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# Genetic insights on sleep schedules: this time, it's *PER*sonal

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**The study of circadian rhythms is emerging as a fruitful opportunity for understanding cellular mechanisms that govern human physiology and behavior, fueled by evidence directly linking sleep disorders to 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 because 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.**

## 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 h, including 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 a circadian rhythm (see [Glossary](#)) ensures the execution of behaviors appropriate for the environment, and this may ultimately enhance evolutionary fitness [1].

Owing to the complexity of human behavior, pioneering work on understanding the regulation of circadian rhythm relied on model organisms. Konopka and Benzer identified *Drosophila melanogaster* with various circadian timing defects in locomotor activity, and 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, and this opened the floodgate for the study of analogous circadian genes in mammalian model systems [3]. However, are human circadian behaviors also genetically 'hard-wired'?

Although humans are by nature diurnal, there is a wide spectrum of sleep–wake time preferences in 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 7.30 pm [9]. This time-frame is significantly advanced compared to the 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] and K.C. Brennan *et al.*, unpublished). These

## 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 h intervals.

**Circadian rhythm:** 'circa diem,' or 'about a day' in Latin. Describes processes that occur or function in approximately 24 h intervals.

**Diurnal:** active during the day and sleeping at night.

**Enhancer box (E 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.

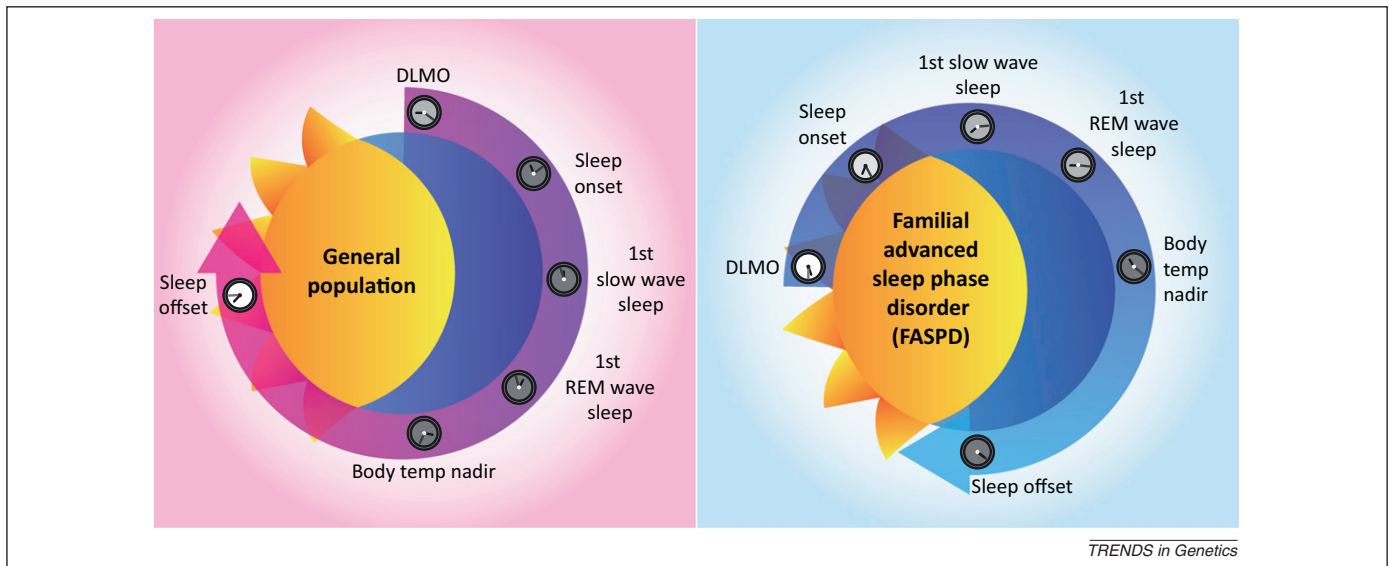
**Subjective time:** Segments of time corresponding to what the organism perceives it to be; in other words, subjective day corresponds to the light phase of the daily light cycle, whereas subjective night corresponds to the dark phase.

