Circadian rhythms: molecular basis of the clock
Lisa D Wilsbacher* and Joseph S Takahashit

Much progress has been made during the past year in the molecular dissection of the circadian clock. Recently identified circadian genes in mouse, Drosophila, and cyanobacteria demonstrate the universal nature of negative feedback regulation as a circadian mechanism; furthermore, the mouse and Drosophila genes are structurally and functionally conserved. In addition, the discovery of brain-independent clocks promises to revolutionize the study of circadian biology.

Introduction
A major goal in the study of circadian biology is to elucidate the molecular mechanisms governing the circadian clock. To date, genetic approaches have yielded the most success in this endeavor: the period (per) and timeless (tim) genes in Drosophila, the frequency (frq) gene in Neurospora, and the Clock gene in the mouse were each cloned following mutagenesis screens for altered circadian phenotypes (Table 1) [1–3]. Studies in Drosophila on circadian mutants that either shorten (per–) , lengthen (per+) or abolish (per0 , tim0) rhythms in behavior and eclosion have provided a compelling molecular model of rhythmicity in that animal (reviewed in detail in [3] and [4] but only briefly below, as space restrictions prevent extensive description and referencing). The per and tim genes oscillate in mRNA expression, protein abundance, and protein localization [5–10]. The periods of these oscillations depend upon the per and tim alleles (i.e. the molecular period matches the behavioral period in a mutant per or tim genetic background). In addition, PER overexpression from an inducible promoter inhibits the endogenous per mRNA rhythm [11]. These observations indicate that the per and tim gene products regulate their own transcription. A ~6 hour delay between transcription and translation occurs in both gene products [6–10] and, furthermore, nuclear localization of PER and of TIM is blocked in tim0 and per0 flies, respectively [8,9]. PER physically interacts with TIM via its PAS domain [11] both in vitro [12] and in cell culture [13]. Finally, light degrades the TIM protein, which provides a mechanism for photic entrainment of the PER–TIM molecular cycle [8–10,14]. Integration of these observations results in the following model (Figure 1): the per and tim genes are transcribed during the subjective day (peak at circadian time [CT] 12–14) (Figure 1a); PER and TIM accumulate slowly until a threshold level of TIM is reached and stabilizes PER (Figure 1b,c). PER–TIM dimers enter the nucleus around CT 21 and inhibit transcription of their own genes (Figure 1d,e), but as the proteins turn over, inhibition is released and transcription begins again in the subjective morning (Figure 1f). Light-induced degradation of TIM during the early subjective night or the late subjective night decreases PER stability, which results in a phase delay or a phase advance in the cycle, respectively.

The current Neurospora model is analogous to that of Drosophila [15]. Similar to PER and TIM, FRQ appears to inhibit its own transcription [16]. In contrast to Drosophila, however, peak frq expression occurs during the day (CT 4–6), and light strongly induces frq transcription [16,17]. These differences indicate that while the negative feedback mechanism of circadian rhythmicity appears to be conserved, the required genes and regulatory pathways may differ from species to species.

In the past year, remarkable progress has been made in discerning the elements of the clock mechanism. Identification of positive elements (i.e. factors which activate rather than inhibit) allows the formal testing of the feedback loop model, and other new circadian genes provide additional information about the regulation of rhythmicity (Table 1). The discovery and functional analyses of these genes, comparison of circadian organization among divergent species, and new approaches in circadian biology are addressed in this review.

Closing in on closing the loop: new clock component genes and new aspects of circadian regulation
The mammalian clock
Molecular analysis of mammalian clock components began in earnest with the identification of the mouse Clock gene [18**]. Clock was cloned by rescue of both the period length and period stability mutant phenotypes using a Clock-containing bacterial artificial chromosome transgene [18**]. Sequence analysis indicated that Clock encodes a putative bHLH-PAS domain transcription factor [19**]. This finding raised the intriguing possibility that CLOCK could act as a positive element within a transcription-translation negative

Abbreviations
bHLH basic helix-loop-helix
Cry Cryptochrome
CT circadian time
DBP D-binding protein
dbt double-time
frq frequency
PAS PER ARNT SIM
per period
SCN suprachiasmatic nuclei
tim timeless
Table 1

