Circadian Gene Expression in the Suprachiasmatic Nucleus

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Introduction

Regulated expression of a core set of clock genes is the basis of circadian timekeeping in vertebrates, as in other organisms. Clock genes are defined as being necessary to generate normal circadian rhythms, near 24 h oscillations in biological processes. With the enlargement of the brain in vertebrates, control of circadian rhythms became centralized. In lower vertebrates, such as fishes, lizards, and some birds, the pineal gland, near the surface of the brain, is directly photosensitive and exerts significant circadian control. In mammals, the pineal is overlain by enlarged cerebral hemispheres and is no longer photoreceptive. The central circadian clock of mammals resides within the suprachiasmatic nucleus (SCN) of the hypothalamus.

Clock genes are under study in the range of vertebrate taxa, but have been most fully studied in the mammalian SCN. Rhythmic expression of clock genes within the SCN is required for the production of circadian rhythms throughout the brain and body. Circadian clock genes also are expressed rhythmically in the cells of most tissues. Clock gene expression modulates myriad processes that oscillate daily at levels from behavior to tissue-specific cellular function. States of sleep and arousal, release of neuroendocrine hormones, autonomic control of organs, contractile strength of muscle, and production of liver enzymes all oscillate with circadian rhythmicity. Regulated clock gene expression within the SCN enables this central clock to act as the conductor to the orchestra of local body clocks. In concert, these circadian clock-driven oscillations enable the organism to anticipate predictable changes in the environment, to coordinate diverse internal processes, and to align itself adaptively with the major variable in its environment, the cycle of night and day.

Circadian rhythms emerge from cell-autonomous processes, posttranslational modifications (PTMs) of clock proteins that regulate transcriptional-translational feedback loops of clock genes. These cellular processes together form a dynamic timekeeping mechanism with a near-24 h period. Interlocking positive and negative feedback loops wherein clock proteins alternately activate or repress transcription is the apparent basis of the circadian clocks in plants and diverse animals, including mammals. Key to the oscillatory process is the tight regulation of proteins encoded by clock genes; during their lives within the circadian cycle, clock proteins associate dynamically with other clock proteins to form macromolecular assemblies, move between different subcellular compartments, and undergo extensive changes in posttranslational state and stability.

The regulated expression of clock genes and proteins underlies the three primary components of the circadian system as it functions within the organism: the central clock, which generates the circadian time base in the SCN; input pathways, by which light entrains the SCN clock to the day–night cycle; and output pathways from the SCN, which transmit timing information beyond the central clock.

1. Within the central clock, clock genes and proteins form the core timekeeping mechanism. Some clock proteins are transcription factors that regulate their own transcription and that of other clock genes. Others are kinases, enzymes that add phosphate groups, reversible PTMs that profoundly alter the functioning and associations of substrate clock proteins.
2. The input pathways, which transmit signals to the SCN, adjust and align the clock's time base. This requires that incoming signaling pathways engage the molecular clockwork. Thus, neurotransmissions to the SCN initiated by light at the retina must activate signal transduction events that target clock proteins and genes.
3. The output pathways, which transmit signals from the clock, are initiated by clock proteins via effects on transcription of genes that are not part of the timekeeping mechanism. These clock-controlled genes enable the central clock to communicate time-of-day information to the brain and body. Outputs include neural and humoral signals from the SCN that are regulated by the clock and bear time-of-day information to peripheral clocks. There, clock proteins drive the expression of tissue-specific output genes throughout the genome. This enables peripheral clocks in the diverse cells of the body to regulate local circadian functions in tissue-specific ways.

This review evaluates present understanding of the expression of circadian clock genes in the mammalian SCN. This is an area of rapid flux; insights are still developing. Extrapolations have been made from the well-studied *Drosophila* model, but must be regarded cautiously, with the recognition that functional differences in some clock elements are emerging. We consider (1) putative circadian clock genes, (2) SCN clockwork mechanisms, (3) the structural and

functional features of clock proteins, (4) patterns of clock gene expression in the SCN, (5) the regulation of clock mRNA/protein expression and function, and (6) the effects of light.

