The Neurobiology of Circadian Timing

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CHAPTER 14

The circadian clock component PERIOD2: From molecular to cerebral functions

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Abstract: The circadian clock is based on a molecular oscillator, which simulates the external day within nearly all of a body's cells. This “internalized” day then defines activity and rest phases for the cells and the organism by generating precise rhythms in the metabolism, physiology, and behavior. In its perfect state, this timing system allows for the synchronization of an organism to its environment and this may optimize energy handling and responses to daily recurring challenges. However, nowadays, we believe that desynchronization of an organism due to its lifestyle or problems with its circadian clock not only causes discomfort but also may aggravate conditions such as depression, metabolic syndrome, addiction, or cancer.

In this review, we focus on one simple cogwheel of the mammalian circadian clock, the PERIOD2 (PER2) protein. Originally identified as an integral part of the molecular mechanism that yields overt rhythms of about 24 h, more recently multiple other functions have been identified. In essence, the PER proteins, in addition to their important function within the molecular oscillator, can be seen not only as integrators on the input side of the circadian clock but also as mediators of clock output. This diversity in their function is possible, because the PER proteins can interact with a multitude of other proteins transferring oscillator timing information to the latter. In this fashion, the circadian clock synchronizes many rhythmic processes.

Keywords: metabolism; mood; synchronization; Per2.

Introduction

The circadian clock generates a projection of the external day into the individual cells of the body (Dibner et al., 2010; Ko and Takahashi, 2006; Liu et al., 2007). However, under normal conditions we may not become aware of the existence of such a timing device in us. The reason for this is that the circadian clock is responsive to external timing cues, the so-called Zeitgebers (german for “time-giver”), such as the light/dark cycle. Hence, the effects of the circadian clock are generally masked...
by external factors. To observe the characteristic properties of a circadian clock, it is necessary to place an organism into conditions without external Zeitgebers (e.g., constant darkness or constant light of constant light intensity). In these free-running conditions, solely the circadian clock governs the rhythmic metabolism, physiology, and behavior of an organism. The advantage of such a process is obvious. It provides a temporal network allowing synchronization of many metabolic pathways that otherwise would run each on their own pace, just driven by substrate availability, for example. Daily separation of metabolic pathways yielding or consuming energy is already observed in simple organisms such as cyanobacteria (Dong et al., 2010; Johnson et al., 2011). Experiments performed with cyanobacteria also provide compelling evidence for the biological advantage of possessing such timing systems: the growth and survival rate of strains with mutations in their circadian clock are generally not affected unless these mutants are kept in coculture with another strain that is in better resonance with the environmental light/dark cycle (Ouyang et al., 1998). Under the latter conditions, the better adjusted one completely wipes out the less adjusted strain. Hence, there is a substantial biological advantage to possess a well functioning timing system, and it is therefore not surprising that circadian clocks are found throughout the bacteria, plant, and animal kingdoms (Hut and Beersma, 2011; Yerushalmi and Green, 2009). However, interesting similarities and common concepts can be found over the borders of the taxa, such as the occurrence of posttranslational circadian oscillations of the redox state of peroxiredoxins, which are observed in human red blood cells (O’Neill and Reddy, 2011) and also the green algae Ostreococcus tauri (O’Neill et al., 2011). The mammalian circadian oscillator is related to the one found in the fruit fly Drosophila melanogaster (Dunlap, 1999). The basic concept is quite simple and is found in essentially all cells of an organism. At the center of the clock is a negative transcriptional feedback loop. A pair of transcriptional activators increases the transcription of a set of repressors. The corresponding repressor proteins accumulate until their concentration is sufficient to shuts down the transcription of their own genes. Consequently, transcription and translation of the repressors cease and the activity of the repressor proteins declines. As soon as the transcription block is relieved, a new cycle of about 24 h restarts, yielding robust oscillations with a periodicity of about a day. Obviously, such a simple mechanism would not be sufficient to produce all of the robust rhythms observed in the organisms. There would be the risk that the oscillations generated by the feedback loop dampen rapidly, nearly reaching equilibrium conditions (Ripperger and Brown, 2010). Hence, there exist regulatory posttranslational mechanisms and an associated network of other feedback loops that stabilizes and reinforces the circadian rhythms. This network also provides input information to the core circadian oscillator to allow synchronization of the self-sustaining loop to the environment. On the other side, most of the primary output generated by the circadian oscillator is mediated primarily by rhythmic transcriptional regulation of target genes.