Circadian clock gene expression, function and effects 1*.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Circadian phenotype</th>
<th>Circadian expression(^\d)</th>
<th>Acute light response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mammals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clock</td>
<td>28 hour period, arrhythmicity</td>
<td>No</td>
<td>n.d.(^2)</td>
<td>[18(^<em>),19(^</em>),21(^*)]</td>
</tr>
<tr>
<td>Per1</td>
<td>n.d.</td>
<td>Yes</td>
<td>mRNA</td>
<td>[21(^<em>),22(^</em>),26(^*)]</td>
</tr>
<tr>
<td>Per2</td>
<td>n.d.</td>
<td>Yes</td>
<td>mRNA</td>
<td>[23(^*)]</td>
</tr>
<tr>
<td>Per3</td>
<td>n.d.</td>
<td>Yes</td>
<td>No</td>
<td>[24(^<em>),25(^</em>)]</td>
</tr>
<tr>
<td>Bmal1</td>
<td>n.d.</td>
<td>No</td>
<td>n.d.(^3)</td>
<td>[25(^*)]</td>
</tr>
<tr>
<td><strong>Drosophila</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>per</td>
<td>Many alleles, including 16 hour period, 19 hour period, 28 hour period, arrhythmicity</td>
<td>Yes</td>
<td>No</td>
<td>[3(^*)]</td>
</tr>
<tr>
<td>tim</td>
<td>Arrhythmicity</td>
<td>Yes</td>
<td>Protein degradation</td>
<td>n.d.(^4)</td>
</tr>
<tr>
<td>dClock (Jrk)</td>
<td>Arrhythmicity; low per, tim expression</td>
<td>Yes</td>
<td>(light-dark cycle)</td>
<td>n.d.(^5)</td>
</tr>
<tr>
<td>cycle (dymbal)</td>
<td>Arrhythmicity; low per, tim expression</td>
<td>n.d.</td>
<td>n.d.(^5)</td>
<td>[37(^<em>),38(^</em>)]</td>
</tr>
<tr>
<td>double-time</td>
<td>18 hour period, 27 hour period, arrhythmicity</td>
<td>No</td>
<td>No</td>
<td>[40(^<em>),41(^</em>)]</td>
</tr>
<tr>
<td><strong>Neurospora</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>frq</td>
<td>Many alleles, including short period, long period, arrhythmicity</td>
<td>Yes</td>
<td>mRNA</td>
<td>[15(^*)]</td>
</tr>
<tr>
<td>white collar-1</td>
<td>Arrhythmicity; low frq expression</td>
<td>n.d.</td>
<td>n.d.(^6)</td>
<td>[51(^*)]</td>
</tr>
<tr>
<td>white collar-2</td>
<td>Arrhythmicity; low frq expression</td>
<td>n.d.</td>
<td>n.d.(^6)</td>
<td>[51(^*)]</td>
</tr>
<tr>
<td><strong>Synechococcus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kaiABC</td>
<td>Many alleles, including short period, long period, arrhythmicity</td>
<td>Yes</td>
<td>n.d.(^6)</td>
<td>[63(^*)]</td>
</tr>
</tbody>
</table>

*Genes* indicates known DNA and protein sequence. Circadian rhythm of mRNA and/or protein expression. n.d., not determined. \(^*\)Mutations originally identified as a blue light blind phenotype.

feedback loop and could be inhibited by a mammalian version of PER via its PAS domain. Furthermore, the Clock mutation, an A→T transversion in the splice donor site of exon 19, results in a 51 amino acid deletion within the proposed activation domain and is consistent with the antimorphic nature of the Clock mutant phenotype [19\(^*\),20\(^*\)].

Following the cloning of Clock, the identification of per orthologs in human and mouse by several independent laboratories underscored the possibility of a Drosophila-like feedback loop in mammals. These genes, prefixed human (h) or mouse (m) Per1 [21\(^*\),22\(^*\)], Per2 [23\(^*\),24\(^*\)], and Per3 [25\(^*\)], all encode proteins that contain a PAS domain. Circadian expression of mPer1, mPer2, and mPer3 in the suprachiasmatic nuclei (SCN), the site of the mammalian clock, suggests that these genes could be circadian clock components [21\(^*\),22\(^*\),23\(^*\),24\(^*\),25\(^*\),26\(^*\)]. Confirmation of these genes as circadian components requires functional evidence: either a mutation within each gene or altered expression of each gene must result in an altered circadian phenotype in mice. In the SCN, mPer1 expression peaks at CT 6 whereas mPer2 peaks at CT 9; mPer3 expression reaches its maximum at CT 6 and remains at that level until after CT 9. In tissues throughout the body, such as retina and skeletal muscle, expression of all mPer genes is delayed 6 hours relative to their phase in the SCN [21\(^*\),22\(^*\),23\(^*\),24\(^*\),25\(^*\),26\(^*\)]. Interestingly, a light pulse administered during the subjective night results in rapid, transient induction of mPer3 expression (maximal induction within one hour) and delayed, transient induction of mPer2 (maximal induction within 1.5 to 2 hours) [23\(^*\),24\(^*\),25\(^*\),26\(^*\)]. In contrast, mPer3 does not acutely respond to a light pulse [25\(^*\)].

