

Research Progress on the Molecular Pathways Regulating Mammalian Sleep–Wake Rhythms

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Abstract. The mammalian brain governs the timing of sleep and wakefulness with considerable precision, yet the mechanism underlying this timing is not a single switch. Work over the past two decades has uncovered a layered molecular architecture in which neurotransmitter systems, neuropeptide signaling, and circadian clock genes play distinct but interacting parts. Three pathways are examined here, and their coordination sustains the sleep–wake rhythm. Sleep onset falls to GABAergic inhibitory circuits organized around the ventrolateral preoptic nucleus (VLPO), a small hypothalamic cell group whose reciprocal inhibition of arousal nuclei has been modeled as a bistable switch. Wakefulness is held in place by orexin (hypocretin) neurons of the lateral hypothalamus, and the translation of this pathway into dual orexin receptor antagonists (DORAs)—suvorexant and daridorexant—has given insomnia pharmacotherapy a new drug class. Beneath both lies a cell-autonomous molecular clock built from CLOCK, BMAL1, PER and CRY, which generates endogenous rhythmicity through a transcription–translation feedback loop (TTFL); across mammalian species, free-running periods usually fall between 22 and 26 hours. Three advances have reshaped the field in the past five years. MARK2 has been identified as the kinase phosphorylating PER2 at S662—the long-sought FASP-site kinase. Single-cell transcriptomics has traced homeostatic sleep pressure to mitochondrial metabolism. Network meta-analysis across more than 5,000 randomized patients has confirmed DORA efficacy and safety.

Keywords: Sleep rhythm, GABAergic circuits, Orexin, Clock genes, Transcription–translation feedback loop

1. Introduction

Of the many physiological rhythms in mammals, the sleep–wake cycle is the most conspicuous and, arguably, the easiest to disrupt. Its maintenance depends on a dynamic balance between excitatory and inhibitory signaling within the central nervous system. When that balance falters, brain function suffers first; sustained dysregulation can culminate in insomnia, narcolepsy, metabolic syndrome, and even neurodegenerative disease. The study of sleep regulation therefore carries weight on both basic and translational grounds. For much of the twentieth century, however, methodology confined the field to behavioral observation and electroencephalography, and progress at the molecular level was slow.

Over roughly the past decade, optogenetics, chemogenetics, single-cell sequencing, and gene editing have pushed sleep research from behavior down to receptors, signaling pathways and gene oscillation. What has become clear is that no single pathway dominates mammalian sleep rhythms. Transmitter–receptor systems, neuropeptide pathways, and gene-expression networks cooperate across levels to sustain a balanced regulatory system [1]. Historically, the field has moved from neurotransmitter effects to specific circuits to endogenous gene-driven oscillation. GABAergic inhibitory circuits operate at the level of circuit execution; orexinergic arousal pathways at the level of state regulation; and clock-gene oscillation at the level of rhythm setting. These regulatory layers are not independent but interact dynamically to shape the timing and stability of mammalian sleep–wake states.

Despite considerable progress in identifying individual components of sleep regulation, the relationship between molecular timing systems, neural circuits, and physiological sleep–wake transitions remain incompletely understood. Existing reviews have often focused on specific regulatory mechanisms separately, leaving the integration of these different levels insufficiently explored. Therefore, this review aims to provide a hierarchical perspective on mammalian sleep–wake regulation by examining how molecular oscillations, neural circuitry, and recent therapeutic advances collectively contribute to the maintenance of sleep–wake rhythms. This review seeks to provide a comprehensive framework for understanding how these mechanisms cooperate to maintain sleep–wake rhythms and to highlight emerging directions for future research.

2. GABAergic circuits: the inhibitory executor of sleep onset

γ -Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter of the central nervous system. Its two receptor families—ionotropic GABA_A, which gates chloride influx and hyperpolarizes the membrane, and metabotropic GABA_B, which acts through Gi/o proteins for slower inhibition—together supply the molecular substrate for sleep generation [2].