Putative Circadian Clock Genes

Cross-species genomic comparisons of putative clock genes suggest striking similarities in the molecular circuits within timekeeping mechanisms across diverse circadian clockworks. The regulatory circuits are proposed to comprise interacting proteins – transcription factors together with their modifying kinases and phosphatases. These clockwork elements form similar transcriptional-translational feedback loops in phylogenetically distant organisms. Elements of such molecular circuits have been found in unicellular photosynthetic blue-green algae (*Cyanobacteria*), the bread mold (*Neurospora*), the fruit fly (*Drosophila*), the flowering plant (*Arabidopsis*), a number of rodents (rat, hamster, and mouse) and humans (*Homo*). Within animal lineages, the principal clock genes are largely conserved. Where differences appear, they can be traced deep into basal lineages; some clock genes are more similar between mouse (*Mus*) and honeybee (*Apis*) than between bee and fruit fly. This indicates that circadian clocks are ancient and that clockwork elements have been under high selective pressure to be conserved during evolution.

Circadian clock genes are identified by phenotypic profiles as those genes necessary for the generation of circadian rhythms. Key loci are defined by gene mutations, genetic deletions, or molecular manipulations that alter the period by $\geq$3 SD (standard deviations) from mean wild-type rhythms or completely abolish circadian rhythms (Table 1). Although our understanding is growing, the roles of putative clock elements in circadian rhythm generation are less well-established in mammals than in more tractable genetic models, such as *Drosophila*. Orthologs of some fly clock genes exist in mammals and probably perform similar roles in timekeeping. However, mammals may express multiple paralogs, distinct genes with high sequence similarity, probably evolved from a single ancestral gene. For example, *Drosophila Period* (*dPer*), the first animal clock gene identified, exists as a single gene, an ortholog of three paralogous mammalian *Period* genes (*Per1, 2, 3*). Of the three, *Per2* is thought to function as the core clock element, whereas *Per1* probably acts in the input pathway and *Per3* contributes to output functions. Other putative mammalian clock genes are ancestrally distant from those in the fly. New evidence indicates that mouse *Cryptochrome* (*mCry*) *and Timeless* (*mTim*) genes are divergent in sequence and expression pattern from related genes in *Drosophila* but are similar to *Apis*. These differences complicate deciphering timekeeping circuits in mammals.

A working model for the SCN clock has been developed based on overall similarities to the fly model of the structure and regulatory features of putative mammalian clock genes and proteins, their temporal expression patterns, and their functional characteristics. The circadian clock in the SCN is predicted to emerge from interactions among 10 transcription factors encoded by putative clock genes: *CLOCK* (*Clk*), *Bmal1/Mop3* (hereafter, *Bmal1*), *Period* (*Per 1, 2, 3*), *Cryptochrome* (*Cry 1, 2*), *Timeless* (*Tim*),*Rev-erbα* (orphan nuclear receptor), and *Ror* (retinoid orphan receptor). Two putative clock genes encode kinases: *casein kinase I epsilon* (*CKIε*) and *glycogen synthase kinase 3 beta* (*GSK3β*). No phosphatases have yet been identified as mammalian clock elements; however, the strong evidence for clock genes encoding protein kinases in mammals predicts that counterbalancing phosphatases also form the core clock mechanism. An additional feature is that clock elements must be expressed appropriately in temporal and spatial domains. Thus, present models are incomplete and limited by our knowledge, but they are

Table 1 Mutation phenotype and SCN expression patterns of clock genes

Gene	*Mutation phenotype*	*Peak mRNA expression*	*Peak protein expression*
Clock	Longer period (+4 h); arrhythmicity in DD	Constitutive	Constitutive
Bmal1/Mop3	Arrhythmicity in DD	CT 15–18	CT 0–8
Per1	Shorter period (0–1.1 h)	CT 4–6	CT 10–14
Per2	Shorter period (−1.5 h), arrhythmicity in DD	CT 6–12	CT 10–14
Per3	Shorter period (0–0.5 h)	CT 4–9	CT 10
Tim	Homozygous embryonic lethal; antisense ODN abolishes circadian rhythm in SCN	CT 8–12	CT 10–16
Cry1	Shorter period (−1 h)	CT 8–12	CT 12–18
Cry2	Longer period (+1 h)	CT 8–16	CT 12–16

DD, constant darkness; ODN, oligodeoxynucleotide; SCN, suprachiasmatic nucleus.

useful for conceptualizing this complex dynamic process.

