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Genetic Insights on Sleep Schedules: This Time, It's *PER*sonal

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Abstract

The study of circadian rhythms is emerging as a fruitful opportunity for understanding cellular mechanisms that govern human physiology and behavior, as fueled by evidence directly linking sleep disorders and genetic mutations affecting circadian molecular pathways. Familial Advanced Sleep Phase Disorder (FASPD) is the first recognized Mendelian circadian rhythm trait, and affected individuals exhibit exceptionally early sleep-wake onset due to altered post-translational regulation of period homolog 2 (PER2). Behavioral and cellular circadian rhythms are analogously affected, as the circadian period length of behavior is reduced in the absence of environmental time cues, and cycle duration of the molecular clock is likewise shortened. In light of these findings, we review the PER2 dynamics in the context of circadian regulation to reveal the mechanism of sleep schedule modulation. Understanding PER2 regulation and functionality may shed new light on how our genetic composition can influence our sleep-wake behaviors.

Keywords

circadian rhythm; post-translational regulation; familial advanced sleep phase disorder; phosphorylation; period homolog 2; casein kinase 1 delta

Genetically hardwired configurations for sleep-wake timing

"I can't help it—it's genetic!" remains a common exclamation, but is our behavior truly etched in our DNA? Although the current view implicates a combination of genetic and environmental factors, an enduring question is how genes alone can regulate innate behavior necessary for survival. This is particularly relevant when considering the evolutionarily advantages for organisms to anticipate and adapt to environmental changes, such as those that fluctuate approximately every 24 hours like the amount of sunlight, temperature, nutritional availability, and the level of predation. For example, organisms optimize certain behaviors, such as sleep, to occur at designated times. Therefore, keeping track of time through circadian rhythm ensures the execution of behaviors appropriate for the environment, which may ultimately enhance evolutionary fitness [1].

Pioneering work on understanding the regulation of circadian rhythm relied on model organisms due to the complexity of human behavior. Konopka and Benzer identified *Drosophila melanogaster* with various circadian timing defects in locomotor activity, and

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these phenotypes were attributed to mutant alleles of the same gene, appropriately named *period (per)* [2]. These studies demonstrated the genetic basis of circadian behaviors, which opened the floodgate for the study of analogous circadian genes in mammalian model systems [3]. However, are human circadian behaviors also genetically "hard-wired ?"

While humans are by nature diurnal, a wide spectrum of sleep-wake time preferences exists throughout the general population, ranging from "morning larks" to "night owls" [4]. With the advent of high-throughput genotyping technologies and the identification of conserved circadian genes, mounting evidence now supports the role of genetics in regulating sleep timing [5]. Nevertheless, the identification of causative genes remains challenging due to complex polygenic interactions, confounding environmental factors, and a lack of human-relevant experimental models. To overcome these difficulties, families with Mendelian sleep traits were identified [6]. FASPD (previously known as FASPS), the first recognized Mendelian circadian rhythm phenotype, is autosomal dominant [7–10]. Individuals affected by FASPD wake up well before sunrise and feel compelled to sleep at around 7:30PM [9]. This timeframe is far more advanced compared to a majority of the population (Figure 1). Therapeutic attempts at delaying sleep are usually unsuccessful, illustrating the ability of genetics to "hardwire" human circadian rhythmicity [11]. Three causative genetic variants have so far been identified [12,13, Brennan, K.C., Bates, E.A., Shapiro, R.E., Zyuzin, J., Hallows, W.C., Lee, H.-Y., Jones, C.R., Fu, Y.-H., Charles, A.C., Ptá ek, L.J. (2012) Casein kinase 1 delta mutations in familial migraine and advanced sleep phase syndrome. *Manuscript under revision*]. These mutations affect the phosphorylation of PER2, highlighting the role of post-translational modifications in regulating human circadian rhythms. Here we review PER2 dynamics and functionality as a model for understanding molecular mechanisms of circadian behavior. As there are nuanced differences between species (Box 1), we will focus on evidence from mammalian model systems.

Box 1

Are cellular mechanisms driving circadian rhythm conserved across species?

The ability to maintain circadian rhythm is highly conserved, and recent discoveries have unveiled analogous clock mechanisms in species from diverse kingdoms. For most species, cellular circadian rhythm is typified by core transcriptional-translational feedback loops stabilized by interlocking loops, and the molecular substrates often share homologous domains. However, notable exceptions do apply—for example, cyanobacteria and unicellular algae maintain circadian rhythm by post-translational regulation without *de novo* synthesis of clock components [76,77]. Remarkably, human red blood cells, which do not have nuclei or perform transcription, undergo rhythmic 24-hour redox cycles [78]. It will not be surprising to find a future paradigm shift towards non-transcriptional-translational mechanisms as more "exceptional" cases continue to be discovered.

Nevertheless, there is a great deal of conservation, and mammalian PER2 was initially discovered through a search for homologous cDNA sequences to PER1, although it bears a greater resemblance to *Drosophila* PER than its paralogs [60,62]. However, there are also notable differences amongst species. For example, mammalian CRY proteins replaced *Drosophila* TIM as a binding partner for PER1/2 [12,79]. In particular, even homologous proteins may function in a different way in higher organisms. For instance, even though transgenic mice carrying an FASPD mutation in CSNK1D recapitulate the phenotype of human subjects, the opposite phenotype was observed in *Drosophila* [10].

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Therefore, it is important to keep in mind the possibility of divergent mechanisms and exercise prudence when comparing findings from different model organisms.

The ins and outs, highs and lows: intracellular dynamics of PER2

Approximately 10–15% of gene transcripts oscillate in a circadian manner, the identity of which vary according to cell type [12]. These oscillations are cell-intrinsic and driven by a core set of transcription factors [12,13], consisting of period homolog 1 (PER1) and PER2, cryptochrome 1 and 2 (CRY1 and CRY2), aryl hydrocarbon receptor nuclear translocator-like (ARNTL, also known as BMAL1) and clock homolog (CLOCK) (Figure 2, inner circle). BMAL1 and CLOCK [or its paralog, neuronal PAS domain protein 2 (NPAS2)] bind to E-box promoter elements of *PER1/2*, *CRY1/2*, and other circadian regulated genes in the nucleus and activate their transcription. This leads to an increase in the levels of PER1/2 and CRY1/2, which subsequently repress BMAL1/CLOCK, leading to the termination of their own transcription. Afterwards, PER1/2 and CRY1/2 are degraded through proteosomal pathways, and the transcription of *PER1/2* and *CRY1/2* are once again activated. This transcriptional/translational negative feedback loop takes approximately 24 hours to complete, and additional negative feedback loops (also known as interlocking loops) of other circadian regulators such as RAR-related orphan receptor A (RORA), nuclear receptor subfamily 1, group D, member 1 (NR1D1, also known as REV-ERBA), basic helix-loop-helix family, members e40 and e41 (BHLHE40/41, also known as DEC1/2), D site of albumin promoter binding protein (DBP), and nuclear factor, interleukin 3 regulated (NFIL3, also known as E4BP4) act to either activate or repress core clock components in a circadian manner [14]. Together, these components provide redundant and additional modes of regulation, reflected by the robustness and the complexity of the molecular clock.

