# Circadian Rhythm Models

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## Introduction

Many organisms, including animals, plants, and cyanobacteria, undergo 24 h rhythms in physiology and behavior, and these rhythms persist in constant dark conditions. Therefore, self-sustaining internal oscillators with periods of approximately 24 h must have evolved in these organisms. The operation of complex biological systems, such as these circadian oscillators, is generally too complex to understand by intuition. Mathematical models are therefore essential to provide a picture of how the system components work together to determine the dynamics and the response to changes in the organism or the environment. Models are also useful to suggest further experiments, to test whether the system responds in ways that the model predicts, or to verify/disprove hypothetical regulatory mechanisms that are predicted to explain specific system behaviors.

Before experiments characterized the molecular mechanisms of these oscillators, attempts were made to describe their dynamics using phenomenological oscillator models from physics. Such relatively simple models are still important. For example, the Van der Pol equations, originally developed to describe oscillating electrical circuits, have proven useful to model light-induced entrainment and phase-shifting of circadian oscillations in humans and cyanobacteria.

## Early Models Illustrated Essential Roles of Negative Feedback and Posttranslational Modifications

Data establishing the molecular nature of the internal circadian oscillators have been obtained for many organisms. Mutations have been found that alter or abolish circadian oscillations in mammals, fruit flies (*Drosophila*), fungi (*Neurospora*), and plants. In all these organisms, circadian oscillations in the rate of transcription of genes and in the levels of gene protein and mRNA products have been characterized. Circadian rhythms of protein phosphorylation and degradation have also been observed. Therefore, the internal oscillators are believed to rely on periodic feedback regulation of gene transcription by gene product proteins, as well as periodic posttranslational modifications. Modeling has proven very useful to characterize more specific schemes of molecular interactions and to suggest experimental tests. Since the 1990s, numerous investigators have developed models that have enhanced the understanding of the ways in which feedback regulation and posttranslational modification are organized to sustain oscillations that are robust to changes in the organism's internal or external environment, and that are entrained by light or temperature cycles.

Sustained oscillations in reaction rates and concentrations can be exhibited by biochemical systems with nonlinear kinetics that operate away from equilibrium due to ongoing energy input (e.g., ATP hydrolysis). Most commonly, to drive oscillations, a particular type of kinetic nonlinearity is required – a negative feedback loop. In a negative feedback loop of chemical reactions, the product of the last reaction inhibits the first reaction. A model proposed by Goodwin in 1965 was the first to simulate circadian oscillations due to negative feedback in which a protein repressed its own gene. This venerable model is still proving useful, for example, to simulate aspects of *Neurospora* oscillations.

*Drosophila* was the first organism for which a negative feedback loop was found essential for circadian oscillations. Experiments established that the period protein (PER) inhibits the transcription of its own gene (*per*). Early modeling by Goldbeter determined that a negative feedback loop in which PER underwent several posttranslational modifications prior to repressing *per* transcription could sustain circadian oscillations in PER levels and formation. Both a critical number of sequential reactions in the loop (at least six or seven) and a significant, sigmoidal nonlinearity in the repression of *per* by PER (e.g., a Hill function of PER with an exponent of 3 or 4) appear necessary to sustain large-amplitude oscillations. Subsequent experiments have confirmed the necessity of this negative feedback loop. PER is multiply phosphorylated, and specific phosphorylations appear to gate PER nuclear entry. PER nuclear entry may also require the TIM protein, and *tim* mutations block rhythmicity. Nuclear PER interacts with a transcriptional activator protein, CLK, and inhibits CLK's binding to DNA. In this way, PER rhythmically inhibits the transcription of not only *per* but also numerous other genes that are activated by CLK, thereby driving numerous physiological/behavioral aspects of rhythmicity. Transcriptional repression by PER persists for several hours, during which PER is progressively phosphorylated. This process is an important factor in setting the long (~24 h) oscillation period. When PER is maximally phosphorylated, it degrades rather abruptly,

and CLK-dependent transcriptional activation of *per* and other genes then resumes, beginning the next cycle. Figure 1 illustrates this negative feedback loop and simulated PER circadian oscillations.