Thus far, relatively few clock components have been found in mammals. We might ask if there are new clock components to be discovered: doubtless there are. New candidate clock genes are being reported as potential components of core clock mechanisms. In addition, microarray data have shown that approximately 10% (hundreds of genes) of all detectable transcripts in mice tissue express circadian patterns. Large-scale genomic approaches will likely reveal new candidate genes for testing functionally, which will no doubt cause us to revise core clock mechanisms.

Suprachiasmatic Nucleus Circadian Clockwork Mechanisms

Fundamental to circadian clocks is that different sets of clock proteins act as positive and negative regulators of clock gene expression (Figure 1). This relationship sets up positive and negative feedback loops, dynamic relationships that characterize biological oscillators of many types. Because circadian clock gene expression requires regulating multiple transcriptional, translational, posttranslational, and translocational processes, the time domain of the circadian clock is long relative to other biological oscillators.

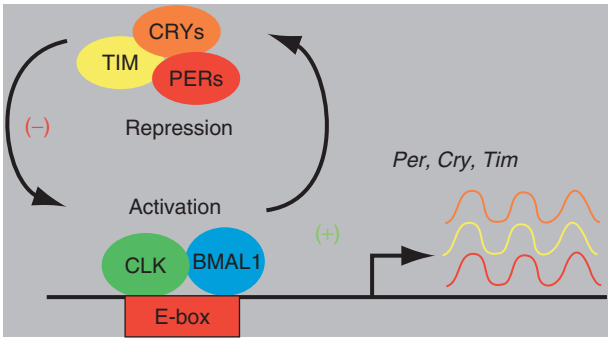

Figure 1 A model of positive and negative transcriptional feedback loops formed by clock proteins within cells of the SCN. When CLK and BMAL1 together bind to E-box sequences in promoters of other genes, they activate transcription. This forms a positive loop, enhancing transcription. In this model, they activate transcription at *Per, Cry,* and *Tim* genes producing mRNAs (small oscillatory lines). These mRNAs are translated in the cytoplasm into PER, CRY, and TIM proteins, which eventually translocate to the cell nucleus. This process is thought to be driven by posttranslational modifications (PTMs) and protein–protein interactions that stabilize the proteins and favor nuclear localization. Within the nucleus, PER, CRY, and TIM associate with the CLK-BMAL1 complex at E-boxes of the *Per, Cry,* and *Tim* gene promoters. This represses transcriptional activity at these genes, closing the negative loop of clock gene regulation. The affinity of the complex changes the association of PER, CRY, and/or TIM, which dissociate from the complex. Upon release, they may be ubiquinated and then degraded. This tips the balance toward the positive feedback loop and the cycle repeats. The period of each cycle is ~24 h.

The proposed mammalian clockwork mechanisms follow. The protein products of the putative clock genes *Clk* and *Bmal1,* CLK and BMAL1, respectively, regulate a positive feedback loop. Under appropriate conditions, these proteins dimerize, translocate to the cell nucleus, bind to E-box (CACGTG)-containing *cis*-regulatory sequences in target genes and initiate transcription. BMAL1 transcriptional activation requires a coactivator, acetylate 3. Recently, CLK itself was found to be a histone acetyl transferase. Among the genes that CLK and BMAL1 activate are the putative clock genes of the *Per* and *Cry* families. In *Drosophila,* target clock genes include *Timeless* (*dTim1*), but it is not known whether they activate mammalian *Timeless* (*mTim*).