**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. Because 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 intensity).

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**Figure 1.** Clinical phase markers for familial advanced sleep-phase disorder (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].

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. Because there are nuanced differences between species (Box 1), we will focus on evidence from mammalian model systems.

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

Approximately 10–15% of gene transcripts oscillate in a circadian manner, the identities of which vary according to

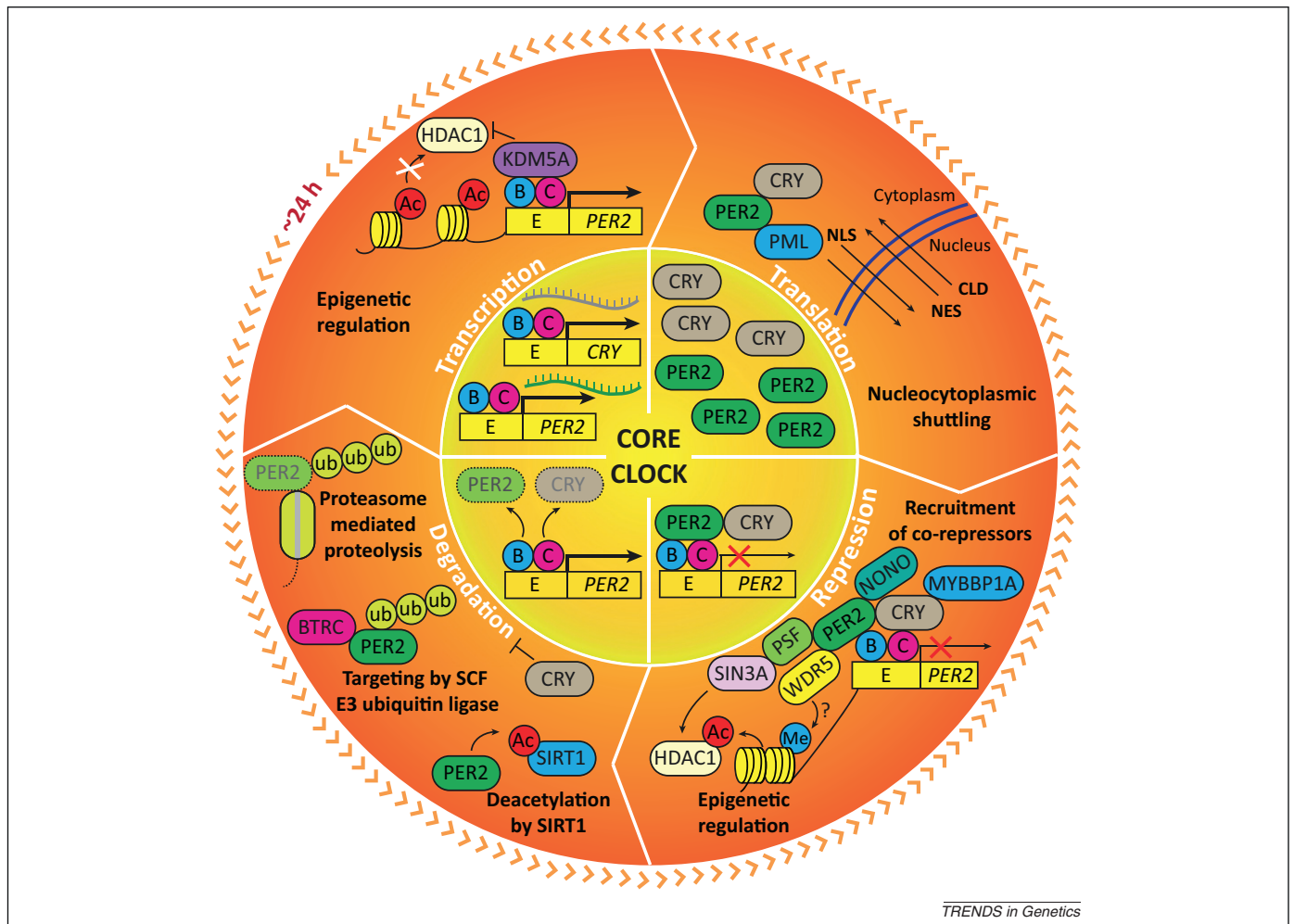
#### Box 1. Are cellular mechanisms driving the circadian rhythm conserved across species?