Experiments have identified analogous negative feedback loops that underlie circadian rhythms in mammals (rhythmic repression of transcription by PER and cryptochrome (CRY) proteins) and in *Neurospora* (rhythmic repression of transcription by FRQ protein) as well as in the flowering plant *Arabidopsis*. Models based on negative feedback have simulated molecular oscillations in these organisms. Indeed, it appears that a generic mechanism of rhythmicity based on a negative feedback loop of transcriptional repression is very widespread. Note, however, that some observations indicate rhythmicity can persist in the absence of these loops, perhaps suggesting that multiple mechanisms of rhythm generation exist. For example, in cyanobacteria, circadian oscillations in the phosphorylation of proteins necessary for circadian rhythms (KaiA–C) persist in the absence of transcription.

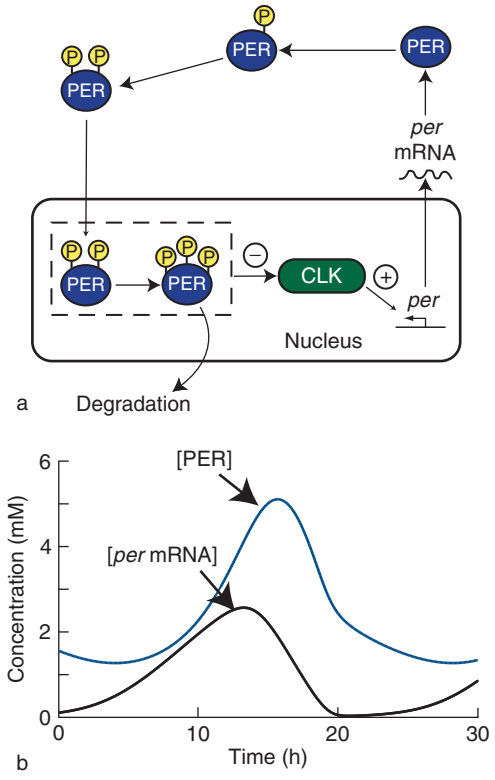


**Figure 1** (a) Schematic of the negative feedback loop which appears to be central to circadian rhythm generation in *Drosophila*. Transcription of *per* is activated by the CLK transcription factor. *Per* mRNA is translated, and PER protein then undergoes multiple phosphorylations. Data suggest some phosphorylation must precede PER's nuclear entry. Nuclear PER represses *per* expression by interfering with CLK, closing the negative feedback loop. Subsequently, PER is further phosphorylated over succeeding hours. Fully phosphorylated PER is then rapidly degraded. PER degradation releases *per* repression, allowing a new cycle to begin. When repression is relieved, *per* expression is again induced. (b) Oscillations of *per* mRNA and protein simulated by using the original model of the negative feedback loop from Goldbeter A (1995) A model for circadian oscillations in the *Drosophila* period protein (PER). *Proceedings of the Royal Society of London, Series B* 261: 319–324.

The models with negative feedback loops also rely on nonlinear repression of transcription to sustain large-amplitude oscillations (e.g., sigmoidal Hill functions of FRQ, CRY, or PER levels). However, experiments have failed to demonstrate cooperative binding of these proteins to regulatory regions of their target genes. Therefore, a mechanism for the hypothesized nonlinear repression has not been found. However, a model of circadian rhythm generation in *Drosophila* by Smolen et al. suggests that multiple sequential chromatin modifications that accompany binding of PER or CLK to DNA could account for nonlinear repression. In mammals, binding of CLK and/or PER is known to be accompanied by such rhythmic chromatin modifications (multiple histone acetylation/methylation events).

## Recent Models Consider Additional Positive Feedback and Simulate the Effects of Mutations

Experiments have revealed additional molecular interactions that modulate the negative feedback interactions discussed previously. In *Drosophila*, the transcriptional activator CLK indirectly represses its own gene. In mammals, an analogous activator, denoted BMAL1, indirectly represses its own gene. In both organisms, a positive feedback loop is also present. This loop is organized as follows for mammals and *Drosophila* (*Drosophila* in parentheses). First, *bmal1* (*clk*) gene expression increases levels of BMAL1 (CLK) protein. Second, the increased BMAL1 (CLK) activates *cry* and *per* expression. Third, the expressed CRY and PER inhibits the action of BMAL1 (CLK). Finally, this inhibition of BMAL1 (CLK) de-represses *bmal1* (*clk*), increasing *bmal* (*clk*) expression and thereby closing the positive feedback loop. A detail of the mammalian loop is that BMAL1 inhibition reduces expression of the REV-ERBα transcriptional repressor, and this is the way in which *bmal1* expression is de-repressed. In *Neurospora*, a similar positive feedback loop involves FRQ and the activator WCC. Figure 2 illustrates the mammalian positive and negative feedback loops.