Transcription-translation of the *Per* and *Cry* genes initiates a negative feedback loop. PER and CRY accumulate in the cell cytoplasm for part of the circadian cycle. Conformational changes induced by protein–protein interactions and/or PTMs expose nuclear localization sequences (NLS) and target the cell nucleus with these proteins. Nuclear transport/shuttling mechanisms for clock proteins are not understood. Within the nucleus, PER and CRY form multimeric complexes with CLK:BMAL1, still bound to the E-boxes in *Per* and *Cry* promoters. This leads to a reduction in histone acetyltransferase activity, which decreases histone H3 acetylation and transcriptional activation, thereby inhibiting the positive loop. This important control point leads to the cessation of production of mRNA from the *Per* and *Cry* genes, and their proteins are degraded. This closes a negative feedback loop. The loss of CRY and PER proteins poises the system to reenter the positive loop phase. TIM can interact with PER1 to inhibit CLK:BMAL1-activated transcription in cultured cells. Although there is evidence that TIM is necessary for SCN circadian rhythms, it has not yet been shown to act within the negative loop in the SCN clock.

A second bidirectional set of feedback loops is present in mammals, as in flies. These positive and negative loops center around the regulation of and by an orphan nuclear receptor, REV-ERBα. In the positive loop, CLK:BMAL1 binding at an E-box enhancer of the *Rev-erbα* gene activates transcription. The resulting protein, REV-ERBα, initiates the negative loop by binding to retinoic acid-related orphan receptor response elements (ROREs) in the promoter of *Bmal1,* and possibly *Clk* and *Cry1*, thereby repressing transcription. PER:CRY complexes also feedback negatively on the CLK:BMAL1 complex at the E-box site, inhibiting transcription of *Rev-erbα*. As levels of REV-ERBα, decrease, ROR is thought to displace

REV-ERBα on RORE sites, turning on *Bmal1* transcription. Rising levels of PER and CRY again lead to increased BMAL1 expression by repressing *Rev-erbα*, completing an oscillatory loop. These loops are thought to enhance the stability and robustness of the circadian oscillator.

Transcriptional-translational feedback loops do not alone account for an oscillatory cellular clockwork. The critical drive for the dynamics of temporal and spatial oscillation in clock-protein transcription factors resides in their PTM. PTMs can include phosphorylation/dephosphorylation, limited proteolysis, ubiquitination, sumoylation, methylation, and glycosylation. PTMs of clock proteins cause conformational changes in three-dimensional protein states that determine key events in the life of the protein: subcellular localization, intermolecular interaction, translocation, and degradation. Each clock protein is likely to have multiple effectors of PTM. PER2 is phosphorylated both by CKIε and GSK3*β*. Mutation within the CKIε recognition site of PER2 significantly alters circadian behavioral rhythms, including human sleep–wake cycles. GSK3*β* is expressed in the SCN and liver of mice, where it exhibits robust circadian oscillation in phosphorylation state, and it phosphorylates PER2 in COS1 cells. Doubtless additional clock genes will be identified that regulate PTM of other clock proteins.

Structural and Functional Features of Clock Proteins

Structural motifs and functional domains of clock proteins are substrates for protein–protein interactions, PTM, and regulation of subcellular distribution (Figure 2). PERs are the most thoroughly studied of the core clock components. In dPER, intermolecular interaction of the two PAS domains (PAS-A and PAS-B) is necessary for PER homodimer formation. All three mammalian PERs contain the PAS-A and PAS-B dimerization domains, nuclear export signal (NES) domains, and CKIε-binding motifs. Studies of a natural period variant in hamster, *tau*, found that it encodes an altered CKIε. CKIε regulates protein stability and subcellular localization of PER. Substituting serine for glycine in the CKIε substrate site of human PER2 shortens the circadian period, possibly by altering degradation or accelerating nuclear entry. CKIε actively regulates the intracellular location of PER by masking or unmasking the nuclear localization signal (NLS) domain, which is adjacent to the CKIε-binding motif in all three PERs. PERs also contain cytoplasmic localization domains (CLD), which are mediators of nucleo-cytoplasmic shuttling. CLDs of PER1 and PER2 are suggested to be nonfunctional. Indeed, mPER2 shows predominantly nuclear staining in the SCN.