**General mechanism of circadian clocks**

Circadian clocks arose probably independently during evolution in different taxa such as cyanobacteria, plants, and animals (Rosbash, 2009). However, interesting similarities and common concepts can be found over the borders of the taxa, such as the occurrence of posttranslational circadian oscillations of the redox state of peroxiredoxins, which are observed in human red blood cells (O’Neill and Reddy, 2011) and also the green algae Ostreococcus tauri (O’Neill et al., 2011). The mammalian circadian oscillator is related to the one found in the fruit fly Drosophila melanogaster (Dunlap, 1999). The basic concept is quite simple and is found in essentially all cells of an organism. At the center of the clock is a negative transcriptional feedback loop. A pair of transcriptional activators increases the transcription of a set of repressors. The corresponding repressor proteins accumulate until their concentration is sufficient to shut down the transcription of their own genes. Consequently, transcription and translation of the repressors cease and the activity of the repressor proteins declines. As soon as the transcription block is relieved, a new cycle of about 24 h restarts, yielding robust oscillations with a periodicity of about a day. Obviously, such a simple mechanism would not be sufficient to produce all of the robust rhythms observed in the organisms. There would be the risk that the oscillations generated by the feedback loop dampen rapidly, nearly reaching equilibrium conditions (Ripperger and Brown, 2010). Hence, there exist regulatory posttranslational mechanisms and an associated network of other feedback loops that stabilizes and reinforces the circadian rhythms. This network also provides input information to the core circadian oscillator to allow synchronization of the self-sustaining loop to the environment. On the other side, most of the primary output generated by the circadian oscillator is mediated primarily by rhythmic transcriptional regulation of target genes.

**The history of Period**

In the early 1970s, Konopka and Benzer performed a mutagenesis screen in *D. melanogaster* to identify mutations in its circadian timing system (Konopka and Benzer, 1971). Surprisingly, the identified mutations provoking shortening or lengthening of
the free-running period length or complete arrhythmicity in constant darkness mapped all to the same gene, which was baptized Period (Per) (Konopka and Benzer, 1971; Reddy et al., 1984; Zehring et al., 1984). Nearly 20 years after the identification of the Per mutants, it was realized that Per regulated the accumulation of its own mRNA and the concept of the feedback loop generating overt rhythms of about 24 h was born (Hardin et al., 1990). However, it was not until about 10 years later that the mammalian Per genes were identified and cloned (Albrecht et al., 1997; Shearman et al., 1997; Sun et al., 1997; Tei et al., 1997), and that the function of these Per genes for the mammalian circadian oscillator (at least of Per1 and Per2) was verified genetically by analyzing the corresponding knockout mouse strains (Bae et al., 2001; Cermakian et al., 2001; Zheng et al., 1999, 2001). At about the same time the mammalian transcriptional activators, CLOCK (Gekakis et al., 1998; King et al., 1997) and BMAL1/MOP3 (Bunger et al., 2000; Hogenesch et al., 1998), and a pair of additional repressors, the CRYPTOCHROME (CRY) 1 and 2 proteins were also found (Griffin et al., 1999; Kume et al., 1999; van der Horst et al., 1999). These proteins cooperate and make up the core loop of the molecular oscillator (Fig. 1). As mentioned above, all of the mammalian genes have their counterparts in Drosophila, maybe with the exceptions that the mammalian CRY proteins

![Diagram of the molecular oscillator](image_url)