Recent models have examined plausible effects of these positive feedback loops. The existence of both positive and negative loops has been suggested to increase the robustness (stability) of the oscillation period and/or amplitude given biochemical parameter

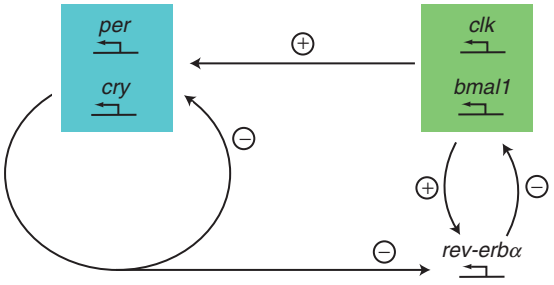


**Figure 2** Schematic of the feedback loops that appear to underlie mammalian circadian rhythm generation. *Per* and *cry* expression is induced by the *bmal1* and *clk* gene products. In the first negative feedback loop, PER and CRY proteins repress expression of their own genes via blocking of transcriptional activation by BMAL1/CLK. In a second negative feedback loop, BMAL and CLK proteins indirectly repress their own genes by inducing *rev-erbα*. The REV-ERBα protein is a repressor. In the positive feedback loop, expression of *bmal1* and/or *clk* leads to induction of *per* and *cry*, which inhibits induction of *rev–erbα* repressor by BMAL1/CLK. *Bmal1* expression is therefore de-repressed and can increase further, closing the positive feedback loop. For clarity, proteins are omitted from the figure and multiple *per* and *cry* isoforms are not distinguished.

variations, such as would occur between individuals in a species. However, others have suggested that positive feedback may not increase robustness. Rather, it may primarily drive oscillations in the expression of 'output' circadian genes that are not part of the core oscillatory mechanism but act to govern physiological and behavioral rhythms. Indeed, data suggest that in *Drosophila*, the level of CLK remains relatively constant.

Another perspective on robustness of oscillations has been obtained from studies that examined a range of simplified model network motifs that simulate oscillations of gene expression. These investigations attempted to determine what type of networks exhibit the greatest stability of oscillation period and/or amplitude to parameter changes. In some of these studies, multiplicity of feedback loops was found to increase oscillation robustness.

Since the 1970s, numerous mutations have been found to alter or abolish circadian rhythms. Arrhythmia can be induced by mutation of *Drosophila per* or *clk*, mammalian *clk*, or *frq* in *Neurospora*. Less drastic mutations of these genes, which preserve partial function, can result in lengthened or shortened free-running circadian oscillations (i.e., in constant darkness the period differs from 24 h). Any viable model of circadian rhythm generation should at least qualitatively simulate the effects of such mutations. For mammals, gene duplication has led to several *per* genes that differ slightly in their importance for rhythmicity (*per1–3*). Two other mammalian transcriptional repressors, CRY1 and CRY2, are also core circadian proteins that function similarly to the PERs.

Complex models attempt to capture most of the currently known complexity of the mammalian oscillator (posttranslational modifications and the roles of additional core circadian genes) and therefore consist of as many as 73 differential equations. However, these models only represent regulation of approximately five core genes, and it is likely that additional genes help drive or shape circadian behavioral oscillations. Although there may be value in increasing the complexity of models to incorporate additional genes, it is clearly also necessary to develop 'reduced' models with fewer variables that capture one or a few of the critical mechanisms underlying rhythm generation and modulation, such as feedback loops, or entrainment by light stimuli. For *Drosophila*, the original model with a single negative feedback loop (Figure 1) remains useful. One- or two-variable reduced models with a time delay have also been developed that represent circadian transcriptional feedback in *Drosophila* and in *Neurospora*, as well as effects of light stimuli. However, a weakness of such models is that introducing explicit time delays generally and strongly promotes oscillations, without representing kinetic details of biochemical reactions.

An alternative approach to model reduction begins with a complex model containing many differential equations and projects the dynamics onto a two- or three-dimensional manifold. For example, the 73-variable model of the mammalian oscillator noted previously has been reduced to a two-variable model that retains most of the qualitative dynamics and is much easier to analyze.