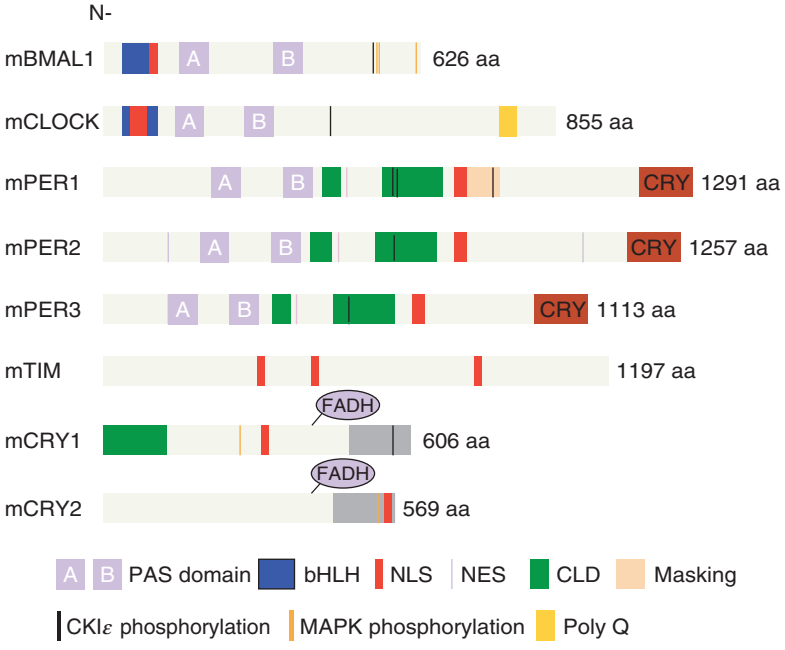

Figure 2 Schematic representation of structural and functional features of mouse clock proteins. A and B represent the PAS-A and PAS-B domains, respectively, sites of protein–protein interactions that enable the formation of dimers and macromolecular assemblies. bHLH, basic helix–loop–helix DNA-binding domains; CKI, casein kinase Iε recognition motif; CLD, cytoplasmic localization domain; CRY, cryptochrome-binding domain; NES, nuclear export signal; NLS, nuclear localization signal; PAS, Period-Arnt-Single-minded transcription factor domain; Poly Q, poly-glutamine-rich sequence.

CRY1 and CRY2 contain N-terminal chromophore-binding domains but exhibit structural divergence in C-terminal extension domains. This suggests that C-terminal domains have unique functions in each CRY. CRY acts as a repressor by directly interacting with the CLK-BMAL1 heterodimer in the nucleus. Thus, the nuclear localization and interaction with this heterodimer are essential for CRY function. An NLS has been found in the N-terminal chromophore-binding sequence in CRY1 and a functional NLS was identified in the C-terminal extension domain in CRY2. How CRY localization is regulated is unknown, but it is likely that PER-binding unmasks the NLS or masks the CLD of CRY.

Intracellular distribution and PTM are also affected by the interactions of PERs with CRYs. The two PAS domains are required for PER–PER and PER–CRY dimerization. PER–CRY dimerization appears important for the nuclear accumulation of both proteins; *Cry1*/*Cry2* double-mutant mice show weak nuclear localization of PER1 and PER2. Moreover, PER–CRY dimerization prevents their degradation through the ubiquitin–proteasome pathway.

CLK and BMAL1 proteins each have one basic helix–loop–helix (bHLH) DNA-binding domain and two PAS dimerization domains. CLK and BMAL1 also contain conserved NES and NLS domains, adjacent to the PAS-A and bHLH domains, respectively. CLK may be regulated by protein kinase G type II (PKG-II) at the end of the night, when they co-immunoprecipitate from SCN extracts. BMAL1 is phosphorylated *in vitro* by two kinases, CKI and mitogen-activated protein kinase (MAPK). MAPK phosphorylates Ser-527, Thr-534, and Ser-599 in BMAL1. A conserved CKI substrate consensus motif exists in the C-terminus of BMAL1, but phosphorylated residues have not been determined. These two kinases may compete to regulate BMAL1 function because their phosphorylation motifs overlap. Transactivation studies support this hypothesis – MAPK-mediated phosphorylation decreases CLK–BMAL1 transcriptional activity, whereas CKI-mediated phosphorylation induces it.