Fig. 1. The network of the mammalian molecular oscillator. The core loop (red shaded circle), responsible for generating rhythms of about 24 h, is composed of activation of the Per genes by BMAL1 (red) and CLOCK (blue) and the increasing repression of Per gene expression by the accumulation of its own gene product. The feedback inhibition by the PER proteins is delayed by posttranslational modifications and interaction with the CRY proteins (not shown). In the stabilizing loop (blue circle), the Rev-erbα gene is also activated by BMAL1 and CLOCK (not shown) and later on repressed by the PER proteins but immediately REV-ERBα starts to inhibit transcription of the Bmal1 and Clock genes and of its own gene. PER2 can interact with REV-ERBα (or PPARα, not shown) to regulate the Bmal1 gene. The overall organization of the network allows for a tight synchronization of the core and stabilizing loops. Extended and adapted from Ko and Takahashi (2006).
are no (or less) important light-sensitive components of the circadian oscillator (Griffin et al., 1999; Okamura et al., 1999), and the function of the Drosophila Timeless protein (which functions similar as the mammalian CRY proteins) in the mammalian circadian clock is less pronounced (Gotter et al., 2000).

**CRYs or PERs?**

Ever since the discovery of the main repressor molecules of the mammalian circadian oscillator, the question was posed, which of both kinds of molecules were the more important ones. The genetics were not really helpful to unravel the precise functions of these supposed repressor molecules. Mutations that affect the free-running period of the circadian oscillator are thought to be integral parts of the clock mechanism or are affecting its integral parts. Single knockout mice deficient for Per1 (Bae et al., 2001; Cermakian et al., 2001; Zheng et al., 2001), Per2 (Bae et al., 2001; Zheng et al., 1999), or Cry1 (van der Horst et al., 1999; Vitaterna et al., 1999) had shorter free-running circadian oscillators and mice deficient for Cry2 (van der Horst et al., 1999; Vitaterna et al., 1999) had a longer free-running circadian oscillator, while the knockout for Per3 did not affect circadian rhythmicity (Bae et al., 2001). Interestingly, depending on the strain, Per2 single knockout mice had a shorter free-running period length but became arrhythmic after 1–2 weeks in constant darkness, suggesting that PER2 has a more prominent function for the circadian clock than PER1. Hence, most of these individual components have an impact on the clock mechanism. The combination of knockout mice for Per1 and Per2 (Zheng et al., 2001) and the combination of knockout mice for Cry1 and Cry2 (van der Horst et al., 1999) had nearly the same phenotype, yielding arrhythmic behavior immediately after placing the mice in constant conditions. Consequently, both classes of proteins appear to be equally important to maintain circadian oscillator function. Interestingly, the combination of any Per knockout with any Cry knockout yielded a whole plethora of phenotypes ranging from a rescue of the Per2’s arrhythmic phenotype in Per2:Cry2 double knockout mice to completely arrhythmic Per2:Cry1 double knockout mice (Oster et al., 2002, 2003a,b). These genetic interactions suggest that the PER and CRY proteins form multiple different complexes with each other to fulfill their function(s). Interestingly, all of the mammalian clock components are redundant (i.e., there exist Bmal1 and Bmal2, Clock and Npas2, Per1 and Per2, Cry1 and Cry2) (Ripperger et al., 2011). A reason for this is unknown yet. Are there two oscillators running in parallel in the same cell or do the possible combinations mediate, for example, tissue-specific gene regulation? At least for CLOCK and Npas2, it was established that CLOCK may be more important in the periphery, and Npas2 may be more important in the brain (DeBruyne et al., 2007a,b).