## Models Have Increased the Understanding of Entrainment and Phase-Shifting by Light Cycles, Light Pulses, and Temperature Changes

Circadian oscillators generically respond to light. Day–night cycles entrain these oscillators. That is, if the constant darkness oscillation period is within a few hours of the light–dark cycle length, then the oscillator will adjust its period to match the light–dark cycle. It is also generically observed that during a background of constant darkness, brief light pulses will shift the phase of the free-running oscillation. A phase–response curve (PRC) is commonly used to present the light pulse data. In plotting this curve, the abscissa represents the time in the free-running cycle at which the pulse is applied, and the ordinate represents the phase shift caused by the pulse. For a variety of organisms, the PRC has comparably sized regions of both positive and negative phase shifts and tends to have a rather abrupt transition from negative to positive phase shifts. Such a curve is a 'type 1' PRC.

For very strong stimuli, type 0 PRCs are occasionally observed. With these PRCs, phases after stimuli cluster near some fixed value and are thus independent of the phase before stimulus.

The biochemical effects of light on core circadian oscillator genes have been examined. In *Drosophila*, light indirectly enhances degradation of the PER protein (via enhanced TIM degradation, which in turn destabilizes PER). In mammals, light enhances expression of *per* genes, and in *Neurospora* light induces *frq* expression. Models of the mammalian and *Drosophila* oscillators have incorporated these effects and have succeeded in simulating light entrainment of oscillations as well as experimental type 1 PRCs. However, given the same model parameter values, these models have usually not succeeded in simulating type 0 PRCs. Figure 3 illustrates simulated light entrainment of *Drosophila* circadian oscillations and a simulated PRC.

Simulations of PRCs and entrainment can help in understanding human circadian rhythm sleep disorders. Advanced and delayed sleep phase syndromes have been identified, and simulations suggest that mutations decreasing or increasing phosphorylation of PER protein could phase-shift the rhythm and account for these syndromes. Experiments have indeed implicated *per* mutations. In other patients, the phase of sleep does not entrain to night and day but, rather, continuously varies. Analogously, in models, modest changes in PER degradation or in other kinetic parameters can alter oscillation period so that entrainment to a 24 h light–dark cycle is no longer possible.

Circadian models incorporating light responses have been seen, for some parameter ranges, to exhibit multiple dynamic behaviors, including coexistence of a steady state and an oscillating solution (hard excitation) or coexistence of two oscillating solutions (birhythmicity). With coexistence, the initial conditions (initial values of the dependent variables) determine which model behavior is observed. For hard excitation, modeling predicts that an appropriate perturbation (e.g., a light pulse of appropriate intensity) could switch the behavior from the oscillating solution to the steady state or vice versa. Indeed, in constant darkness, a single light pulse has been observed to abolish rhythmic oscillations, and oscillations can be restored by a second pulse. This observation is an example of a behavior that can only be understood or predicted with the help of a mathematical model. For certain parameter ranges in some models, chaotic behavior occurs (i.e., seemingly random variable trajectories without periodic behavior). However, chaotic dynamics have not been confirmed experimentally.

In many organisms, circadian rhythms are observed to be temperature compensated. That is, if the environmental temperature is varied within a limited but substantial range (typically 10–15 °C above or below normal), the period of the free-running rhythm in constant darkness is observed to remain almost constant, and near 24 h. Temperature compensation is surprising because the rates of all biochemical reactions increase with temperature, typically by a factor of approximately two for a temperature increase of 10–15 °C. Models have attempted to simulate