Patterns of Clock Gene Expression in the Suprachiasmatic Nucleus

Temporal patterns of putative clock mRNA and protein expression have characteristic relationships to phases of the day–night cycle (Figure 3). Most show oscillatory circadian patterns that map to specific phases of the ~24 h cycle. Time of the SCN clock in animals under light–dark conditions is expressed as zeitgeber time (ZT) because the regular daily appearance of light acts as a time-giver. Subjects usually are entrained to a 24 h cycle with 12 h of light (day) and 12 h of darkness (night). Under constant environments, including darkness, phase-relationships persist. The period of these clock-driven oscillations is *circa*, not exactly, 24 h. Reckoning circadian time (CT) includes correcting for the non-24-h period.

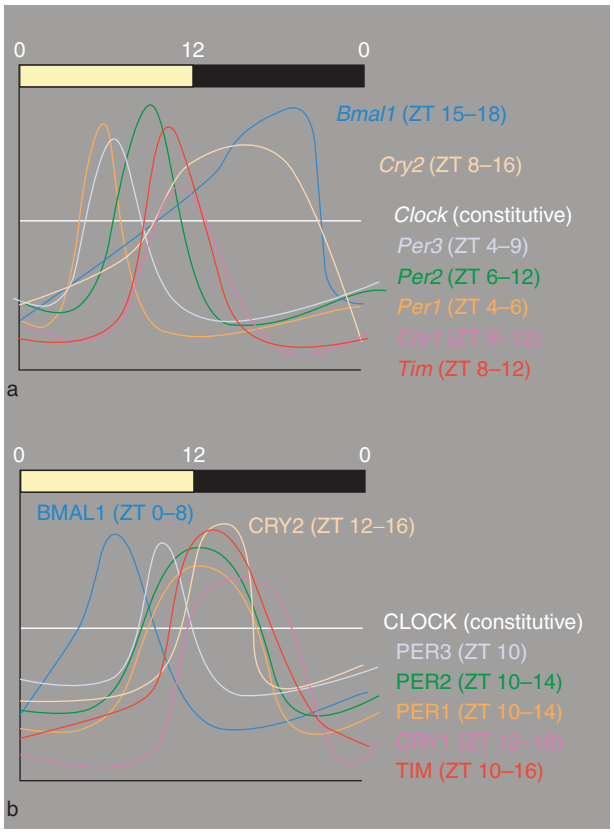

Figure 3 Schematic of circadian expression patterns in the SCN: (a) clock gene mRNAs; (b) clock proteins. Relative mRNA and protein levels of clock gene transcription factors oscillate over the circadian cycle. An exception is those products of the gene *Clock*, which are constitutive. The mRNAs of negative elements are generally high in the daytime; transcripts of *Bmal1*, a positive element, peak in the nighttime. The mRNAs of most clock genes are nearly immediately translated, so the oscillations in clock proteins are offset from mRNA rhythms by ~4 h. BMAL1 peaks midday, whereas proteins of negative elements peak from late day to early night. Horizontal bars: yellow indicates daytime (ZT 0–12 h); black indicates nighttime (ZT 12–24 h).

Proteins of the positive loop exert primary influence during the daytime. In anticipation, *Bmal1* mRNA increases during the nighttime (CT 15–18), peaks just before dawn (CT 23), and then drops sharply. BMAL1 protein is present in the first half of the day (CT 0–8). *Clk* mRNA and protein are constitutively expressed in the SCN. For CLK and BMAL1 to dimerize, they need to be in the same physical space. Dimerization dynamics are unknown; however, shortly after BMAL1 starts to accumulate, mRNAs of the elements of the negative loop appear (Figure 3). This is

consistent with a model wherein CLK–BMAL1-driven transcription forms the positive loop, activating the transcription of clock genes in the negative loop (Figure 1).

