other and the external environment, which is achieved by coordination by a central pacemaker and integration with environmental cues [35, 36]. In fruit flies and mice, circadian clock neurons (~150 distributed neurons in flies and the densely packed suprachiasmatic nucleus in mice) receive environmental inputs ("**zeitgebers**") and set the pace of the **circadian rhythms** of the animal by directly or indirectly inducing release of secreted signals including neurotransmitters, neuropeptides, and hormones [37–40]. The crucial role of these circadian pacemaker neurons in sleep regulation is demonstrated by loss of cycling of sleep/wake behavior in flies and mice when these neurons are ablated [41–43]. Zebrafish also have a region in the hypothalamus containing several neuropeptide-expressing cell populations, similar to that seen in the mammalian SCN; however, it is not required for pineal rhythmicity in larval zebrafish and thus may not play a key circadian pacemaking role [44]. Instead, it may be that light serves as a master organizer of circadian clocks in zebrafish [45].

Mutations in Core Clock Genes Cause Human Circadian Rhythm Sleep Disorders

The genetic pathways by which the circadian clock regulates sleep has been addressed at both the levels of the CGs themselves, as well as CCGs. Work over the past two decades has shown that knockout of canonical CGs in mice and flies leads to marked and varying effects on sleep amount and timing [46–53]. However, perhaps the most compelling evidence for an important role for CGs in regulating sleep comes from the analysis of the genetic basis of human circadian rhythm sleep disorders, specifically Familial Advanced Sleep Phase Syndrome (FASPS) and more recently Delayed Sleep Phase Syndrome (DSPS). Over the past ~15 years, several studies have identified a number of clock genes (*Casein Kinase 1 delta/epsilon, Period2, Cryptochrome2, Period3*) that lead to FASPS [54–57], where affected individuals exhibit markedly earlier bedtimes and wake times, compared to the general population. On the other hand, DSPS is characterized by delayed sleep initiation and trouble awakening at an appropriate hour. The prevalence of DSPS in the general population is

significantly higher than that for ASPS, which raised the possibility that DSPS mainly results from voluntary behaviors (e.g, staying up at night with electronic devices or media). However, mutations in 2 clock genes, *Period3* [58] or *Cryptochrome1* [59], have recently been shown to cause this disorder.

In contrast to clock null mutants in animal models, which result in loss of rhythmic cycling of sleep behavior under constant conditions [49], the mutations causing ASPS and DSPS alter the phase of the consolidated sleep period of the individual. In addition, these mutations generally do not affect sleep quantity, although clinically, these disorders can restrict sleep amount because of forced wake-up times or bed times from social or work-related cues. At a molecular level, the ASPS mutations destabilize core clock genes. For example, the P415A/H417R variants of *Per3* induce more rapid degradation of the PER3 protein[57], which facilitates degradation of PER1 and PER2 proteins, shortening the cycle length of the core oscillator. Similarly, a shortened period was observed in human subjects and transgenic mice carrying the point mutation A260T in *Cry2* [56]. This genetic variant leads to enhanced affinity of CRY2 for FBXL3, a key ubiquitin ligase which targets CRY2 for degradation.

The recently identified 1657+3A>C *Cry1* variant [59] causing DSPS illustrates another way by which the clock machinery may be perturbed: by slowing down rather than speeding up the core oscillator. Instead of affecting stability, this variant, which results in the skipping of exon 11 and a truncation of the C-terminus, enhances the repressor activity of CRY. This significantly lengthens the timing of feedback onto the CLOCK:BMAL1 heterodimer, and hence lengthens the **circadian period**. Because humans with these ASPS and DSPS mutations have intact circadian **entrainment**, the changes in their period length do not lead to non-24 hour sleep/wake disorder, where their wake-up times free-run, shifting progressively each day until misaligned with the environment. Instead, external cues such as light serve to reset their phase, inducing a consistent advance or delay of their sleep time each day, making them appear as extreme ‘early birds’ or ‘night owls’.