Transcriptional activation by bHLH factors such as CLOCK require dimerization and binding to a DNA promoter element called the E box (consensus 5’-CANN[T-G]-3’) [27]. The CLOCK dimerization partner, Bmal1 (for brain and muscle ARNT-like factor), was identified using a two-hybrid screen [28\(^*\),29\(^*\)]; this gene of previously unknown function [30] also encodes a bHLH-PAS protein and is expressed in the SCN [29\(^*\)]. Interestingly, an E box present in a 69 bp enhancer of the Drosophila per promoter is required for per mRNA cycling, which suggests a role for bHLH transcription factors in the circadian mechanism [31\(^*\)]. Using a proximal fragment of the mPer1 promoter which contains three E boxes, Gekakis et al. [29\(^*\)] find that CLOCK and BMAL1 heterodimers bind the mPer1 promoter and activate transcription. Furthermore, CLOCK-BMAL1 heterodimers can activate transcription from the three E boxes alone, whereas mutation of the E boxes abolishes DNA binding
Current molecular model of rhythm generation in Drosophila. The succession of events (a–f) occur over the course of ~24 hours.

(a) CLOCK-BMAL heterodimers bind the per and tim promoters and activate mRNA expression from each locus; peak expression occurs ~CT 12. CLOCK-BMAL may also activate transcription other circadian-regulated genes (not shown). (b) per and tim mRNA are transported to the cytoplasm and translated into PER and TIM protein, respectively. (c) Regulation of protein levels occurs by two mechanisms: DBT protein phosphorylates and destabilizes PER, and light destroys TIM. Light during the early subjective night can phase-delay the clock. Small 'blobs' indicate degraded proteins. (d) PER and TIM levels slowly accumulate during the early subjective night; TIM stabilizes PER and promotes nuclear transport. Peak PER and TIM levels in the cytoplasm occur ~CT 19. (e) PER and TIM dimers enter the nucleus and inhibit CLOCK-BMAL-activated transcription; peak nuclear PER/TIM levels occur ~CT 21. (f) Protein turnover (combined with the lack of new PER and TIM synthesis) leads to derepression of per and tim mRNA expression; the cycle begins again (a) ~CT 2. Light during the late subjective night can phase-advance the clock.

Current molecular model of rhythm generation in Drosophila. The succession of events (a–f) occur over the course of ~24 hours.

(a) CLOCK-BMAL heterodimers bind the per and tim promoters and activate mRNA expression from each locus; peak expression occurs ~CT 12. CLOCK-BMAL may also activate transcription other circadian-regulated genes (not shown). (b) per and tim mRNA are transported to the cytoplasm and translated into PER and TIM protein, respectively. (c) Regulation of protein levels occurs by two mechanisms: DBT protein phosphorylates and destabilizes PER, and light destroys TIM. Light during the early subjective night can phase-delay the clock. Small 'blobs' indicate degraded proteins. (d) PER and TIM levels slowly accumulate during the early subjective night; TIM stabilizes PER and promotes nuclear transport. Peak PER and TIM levels in the cytoplasm occur ~CT 19. (e) PER and TIM dimers enter the nucleus and inhibit CLOCK-BMAL-activated transcription; peak nuclear PER/TIM levels occur ~CT 21. (f) Protein turnover (combined with the lack of new PER and TIM synthesis) leads to derepression of per and tim mRNA expression; the cycle begins again (a) ~CT 2. Light during the late subjective night can phase-advance the clock.

and transcriptional activation [28*,29**]. Finally, the exon-19-deleted mutant form of CLOCK failed to activate transcription, a result fully consistent with the antimorphic Clock mutant allele [29**]. These results identify CLOCK and BMAL as positive elements in the transcription–translation loop; whether mPER1 inhibits CLOCK/BMAL activity awaits additional experimentation.