**Function of PER proteins on the core loop of the circadian oscillator**

Considering the role of Per within the Drosophila circadian oscillator (Hardin et al., 1990), it was tempting to speculate that the mammalian PER proteins fulfilled a similar function to create a transcriptional feedback loop. However, cotransfection experiments in vitro revealed that, by contrast to the CRY proteins (Kume et al., 1999), the impact of the PER proteins on BMAL1 and CLOCK-mediated transcription was quite subtle (Kume et al., 1999). This was surprising because the biochemical analysis clearly indicated that PER and CRY proteins can be coimmunoprecipitated and consequently they are in the same (repressor?) complex (Brown et al., 2005; Duong et al., 2011; Lee et al., 2001). Indeed, the regions within both classes of proteins mediating the interaction were biochemically mapped (Fig. 2) (Langmesser et al., 2008). Nevertheless, nonrhythmic overexpression of PER2 but not CRY1 strongly interfered with
normal circadian oscillator function, which is a strong indicator for a prominent function of the PER2 protein for the circadian oscillator (Chen et al., 2009). The more detailed analysis suggested that the PER proteins form a kind of scaffold for the CRY proteins to bind to their target sequences in the genome (Chen et al., 2009). Interestingly, both kinds of proteins can interact with other proteins that repress transcription via the modification of the chromatin structure. For CRYs, these include histone deacetylases (HDAC) (Naruse et al., 2004), which attenuate local transcription by removing specific acetyl groups from 5′-termini of histones, and for PER deacetylases (Duong et al., 2011), but also proteins that remove methyl groups from (DiTacchio et al., 2011) or add methyl groups to 5′-termini of histones (Brown et al., 2005; Etchegaray et al., 2006). However, it is necessary to decipher the precise biochemical function(s) and their impact on the molecular oscillator of both classes of repressor molecules in more detail. Taken together, the available literature on the function of PER proteins in vivo and in vitro suggests that it is not the protein itself that acts as a regulator within the circadian oscillator mechanism, but it is the interaction of PER proteins with other proteins, which mediates the effect(s).

Fig. 2. Protein–protein interaction regions of PER2. The protein belongs to the class of PAS domain-containing factors. The PAS A and B domains and the associated PAC domain mediate the interaction with other PER proteins and the BMAL1 and CLOCK heterodimer. The region is flanked by binding sites for the regulatory kinase GSK3β on the left and the ubiquitin ligase β-TrCP on the right. Both enzymatic activities affect the half-life of PER2. The basic helix-turn-helix (HLH) motif resembles a DNA-binding motif but is probably not functional. The protein has three nuclear-export sites (NES), one nuclear-localization site (NLS) and a cytoplasm-localization domain (CLD), which influence the nuclear localization of the protein. Interaction with the CRY proteins (and E4BP4) is mediated by the C-terminus. The protein contains a sequence of casein kinase 1 (CKI) phosphorylation sites organized as a relay, from which the priming site is affected in FASPS. PER2 also harbors some potential motifs, which resemble binding sites for nuclear receptors (LXXLL and CoRNR), but only for the most N-terminal LXXLL motif an interaction with nuclear receptors was identified. Finally, the protein contains potential interaction motifs resembling proline-rich (Pro-rich) motifs and coiled-coil interaction motifs. Interactions with further kinds of proteins have been described, such as with WDR5 (an adaptor for the histone methyl transferase Set1), NONO and SFPQ1, and histone demethylases of the Jarid1 family; however, these were mainly identified in complexes containing also PER1 and a direct interaction has not been demonstrated yet. Adapted from Albrecht et al. (2007).
Role of PER2 in the stabilizing loop