Clock-Controlled Genes Regulate Sleep via Extracellular Signaling Pathways

Compared with our understanding of the molecular basis of the core circadian oscillator, significantly less is known about the genetic mechanisms by which the circadian clock regulates rhythmic expression of behaviors such as sleep. Starting with classical SCN transplantation studies in the 1990s, a prevailing model for how the SCN exerts controls over behaviors is via the release of diffusible factors [60, 61]. In addition, circadian pacemaker neurons in flies and mice also project, directly or indirectly, to structures that regulate sleep (e.g., the arousal-promoting pars intercerebralis in flies and the sleep-promoting ventrolateral preoptic nucleus in mice) [62–64]. In this section, we will focus on the former process and discuss how circadian clocks regulate sleep via release of diffusible factors. A few such factors like Prokineticin2 (Prok2), transforming growth factor-α, and cardiotrophin-like cytokine have been previously suggested to act as circadian output molecules that regulate sleep timing in mammals [65–71]. However, functional evidence in support of this notion is relatively sparse, and recent evidence from zebrafish suggests

instead that it acts to mediate light-dependent regulation of sleep [72]. Here, we discuss several recent studies that have re-examined the roles of melatonin and histamine, and identified a function for DH31 (a neuropeptide in *Drosophila*) in regulating the circadian timing of sleep.

Melatonin is required for circadian timing of sleep

The role of melatonin in sleep regulation has been somewhat controversial. Melatonin, the so-called “darkness hormone”, is released at night [73] in both diurnal and nocturnal animals [74] . Its synthesis in the pineal gland is gated by the circadian system in mammals and potently inhibited by light [75]. Although the timing of melatonin release is strongly linked to **circadian phase** [76, 77], via autonomic innervation by the SCN [78], studies in humans have generally found mild effects of exogenous melatonin administration on nighttime sleep behavior [79, 80]. Moreover, melatonin is not considered soporific in nocturnal rodents, and the most widely used mouse background for sleep/circadian studies, C57BL/6, lacks melatonin owing to loss of a key biochemical synthesis enzyme [81]. These observations raise the question of whether melatonin is dispensable for sleep regulation.

To address this issue, one study examined zebrafish larvae that lack melatonin due to a mutation in *arylalkylamine-N-acetyltransferase 2* (*aanat2*) [82]. *aanat2* mutants exhibit a significant reduction in night-time sleep, consistent with a sleep-promoting role for melatonin. Moreover, under constant darkness conditions, the normal rhythmic sleep behavior observed in wild-type zebrafish larvae is lost in *aanat2* mutants. This phenotype is reminiscent of loss of rhythmic sleep behavior observed with loss of core clock molecules [46, 48–49], suggesting that melatonin plays a key role in the circadian regulation of sleep behavior. How do we reconcile the divergent findings regarding melatonin and sleep from zebrafish, mice, and humans? The weak sleep-promoting effects of melatonin in humans may reflect reduced effectiveness of exogenous melatonin due to the presence of significant endogenous melatonin signaling at night. Indeed, when given during the subjective day (when endogenous levels are low), melatonin has a more robust sleep-promoting effect in both fish and humans [83, 84]. Melatonin may be dispensable for circadian regulation of sleep in mice, because they are nocturnal animals and tend to sleep during the day.

The DH31 neuropeptide promotes wakefulness at the end of the night

In *Drosophila*, a recent study has identified a neuropeptide that is secreted from clock neurons to specifically regulate sleep [85]. Diuretic hormone 31 (DH31) is the fly homolog of calcitonin-related peptide in vertebrates, and loss-of-function mutations in this gene result in increased late-night sleep, without affecting circadian locomotor rhythmicity. These data suggest that DH31 normally acts to promote wakefulness towards the end of the night. This phenotype depends upon the expression of DH31 in a subset of clock neurons in *Drosophila*, the DN1 subgroup, and functional imaging experiments reveal that the activity of the DN1 neurons peaks towards the end of the night. Together, these data support a role for DH31 as a clock output molecule of DN1 neurons that acts specifically in the late night to promote arousal, in preparation for morning awakening.