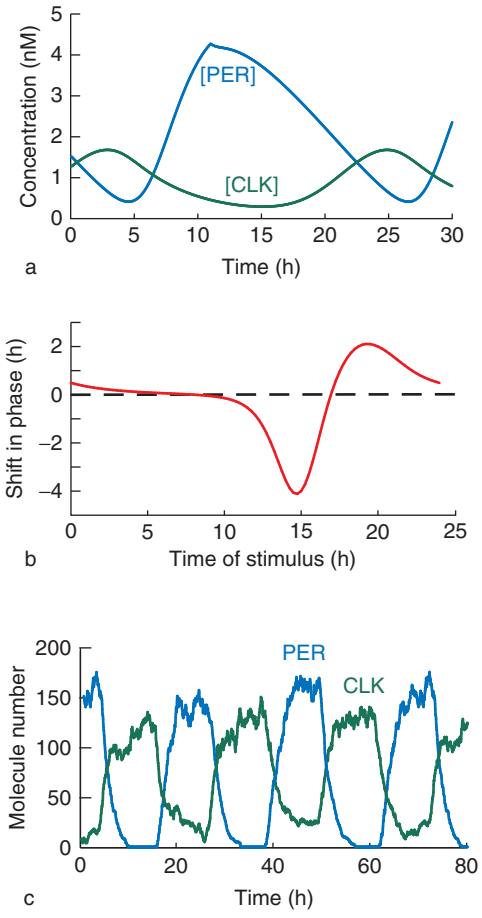


**Figure 3** (a) Simulated circadian oscillations of *Drosophila* PER and CLK entrained to a short 22 h light–dark cycle. Note the accelerated degradation of PER at light onset ($t = 11$ h). (b) A simulated *Drosophila* PRC. Constant darkness circadian oscillations were phase-shifted by a brief light pulse, simulated as a 3 h enhancement of the rate of PER degradation. To generate the curve, light pulses were applied at various times during the circadian oscillation. (c) Simulated stochastic oscillations of PER and CLK suggest that relatively reproducible oscillations, with little variation in period, can be sustained when copy numbers of core clock proteins are on the order of 100. (a, b) model used from Smolen P, Hardin PE, Lo BS, Baxter DA, and Byrne JH (2004) Simulation of *Drosophila* circadian oscillations, mutations, and light responses by a model with VRI, PDP-1, and CLK. *Biophysical Journal* 86: 2786–2802. (c) Model used from Smolen P, Baxter DA, and Byrne JH (2002) A reduced model clarifies the role of feedback loops and time delays in the *Drosophila* circadian oscillator. *Biophysical Journal* 83: 2349–2359.

temperature compensation. To date, the only explanation supported by modeling relies on simultaneous acceleration of opposing reactions. For example, sequential phosphorylations of PER protein must be completed during a circadian cycle in *Drosophila* or mammals. At each phosphorylation site, there are opposing phosphorylation and dephosphorylation reactions. If, at each site, the rates of the opposing reactions are increased by similar amounts, then simulations have illustrated that the time required for progression through the sequence of phosphorylations can remain almost constant. This sequence of PER phosphorylations, in turn, appears to be an important determinant of oscillation period length, and as a result, oscillation period does not change appreciably upon a temperature increase.

The above explanation of temperature compensation does rely on 'fine-tuning' of kinetic parameters in the opposing reactions. However, it appears plausible that molecular evolution could lead to such fine-tuning if maintaining a circadian rhythm of proper period in different seasonal temperatures is evolutionarily advantageous to an organism. Indeed, a rhythm of proper period is important for the organism's fitness to ensure that active behaviors (food seeking) and rest/sleep occur at the correct phases of the day–night cycle.

Despite temperature compensation, temperature cycles are observed to entrain circadian rhythms, and temperature pulses can phase-shift rhythms. Models in which temperature affects the rates of opposing reactions can show these effects as well, but the phenomena have not been studied in detail.

## Models Illustrate Sensitivity of Rhythms to Stochastic Molecular Noise and Suggest Mechanisms to Enhance Noise Resistance

Models of biochemical systems can be extended to include random variability in the times of individual macromolecular synthesis and degradation events. Observations indicate that the timing of transcription of individual mRNAs is indeed quite random. Also, for some macromolecules, such as specific mRNAs or transcription factors, average molecule numbers per cell can be modest ($\sim$100). In this case, variability in the times of single-molecule synthesis or degradation will lead to significant stochastic fluctuations in macromolecule numbers. These fluctuations can considerably alter the dynamics of biochemical pathways.

It is difficult to determine absolute copy numbers of proteins or mRNAs in neurons, and for circadian gene products, these data are generally not available. Therefore, models including molecule number fluctuations have focused on qualitative predictions of the minimal average copy numbers needed to sustain oscillations of reproducible period and amplitude. Simulations predict that a few hundred copies of PER and other key proteins should be present on average, and average numbers of mRNAs can be somewhat less. Figure 3(c) illustrates *Drosophila* model simulations that sustain reproducible molecular oscillations when the average copy number of key proteins is on the order of 100.