An earlier view held that GABA suppressed sleep through broad, whole-brain inhibition. Modern neuroanatomy and functional studies have overturned that picture. The ventrolateral preoptic nucleus (VLPO) of the hypothalamus is now recognized as the key nucleus for sleep initiation. It is densely populated by GABAergic neurons, most of which co-express galanin, and their firing rate climbs sharply during non-rapid-eye-movement (NREM) sleep [3]. Their axons project specifically to multiple wake-promoting nuclei—the tuberomammillary nucleus (TMN, histaminergic), the locus coeruleus (LC, noradrenergic), and the dorsal raphe (serotonergic)—releasing GABA to suppress arousal signals. Using rabies-virus-mediated tracing, Prokofeva and co-workers reported in 2023 that VLPO GABAergic neurons also project directly to the lateral hypothalamic area (LHA), forming synapses with orexin neurons [3]. Unexpectedly, optogenetic activation of the VLPO→LHA GABAergic projection induced arousal rather than sleep, pointing to functionally distinct subpopulations within the VLPO.

The reciprocal inhibition between the VLPO and monoaminergic arousal nuclei is the basis of the widely cited "sleep–wake switch" model. During wakefulness, monoaminergic neurons inhibit the VLPO; at sleep onset, the activated VLPO inhibits these arousal centers. The arrangement is bistable and seesaw-like—once it tips toward one state, it resists being pulled back. In 2020, Cheng and colleagues microinjected a GABA_A antagonist and a histamine H1 inhibitor into the VLPO and TMN, respectively. Their results confirmed that VLPO GABAergic neurons inhibit TMN histaminergic neurons via GABA_A receptors to induce sleep, while the activated TMN reciprocally inhibits the VLPO via H1 (HRH1) receptors to maintain wakefulness [4]. GABA thus acts through highly specific circuit inhibition, not indiscriminate global suppression. A 2026 review by Wang and

co-workers goes further, arguing that the VLPO is far more than a simple switch: it integrates diverse transmitters, neuropeptides, and glial signals, functioning as a multisystem integrator whose neuronal subpopulations differ substantially in molecular identity and circuit engagement [5]. We are inclined toward this broader view. The bistable-switch model remains a useful first approximation, but the accumulating evidence for molecularly distinct VLPO subpopulations—with different projection targets and different neuromodulatory responses—makes it hard to defend as a complete account.

The GABAergic circuit is the key executor of sleep initiation and maintenance, yet it does not itself decide when to fall asleep. VLPO activity is governed both by upstream circadian signals and by homeostatic sleep pressure. Adenosine illustrates the point. During wakefulness, astrocytes continuously release ATP, which tissue-nonspecific alkaline phosphatase (TNAP) hydrolyzes to adenosine. As Bellier and colleagues reviewed in 2025, astrocytes play multiple roles in sleep regulation [6]. Adenosine activates sleep-promoting VLPO neurons via A2A receptors and, at the same time, inhibits local interneurons via A1 receptors; the two actions together raise sleep drive. This astrocyte–adenosine–VLPO pathway links glial signaling, homeostatic pressure, and circuit execution, showing that sleep initiation is embedded within a broader signaling network rather than operating in isolation [6]. The GABAergic circuit alone cannot account for the timing of sleep, and higher-level regulation must be invoked.

3. The orexinergic pathway: targeted control of sleep-wake transitions

Active maintenance of wakefulness relies chiefly on another hypothalamic peptide system—the orexin (hypocretin) pathway. Orexin neurons of the lateral hypothalamus release two isoforms, orexin-A and orexin-B, acting on Gq-coupled receptors (OX1R, OX2R) whose distribution is markedly directional: OX1R is enriched in the LC and OX2R in the TMN, both monoaminergic arousal centers [7].