The ability to maintain a 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, there are notable exceptions – for example, cyanobacteria and unicellular algae maintain circadian rhythm by post-translational regulation without *de novo* synthesis of clock components [75,76]. Remarkably, human red blood cells, which do not have nuclei or perform transcription, undergo rhythmic 24 h redox cycles [77]. 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 [59,61]. However, there are also notable differences amongst species. For example, mammalian CRY proteins replaced *Drosophila* TIM as a binding partner for PER1/2 [12,78]. 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]. Therefore, it is important to keep in mind the possibility of divergent mechanisms and exercise prudence when comparing findings from different model organisms.

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), ARNTL (aryl hydrocarbon receptor nuclear translocator-like; also known as BMAL1), and clock homolog (CLOCK) (Figure 2, inner circle). BMAL1 and CLOCK [or its paralog, NPAS2 (neuronal PAS domain protein 2)] 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* is once again activated. This transcriptional/translational negative feedback loop takes approximately 24 h to complete, and additional negative feedback loops (also known as interlocking loops) of other circadian regulators, such as RORA (RAR-related orphan receptor A), NR1D1 (nuclear receptor subfamily 1, group D, member 1; also known as REV-ERBA), BHLHE40/41 (basic helix-loop-helix family, members e40 and e41; also known as DEC1/2), DBP (D site of albumin promoter-binding protein), and NFIL3 (nuclear factor, interleukin 3 regulated; 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 crucial feature of the core clock [15,16]. The coordination of circadian output by PER2 is partially mediated through interaction with nuclear receptors including peroxisome PPAR $\alpha$  (proliferator-activated receptor  $\alpha$ ) and REV-ERBA [17]. Through a series of overexpression experiments, *in vitro* and *in vivo*, it was shown in mice that



**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 the 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 h to complete. Abbreviations: Ac, acetyl group; B, BMAL1; BTRC,  $\beta$ -transducin repeat-containing E3 ubiquitin protein ligase; CLD, cytoplasmic localization domain; C, CLOCK; E, E-box promoter elements; HDAC1, histone deacetylase 1; KDM5A, lysine (K)-specific demethylase 5A; MYBBP1A, MYB binding protein (P160) 1a; NES, nuclear export sequence; NLS, nuclear localization sequences; NONO, non-POU domain containing, octamer-binding; PML, promyelocytic leukemia protein; PSF, polypyrimidine tract-binding protein-associated splicing factor; SIN3A, SIN3 transcription regulator homolog A; SIRT1, Sirtuin 1; ub, ubiquitin; WDR5, WD repeat domain 5.

PER2 oscillation, rather than its absolute quantity, is crucial 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 wherein a protein is transcribed, translated, and represses its own transcription over approximately 24 h. Because these processes occur continuously it would be inaccurate to state definitively 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]. Similarly to 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 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 KDM1A [lysine (K)-specific demethylase 5A; 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 HDAC1 (histone deacetylase 1) 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*. Because 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]. In addition, 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* protein is thought to accumulate in the cytoplasm. However, *PER2* is shuttled between the cytoplasm and the nucleus through a number of regulatory mechanisms. First, *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 because 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 *PER2* translocation [12,26]. Interestingly, *PER2* localization in the nucleus is unimpaired in *CRY1/2* knockout cells [22,24,27], and recent evidence proposes that *CRY1/2* may instead mitigate *PER2* degradation in addition to its collaborative function in repressing BMAL1/CLOCK [22,28]. 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 PML (promyelocytic leukemia protein) because *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 because 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.