Although numerous molecular components coordinate to drive circadian oscillations, precise rhythmicity of PER2 stands out as a critical feature of the core clock [15,16]. The coordination of circadian output by PER2 is partially mediated through interaction with nuclear receptors including peroxisome proliferator-activated receptor alpha (PPARA) and REV-ERBA [17]. Through a series of overexpression experiments *in vitro* and *in vivo*, it was shown in mice that PER2 oscillation, rather than its absolute quantity, is critical for both cellular circadian oscillations and behavioral rhythmicity [15]. To understand how this oscillation is regulated, we outline the progression of events responsible for the production, nucleocytoplasmic shuttling, and subsequent repression and degradation of PER2. Together, the timing of these events provides the cadence necessary for the circadian expression of PER2, which in turn contributes to the circadian rhythmicity of cellular processes and behavior.

From the beginning of time: transcriptional regulation of PER2

The molecular core clock is defined as a negative feedback mechanism whereby a protein is transcribed, translated, and represses its own transcription over approximately 24 hours. As these processes occur continuously it would be inaccurate to definitively state when the cycle starts, but for the sake of clarity we begin by discussing *PER2* transcription (Figure 2, outer circle). Human *PER2* resides on Chr2q37.3, and mouse *Per2* is found on Chr1qD. These genes contain 23 exons, and their encoded proteins are homologous to *Drosophila* PER [16]. Like other core clock genes, *PER2* is regulated by a combination of enhancer elements including E Boxes (recognized by BMAL1/CLOCK) and D Boxes (recognized by DBP/E4BP4) [14]. These transcriptional binding elements work in tandem to drive circadian oscillations of *PER2* and other core clock components. However, these transcription factors are themselves regulated by PER2 and other core clock components, which introduces further complexity to the circadian transcriptional machinery due to implicated

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dependencies between these circadian pathways. Moreover, transcriptional regulation is intimately tied to epigenetic mechanisms that activate and silence genes in a circadian context, as discussed in the following section.

(Un)winding the clock: epigenetic regulation of circadian rhythms

The pervasive role of epigenetics is now widely recognized across biological fields of study, and circadian rhythmicity is no exception. Early evidence demonstrated the rhythmic acetylation of histone 3 and binding of RNA polymerase II at the promoter regions of *Per2* along with *Per1* and *Cry1*, suggesting that circadian rhythms are also epigenetically regulated [18]. In agreement with these findings, recent evidence reveals lysine (K)-specific demethylase 5A (KDM1A, also known as JARID1A) as a novel binding partner of BMAL1/CLOCK. Reduction of KDM5A shortens the circadian period of *Per2* transcription *in vitro*, and this change is attributed to the activation of *Per2* transcription by KDM5A through inhibition of histone deacetylase 1 (HDAC1) activity [19]. Hence, post-translational modifications of histone proteins such as acetylation, methylation, SUMOylation, and poly(ADP)-ribosylation may likewise affect the transcription of core clock components such as *Per2*. As core clock proteins are also directly post-translationally modified, future research should strive to distinguish between direct effects on protein function versus indirect effects mediated by epigenetic mechanisms in regulating circadian rhythms [20]. Additionally, there may be links between histone modifications and metabolic regulation of the circadian clock, as reviewed by others [21].

Around the cell in one day: PER2 moves from the nucleus to the cytoplasm and back

After *PER2* is transcribed and translated, PER2 is thought to accumulate in the cytoplasm. However, PER2 is shuttled between the cytoplasm and the nucleus through a number of regulatory mechanisms. Firstly, PER2 contains a putative cytoplasmic localization domain (CLD) with 43% homology to *Drosophila* PER. However, this is not sufficient for cytoplasmic retention of PER2, suggesting the presence of other bona fide CLDs and/or the overriding importance of other mechanisms in higher organisms [22]. Similarly, the presence of putative nuclear export sequences (NES) flanking the CLD allows PER2 to be transported into the cytoplasm via an exportin-mediated nuclear export system [22–24]. These NES seem to be redundant, as the removal of all three is necessary to retain PER2 in the nucleus [22]. Directing PER2 in the opposite direction, nuclear localization sequences (NLS) are sufficient for nuclear entry [25]. In addition, these regulatory sequences act in concert with chaperone proteins, including CRY1/2 in mammals, for translocating PER2 [12,26]. Interestingly, PER2 localization in the nucleus is unimpaired in CRY1/2 knockout cells [22,27,28], and recent evidence proposes that CRY1/2 may instead mitigate PER2 degradation in addition to its collaborative function in repressing BMAL1/CLOCK [22,29]. Taken together, these observations suggest additional chaperone proteins or other mechanisms for PER2 translocation, the identification of which will be an important step toward understanding cellular circadian oscillations. One candidate is promyelocytic leukemia protein (PML), as PER2 is found primarily in the cytoplasm or around the nucleus in the absence of PML, and PML knockout mice exhibit defective circadian wheel-running behavior [26]. However, the effect of PML on BMAL1/CLOCK transcription is paradoxical, as it enhances, rather than represses, *Per2* transcription [26]. Therefore, the exact mechanism of PML regulation of PER2 still needs to be investigated. Overall, nucleocytoplasmic shuttling of PER2 appears to rely on regulatory mechanisms working together to shift the equilibrium of transport in and out of the nucleus. Further research is necessary to clarify how PER2 translocation is integrated with other circadian regulatory events.