Loss- and gain-of-function evidence converges on the same conclusion: orexin is both necessary and sufficient for arousal. Knockout of the orexin gene, or ablation of orexin neurons, produces a narcolepsy-like phenotype in mice; a natural mutation of the canine OX2R gene does the same in dogs [7]. Optogenetic activation of lateral hypothalamic orexin neurons during the inactive phase wakes animals from NREM or REM sleep within seconds, while chemogenetic inhibition prolongs sleep [8]. Melanin-concentrating hormone (MCH) neurons, which share the lateral hypothalamus, mainly drive the NREM-to-REM transition and counterbalance orexin neurons to sustain the sleep–wake cycle [8].

What sets orexin apart from the GABAergic circuit is the level at which it operates. Orexin neurons do not directly innervate the core sleep-promoting neurons of the VLPO. They first activate GABAergic interneurons within the VLPO, and those interneurons in turn inhibit the GABA/galanin-co-expressing sleep-promoting cells, keeping the sleep circuit suppressed while wakefulness is required [8]. State switching can therefore be achieved efficiently, without compromising motor or sensory function. This indirect mode of control is precisely what made the pathway attractive for drug development. Dual orexin receptor antagonists (DORAs)—suvorexant, lemborexant, and daridorexant—have each received FDA approval. The 2025 network meta-analysis by Kishi and colleagues, pooling eight randomized controlled trials and 5,198 patients, found all three DORAs superior to placebo in shortening sleep-onset latency, increasing total sleep time, and improving sleep maintenance, with no physiological tolerance or rebound insomnia [9]. In our view, the orexin–DORA arc is the cleanest example of translational success in sleep biology. Selective OX2R agonists for narcolepsy and partial antagonists with reduced next-day somnolence are already

moving through clinical development, and the pathway will likely remain the most active front in sleep pharmacology for some years.

4. The clock-gene loop: molecular timing of the endogenous circadian rhythm

The deepest phase information for the sleep–wake rhythm comes from the molecular oscillation of intracellular clock genes. The field traces back to 1971, when Benzer's laboratory identified the first clock gene, *Period*, in *Drosophila*. Subsequent cloning of core genes such as *Clock*, *Bmal1*, and *Cryptochrome* established that circadian rhythms are driven by a cell-autonomous transcription–translation feedback loop (TTFL). Hall, Rosbash, and Young received the 2017 Nobel Prize in Physiology or Medicine for elucidating this mechanism.

The core TTFL works as follows. In the nucleus, CLOCK and BMAL1 heterodimerize through their bHLH-PAS domains and bind E-box enhancer elements in the promoters of *Per* and *Cry* to drive transcription [1]. *Per* and *Cry* mRNAs are translated in the cytoplasm into PER and CRY proteins, which accumulate and assemble into multimeric complexes. Post-translational modification supplies the precise temporal control, and phosphorylation of PER by casein kinase I δ/ϵ (CK1 δ/ϵ) is especially critical. CK1 δ/ϵ mainly phosphorylates the phosphodegron region at S478 of PER2, promoting its ubiquitin-dependent degradation and shortening the circadian period.

We can think of PER2 phosphorylation as a molecular dial that sets the duration of the circadian cycle. Two regions on the protein pull in opposite directions, and the balance between them is what researchers now call the phosphoswitch. One of these regions, known as FASP, begins at serine 662. When S662 is phosphorylated, PER2 becomes more stable and the circadian period lengthens. The other region—the phosphodegron—sits at S478, and its phosphorylation has the opposite consequence: it recruits the E3 ubiquitin ligase β -TrCP, which tags PER2 for degradation and thereby shortens the period [10, 11].

The two sites, though both on PER2, are not phosphorylated by the same enzyme, and identifying the responsible kinases became a central problem in the field. Work over several years established that CK1 δ/ϵ acts preferentially at the phosphodegron (S478), while a different kinase, MARK2, acts at the FASP site (S662). The two enzymes are not interchangeable; removing one cannot be compensated for by increasing the activity of the other [10, 11]. The net period length thus reflects the balance of phosphorylation at these two opposing sites.