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

As previously mentioned, *PER1* and *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 [29]. Moreover, MYBBP1A, a transcription cofactor 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 [30]. Further emphasizing the codependence between epigenetics and transcriptional regulation, histone modifiers have been shown to participate in *PER*-mediated transcriptional repression as well. SFPQ (polypyrimidine tract-binding protein-associated splicing factor; 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 [31]. In addition, WDR5, a member of a histone methyltransferase complex, binds to *PER1/2*, and knockdown of WDR5 reduces histone methylation at *PER1*-regulated promoters, implicating it in circadian regulation [29]. However, reduction of WDR5 does not affect the circadian period of luciferase driven by circadian reporter genes [29], 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.

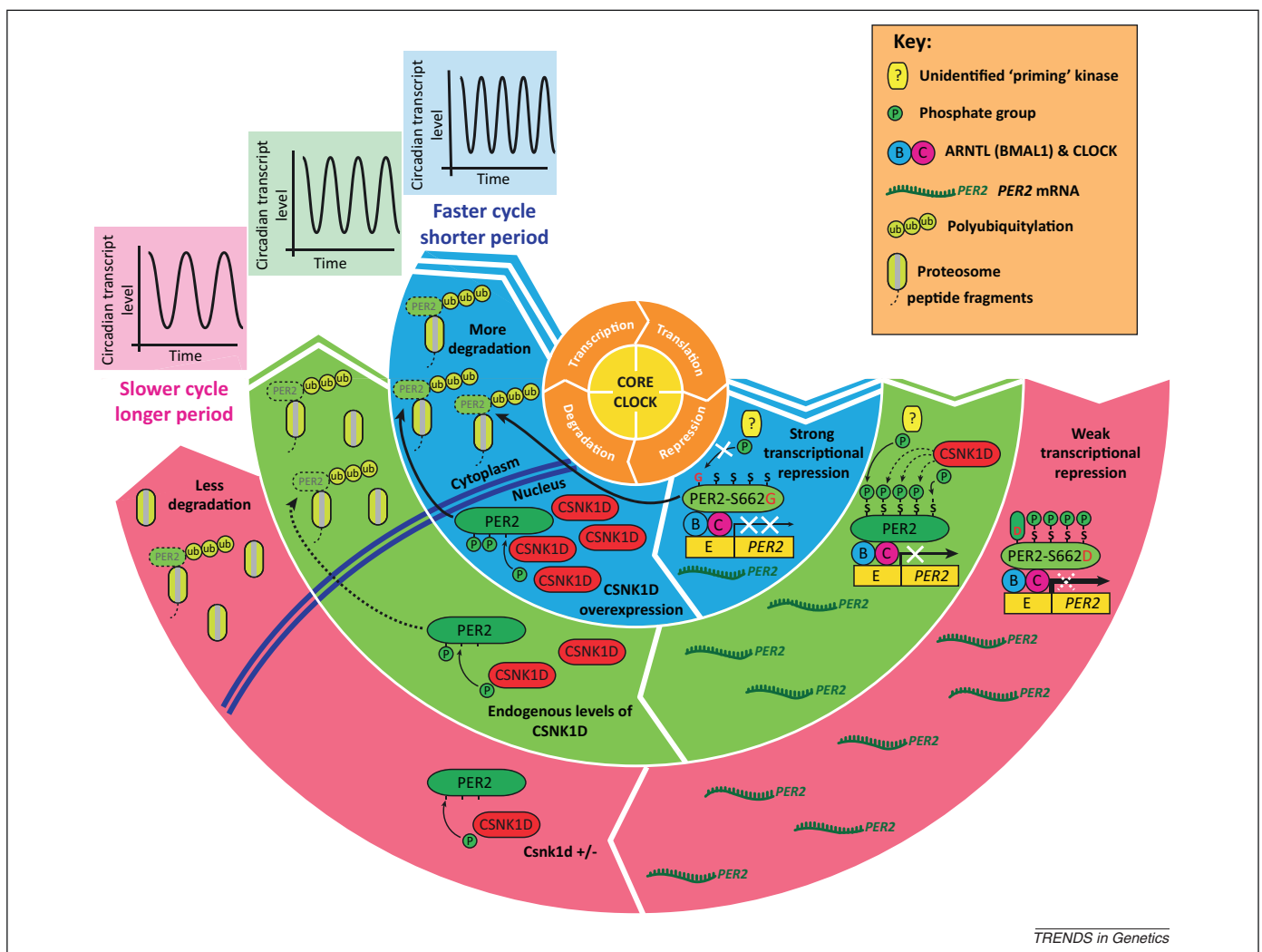
After binding and repressing its own transcription, *PER2* is degraded through an ubiquitin-dependent proteosomal pathway. Protein degradation is crucial for maintaining circadian period because proteasome inhibitors can affect period length [32]. As mentioned above, *PER2* degradation may be prevented by binding to *CRY1/2* [22]. The stability of *PER2* is also dependent on its acetylation, which occurs once it binds to BMAL1/CLOCK [33]. Sirtuin 1 (*SIRT1*) then binds to BMAL1/CLOCK and deacetylates *PER2*, promoting its degradation [34,35]. 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* [36–38]. Similarly, BTRC ( $\beta$ -transducin repeat-containing E3 ubiquitin protein ligase) may play a corresponding role in the timely degradation of *PER2* because deleting the BTRC-interacting domain of *PER2* results in its stabilization