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Turning in for the night: PER2 repressor activity and proteosomal degradation

As previously mentioned, PER1/2 repress their own transcription by inhibiting BMAL1/CLOCK. This process involves the recruitment of numerous protein complexes. For example, NONO, an RNA- and DNA-binding protein that may be involved in RNA export and splicing in addition to transcriptional repression, binds to PER1/2, and its reduction results in dampened circadian rhythms in mammalian cells [30]. Moreover, MYBBP1A, a transcription co-factor with potential roles in histone regulation and the recruitment of other transcription factors, is proposed to complex with CRY1, which also binds to PER1/2 to co-repress transcription [31]. Further emphasizing the co-dependence between epigenetics and transcriptional regulation, histone modifiers have been shown to participate in PER-mediated transcriptional repression as well. Polypyrimidine tract-binding protein-associated splicing factor (SFPQ, also known as PSF) and SIN3A, a scaffold for transcription inhibitory complexes, interact with PER1 to drive rhythmic recruitment of HDAC1 and subsequent deacetylation of histones 3 and 4 at the *Per1* promoter, suggesting that the same mechanism may be true for PER2, which also complexes with PSF and SIN3A [32]. In addition, WDR5, a member of a histone methytransferase complex, binds to PER1/2, and knock down of WDR5 reduces histone methylation at PER1-regulated promoters, implicating it in circadian regulation [30]. However, reduction of WDR5 does not affect the circadian period of luciferase driven by circadian reporter genes [30], indicating additional compensatory mechanisms such as histone acetylation or other methods of methylation may be in place to ensure accurate circadian periodicity. Together, this evidence demonstrates the complexity and the resulting robustness of transcriptional negative feedback and the ubiquitous involvement of epigenetics in circadian regulation.

After binding and repressing its own transcription, PER2 is degraded through an ubiquitin-dependent proteosomal pathway. Protein degradation is crucial for maintaining circadian period, because proteasome inhibitors can affect period length [33]. As mentioned above, PER2 degradation may be prevented by binding to CRY1/2 [22]. The stability of PER2 is also dependent on its acetylation, which occurs once it binds to BMAL1/CLOCK [34]. Sirtuin 1 (SIRT1) then binds to BMAL1/CLOCK and deacetylates PER2, promoting its degradation [35,36]. The degradation process requires specific targeting via ubiquitylation. FBXL3, a member of the SCF-containing ubiquitin ligase complex, is important for CRY degradation, which in turn regulates circadian period *in vitro* and *in vivo* [37–39]. Similarly, beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC) may play a corresponding role in the timely degradation of PER2, as deleting the BTRC-interacting domain of PER2 results in its stabilization [40,41]. In addition to proteosomal targeting, inhibiting nuclear export indirectly stabilizes PER2, suggesting that degradation occurs after PER2 is exported back into the cytoplasm [29]. Additional coupling mechanisms, such as those linking protein translocation and degradation, may be required to maintain circadian rhythm. Moreover, PER2 intracellular dynamics are further regulated by post-translational mechanisms, and they reveal the substantial influence of genetic mutations on circadian rhythmicity of cells and behavior, as discussed in the following section.

Using human genetics to decode post-translational circadian regulation

While model systems have revealed the molecular substrates of circadian rhythmicity, one of the most salient questions is whether these findings apply to human circadian behavior. Serendipitously, the first recognized human Mendelian circadian rhythm genetic variant was a mutation in *PER2* at position 662 from serine to glycine [9]. Transgenic mice carrying this human mutation phenotypically recapitulate FASPD, including both the advanced activity phase onset and the shortening of behavioral and cellular circadian period length [28] (Figure 3). The latter characteristic is attributed to enhanced repressor activity, as mRNA levels of endogenous mouse *Per2* and introduced human *PER2* both decrease, suggesting

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enhanced promiscuous transcriptional repression [28]. This is caused by a disruption of phosphorylation at S662, which normally results in a phosphorylation cascade of four downstream serine sites and weakens transcriptional repression [28]. Conversely, mutating the S662 to aspartic acid (which mimics constitutive phosphorylation) results in lengthened circadian period and decreased repression (increased PER2 expression) [28]. The enhancement of repressor activity through PER2-S662G would lead to decreased expression levels, although it is also possible that the changes in PER2 phosphorylation result in deficient nuclear retention, promoting PER2 degradation in the cytoplasm [29,42]. Likewise, studies using phospho-specific antibodies demonstrate that PER2-S662 phosphorylation stabilizes protein half life [42].

Together, these results suggest the involvement of specific kinases and phosphatases in regulating circadian pathways. Indeed, casein kinase 1 delta (CSNK1D) is involved in regulating transcription repressor activity and degradation of PER2 through separate phosphorylation events. *Csnk1d* heterozygosity counteracts the shortened circadian period observed in PER2-S662G mice; the increase in repressor activity is maintained by the PER2 mutation, but there is less degradation of PER2, which acts to correct the shortened period [28]. Consistent with these findings, pharmacological inhibition of CSNK1D lengthens the circadian period of locomotor activity, attributed to increased PER2 nuclear retention [43]. Furthermore, additional mutations underlying FASPD affect *CSNK1D* directly. These mutations occur on amino acid 44 (from tyrosine to alanine) or on amino acid 46 (unpublished), both of which decrease CSNK1D enzymatic activity *in vitro* [13]. Surprisingly, CSNK1D is not directly responsible for the phosphorylation of PER2-S662, as *in vitro* kinase assays reveal that S662 has to be phosphorylated by an as of yet unidentified priming kinase prior to the addition of phosphate to downstream sites by CSNK1D [28]. Hence, these experiments reveal the temporal complexity and site specificity of phosphorylation cascades involved in core clock regulation. Future research defining the stoichiometry of these events may provide insight into circadian rhythmicity beyond transcriptional-translational feedback. For instance, recent findings on the dynamics of casein kinase 1, epsilon (CSNK1E) and protein phosphatase 1A (PP1A) reinforce the idea that a delicate balance of phosphorylation kinetics is required for regulating circadian periodicity [44,45].

In addition to phosphorylation, other forms of post-translational modification may also regulate circadian rhythms. As other enzymes, such as glycogen synthase kinase 3 beta (GSK3B) [46], protein kinase, AMP-activated, alpha 2 catalytic subunit (PRKAA2, also known as AMPK) [47,48], an additional function of CLOCK as an acetyltransferase [49–51], and poly (ADP-ribose) polymerase 1 (PARP1) [52], are all implicated in post-translational circadian regulation, future discoveries of human mutations affecting these genes may reveal mechanisms directly relevant to human biology. In addition, small chemical molecular activators and inhibitors are often able to target circadian enzymatic pathways, and understanding their underlying mechanistic effects will be valuable for developing therapeutics aimed at sleep phase disorders [53,54]. In particular, drugs specifically aimed at correcting circadian period length may prove to be useful for treating FASPD. In the next section, we consider potential mechanisms linking circadian period length and the timing of sleep-wake onset.