Histamine signaling is regulated by local clocks to rhythmically promote arousal

In addition to the secretion of signaling molecules under control of central pacemakers described above, the cycling of local cell-endogenous circadian clocks also tunes the signaling of independent circuits to amplify information received from central pacemaker cells. Histaminergic cells of the tuberomammillary nucleus (TMN) in mice promote arousal, firing rapidly after wake onset but quieting during sleep. Expression of histidine decarboxylase (HDC), the rate limiting enzyme for histamine biosynthesis, cycles within these cells in phase with their electrical activity. Interestingly, selectively abolishing the local core clock via conditional BMAL1 knockout in only these cells eliminates HDC cycling and results in increased histamine levels during the daytime rest period. These animals exhibit significantly decreased daytime NREM sleep, in addition to greater sleep fragmentation and increased transitions between REM and NREM sleep. Although whole-animal measures of circadian rhythmicity remained unaffected, the decrease in regular and recovery SWA induced learning and memory deficits [86]. Thus, in histaminergic TMN cells, local cycling of the circadian transcriptional translation feedback loop directly influences the biosynthetic pathway of an arousal promoting neurotransmitter, and in this manner regulates various aspects of sleep *in vivo*.

Clock-Controlled Genes Regulate Sleep By Intrinsic Modulation of Neuronal Excitability

Wide Awake and Fbxl4 coordinate rhythmic GABA sensitivity in clock neurons to regulate sleep

Another process by which the circadian clock modulates sleep is via tuning of electrical activity of sleep/wake circuits. In *Drosophila*, a subset of clock neurons has been shown to promote arousal (l-LNvs), and a clock output molecule, named WIDE AWAKE (WAKE) acts in these cells to regulate the timing of sleep onset [87]. WAKE levels cycle under circadian clock control in the l-LNvs, rising in the early night to increase the levels and membrane targeting of a $GABA_A$ receptor (RDL). This enhanced GABA sensitivity of the arousal-promoting l-LNvs at dusk inhibits spontaneous firing and evoked excitability of these neurons to facilitate the switch from wakefulness to sleep. The absence of WAKE (as seen in *wake* mutants) results in greater l-LNv firing at dusk and specifically prolongs sleep latency (time from lights out to first sleep bout) [87], similar to the phenotype observed in patients with sleep-onset insomnia. The clock-dependent cycling of l-LNv excitability in sleep regulation not only depends on WAKE, but also a specific E3 ubiquitin ligase (Fbxl4), whose expression is also CLOCK-dependent [88]. Fbxl4 acts in an opponent manner to WAKE, by rhythmically degrading RDL at dawn to promote arousal, with mutant flies exhibiting significantly increased sleep and reduced sleep onset latency [88]. Strikingly, there is a single homolog of WAKE in mice and humans that is correspondingly enriched in the suprachiasmatic nucleus in mice [87], suggesting that these mechanisms are conserved in mammals.

A Clock-regulated microRNA regulates sleep by tuning excitability of sleep/wake circuits

The core circadian oscillator modulates the expression of many downstream genes, by not only directly regulating gene transcription, but also indirectly via control of non-coding RNAs, including microRNAs (miRs) [89–91]. Recently, one of these miRs, miR-92a, was shown to regulate neural excitability and also sleep in *Drosophila* [92]. Levels of miR-92a in fly clock neurons are elevated at night, and, like WAKE, miR-92a acts to suppress neural excitability of these cells. In arousal-promoting dopaminergic neurons, overexpression and knockdown of miR-92a levels led to an increase and decrease in sleep-bout duration, respectively, exclusively during the daytime. Conversely, overexpression of miR-92a in a sleep-promoting circuit increased daytime sleep. miR-92a therefore is an example of a post-transcriptional mechanism by which the circadian clock tunes the excitability of sleep/wake circuits to regulate both sleep and arousal across 24 hours.

Concluding Remarks and Future Perspectives

Our review of recent literature updates the varied mechanisms by which circadian clocks regulate sleep: from actions of the central clock and local clocks on rhythmic release of secreted molecules to cyclical tuning of intrinsic neural excitability. Given the paucity of knowledge regarding clock output mechanisms, it is likely that many more such genes will be identified in the future. Why are so many different clock-dependent genetic mechanisms necessary for regulating sleep? Sleep has been proposed to serve multiple functions [93]. These different sleep-related processes likely require spatial and temporal segregation. Related to the latter point, recent work using next generation sequencing technologies [for review see 31] demonstrates that, by using a variety of genetic regulatory mechanisms, the transcriptional network governed by the circadian clock is able to temporally segregate distinct cellular functions across nearly any phase of the 24 hr cycle. This fine-grained temporal resolution potentially enables different clock output genes to regulate the timing of various sleep processes. As an example, in *Drosophila*, WAKE promotes sleep onset in the early night, while DH31 promotes arousal in the late night. Further work is needed to not only delineate the functions of sleep, but also how timing of specific sleep-related processes may support these functions.