Initially, the characterization of the available circadian oscillator mutant and knockout mice uncovered a surprise (Shearman et al., 2000). The PER2 protein, but not PER1, was suggested as activator of circadian Bmal1 transcription. This puzzle resolved itself about 2 years later, when an additional feedback loop was discovered based on the transcriptional repressor REV-ERBα (Preitner et al., 2002; Ueda et al., 2002). Hence, the model of the circadian transcriptional feedback loop was extended by the stabilizing loop (Fig. 1). In the core loop, BMAL1 and CLOCK activate transcription of the Per genes until the PER proteins (together with the CRY proteins) feed back onto their own synthesis. To achieve this, there is a typical delay in the action of the repressor molecules as a prerequisite for the near 24 h oscillations. In parallel, BMAL1 and CLOCK also activate transcription of Rev-Erbα. The accumulating REV-ERBα subsequently represses transcription of the Bmal1 and also the Clock genes by replacing the transcriptional activator RORα (Akashi and Takumi, 2005; Sato et al., 2004) or PPARα (Canaple et al., 2006), dependent on the cell type, and also by recruiting HDAC3 activity to the target genes (Feng et al., 2011). Because the action of REV-ERBα does not involve a delay mechanism typical for the action of the PER proteins, the phases of transcription of the Per and Rev-Erbα genes on one hand and of the Bmal1 and Clock genes on the other hand are separated by about 12 h. As conclusion, loss of repression of Bmal1 transcription as observed in Per2 knockout mice was probably due to the reduction of REV-ERBα and a concomitant increase in Bmal1 expression. The story became even more complicated about 2 years ago. It was found that PER2 rather than PER1 directly interacted with both REV-ERBα and PPARα, contributing to about 10% of the repression by REV-ERBα and 20–30% of activation by PPARα of the Bmal1 gene in the liver (Schmutz et al., 2010). This interaction was due to a specific amino acid motif in PER2, which resembles a typical nuclear receptor/coregulator binding site (Fig. 2). However, the functional extension of PER2 due to its interaction with nuclear receptors appears not to be restricted to the core circadian oscillator alone (see below).

The rhythmic metabolism of the liver

As revealed by DNA-microanalysis experiments, many metabolic processes in the liver occur rhythmically (Miller et al., 2007; Panda et al., 2002; Storch et al., 2002; Vollmers et al., 2009). At the center of this regulation is glucose homeostasis to keep the blood glucose concentration at a constant level (Lamia et al., 2008). Hence, over the day, the liver has to switch steadily from glucose uptake from the blood and the storage of glucose as glycogen to the mobilization of glucose from its glycogen stores and its secretion into the blood stream. About 15% of the transcripts found in the liver accumulate in rhythmic fashion (Vollmers et al., 2009); however, only about 10% of those rhythmic transcripts can be assigned to the circadian oscillator. This means that the bulk of rhythmic transcripts is induced solely by the presence of their substrates. What is the advantage of either way of regulation by induction through substrates or by the circadian clock? Induction through substrates necessitates the presence of substrates, but the response is normally fairly proportional to the substrate concentration. Hence, it is a quite economical way of gene regulation according to the needs. On the other hand, the circadian clock allows the expression of genes before the substrates become available, obviously with the drawback that the gene is expressed even if the substrates never come. However, the anticipation of daily recurring events may be the main biological advantage of possessing a circadian clock as evidenced by the increase of fitness of cyanobacteria that are in better resonance with the environment. The circadian oscillator provides stable rhythms of about 24 h, but over the course of a
year the photoperiod (i.e., the day/night ratio) changes with an impact on the time when the animal gets active and starts eating (Schultz and Kay, 2003). Consequently, there exist mechanisms in the liver that uncouple anticipation mechanisms from the circadian oscillator to keep the anticipation potential intact (Stratmann et al., 2010). As example, some detoxification enzymes of the P450 family are rhythmically regulated by the circadian transcription factor CAR, which itself is regulated by the circadian transcription factors of the PAR-Zip family (Gachon et al., 2006). The reason may be that those detoxification enzymes, in the absence of their substrates, produce radical oxygen species, which may cause damage to the cell. Hence, basal expression of these enzymes is restricted to a phase when the occurrence of substrates is probable to provide some basic protection. In addition, the genes of these enzymes can be super-induced by the nuclear receptor CAR according to the presence of their substrates. Consequently, the risk of damage to the cell is optimally reduced. The expression of CAR is adjusted to the photoperiod by the PAR-Zip factors, demonstrating that the precise temporal action of these regulators and their target genes relative to the activity phase of the animals is important (Stratmann et al., 2010). In conclusion, temporal organization of metabolic processes in the liver optimizes not only energy expenditure but also protects the organ from excessive damage. The circadian clock represents an elegant means to fulfill such a coordinating function. Interestingly, the analysis of Per knockout mice revealed a function of PER proteins in the concerted action of DNA damage repair enzymes and proto-oncogenes, rendering the Per knockout animals more prone to tumor formation under certain circumstances (Fu et al., 2002).