How can FASPD mutations affecting PER2 lead to circadian phase advancement?

Although individuals with FASPD exhibit altered sleep timing patterns, it is important to note that sleep homeostasis remains unaffected (the amount of sleep required), distinguishing this syndrome from sleep disorders such as narcolepsy [55] or the familial

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natural short sleeper (FNSS) phenotype [56]. In some cases, FASPD manifests itself behaviorally through shortening the duration of free-running activity period and advancing sleep and wake onset [9]. Likewise, circadian pathways are altered in terms of both circadian period and phase as well: transcript levels of core and interlocking clock genes peak earlier for PER2-S662G liver and fibroblast cells [28]. In addition, temperature-synchronized fibroblasts exhibit an earlier circadian phase when transfected with *PER2* carrying the FASPD mutation [29]. This evidence supports the direct role of PER2-S662G in advancing the phase of cellular circadian pathways. However, a long-standing question that remains is how circadian phase and circadian period are connected.

Whereas circadian period is correlated with the time required for each cycle of the core clock, the molecular mechanisms underlying circadian phase are not well understood. As the human cellular and behavioral circadian period is not exactly 24 hours and varies between individuals [9,57], it is thought that circadian phase is adjusted by external cues in order to predict environmental fluctuations. In addition, circadian behavior should ideally adapt to gradual environmental variations such as seasonal changes in daylight availability, or acute changes such as relocation to a different time zone. Light is the primary source of time information, or zeitgeber, and the molecular circadian clock adjusts itself to light (a process known as photoentrainment) in order to maintain an appropriate circadian phase [1]. Indeed, some blind individuals exhibit a consistent delay in sleep/wake onset relative to the previous day, similar to that observed in humans under experimental free-running conditions dominated by constant darkness [11]. Recent studies have illuminated cellular mechanisms underlying our ability to photoentrain, with focus placed on the role of intrinsically photosensitive melanopsin-expressing retinal ganglion cells (ipRGCs) in transmitting light levels to the suprachiasmatic nucleus of the hypothalamus (SCN) [58,59]. Interestingly, it appears that the timing of light administration differentially affects *Per2*; notably, expression in the SCN is upregulated by light in early subjective night, but not late subjective night [60–63]. In addition, light pulses administered in artificial “dawn” versus “dusk” conditions differentially affect *Per2* mRNA levels, with the latter resulting in increased amplitude and duration of transcript expression [64]. Similarly, phase-response curves to light for humans traditionally demonstrate that morning light tends to phase advance, whereas evening light tends to phase delay, with variations between individuals for the timing of maximum light sensitivity [11]. Together, these findings point to distinct effects of light on *PER2* expression, depending on when it is administered in relation to subjective time. Although these results are only correlative, and *PER2* expression is likely one of many changes imposed on the circadian molecular machinery in response to light, it is an important first step toward deciphering the relationships between entrainment cues and the molecular clock. Understanding the molecular underpinnings of these variations may reveal fresh insight into circadian rhythm sleep disorders (CRSDs). Supporting this notion, successful phototherapy for remediating CRSDs such as FASPD may need to take into consideration the differential effects of light at different times of day for maximum efficacy [11].

Concluding remarks

Akin to how one would disassemble a mechanical timepiece to study its inner workings, disrupting and restoring the molecular clock allows us to infer mechanisms driving circadian behavior. Recent work addressing the intracellular dynamics of PER2 based on human mutations has revealed a prominent role for post-translational regulation in driving cellular circadian oscillations. These results also underscore the importance of studying the relationship between circadian period and phase in the context of PER2 to gain further information about its myriad roles. The identification of additional genes and alleles for FASPD and other sleep disorders may reveal additional regulatory mechanisms for PER2 and novel human-relevant components of the circadian pathway.

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Aside from FASPD, PER2 is also implicated in cancer [65,66], food anticipatory behavior [67], metabolism [68,69], immune responses [70], and alcohol intake [71]. How can the dysregulation of PER2 ultimately lead to distinct physiological and behavioral outcomes? One possibility is that some aspects of PER2 regulation and function are cell-type specific. Indeed, knocking out *Bmal1* in the liver does not affect *Per2* expression, whereas the absence of *Bmal1* in other tissues significantly reduces *Per2* at normal peak time [85]. Future studies using cell-type specific activation of *PER2* human mutations may discern the cellular origins of behavioral rhythmicity. Recent findings have identified PER2 oscillation in other anatomical regions besides the SCN, and understanding these connections may expand our knowledge of the neural circuitry for determining the timing of behavior [73]. Moreover, aside from neurons, PER2 is also expressed in glia, which may also contribute to circadian behavioral timing in the nervous system [74]. Finally, although brain-specific PER2 oscillation is sufficient for rhythmic wheel-running in mice, one cannot discount the possibility of feedback mechanisms from peripheral organs and other parts of the nervous system for mediating circadian outcomes [75].

As evidenced by the intricacies of PER2 intracellular dynamics and the importance of post-translational circadian regulation, it becomes clear that the "core" transcriptional-translational loop may be only one facet of the biological clock. Therefore, it is important to keep an open mind and consider mechanisms beyond the canonical circadian pathway. As Sir Francis Bacon once said, "truth is the daughter of time, not of authority." Perhaps the rigorous study of biological time may also lead us closer to the truth behind genetic regulation of innate behavior.

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Glossary

Circadian period	total time required for one complete daily cycle or oscillation
Circadian phase	a distinct stage of an event that occurs or functions in approximately 24-hour intervals
Circadian rhythm	"circa diem," or "about a day" in Latin. Describes processes that occur or function in approximately 24-hour intervals
Diurnal	active during the day and sleeping at night
E box	"Enhancer box", transcription factor binding site upstream of a gene in promoter regions with a consensus DNA sequence of CANNTG
Entrainment	a process of synchronizing circadian rhythm to a zeitgeber
Free-running conditions	the state of self-sustaining circadian rhythm in the absence of zeitgebers
Phase-response curve	a graph showing the magnitude of circadian phase shift depending on when the stimulus (e.g. light) is applied
Subjective time	Segments of time corresponding to what the organism perceives it to be, i.e. subjective day corresponds to the light phase of the daily light cycle, whereas subjective night corresponds to the dark phase

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Wheel-running behavior	a method of estimating the circadian periodicity and phase on an organismal level for rodents. A running-wheel is placed in a recording cage and the number of revolutions approximates activity levels. As mice are nocturnal (active at night and sleeping during daytime), wheel-running behavior usually occurs during the dark phase of the daily light cycle
Zeitgeber	a source of information about the time of day, capable of entraining circadian rhythm e.g. light