Once the PER/CRY complex reaches a threshold concentration, it enters the nucleus, binds CLOCK/BMAL1, and silences its own transcription. Degradation of the complex then lifts this inhibition, and the cycle restarts. A single turn takes about 24 hours, although when animals are housed in constant darkness and freed from external cues, their free-running periods drift across a range of roughly 22 to 26 hours depending on species [1]. The TTFL does not exhaust the clock mechanism: non-TTFL components such as RUVBL2 point to a more ancient evolutionary layer of circadian regulation that predates the transcriptional loop [1].

An accessory loop involving the nuclear receptors REV-ERB α and ROR α adds stability to the core oscillator. CLOCK/BMAL1 drives REV-ERB α expression, and REV-ERB α in turn binds the *Bmal1* promoter to repress BMAL1 transcription; ROR α competes for the same response elements but activates *Bmal1* instead. The resulting push–pull between REV-ERB α and ROR α superimposes a circadian oscillation on BMAL1 itself and sharpens the precision of the core loop.

Genetic evidence strongly supports the conclusion that this molecular oscillator determines the phase of the sleep–wake rhythm. Families with familial advanced sleep-phase syndrome (FASP) carry an hPER2 S662G missense mutation: the FASP site cannot be phosphorylated, PER2 is degraded faster, and the behavioral period contracts to about 22 hours [10].

The reverse experiment came in 2020, when Masuda and colleagues generated PER2-S478A knock-in mice. Mutating the phosphodegron site stabilized PER2 and lengthened the behavioral period, validating the phosphoswitch model in a living animal [10]. Clock-mutant mice show a progressive lengthening of their free-running period and eventually lose rhythmicity altogether; whole-body Bmal1-knockout mice lose essentially every physiological rhythm they are tested for [1]. Molecular oscillation of the gene loop, in short, directly specifies the phase and period of the sleep–wake cycle.

Until recently, however, the identity of the kinase responsible for S662 phosphorylation in vivo remained unknown. In 2026, the Rao Yi group addressed it using a biochemical purification strategy in HEK293 cells and identified MARK2, a member of the AMPK-related kinase family, as the responsible enzyme [12]. MARK2 phosphorylates S662 directly and delays PER2 degradation. Knocking out MARK2 shortens the circadian period, and this effect depends on S662 being intact. Neuron-specific Mark2-knockout mice show advanced activity onset and a shortened period—a phenotype that closely mirrors human FASP. The result names the FASP-site kinase. We suspect, however, that MARK2 will not tell the whole story—post-translational regulation of PER2 is highly intricate, and the possibility that other AMPK-related kinases act redundantly or in a tissue-specific manner at S662 cannot yet be excluded. Even so, MARK2 is now the most concrete entry point for pharmacological modulation of circadian period.

The 24-hour oscillation described above is not a property of isolated cells alone. In the hypothalamus, the suprachiasmatic nucleus (SCN) serves as the mammalian master clock, and its neuronal population is driven into synchronized oscillation by the same gene loop. The SCN has a refined internal division of labor. Core-region VIP neurons pick up photic input from the retinohypothalamic tract and mediate both photic entrainment and interneuronal synchronization; shell-region AVP neurons are more concerned with holding the circadian period to its precise length [13]. Rhythm information leaves the SCN through neural projections and humoral signals—among them the cortisol rhythm and melatonin secretion—and reaches downstream sleep–wake centers such as the VLPO and orexin neurons. That is the route by which gene oscillation is converted into a physiological rhythm.