Forward genetic approaches in model organisms have been a fruitful avenue to identify genes involved in circadian rhythms and sleep [94–97], and will likely continue to be so into the future. However, the circuit mechanisms by which master circadian clocks regulate outputs such as sleep remain poorly understood. Advances in optogenetic and chemogenetic interrogation, as well as whole-brain functional imaging, should accelerate progress in these areas. These studies are also being coupled with single-cell sequencing methods to further refine our understanding of the circuit mechanisms regulating sleep [98]. Delineation of these output circuits will in the future not only establish the neural basis for these behaviors, but will also be critical for understanding the precise functions of the clock-regulated genes that impact sleep.

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Glossary Box – Key sleep- and circadian-related terms

Circadian Clock/Core Oscillator
The central molecular mechanism, consisting of a transcriptional-translational feedback loop (defined below), by which animals endogenously keep time, and align their physiological and behavioral patterns to the 24-hour day

Transcriptional-Translational Feedback Loop (TTFL)
See Figure 1. Core clock transcription factors (CLOCK/NPAS2 and BMAL) drive expression of repressors (PERIOD and CRYPTOCHROME), which translocate back into the nucleus to inhibit the core clock transactivators, thus suppressing their own expression. In this manner, many genes regulated by these transcription factors are subject to ~24-hour oscillation in expression levels

Circadian Period
The length of time required to complete one full cycle of rhythmic molecular oscillations or behavior. Circadian periods ('circa' = about, 'diem' = day), occur with approximately 24-hour regular intervals

Circadian Phase
The phase of a circadian rhythm is defined relative to a reference point of the rhythm, such as the temperature nadir in mammals

Circadian Rhythms
Biological rhythms that oscillate with a ~24 hr period that are regulated by the core circadian clock. Examples include cycling of sleep/wake behavior, feeding behavior, core body temperature, and blood cortisol levels

Entrainment
The ability of the circadian clock to adjust its phase, based upon environmental cues, including light, temperature, and food availability

Zeitgeber
German for "time-giver." Environmental cues that adjust the phase of the circadian clock. Light is the most powerful zeitgeber, but food access, temperature, and social cues also serve to entrain the clock

Electroencephalography (EEG)
An assay that measures brain activity, in which voltage changes resulting from the synchronized firing of thousands of cortical neurons are detected at the scalp, amplified, and recorded. EEG is a commonly used method for measuring sleep in mammals, including humans

Non-REM (NREM) Sleep
A state of sleep defined by characteristic EEG waveforms with increasing cohesiveness and amplitude and reduced frequency. In deep NREM sleep, high amplitude delta waves (0.5–4

Hz) are prominent (Slow-Wave Activity, SWA), and their spectral power correlates with sleep need

Rapid Eye Movement (REM) Sleep

also known as 'Paradoxical Sleep,' REM is defined by low amplitude, mixed frequency EEG waveforms, similar to that seen in wakefulness, coupled with muscle atonia and characteristic horizontal eye movements. In humans, REM is the sleep sub-state associated with vivid dreams, but its specific function remains contested

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Highlights

- Work over the past few decades has led to detailed understanding of the genetic mechanisms underlying the core circadian clock, culminating in the Nobel Prize in Physiology or Medicine in 2017
- Much less is known about how this core clock regulates output behaviors, such as sleep
- Emerging data in fruit flies, zebrafish, mice, and humans reveal that central and local clocks use diverse mechanisms to regulate distinct aspects of sleep

Outstanding Questions Box

- What is the role of local clocks within specific sleep/wake circuits in sleep regulation? Do local clocks regulate transcriptional networks altering electrical activity, metabolism, or even sleep need in these neurons in a circuit-specific manner?
- Astrocytes express circadian clock genes from flies to mammals. Do local clocks in these cells impact cortical function and sleep need or sleep quality?
- Recent GWAS studies have identified human genomic loci associated with sleep and circadian rhythms phenotypes. Do these sequences contain new genes and/or non-coding RNAs which are relevant to the circadian regulation of sleep? Can model organisms help clarify the role of these molecules?
- Given the increasing recognition that disordered sleep and circadian rhythms may contribute to the development or progression of neurodegenerative diseases in humans, do alterations in the mechanisms mediating the circadian regulation of sleep play a role in diseases of aging in humans?