Further delineation of circadian output and input pathways in mammals has also been achieved recently. Expression of the basic leucine zipper transcription factor albumin site D-binding protein (DBP) oscillates under constant conditions in several tissues, including the SCN and the liver; interestingly, the phase of the rhythm in the SCN is advanced four hours relative to that in peripheral tissues [32*]. Animals homozygous for a targeted DBP-null mutation are less active than wild-type littermates but still display free-running rhythms, although period is ~30 minutes shorter than wild-type [32*]. In addition, the dbp gene does not require the DBP protein for its own expression [32*]. This
information suggests that dhp is an output gene rather than a part of the central oscillator mechanism. At the input level, targeted disruption of the melatonin 1a (Mel1α) receptor showed that this receptor mediates the melatonin-induced inhibition of SCN neural activity, but that the phase-shifting effects of melatonin may be mediated by the Mel1b receptor or by an unidentified receptor [33]. In addition, a putative input gene was identified in a cDNA subtraction screen for light-induced gene expression in the SCN; this gene, the zinc-finger transcription factor eyr-3, is expressed in the ventral SCN and is gated by the circadian clock [34]. Finally, the mammalian blue-light photoreceptors cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2) are expressed in the retina (CRY1 and CRY2) and the SCN (CRY only), thereby providing a new class of photopigments as candidates for the photic entrainment pathway in mammals [35*].

**The Drosophila clock**

Genetic and molecular approaches in *Drosophila* have led to recent discoveries that maintain this species as the best understood in the circadian field. Mutagenesis screening revealed the semidominant mutation *jrk* and the gene dose-dependent mutation cycle ( cyc ), both of which either alter or abolish rhythms in behavior, per expression, and tim expression [36*,37*]. Concomitantly, elock was isolated in a low-stringency screen with *mClock*, and a *Drosophila-*expressed sequence tag clone with homology to hBmal1 was identified [38*]; elock and dBmal were found to map to the *jrk* and cylo loci, respectively. Darlington et al. [38*] have shown that dCLOCK (presumably with dBMAL) binds E box sequences to activate transcription of *per* and *tim*. Furthermore, co-expression of PER and TIM inhibits transcriptional activation by dCLOCK [38*]. These results thus appear to close the circadian loop: the positive elements dCLOCK and dBMAL activate transcription of the negative elements *per* and *tim*, the products of which eventually inhibit their own transcription via interaction with dCLOCK-dBMAL (Figure 1). The precise molecular interactions that mediate this inhibition are unknown; however, the action of PER-TIM on dCLOCK-dBMAL is relatively direct, as the E box element is necessary and sufficient for activation and inhibition of transcription [38*].

Regulation of the *Drosophila* circadian loop appears to occur at both the post-transcriptional and the post-translational levels. Using nuclear run-on experiments, So and Roshbash [39*] have demonstrated that *per* and *tim* are transcribed at high levels several hours before an RNase protection assay can detect their mRNA species. In addition, no rhythm in transcription rate was detected from a protection assay can detect their mRNA specific. In addition, a putative input gene was identified in a cDNA subtraction screen for light-induced gene expression in the SCN; this gene, the zinc-finger transcription factor eyr-3, is expressed in the ventral SCN and is gated by the circadian clock [34]. Finally, the mammalian blue-light photoreceptors cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2) are expressed in the retina (CRY1 and CRY2) and the SCN (CRY only), thereby providing a new class of photopigments as candidates for the photic entrainment pathway in mammals [35*].

**The Neurospora clock**

Continued analysis of the *Neurospora* *frq* gene in the past year has resulted in the identification of novel regulatory mechanisms. As in *Drosophila*, post-translational regulation plays a role in the *Neurospora* circadian clock: *frq* mRNA contains alternative translation initiation sites, the choice of which is mediated by environmental temperature [46*,47*]. At moderate temperatures (25°C), two forms are expressed, and each can support rhythmicity; however, at high temperatures (30°C) the short form of FRQ (FRQ SHORT) is unable to maintain rhythmicity, whereas at low temperatures (18°C) the full-length form cannot drive rhythms [47*]. In addition, temperature-shifting experiments in *Neurospora* have demonstrated that a shift from low temperature (21°C) to high temperature (28°C) strongly resets the clock to CT 0, whereas the opposite shift resets the clock to -CT 12 [48*]. Finally, FRQ protein was shown to translocate to the nucleus and repress *frq* mRNA expression within 4 hours of the *frq* mRNA peak, with recovery from this repression taking the remainder of the circadian day [49*,50]. Thus, the details of circadian regulation between *Neurospora* and *Drosophila* continue to differ, as translational delay of PER and TIM in *Drosophila* versus long recovery period in *Neurospora* are used to maintain a 24-hour period.