5. Multi-level integration: from molecular oscillation to sleep–wake transitions

The three pathways described above do not operate in isolation. The clock-gene loop sits most upstream: its molecular oscillation sets the phase of the endogenous rhythm and determines when the sleep window opens. Orexin occupies the middle position, receiving circadian signals from the clock on one side and homeostatic sleep-pressure signals on the other, and regulating how stably wakefulness is maintained. The GABAergic circuit is the most downstream element, initiating and maintaining sleep through reciprocal inhibition between the VLPO and arousal nuclei. A fault at any of these nodes destabilizes the rhythm.

The SCN sits at the core of this architecture [13]. Through indirect polysynaptic projections and humoral signals, it entrains the circadian activity of the VLPO and orexin neurons, so that sleep- and wake-promoting signals peak at opposite phases. Homeostatic sleep pressure, marked by adenosine, accumulates with time awake and, via A2A receptors, independently potentiates VLPO activity [6], jointly determining sleep timing with the circadian system. In 2025, Samataro and colleagues reported in *Nature* that sleep pressure could be traced to the mitochondrial level using single-cell transcriptomics [14]. After sleep deprivation, transcriptomic changes in sleep-control neurons clustered almost entirely around mitochondrial respiration and ATP synthesis. The mitochondria themselves were remodeled—fragmented, with enhanced mitophagy and more mitochondria–ER

contacts. When mitochondria were excessively fused, neuronal excitability dropped and sleep followed; fragmentation did the reverse. Samataro's group argues that sleep may be an inescapable consequence of aerobic metabolism. We are not yet convinced that the mitochondrial hypothesis, as stated, closes the question of sleep pressure—the evidence is correlational in important respects—but it is the most provocative lead in years. Coupling of this kind, among distinct systems, is what allows sleep–wake behavior to obey endogenous rhythms while still responding flexibly to immediate physiological demands.

From a multi-level perspective, the etiology of sleep disorders is rarely confined to a single level. Insomnia may arise from gene polymorphisms in rhythm setting, from hyperactivity of the arousal pathway, from insufficient GABAergic function, or from faulty coupling between levels. A 2025 review by Ren and colleagues summarized the associations of clock-gene polymorphisms such as PER3 VNTR and CLOCK 3111T/C with individual differences in chronotype, and noted that these variants may induce insomnia by affecting the rhythmic expression of the melatonin, serotonin and GABA systems [15]. Precise intervention, accordingly, requires level-specific treatment: melatonin-receptor agonists to advance phase, REV-ERB ligands to enhance rhythm amplitude, and DORAs to suppress the overactive arousal pathway [15].

6. Conclusion

Research on the molecular mechanisms of sleep regulation has traced a trajectory from single transmitters to multi-level networks. The GABAergic circuit answers the question of sleep-onset execution; the orexin pathway offers a targeted means of regulating the arousal state; and the clock-gene loop reveals, at the genetic level, the essence of the endogenous rhythm. We would argue that the field's center of gravity has shifted, over the past five years, toward the clock-gene layer—MARK2, the phosphoswitch, and the mitochondrial origin of sleep pressure have all changed what counts as a "sleep molecule." DORA drug development along the orexin pathway, for its part, stands as the rare completed translation from basic research to clinical application [9].

For future research, some dialogue between the GABAergic and orexin pathways is already visible—direct VLPO GABAergic projections to orexin neurons [3], and indirect orexinergic inhibition of the VLPO [8]. How the clock-gene loop "commands" these two pathways, however, is still explained mainly through indirect SCN projections and humoral signals [13], and the intermediate steps remain murky.

The identification of MARK2 as the physiological kinase for PER2 [12] raises an intriguing possibility: post-translational modification of clock genes may also operate in extra-SCN brain regions, rather than merely setting the global SCN rhythm. Put differently, each brain region may possess its own local clock, one that does not passively receive SCN commands but actively tunes its sensitivity to sleep–wake signals. How these local clocks cooperate or compete with the central clock is, in our view, the single most important open question in sleep-rhythm biology. It is also the question most likely to require tools we do not yet have—region-specific genetic perturbation of clock components with minute-scale temporal resolution, in behaving animals.

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