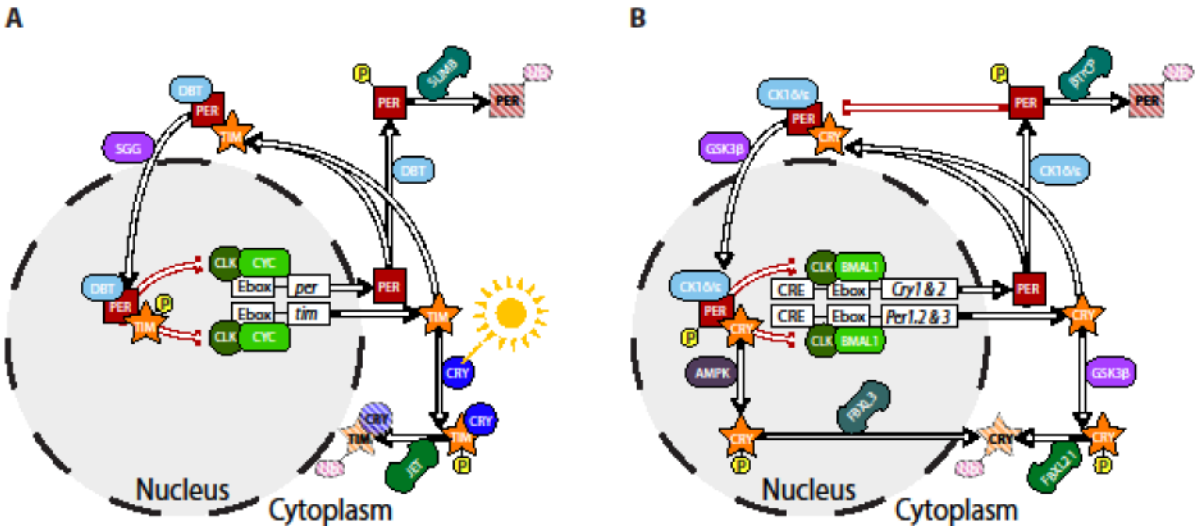

Figure 1. Conservation of canonical circadian clock from invertebrates (A) to vertebrates (B)

A) In a highly simplified model of the invertebrate circadian clock *Clock* and *cycle* genes drive transcription of *period* and *timeless* via enhancer boxes (Ebox) found upstream of the genes. PER and TIM proteins form heterodimers which translocate back to the nucleus to inhibit their own translation. While CRY-mediated ubiquitination and subsequent proteosomal degradation of TIM drives daily resetting by light, clock speed is controlled by the rate of degradation of PER proteins. B) Similar feedback and cycling occurs in mammals with the *Cryptochrome* genes substituting for *timeless* in the core oscillator. Cytosolic PER and CRY proteins are ubiquitinated by two separate mechanisms when not heterodimerized. PER is flagged for degradation by the *doubletime* orthologues CK1δ/ε leading to ubiquitination by βTrCP, while phase-resetting occurs via intracellular signaling pathways AMPK (Cry1) and GSK3β (Cry2) triggering FBXL3 ubiquitination and proteosomal degradation mediated intracellular signaling. In these figures, several accessory loops in both flies and mammals are omitted to focus on the balance of synthesis and degradation of clock proteins, which has been implicated in the circadian timing of sleep in humans. Indeed, all mammalian genes presented here, except *Clk* and *Bmal1*, have at least one allelic variant associated with a sleep-wake disorder. **Abbreviations:** AMPK - AMP-activated protein kinase; BMAL1 – *Brain and muscle arntl-like protein 1*; βTrCP – *β-transducin repeat-containing protein;* CK1δ/ε – *Casein kinase 1 delta/epsilon*; CLK – *Clock*; CRE – cAMP response element; CRY – *Cryptochrome*; CYC – *Cycle*; Ebox – promoter enhancer box; FBXL – *F-box and leucine rich repeat protein* ; GSK3β – *Glycogen synthase kinase-3*; PER – *Period*; SGG – *Shaggy*; TIM – *Timeless*; Ub – post-translational ubiquitination.

Table 1

Genes implicated in circadian regulation of sleep.