References

1. Hut RA, Beersma DGM. Evolution of time-keeping mechanisms: early emergence and adaptation to photoperiod. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 2011; 366:2141–54.
2. Konopka RJ, Benzer S. Clock mutants of Drosophila melanogaster. Proceedings of the National Academy of Sciences of the United States of America. 1971; 68:2112–6. [PubMed: 5002428]
3. Lowrey, PL.; Takahashi, JS. Genetics of circadian rhythms in Mammalian model organisms. 1. Elsevier Inc; 2011. p. 74
4. Roenneberg T, et al. Epidemiology of the human circadian clock. Sleep medicine reviews. 2007; 11:429–38. [PubMed: 17936039]
5. Sehgal A, Mignot E. Genetics of sleep and sleep disorders. Cell. 2011; 146:194–207. [PubMed: 21784243]
6. Zhang L, et al. The Genetics of the Human Circadian Clock. Advances in Genetics. 2011:74.
7. Satoh K, et al. Two pedigrees of familial advanced sleep phase syndrome in Japan. Sleep. 2003; 26:416–7. [PubMed: 12841366]
8. Reid KJ, et al. Familial advanced sleep phase syndrome. Archives of neurology. 2001; 58:1089–94. [PubMed: 11448298]
9. Jones CR, et al. Familial advanced sleep-phase syndrome: A short-period circadian rhythm variant in humans. Nature medicine. 1999; 5:1062–5.
10. Xu Y, et al. Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. Nature. 2005; 434:640–4. [PubMed: 15800623]
11. Dodson ER, Zee PC. Therapeutics for Circadian Rhythm Sleep Disorders. Sleep medicine clinics. 2010; 5:701–715. [PubMed: 21243069]
12. Mohawk JA, et al. Central and peripheral circadian clocks in mammals. Annual review of neuroscience. 2012; 35:445–62.
13. Brown SA, et al. (Re)inventing the Circadian Feedback Loop. Developmental Cell. 2012; 22:477–487. [PubMed: 22421040]
14. Ueda HR, et al. System-level identification of transcriptional circuits underlying mammalian circadian clocks. Nature genetics. 2005; 37:187–92. [PubMed: 15665827]
15. Chen R, et al. Rhythmic PER abundance defines a critical nodal point for negative feedback within the circadian clock mechanism. Molecular cell. 2009; 36:417–30. [PubMed: 19917250]
16. Albrecht U, et al. The multiple facets of Per2. Cold Spring Harbor symposia on quantitative biology. 2007; 72:95–104.
17. Schmutz I, et al. The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. Genes & development. 2010; 24:345–57. [PubMed: 20159955]
18. Etchegaray, J-pierre, et al. Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. Nature. 2003; 421:177–182. [PubMed: 12483227]
19. DiTacchio L, et al. Histone Lysine Demethylase JARID1a Activates CLOCK-BMAL1 and Influences the Circadian Clock. Science. 2011; 333:1881–1885. [PubMed: 21960634]
20. Brown SA. Circadian rhythms. A new histone code for clocks? Science (New York, NY). 2011; 333:1833–4.

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21. Bellet MM, Sassone-Corsi P. Mammalian circadian clock and metabolism - the epigenetic link. Journal of cell science. 2010; 123:3837–48. [PubMed: 21048160]
22. Yagita K, et al. Nucleocytoplasmic shuttling and mCRY-dependent inhibition of ubiquitylation of the mPER2 clock protein. The EMBO journal. 2002; 21:1301–14. [PubMed: 11889036]
23. Vielhaber EL, et al. Nuclear export of mammalian PERIOD proteins. The Journal of biological chemistry. 2001; 276:45921–7. [PubMed: 11591712]
24. Yagita K, et al. Dimerization and nuclear entry of mPER proteins in mammalian cells. Genes & Development. 200010.1101/gad.14.11.1353
25. Miyazaki K, et al. Nuclear Entry Mechanism of Rat PER2 (rPER2): Role of rPER2 in Nuclear Localization of CRY Protein. Molecular and cellular biology. 2001:2.
26. Miki T, et al. PML regulates PER2 nuclear localization and circadian function. The EMBO journal. 2012; 2:1–13.
27. Yagita K, et al. Dimerization and nuclear entry of mPER proteins in mammalian cells. Genes & Development. 200010.1101/gad.14.11.1353
28. Xu Y, et al. Modeling of a human circadian mutation yields insights into clock regulation by PER2. Cell. 2007; 128:59–70. [PubMed: 17218255]
29. Vanselow K, et al. Differential effects of PER2 phosphorylation: molecular basis for the human familial advanced sleep phase syndrome (FASPD). Genes & development. 2006; 20:2660–72. [PubMed: 16983144]
30. Brown SA, et al. PERIOD1-associated proteins modulate the negative limb of the mammalian circadian oscillator. Science (New York, NY). 2005; 308:693–6.
31. Hara Y, et al. Molecular characterization of Mybbp1a as a co-repressor on the Period2 promoter. Nucleic acids research. 2009; 37:1115–26. [PubMed: 19129230]
32. Duong HA, et al. A molecular mechanism for circadian clock negative feedback. Science (New York, NY). 2011; 332:1436–9.
33. Eide EJ, et al. Control of Mammalian Circadian Rhythm by CKIε-Regulated Proteasome-Mediated PER2 Degradation. Molecular and cellular biology. 200510.1128/MCB.25.7.2795
34. Belden WJ, Dunlap JC. SIRT1 is a circadian deacetylase for core clock components. Cell. 2008; 134:212–4. [PubMed: 18662537]
35. Asher G, et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell. 2008; 134:317–28. [PubMed: 18662546]
36. Nakahata, Y., et al. The NAD +-Dependent Deacetylase SIRT1 Modulates CLOCK-Mediated Chromatin Remodeling and Circadian Control. 2008.
37. Busino L, et al. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science (New York, NY). 2007; 316:900–4.
38. Godinho SIH, et al. The after-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period. Science (New York, NY). 2007; 316:897–900.
39. Siepka SM, et al. Circadian mutant Overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. Cell. 2007; 129:1011–23. [PubMed: 17462724]
40. Shirogane T, et al. SCFbeta-TRCP controls clock-dependent transcription via casein kinase 1-dependent degradation of the mammalian period-1 (Per1) protein. The Journal of biological chemistry. 2005; 280:26863–72. [PubMed: 15917222]
41. Reischl S, et al. Beta-TrCP1-mediated degradation of PERIOD2 is essential for circadian dynamics. Journal of biological rhythms. 2007; 22:375–86. [PubMed: 17876059]
42. Shanware NP, et al. Casein kinase 1-dependent phosphorylation of familial advanced sleep phase syndrome-associated residues controls PERIOD 2 stability. The Journal of biological chemistry. 2011; 286:12766–74. [PubMed: 21324900]
43. Meng, Q-jun, et al. Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. 2010. p. 1
44. Meng Q-J, et al. Setting clock speed in mammals: the CK1 epsilon tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. Neuron. 2008; 58:78–88. [PubMed: 18400165]