**Role of PER2 in the liver metabolism**

Many of the metabolic processes in the liver are regulated by members of the nuclear receptor superfamily (Yang et al., 2006). There are 49 members of this family, of which about one half is regulated in a circadian fashion. In essence, rhythmic expression of the transcriptional regulators of the nuclear receptor family suffices to regulate their target genes in a rhythmic fashion. In addition, many of these nuclear receptors require specific ligands for their activity. Some of these ligands are produced and secreted in a rhythmic fashion. Under normal conditions, this system could stably regulate rhythmic processes but appears to be circuitous and inflexible to respond rapidly to changes of the circadian clock. The extended capacity of PER2 to directly interact with these nuclear receptors provides an elegant way to transmit circadian clock information to the nuclear receptor regulated target genes (Fig. 3) (Schmutz et al., 2012). Nuclear receptors involved in the regulation of metabolic pathways and able to interact with PER2 in vitro include REV-ERBα, PPARγ and PPARβ, and HNF4α and affect the lipid and glucose metabolism, respectively. Interestingly, PER2 acts as nuclear receptor coregulator, context-dependent as coactivator or corepressor. However, further experiments are required to fully understand these differences in its action. Nevertheless, PER2 transmits circadian oscillator information directly to nuclear receptor-mediated metabolic pathways.

**Function of PER2 in the brain**

Per2 expression was initially observed in the suprachiasmatic nuclei in the ventral part of the hypothalamus and various other brain regions as well as in peripheral tissues (Albrecht et al., 1997). Because of its inducibility by light, food restriction, and temperature pulses, Per2 was recognized soon as a link between signals from the environment (the input) and the clock mechanism. Per2 acts as a responder to environmental signals and is a component of the core clock mechanism, but it can also affect physiological pathways downstream of the clock (the output, Fig. 3).
Hence, it appeared that *Per2* is an important player in determining the transcription status of the genome in a specific environmental context. Therefore, it was not astonishing to find that *Per2* plays a role in many brain related functions. The reward system regulating addiction appeared to be at least partially affected when the *Per2* gene was mutated (Abarca et al., 2002; Hampp et al., 2008; Spanagel et al., 2005). In particular, it appeared that the rate-limiting step in dopamine degradation involving the monoamine oxidase A was under the control of the circadian clock mechanism including the *Per2* gene (Hampp et al., 2008). In line with this view is the observation that PER2 variation in humans is associated with depression vulnerability (Lavebratt et al., 2010; Partonen et al., 2007). Recent findings indicate that the reward system and addiction processes share neurobiological mechanisms with overeating and obesity (Simerly, 2006). Interestingly, a mutation in the *Per2* gene of mice resulted in the loss of food anticipatory activity, when animals were kept under a timed feeding schedule (Feillet et al., 2006). Taken together, it appears that the *Per2* gene is at the crossroads of the neurobiological circuitry that is common to feeding signals and drugs of abuse. In support of this view is the fact that *Per2* is expressed in regions of the midbrain such as the arcuate nucleus and the ventral tegmental area, which regulate food uptake and reward processing, respectively. Many of the metabolic processes in the brain involve also nuclear receptors such as Nurr1, which can physically interact with PER2 (Ripperger et al., 2010; Schmutz et al., 2010), and hence, brain function is at least partially regulated by this avenue.

**Function of PER2 in sleep**

*Per2* appears to play a role in various parameters of sleep. Loss of *Per2* affected proper sleep timing (Kopp et al., 2002), which is also evidenced in...
advanced sleep-phase syndrome (Jones et al., 1999). Per2 may affect parameters of sleep homeostasis as well (Franken et al., 2007), especially sleep deprivation and recovery sleep appear to involve PER2. However, whether this function of PER2 is only attributable to its role in the brain is not known, because metabolic demands in the liver involving this protein may also impact on sleep mechanisms (Albrecht, 2011). Sleep appears to be regulated by remodeling of neuronal connectivity either strengthening or weakening synaptic connections. The finding that PER2 is involved in gating the light/dark information to vesicular glutamate transporter 1 (vGLUT1) content on synaptic vesicles (Yelamanchili et al., 2006) was of great interest, because the number of vGLUT1 molecules on the synaptic vesicle correlates with the glutamate filling state of this vesicle and hence affects glutamate release potential, which may impinge on synaptic strength. Hence, light may impact via PER2 on brain function and therefore, alterations in lighting schedule as experienced in jet-lag and shift work may affect behavior.