Daytime activation of transcription of elements of the negative loop imposes critical negative feedback that enables the oscillation. The transcription of *Per, Cry,* and *Tim* peaks during the mid- to late circadian day, in antiphase to the *Bmal1* peak (Figure 3). *Per* mRNAs and proteins exhibit robust circadian patterns, with protein lagging mRNA rhythms by ~4 h. Proteins of the negative loop – PER 1, 2, and 3; CRY 1 and 2; and possibly TIM – appear in the late day, with most peaking near the day–night transition. PER-CRY heterodimers are reported to accumulate in the nucleus at night, when they are predicted to interact with CLK–BMAL1 to repress *Per* and *Cry* transcription. However, most *Per* and *Cry* mRNA appears to decrease in the second half of the day. This inconsistency has not been resolved, but it may reflect sampling methods.

Within the SCN, clock gene expression varies regionally. Regions are distinguished as (1) the ventromedial core, receiving inputs from the retina and other afferent regions and expressing vasoactive intestinal peptide (VIP); and (2) the dorsomedial shell, projecting from SCN to the nearby hypothalamus and expressing arginine vasopressin (AVP). In mouse, *Per1* mRNA undergoes circadian oscillation in the shell region, preceding shell *AVP* mRNA rhythms by ~4 h. PER2 rhythms in diurnal rodents peak between ZT 10–18 in the dorsal, ventral, and lateral regions but not centrally. At trough times, PER2 is primarily in the dorsal SCN. PER1 is more widely distributed at its peak, but, as with PER2, it is less dense in the core.

Regulation of Clock mRNA and Protein Expression and Function

Dynamic regulation of clock gene mRNA and protein state is necessary to generate ~24 h oscillations. Regulation of mRNA may include RNA processing (capping, splicing, and polyadenylation), export, decay, and translation, as well as alternative transcriptional start sites. Changes in RNA processing could change clock state by altering the RNA expression pattern or RNA stability. Multiple transcriptional initiation sites and splice variants have been identified for mammalian *Bmal1* and *Bmal2*. Human BMAL2 isoforms exhibit differential transcriptional activity *in vitro*, suggesting that alternative splicing may regulate clock function. In addition, *Tim* may have multiple splice forms because multiple transcript and protein species have been reported.

Protein state is regulated by PTM and protein–protein interactions. Of possible PTMs that can regulate proteins, phosphorylation has been studied most. Changing the equilibrium between phosphorylation–dephosphorylation states may be a key determinant of clock proteins by altering (1) stability in the cytoplasm, (2) transcriptional activator-repressor activity, and (3) nuclear entry. CKIε–mediated phosphorylation of PER2 affects its stability. The phosphorylation state increases PER turnover by altering the rate of ubiquitination, which targets proteins for degradation. In addition to stabilizing clock proteins, phosphorylation is important for regulating transcriptional activator–repressor activity. In cultured mammalian cells, repressing CKI expression decreases BMAL1-dependent transcriptional activity at the *Per1* promoter. MAPK also alters the BMAL1-dependent transcription. Not surprisingly, dephosphorylation has been reported to impact activity of dPER; increased phosphatase PPA2 activity leads to stable, constitutive nuclear expression, but has not been examined in mammals. Nuclear localization of PER2 is affected by CKIε-mediated phosphorylation. In COS1 cells, GSK3β also promotes nuclear translocation of PER2. Thus, these kinases may converge on PER2 in regulating nuclear translocation.

The timing of phosphorylation and oscillating protein–protein interactions shape the period length of circadian clock. PER proteins show similar circadian phosphorylation patterns, with peak phosphorylation levels near CT 21, when most of the PER is nuclear. Phosphorylated CLK is detectable in the nuclear fraction throughout the circadian cycle, with hypophosphorylated forms in the nucleus during the day. A similar pattern of phosphorylation is detected for BMAL1. At the time of negative transcriptional feedback, phosphorylated forms of mammalian clock proteins form complexes in the nucleus, but the significance of nuclear phosphorylated forms of clock proteins is unclear. CKIε is at least partly involved in regulating the nuclear stability of PER proteins and the transcriptional activity of BMAL1.