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45. Lee, H-min, et al. The period of the circadian oscillator is primarily determined by the balance between casein kinase 1 and protein phosphatase 1. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:16451–6. [PubMed: 21930935]
46. Hirota T, et al. A chemical biology approach reveals period shortening of the mammalian circadian clock by specific inhibition of GSK-3B. PNAS. 2008:105.
47. Um JH, et al. Activation of 5′-AMP-activated kinase with diabetes drug metformin induces casein kinase Iepsilon (CKIepsilon)-dependent degradation of clock protein mPer2. The Journal of biological chemistry. 2007; 282:20794–8. [PubMed: 17525164]
48. Zhang J, et al. Constant darkness is a circadian metabolic signal in mammals. Nature. 2006; 439:340–3. [PubMed: 16421573]
49. Doi M, et al. Circadian regulator CLOCK is a histone acetyltransferase. Cell. 2006; 125:497–508. [PubMed: 16678094]
50. Hardin PE, Yu W. Circadian transcription: passing the HAT to CLOCK. Cell. 2006; 125:424–6. [PubMed: 16678086]
51. Sehgal A. Ac-ing the clock. Neuron. 2008; 57:8–10. [PubMed: 18184560]
52. Asher G, et al. Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. Cell. 2010; 142:943–53. [PubMed: 20832105]
53. Hirota T, et al. High-throughput chemical screen identifies a novel potent modulator of cellular circadian rhythms and reveals CKIα as a clock regulatory kinase. PLoS biology. 2010; 8:e1000559. [PubMed: 21179498]
54. Chen Z, et al. Identification of diverse modulators of central and peripheral circadian clocks by high-throughput chemical screening. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:101–6. [PubMed: 22184224]
55. Tafti M, et al. Narcolepsy and familial advanced sleep-phase syndrome: molecular genetics of sleep disorders. Current opinion in genetics & development. 2007; 17:222–7. [PubMed: 17467264]
56. He Y, et al. The transcriptional repressor DEC2 regulates sleep length in mammals. Science (New York, NY). 2009; 325:866–70.
57. Brown SA, et al. The period length of fibroblast circadian gene expression varies widely among human individuals. PLoS biology. 2005; 3:e338. [PubMed: 16167846]
58. Chen S-K, et al. Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. Nature. 201110.1038/nature10206
59. Schmidt TM, et al. Melanopsin-Positive Intrinsically Photosensitive Retinal Ganglion Cells: From Form to Function. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2011; 31:16094–16101. [PubMed: 22072661]
60. Albrecht U, et al. A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. Cell. 1997; 91:1055–64. [PubMed: 9428527]
61. Zylka MJ, et al. Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. Neuron. 1998; 20:1103–10. [PubMed: 9655499]
62. Shearman LP, et al. Two period homologs: circadian expression and photic regulation in the suprachiasmatic nuclei. Neuron. 1997; 19:1261–9. [PubMed: 9427249]
63. Yan L, Silver R. Differential induction and localization of mPer1 and mPer2 during advancing and delaying phase shifts. European Journal of Neuroscience. 2002; 16:1531–1540. [PubMed: 12405967]
64. Schwartz WJ, et al. Distinct patterns of Period gene expression in the suprachiasmatic nucleus underlie circadian clock photoentrainment by advances or delays. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:17219–17224. [PubMed: 21969555]
65. Fu L, et al. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. Cell. 2002; 111:41–50. [PubMed: 12372299]
66. Gu X, et al. The circadian mutation PER2(S662G) is linked to cell cycle progression and tumorigenesis. Cell death and differentiation. 201110.1038/cdd.2011.103

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67. Feillet CA, et al. Lack of food anticipation in Per2 mutant mice. Current biology: CB. 2006; 16:2016–22. [PubMed: 17055980]

68. Kornmann B, et al. System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. PLoS biology. 2007; 5:e34. [PubMed: 17298173]

69. Grimaldi B, et al. PER2 controls lipid metabolism by direct regulation of PPARγ. Cell metabolism. 2010; 12:509–20. [PubMed: 21035761]

70. Liu J, et al. The circadian clock Period 2 gene regulates gamma interferon production of NK cells in host response to lipopolysaccharide-induced endotoxic shock. Infection and immunity. 2006; 74:4750–6. [PubMed: 16861663]

71. Spanagel R, et al. The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. Nature medicine. 2005; 11:35–42.

72. McDearmon EL, et al. Dissecting the functions of the mammalian clock protein BMAL1 by tissue-specific rescue in mice. Science (New York, NY). 2006; 314:1304–8.

73. Amir S, Stewart J. Motivational Modulation of Rhythms of the Expression of the Clock Protein PER2 in the Limbic Forebrain. Biological psychiatry. 2009; 65:829–34. [PubMed: 19200536]

74. Cheng H-YM, et al. Segregation of expression of mPeriod gene homologs in neurons and glia: possible divergent roles of mPeriod1 and mPeriod2 in the brain. Human molecular genetics. 2009; 18:3110–24. [PubMed: 19477955]

75. Albrecht U. Timing to Perfection: The Biology of Central and Peripheral Circadian Clocks. Neuron. 2012; 74:246–260. [PubMed: 22542179]

76. Hosokawa N, Hatakeyama T. Circadian transcriptional regulation by the posttranslational oscillator without de novo clock gene expression in Synechococcus. Proceedings of the. 2011; 108:15396–15401.

77. O'Neill JS, et al. Circadian rhythms persist without transcription in a eukaryote. Nature. 2011; 469:554–8. [PubMed: 21270895]

78. O'Neill JS, Reddy AB. Circadian clocks in human red blood cells. Nature. 2011; 469:498–503. [PubMed: 21270888]

79. Gotter A. A Timeless debate: resolving TIM's noncircadian roles with possible clock function. neuroreport. 2006; 17:11–15.