The genes white collar-1 (*wc-1*) and white collar-2 (*wc-2*), which encode zinc-finger proteins, appear to be involved in circadian clock regulation [51*]. In particular, *wc-1* is necessary for light-induced *frq* expression, whereas *wc-2* may be
required for circadian fri expression [51*]. However, wc-1 and wc-2 have not been shown to bind the fri promoter or directly activate fri transcription. Sequence analysis indicates that FRQ itself may be a transcription factor, as FRO shares moderate homology with known helix-turn-helix transcription factors [52]; but, again, no functional evidence in support of this hypothesis is available.

The cyanobacteria clock

The most recent circadian model system was established for cyanobacterium Synechococcus strain PCC 7942, which displays circadian rhythms in bioluminescence despite a replication time of five to six hours [33*. Using genetic complementation, a three-gene locus named kaiABC was shown to drive all circadian rhythms in this organism [54**]. These genes share no homology with any known genes. The KaiC product represses activity of the cluster, and overexpression of this gene can reset the phase of the clock. KaiA is required to drive kaiC expression. In effect, a single gene cluster in cyanobacteria appears to contain both negative and positive elements for a circadian negative feedback loop. These results demonstrate that circadian transcription-translation negative regulatory loops are conserved among living systems but the underlying genes differ among phyla.

Circadian organization: central pacemakers and peripheral oscillators

Conventional wisdom has it that the circadian clock resides in the brain in higher animal organisms. The lateral neurons in Drosophila appear to be important for circadian regulation [47*55], and the suprachiasmatic nuclei are the site of the circadian clock in mammals [56]. In the past year, however, brain-independent circadian oscillators (cells capable of self-sustained rhythmic output) have been detected in many peripheral tissues of Drosophila and within cultured cell lines in mammals [57,58,59**,60**]. For example, the Malpighian tubules of both decapitated flies and non-decapitated control animals displayed identical circadian rhythms of PER-lacZ reporter expression and nuclear localization [57]. Kay and colleagues have extended this observation using a real-time luciferase reporter assay to show that the Drosophila body as a whole and in cultured segments sustains circadian rhythms in per-driven expression [58,59**]. Furthermore, every cultured tissue could be entrained by light, indicating that non-neural Drosophila cells are photosensitive [59**].

Significant new evidence has been found for the existence of oscillators throughout the mammalian body. As discussed above, rhythms in mPer1, mPer2, and mPer3 can be found in many non-neural body tissues [22*,23*,25**]. In cell culture, serum stimulation of rat-1 fibroblasts and H135 hepatoma cells elicits expression of several genes, including rat (t) Per1, tPer2, dhp, and tef (thyroid embryonic factor). Remarkably, the expression patterns of these genes then oscillate in a circadian manner in the presence of the cell cycle inhibitor cytosine β-D arabinofuranoside [60**]. This discovery provides definitive evidence of brain-independent mammalian clock cells. Furthermore, the relative phases of tPer1 and tPer2 expression in cell culture match those found in the liver in vivo [60**]. Finally, the tPer1 and tPer2 genes fulfill the criteria for immediate-early genes in that serum induction is rapid and independent of new protein synthesis [60**]. This finding is reminiscent of the immediate-early expression of c-fos and jun-B in the SCN in response to light and suggests that immediate-early signaling pathways may play a role in conveying photic information to the circadian clock in the SCN [61].