**Posttranslational modifications of PER2**

How is the interaction potential of PER2 with other proteins regulated? One possibility is the accumulation of the PER2 protein over the circadian cycle (Lee et al., 2001). The peaks of transcription and translation of the Per genes are characteristically separated by a couple of hours. In addition, the accumulation of the PER proteins in the nucleus occurs with high amplitude every day and consequently, the interaction of PER proteins with other proteins would follow their affinity of interaction (i.e., first the high affinity binders and then the low affinity binders). Exchange of interaction partners would occur by simple competition mechanisms. This simple affinity model may be extended by posttranslational modifications (Vanselow and Kramer, 2010). In the extended model, the dynamic combination of phosphorylation of the PER2 protein reflects directly oscillator time and each phosphorylation site may represent a docking site for a specific protein. Specific phosphorylation of a particular site is achieved by equilibrium of the action of kinases on one side and of phosphatases on the other side. For PER2, about 21 phosphorylation sites have been identified in fibroblasts although the kinetics of phosphorylation of the individual sites remains to be established (Maier et al., 2009). Some sites when phosphorylated by casein kinase 1 (CK1) δ or ε serve as binding sites for β-TrCP1/2, which add ubiquitination marks to the PER2 protein, rendering it unstable and finally provoke degradation by the proteasome (Reischl et al., 2007; Shirogane et al., 2005). An interesting case is the familial advanced sleep-phase syndrome (FASPS) (Jones et al., 1999). Individuals with specific mutations in either CK1δ (Xu et al., 2005) or the CK1δ binding site on PER2 display sleep rhythms advanced by several hours (Toh et al., 2001) (a similar mutation in CK1ε is provoking the familial delayed sleep-phase syndrome, (Takano et al., 2004)). Detailed analysis revealed that the ratio of kinase to substrate was important for the free-running period length of the molecular oscillator (Xu et al., 2007). On the phosphatase side, regulatory subunits of the protein phosphatase 1 were shown to regulate the nuclear-localization PER2 and the free-running period length (Lee et al., 2011; Schmutz et al., 2011). Are the interactions of nuclear receptors affected by reversible phosphorylation? At the moment, there is no direct evidence in favor of this hypothesis. The interaction motif appears not to be adjacent to a known phosphorylation side. However, it could be that phosphorylation at a distant site affects the accessibility of the interaction motif. As speculation, the region between the two PAS domains is flexible to dynamically shift from a conformation facilitating interaction with BMAL1 and CLOCK to another, which confers interaction with nuclear receptors. Phosphorylation mediating interaction with PER2 could also be present on the nuclear receptors, possibly regulated by the presence of specific ligands.
Conclusions

Synchronization of the individual branches of the circadian oscillator and the different routes of input and output might necessitate more effort than simple transcriptional feedback loops can provide. Temporally controlled protein–protein interactions enable the coupling of the high-precision timer of the circadian clock to, for example, nuclear receptor-target genes. The high flexibility of these interactions allows PER2 to act as coactivator or corepressor depending on the regulatory context and hence to fine-tune the activity of these nuclear receptors. To fully understand the interaction potential of PER2 and its regulation by posttranslational modifications, however, requires further experiments. These interactions may form the base of the many additional functions of PER2 in the metabolism and the brain. Other members of the core circadian oscillator can interact with a variety of other proteins and regulators, and it is tempting to speculate that the overall function(s) of these interactions are similar to those of PER2. However, it is also tempting to speculate that PER2 can directly interact with many more classes of transcriptional regulators.

Acknowledgments

We thank for the support of our laboratory by the Swiss National Science Foundation.

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