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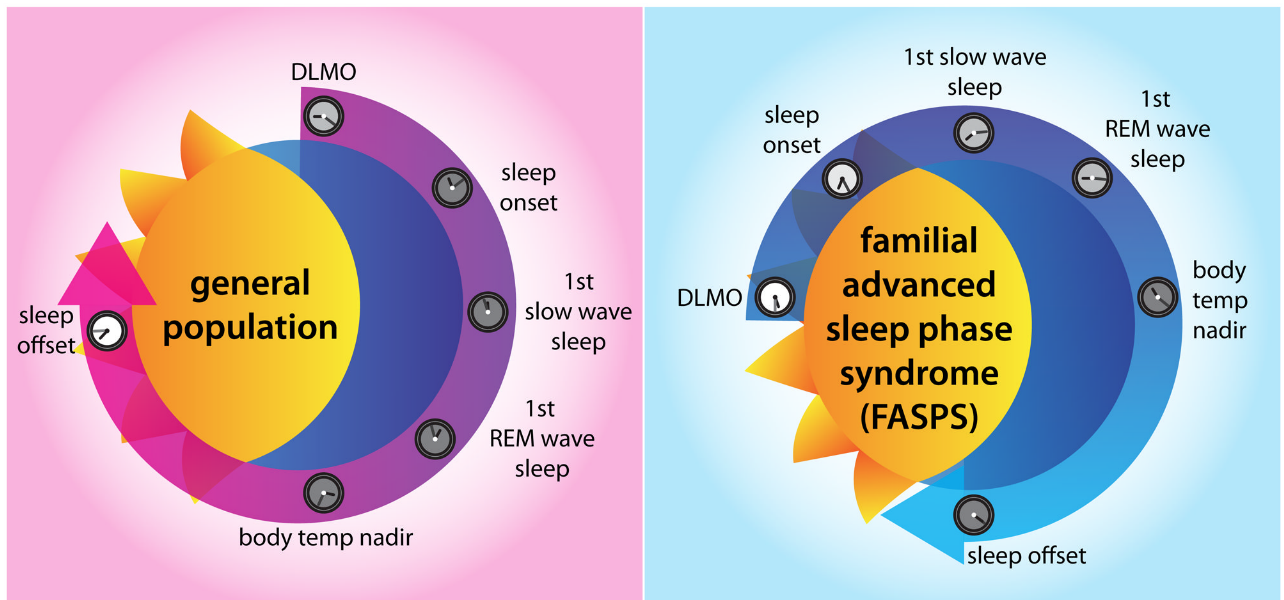

Figure 1. Clinical phase markers for familial advanced sleep phase syndrome (FASPD)
FASPD-affected individuals (right) exhibit a marked advancement in phase markers of overt rhythms related to sleep physiology as compared to the general population (left). Clock faces indicate the mean time of occurrence for each event, with lighter clock faces indicating morning hours and darker clock faces indicating evening hours. Abbreviations: DLMO, dim light melatonin onset. REM, rapid eye movement. Temp, temperature. Data from [9].

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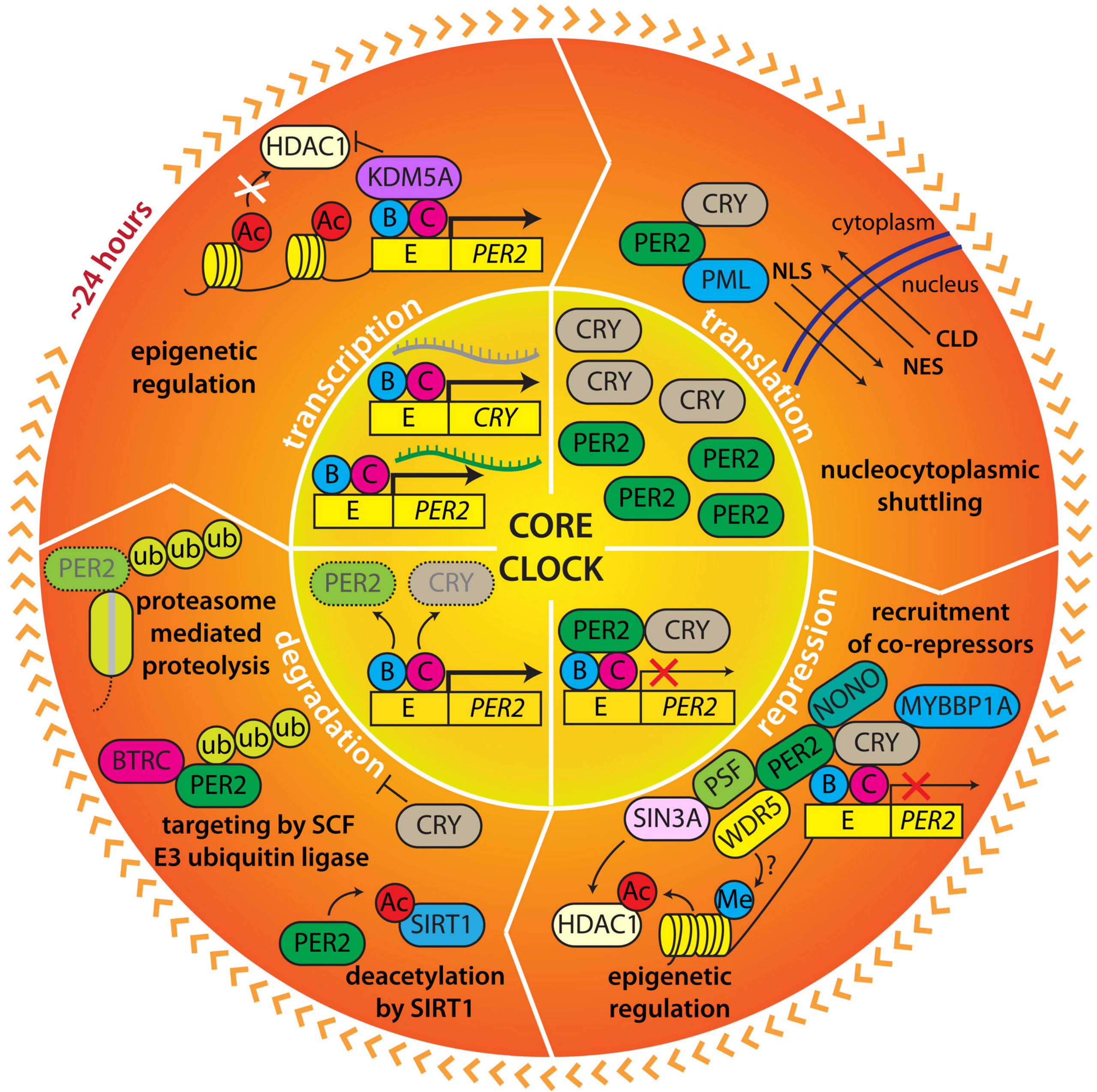