Comparison of mammalian per expression data shows that the phase of these circadian genes is advanced between three and nine hours (depending on lighting conditions, species, and laboratory) in the SCN relative to the rest of the body [22*,23*,25**,32,60**], suggesting that the SCN play a special role within the collection of cellular oscillators. Indeed, twenty-five years of physiological evidence has demonstrated that the SCN contains the required mammalian pacemaker — the oscillator that drives period and phase in other oscillating cells [62]. The method by which the SCN directs circadian rhythmicity throughout the body is unknown, but two general mechanisms are possible: the SCN could drive rhythms in passive, non-oscillating cells, or, conversely, the SCN could coordinate cell-autonomous oscillators. The discovery of peripheral oscillators strongly supports the latter model. Indeed, the physiological organization of circadian rhythmicity can be compared to the hierarchy of cardiac pacemakers: in the heart, the sinoatrial (SA) node controls the period of cardiac rate but, in the absence of the SA node, the atrioventricular (AV) node regulates rhythm. In the absence of either node, individual cardiac cells are capable of rhythmic contraction. This analogy could be applied to the circadian system, where some unknown factor(s) place the SCN at the top of the circadian hierarchy to coordinate cells in the body as a precisely functioning unit.

Conclusions

The tremendous progress towards the molecular dissection of the circadian clock places the circadian field in an exciting era. The identification of new circadian genes and the delineation of regulatory mechanisms in diverse model organisms have underscored the universal nature of the circadian clock yet also suggested phylogenetic differences in its assembly. Future studies will undoubtedly focus on each gene’s functional role and interactions with other circadian genes within the organism. Finally, the discovery of brain-independent circadian clocks should allow the elucidation of the molecular circadian mechanism and provide a better understanding of the physiological circadian hierarchy.

Acknowledgements

We thank M Young, U Schibler, SM Reppert, S Golden, and J Dunlap for providing unpublished manuscripts or sharing unpublished information. JS Takahashi is an Investigator of the Howard Hughes Medical Institute.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


16. Complete rescue of the Clock mutant phenotypes is achieved using bacterial artificial chromosome transgenes, which shows that functional complete rescue of the Clock mutant phenotypes is achieved using bacterial artificial chromosome transgenes, which shows that functional.


18. Genetic and physical mapping is combined with a threedimensional molecular approach to transcription unit analysis leading to the identification of the Clock gene sequence. Sequence analysis indicates that Clock encodes a bHLH-PAS transcription factor. The Clock mutation results in deletion of a portion of the putative activation domain, which is consistent with the anteromorph nature of the mutation.


21. A novel PCR-based approach, which took advantage of relatively conserved sequence within the functionally significant PAS domain, is used here to identify a mammalian ortholog of the Drosophila period gene; significant sequence homology occurs throughout the entire gene in human, mouse, and Drosophila. Mouse (m) Per expression cycles in a circadian manner in the SCN.


23. This group cloned human RIGI [now renamed hper1] in a chromosome 17 transcript mapping project. mPer1 (called mRIGI here) expression displays a circadian rhythm in the SCN as well as in the retina, the pars tuberalis, and the Purkinje neurons; interestingly, expression in the SCN is advanced 0-12 hours relative to the other tissues.


27. These papers [23*, 24*, 25*, 26?] collectively illustrate several new, functionally important resident mammalian circadian biology. First, at least three mammalian per homologs exist. Second, all three genes respond differently to light directed simultaneously during the subjective night; mPer1 expression increases and decreases rapidly, much like the immediate-early gene response; mPer2 expression increases in a delayed fashion, then decreases rapidly; and mPer3 expression does not change in response to light. Finally, the phase of each mPer is advanced 3-9 hours in the SCN relative to other body tissues including the retina, skeletal muscle, and testis.


31. CLock is shown to be a dimersization partner of MOP3 (BMAL1), and the E-box promoter element is shown to direct reporter expression in the presence of CLOCK/MOP3 (BMAL1). In addition, MOP3 forms transcriptionally functional dimers with MOP4 (NPAS2), HIF1α, and HIF2α.

32. Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DT, Takahashi JS, Weitz CJ: Role of the Clock protein in the mammalian circadian clock mechanism: CLOCK and BMAL1 activate mPer1 expression directly. The CLOCK/BMAL1 dimer can bind both the Drosophila per and the mPer promoter E boxes to drive expression of a reporter gene: furthermore, the mutant Clock protein fails to transactivate the mPER1 promoter.


*References cited in this document are available in the full-text version of the review.*

The first indication that a bHLH transcription factor may be involved in the circadian mechanism came from this work. A 69 bp enhancer in the Drosophila Per promoter that is necessary and sufficient for rhythmic expression of Per was isolated. This enhancer contains an E box, the DNA element that bHLH transcription factors bind. Mutation of the E box abolished rhythmic per expression, which suggested that bHLH factors are a part of the circadian loop.