Protein–protein interactions can regulate receptiveness to PTMs that alter clock protein localization and state. PER appears essential to controlling the subcellular distribution and CKI-dependent phosphorylation of CRYs. CKI and CRYs do not initially bind directly. Rather, both bind to PER simultaneously to form a ternary complex in which CKI can phosphorylate CRY1 or 2. Additional PTMs may be contributed by other kinases. A recent report showed that MAPK directly interacts with and phosphorylates CRY1 and CRY2 *in vitro*, and suggested that MAPK-mediated phosphorylation reduces the repressor activity of CRY.

Regulation of Clock mRNA and Protein Expression by Light

The cell-autonomous SCN clock repeats each cycle in about 1 day. Over successive cycles, the circadian clock drifts away from the 24 h solar cycle on earth. When this desynchronization causes daytime light signals to impinge on the nighttime state of the SCN, the time base of the clock adjusted, or reset, realigning the internal state with the external environment. This process requires that the signaling pathways that transduce neurochemical signals of light from the retina to the SCN engage the molecular clockwork. Light signals in early and late night cause the delay and advance of clock phasing, respectively. In rats, *Per1* mRNA increases in the SCN at 60 min after the light pulse at either time. *Per2* mRNA induction can be observed after light exposure at early, but not late, night. Functionally, light-induced phase delays are attenuated by the co-injection of antisense oligodeoxynucleotide (αODN) to mRNA for *Per1* or *Per2*, whereas only *Per1* αODN blocks phase advance. This indicates that the expression of both these genes is necessary for phase delay of the clock mechanism, but only *Per1* is necessary for phase advance.

Nocturnal light exposure causes changes in PER levels in mouse SCN. PER1 and PER2 increase within 4–6 h after brief light pulse in the early night, when light signals delay clock phase, effectively extending the clock reckoning of daytime. However, during late night, only PER1 expression increases significantly. Light in late night signals an early morning, advancing the clock phase. PER1 peaks ~4 h after this light signal and also early in the next cycle, at 10 h post-stimulus, rather than the usual time, ~CT 12. This interval reflects the amplitude of the advance in clock phase. These data support the hypothesis that both PER1 and PER2 are important for phase delay, whereas only PER1 contributes to phase advances. However, questions remain as to how the light-induced increase in PER affects the negative transcriptional loop and what roles other clock proteins might play.

Light-stimulated changes in regional distributions of PER1 and PER2 within the SCN follow their respective mRNAs and depend on the circadian time of light pulse administration. After a phase-delaying light pulse in early night, the number of PER1-positive cells increases primarily in the ventrolateral core region, whereas PER2-positive cells show greatest increase in the shell. After a phase-advancing light pulse, PER1 expression increases in both the core and shell regions, without significant PER2 increase in either core or shell region. Thus, increased PER2 expression in the shell SCN region correlates with phase delay, whereas the induction of PER1 in the shell correlates with phase advance. Effects of light on other clock elements have not been established.

Summary

Circadian rhythms in mammals are orchestrated by a central clock in the suprachiasmatic nucleus (SCN). The clock emerges from cell-autonomous processes. Clock proteins cause the rhythmic expression of clock genes as well as output genes. Structural features and posttranslational modifications of clock proteins determine their intermolecular associations, subcellular localization, transcriptional activity, and stability. Some clock proteins positively and negatively regulate their own rhythmic expression via feedback on transcription. Patterns of clock gene expression within SCN cells and tissue are complex. The understanding of mammalian clock mechanisms critical to timekeeping is as yet incomplete.

See also: Circadian Oscillations in the Suprachiasmatic Nucleus; Circadian Regulation by the Suprachiasmatic Nucleus; Circadian Metabolic Rhythms Regulated by the Suprachiasmatic Nucleus; Circadian Genes and the Sleep–Wake Cycle; Clock Gene Regulation of Endocrine Function; Clock Genes and Metabolic Regulation; Genetic Regulation of Circadian Rhythms in Drosophila; Sleep and Waking in Drosophila.

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