Figure 2. Regulation of PER2 intracellular dynamics plays a crucial role in core clock function

The core clock (inner circle, yellow) consists of a negative feedback loop driven by the transcription, translation, repression, and degradation of core clock components PER2 and CRY, which together drive the circadian rhythms of a cell. For the sake of clarity, pathways pertaining to PER2 are emphasized in this figure, but interlocking negative feedback loops of other circadian genes such as *CRY1/2* also mediate the maintenance of robust circadian rhythmicity. The outer circle in orange illustrates molecular mechanisms pertaining to PER2 intracellular dynamics, which in turn affect core clock events. Together, these processes take ~24 hours to complete. Abbreviations: Ac-acetyl group, B-BMAL1, C-CLOCK, E-E-box promoter elements, ub-ubiquitin. HDAC1- histone deacetylase 1. KDM5A-lysine (K)-specific demethylase 5A. PML-promyelocytic leukemia protein. NLS-nuclear localization

sequences, BTRC-beta-transducin repeat containing E3 ubiquitin protein ligase, NONO-non-POU domain containing, octamer-binding, SIN3A-SIN3 transcription regulator homolog A, SIRT1-Sirtuin 1, CLD-cytoplasmic localization domain, NES-nuclear export sequence, PSF-Polypyrimidine tract-binding protein-associated splicing factor, WDR5-WD repeat domain 5, MYBBP1A-MYB binding protein (P160) 1a.

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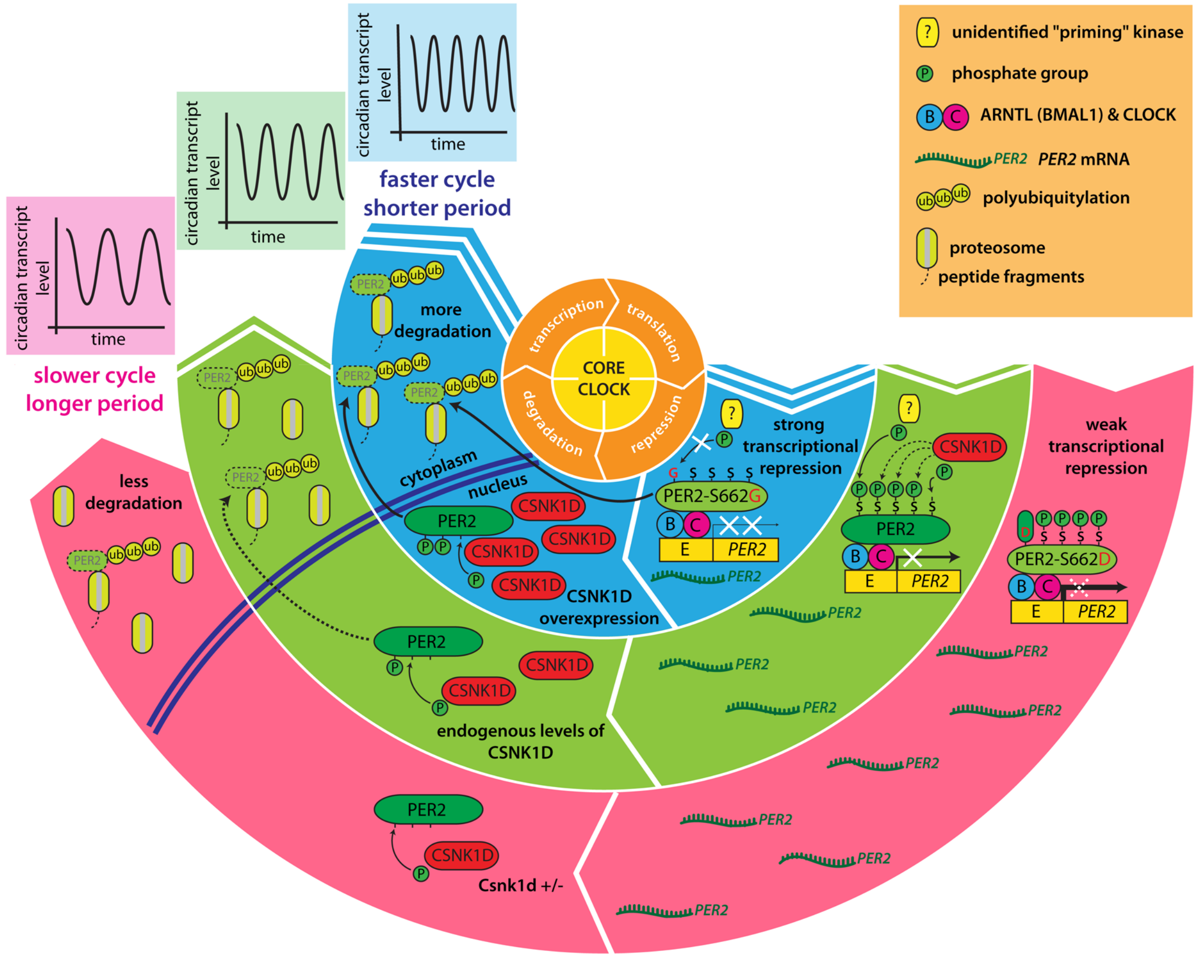

Figure 3. Post-translational mechanisms affect circadian period length through altering transcriptional repression or degradation of PER2

As revealed by studies using causative mutations for FASPD, altering the transcription repressor activity or degradation of PER2 both affect the circadian period length of cells and wheel-running behavior. PER2-S662G prevents phosphorylation of PER2 by an as of yet unidentified priming kinase, resulting in enhanced transcriptional repression of PER2 and increased translocation from the nucleus to the cytoplasm. This leads to a shorter circadian period. The PER2-S662D mutation has the opposite effect, resulting in weaker transcriptional repression and a longer circadian period. CSNK1D levels exacerbate the effects of PER2-S662G on circadian period, as transgenic overexpression of CSNK1D in combination with PER2-S662G increases PER2 degradation through other putative phosphorylation sites and shortens circadian period even more, whereas *Csnk1d* heterozygosity counteracts PER2-S662G, resulting in relatively longer circadian period attributed to reduced PER2 degradation.