Several theoretical studies have identified mechanisms capable of increasing resistance to either stochastic and environmental fluctuations or noise in genetic oscillators. One conclusion is that positive feedback can enhance noise resistance by generating oscillator dynamics that are characterized by abrupt and large transitions states of high and low gene expression. A related conclusion is that noise resistance is increased in oscillators for which the limit cycle is strongly attracting (i.e., for which perturbations away from the normal periodic time course decay very quickly).

## Models Examine the Efficacy of Proposed Mechanisms of Intracellular Synchronization

In the brains of mammals and in *Drosophila*, specific small groups of neurons are responsible for generating the circadian rhythm. This central oscillator, in turn, entrains peripheral oscillators localized in other organs (e.g., the mammalian liver). Two types of synchronization are required in this scheme. Rhythm-generating neurons must synchronize with each other, and the central oscillator must synchronize (entrain) the peripheral oscillators.

In mammals, rhythmogenic neurons are located within the suprachiasmatic nucleus (SCN). Synchronization of these rhythmogenic neurons (and of analogous *Drosophila* neurons) appears to rely on coordinated release of a diffusible neurotransmitter, such as GABA or vasoactive intestinal polypeptide. Electrical coupling of neurons by gap junctions may also play a role. Diffusible neurotransmitters and electrical coupling modulate neuronal electrical activity, and data indicate that variations in electrical activity and $Ca^{2+}$ influx regulate *per* expression. These data therefore suggest a mechanism for rhythm synchronization within the central oscillator. Synchronization of central and peripheral oscillators relies on pulsatile secretion of hormones, such as gonadotropin-releasing hormone, from neurons within or coupled to the SCN.

Models have simulated synchronization of SCN neurons by diffusible neurotransmitters. A plausible

model should also make experimental predictions. One prediction is that efficient synchronization may be achieved even if the average neurotransmitter concentration would dampen individual oscillating neurons. In this case, due to the global neurotransmitter oscillation in the SCN, cells are effectively synchronized. As experimental characterization of SCN neurotransmitter release and possible gap junction coupling proceeds, model refinement will be important to help understand which synchronization mechanisms are most important and to predict how drugs or other environmental changes could alter synchrony.

## Directions for Research

Experimental data characterizing the mechanisms of circadian oscillations continue to accumulate rapidly. As a result, no published model of circadian rhythms in mammals, *Drosophila*, or other organisms represents all the known details of rhythm generation. Revision of current models will be necessary as new data become available. However, it is likely that core mechanistic elements represented in current models will remain essential, such as negative feedback loops based on rhythmic repression of clock genes such as *per*, cycle lengths set largely by the time required for multiple phosphorylations of proteins regulating transcription, and relatively simple representations of the known responses to light and temperature.

## Conclusion

Models of circadian rhythm generation have helped to determine that circadian oscillators in organisms including animals, fungi, and some prokaryotes share a common element of a negative feedback loop. Due to this loop, core circadian genes are rhythmically repressed by some of their protein products. The 24 h oscillation period is largely required for multiple posttranslational modifications of these core gene products, which are then degraded, allowing the next cycle of transcription. In some organisms, positive feedback loops modulate oscillation phase and amplitude. Multiple feedback loops may increase oscillation robustness to stochastic fluctuations and to mutations. Whether oscillations can be sustained by mechanisms other than transcriptional feedback is still being studied. Models have also helped in understanding some human sleep disorders, predicting mutations in *per* as one causative factor.

Negative feedback loops in which proteins act (directly or indirectly) to repress the transcription of their own genes appear to be a common motif for sustaining biologically important oscillations. Some examples are the cyclical development of somites of the vertebrate embryo, driven by a genetic oscillator in the mesoderm; the oscillatory response of p53 tumor suppressor expression to DNA damage; and oscillations in nuclear levels of the transcription factor NF-$\kappa$B. Artificial gene circuits with oscillating expression are also being developed and might prove useful for gene therapies that seek to normalize pulsatile release of hormones or other proteins. Models similar to those discussed for circadian rhythms are being applied to characterize these diverse oscillations and predict their responses to environmental and pharmaceutical stimuli in healthy and diseased states.

*See also:* Circadian Oscillations in the Suprachiasmatic Nucleus; Circadian Regulation by the Suprachiasmatic Nucleus; Circadian Metabolic Rhythms Regulated by the Suprachiasmatic Nucleus; Genetic Regulation of Circadian Rhythms in Drosophila; Genetics of Circadian Disorders in Humans; Learning and Memory in Invertebrates: *Drosophila*; Sleep and Waking in Drosophila.

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