Gene	*Function*	*Manipulation*	*Organism*	*Phenotype*	*Refs*
Aanat2	Melatonin biosynthesis	Null	Fish	↓ Nighttime sleep (L:D), Arrythmic (D:D)	[82]
Bmal1	Transcription factor	KO	Mouse	↑ Total sleep	[28]
cycle	Transcription factor	Null	Fly	↑ Sleep latency, Locomotion	[52, 87]
βTrcp2	F-box WD40 repeat	KO	Mouse	Destabilized rest/activity timing	[99]
slimb	F-box WD40 repeat	Overexpression	Fly	↓ Rest/activity amplitude, ↑ % arrhythmic	[100]
CK1δ/ε	Kinase	*Ck1δ* T44A	Human	ASPS	[54]
		Ck1δ T44A	Mouse	Shortened period	[54]
		Ck1δ T44A	Fly	Lengthened period	[54]
		Ck1ε tau	Mouse	↑ REM sleep time	[101]
Clc	Cytokine	Infusion	Mouse	Suppressed locomotor activity	[71]
Cntfr	CLC receptor	Ab infusion	Mouse	↑ Daytime locomotion	[71]
Clock	Transcription factor	3111C allele	Human	“Night owl” tendency	[102]
		Antimorph	Mouse	↓ Total sleep time	[46]
		Null	Fly	↓ sleep, ↑ locomotion	[52, 87]
Npas2	Transcription factor	KO	Mouse	↓ Nighttime SWS+REM, ↓ Rebound sleep	[103, 47]
Cryptochrome	Transcriptional repressor	*Cry1* 1657+3A>C	Human	DSPS	[59]
		Cry2 A260T	Human	ASPS, ↑ *Cry* degradation by *Fbxl3*	[56]
		Cry1/2 KO	Mouse	↑ Homeostatic sleep drive	[50]
Dec2	Transcriptional repressor	P385R	Human	↓ Total sleep, ↓ NREM and REM sleep	[104]
		P385R	Mouse	↓ Total sleep, ↓ NREM and REM sleep	[104]
		Y362H	Human	↓ Baseline sleep, ↓ sleep	[105]
		Morpholino	Fish	↓ Sleep time, ↑ Sleep fragmentation	[106]
Sharp	Transcriptional repressor	*Sharp1/2* KO	Mouse	↑ Nighttime NREM, ↓ daytime REM	[107]
Dh31	Neuropeptide	Null	Fly	↑ Sleep, ↓ Arousal threshold	[85]

Gene	*Function*	*Manipulation*	*Organism*	*Phenotype*	*Refs*
		Overexpression	Fly	↓ Nighttime sleep	[85]
Fbxl	F-box leucine-rich repeat	*fbxl4Δ45* Null	Fly	↓ sleep latency, ↑ total sleep	[88]
miR-92a	Neural excitability	Overexpression	Fly	↑ Daytime sleep	[92]
		KO	Fly	↓ Daytime sleep	[92]
Period	Transcriptional repressor	*Per2* hypomorph	Human	ASPS	[55]
		Per3 haplotype	Human	DSPS	[58]
		Per3 P415A/H417R	Human	ASPS	[57]
		Per3 P415A/H417R	Mouse	Period lengthening in constant light	[57]
		Per3 P415A/H417R	Fly	Advanced activity offset, Shortened period	[57]
		Per1/2 KO	Mouse	↓ NREM sleep (L:D)	[49]
		Null	Fly	↓ Sleep	[87]
Prok2	Neuropeptide	Infusion	Mouse	↓ Rest/activity amplitude	[65]
		Overexpression	Fish	↓ Rest/activity amplitude	[72]
Prokr2	PRK2 Receptor	KO	Mouse	↓ Nighttime locomotion	[68]
Rev-ERB α/β	Transcriptional repressor	*Rev-ERBα* KO	Mouse	↓ Homeostatic sleep, Advanced sleep/wake	[53]
TGFα	Neuropeptide	Infusion	Mouse	↓ Locomotion	[69, 70]
Egfr	TGFα receptor	Null	Mouse	↑ Daytime activity	[69]
rho	EGF receptor	Receptor activation	Fly	↑ Sleep	[38]
wide awake	Neural excitability	Null	Fly	↑ Sleep latency, ↓ Total sleep	[87]