These authors made the first observation of a gene, dbp (albumin site D binding protein), whose phase of expression is advanced in the SCN relative to peripheral tissues. Targeted deletion of the dbp gene does not abolish circadian rhythms in behavior but period length decreases by ~30 minutes.


Expression of new photopigments in the retina and SCN suggest the existence of an entrainment-specific photoresponse pathway.


The circadian mutation Jrk severely disrupts rhythmicity in behavior, per expression and tim expression in the heterozygous state and abolishes these rhythms in the homozygous state. Jrk is the Drosophila ortholog of mammalian Clock: the Jrk mutation results in truncation of a large portion of the putative activation domain.


The circadian mutation cycle lengths behavioral period in the heterozygous state and abolishes behavioral, per expression, and tim expression rhythms in the homozygous state, cycle is the Drosophila ortholog of Bmal1; a nonsense mutation in Bmal1 downstream of the PAS-B region corresponds well with the circadian phenotype.


This work independently identified the Drosophila ortholog of Clock and bmal1, then demonstrated the activities of the known circadian genes: first, dCLOCK activates expression of dper and dtim through the E box present in each gene's promoter; and second, PER and TIM directly inhibit transcription in the heterozygous state and abolishes these rhythms in the homozygous state. Jrk is the Drosophila ortholog of mammalian Clock: the Jrk mutation results in truncation of a large portion of the putative activation domain.


Using the nuclear run-on assay, these authors confirm that per and tim mRNA transcription rates cycle in a circadian manner but that the amplitude and phase of these rhythms differ unexpectedly from amplitude and phase in their mRNA abundance rhythms. This finding suggests that post-transcriptional regulation, in addition to transcriptional activation, affects mRNA cycling of circadian genes.


See annotation [41*]


The isolation of a new circadian mutant in Drosophila and the positional cloning of its gene introduce an important regulatory mechanism of the circadian clock. double-time encodes a protein closely related to human casein kinase 1; this homology combined with the lack of PER phosphorylation in dbt mutants suggests that the dbt product phosphorylates PER, which destabilizes PER and possibly contributes to its translational delay.

42. Kaneko M, Helfrich-Forster C, Hall JC: Spatial and temporal expression of the period and timeless genes in the developing nervous system of Drosophila larvae. Expression of these genes was found in the larval neurons and a subset of the larval dorsal neurons; surprisingly, two larval dorsal neurons express PER and TIM in antiphase to the other neurons.


These authors provide the first demonstration of FRO protein oscillations in both abundance and reactivity. In addition, alternative initiation events leading to two forms of FRO are described.


This paper introduced a new regulatory mechanism in Neurospora alternation translational initiation mediated by temperature. This work provides a molecular entry into the study of temperature compensation in Neurospora.


An elegant paper that details how temperature can reset a circadian oscillator by its effects on FRO protein levels.


Using an inducible frq construct in a frq loss-of-function genetic background, these authors demonstrate that FRO protein rapidly inhibits frq transcription and that recovery from this inhibition requires the rest of the circadian day.


Evidence of zinc-finger proteins as positive elements provided in this paper. The wc-1 gene is required for response to light, and the wc-2 gene appears to be essential in circadian rhythm generation.


The unexpected presence of circadian rhythms in bioluminescence and mRNAs expression in an organism with a lifespan of five to six hours suggests the existence of a universal circadian mechanism, but the details of this mechanism in prokaryotes are expected to differ significantly from that in eukaryotes.


The cloning and characterization of the genes responsible for all circadian phenotypes in cyanobacteria indicate that the negative regulatory loop is indeed conserved throughout evolution; however, the nature of the gene products differ among phyla.


Both whole bodies and cultured tissue segments of Drosophila are shown to contain endogenous circadian oscillators. Remarkably, all per-expressing cells are photoreceptive, an observation that challenges the somewhat narrow view that only the brain can regulate rhythms in the body.


Both whole bodies and cultured tissue segments of Drosophila are shown to contain endogenous circadian oscillators. Remarkably, all per-expressing cells are photoreceptive, an observation that challenges the somewhat narrow view that only the brain can regulate rhythms in the body.