[39,40]. In addition to proteosomal targeting, inhibiting nuclear export indirectly stabilizes PER2, suggesting that degradation occurs after PER2 is exported back into the cytoplasm [28]. 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

Although 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 serine to glycine mutation in *PER2* at position 662 [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 [27] (Figure 3). The latter characteristic is attributed to enhanced repressor activity because mRNA levels of endogenous mouse *Per2* and introduced human *PER2* both decrease, suggesting enhanced promiscuous transcriptional repression [27]. 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 [27]. Conversely, mutating S662 to aspartic acid (which mimics constitutive phosphorylation) results in a lengthened circadian period and decreased repression (increased *PER2* expression) [27]. 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 [28,41]. Likewise, studies using



**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. The *PER2*-S662G mutation prevents phosphorylation of PER2 by an as 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 because 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.

phospho-specific antibodies demonstrate that PER2-S662 phosphorylation stabilizes protein half-life [41].

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, and this acts to correct the shortened period [27]. Consistent with these findings, pharmacological inhibition of CSNK1D lengthens the circadian period of locomotor activity, attributed to increased PER2 nuclear retention [42]. Furthermore, additional mutations underlying FASPD affect *CSNK1D* directly. These mutations occur at amino acid 44 (from tyrosine to alanine) or at 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 because *in vitro* kinase assays reveal that S662 has to be phosphorylated by an as yet unidentified priming kinase before the addition of phosphate to downstream sites by CSNK1D [27]. 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 [43,44].

In addition to phosphorylation, other forms of post-translational modification may also regulate circadian rhythms. Because other enzymes, such as GSK3B (glycogen synthase kinase 3 $\beta$ ) [45], PRKAA2 (protein kinase, AMP-activated,  $\alpha$ 2 catalytic subunit; also known as AMPK) [46,47], and an additional function of CLOCK as an acetyltransferase [48–50], as well as PARP1 [poly (ADP-ribose) polymerase 1] [51], 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 [52,53]. 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 [54] or the familial natural short-sleeper (FNSS) phenotype [55]. 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 also altered in terms of both circadian period and phase as: transcript levels of core and interlocking clock genes peak earlier for PER2-S662G liver and fibroblast cells [27]. In addition, temperature-synchronized fibroblasts exhibit an earlier circadian phase when transfected with *PER2* carrying the FASPD mutation [28]. This evidence supports the direct role of PER2-S662G in advancing the phase of cellular circadian pathways. However, a longstanding 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. Because the human cellular and behavioral circadian period is not exactly 24 h, and varies between individuals [9,56], it is thought that circadian phase is adjusted by external cues 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) 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) [57,58]. 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 in late subjective night [59–62]. 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 [63]. 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 probably 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.

Aside from FASPD, PER2 is also implicated in cancer [64,65], food anticipatory behavior [66], metabolism [67,68], immune responses [69], and alcohol intake [70]. 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 [71]. 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 [72]. Moreover, aside from neurons, PER2 is also expressed in glia, which may also contribute to circadian behavioral timing in the nervous system [73]. 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 [74].

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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