Genetics of Circadian Rhythms

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KEYWORDS

• Circadian • Genetics • Entrainment • Clock

KEY POINTS

- Circadian rhythms at the organismal level are driven by rhythmic expression of genes at the molecular level.
- The conserved architecture of these circadian clocks is based on a transcriptional feedback loop with posttranscriptional and posttranslational regulation.
- Tissue-specific clocks are synchronized to local time by environmental cues, such as light and food.
- Dysregulation of circadian rhythms through mutation or misalignment with environmental time is shown to contribute to a wide range of disease states.

INTRODUCTION

Almost all organisms organize their physiology, behavior, and metabolism according to the 24-hour solar cycle. Time-dependent changes in these parameters have evolved to allow plants and animals to maximize their fitness according to external cues. Internal "clocks" evoke a set of anticipatory responses to changes in their environment, which are referred to as circadian rhythms.

Circadian rhythms have four unique properties. First, these rhythms closely mirror the 24-hour solar day, hence the word "circadian," which comes from the Latin words "circa" (close or about) and "dian" (day). The second principle dictates that even if there are no exogenous cues present, periodic patterns are still shown[1] indicating that these rhythms result from an internal time-keeping system. Third, although circadian activity rhythms are derived from an endogenous clock, they can adjust to exogenous signals, such as light or heat. Finally, the periodicity of rhythms is stable across a wide range of temperatures, a property referred to as "temperature compensation."[2] For humans, the most prominent circadian rhythm is the 24-hour rhythm in the sleep-wake cycle.

Four widely divergent model systems have been historically used to study the genetics underlying circadian rhythms: (1) fruit flies (*Drosophila*), (2) fungi (*Neurospora*), (3) cyanobacteria, and (4) mouse.[3] The discoveries found using these models have facilitated the understanding of the mechanism behind circadian rhythms and their significance to biology and disease. Remarkably, animal clocks are well conserved from insects to mammals, revealing an important role in basic animal models to understand the mechanistic basis of human circadian rhythms.

THE MOLECULAR CLOCK

Phasic expression of genes drives the physiologic and behavioral manifestations of circadian rhythms at the organismal level. Nearly half of all protein-coding genes show circadian-dependent transcription in at least one tissue in mammals.[4] Although it is known that the specific genes that cycle are variable across species and tissue

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dependent,[5,6] the mechanism that drives their phasic expression is consistent throughout the body and well conserved between species.[7,8]

Rhythmic expression of genes is accomplished through a cell intrinsic transcriptional/translational feedback loop (TTFL). The forward portion of the loop consists of transcription factors whose activity promotes the expression of genes that are the "negative" elements of the loop. Over the course of a day, levels of the negative arm increase until they are capable of translocating into the nucleus and repressing the activity of the positive arm. Once they are degraded, the positive arm is freed to restart the cycle. The activity of this loop creates a self-perpetuating oscillating pattern of gene expression with a near 24-hour period. Here we present the mechanistic details of the fruit fly clock because it is where the clock mechanism was first discovered, is highly conserved with the human clock, and led to the discovery of the first genes involved in human circadian rhythms.

The Drosophila Molecular Clock

In *Drosophila* the forward transcription loop consists of master transcription factors clock (dCLK) and cycle (dCYC), which heterodimerize and initiate transcription.[9,10] This is accomplished through binding to the E-boxes (CACGTG) at target promoters and activating them (Fig. 1).[7] The negative loop consists of period (PER)[11] and timeless (TIM),[12] which dimerize and accumulate slowly in the cytoplasm. In the morning, light causes TIM degradation and without TIM, PER is less stable and both are degraded by a proteasome-dependent pathway.[13] During the evening, however, PER/TIM accumulate and translocate into nucleus.[7,14,15] Once in the nucleus PER-TIM dimer binds to CLK-CYC and inhibits their transcriptional activity (see Fig. 1).[15] This simple negative feedback loop serves as the core principle and primary feedback loop of a 24-hour biologic clock.

In addition to this canonical loop, there are secondary loops that interact with and modulate clock activity. Clockwork orange (CWO) binds directly to E boxes, preventing CLK/CYC enhanced gene expression.[7] PAR-domain protein 1ε (PDP1ε) and vrille (VRI) are two proteins that also act to regulate the forward loop.[16] These circadian proteins feedback to act on the *Clk* promoter with PDP1ε serving as an activator and VRI as an inhibitor (see Fig. 1). Lastly, the transcription factor Ecdysone-induced protein 75 has been found to repress *Clk* expression[17] and time-dependently enhance PER transcription.[18] These additional feedback loops support the function of the primary feedback loop.

The Mammalian Molecular Clock

Mammalian and *Drosophila* TTFLs are similar.[6] In mouse and human the forward loop consists of CLOCK (homolog of dCLK) and brain and muscle Arnt-like protein-1 (BMAL1, homolog of dCYC), which also form a heterodimeric transcriptional activator (see Fig. 1). PER and cryptochrome

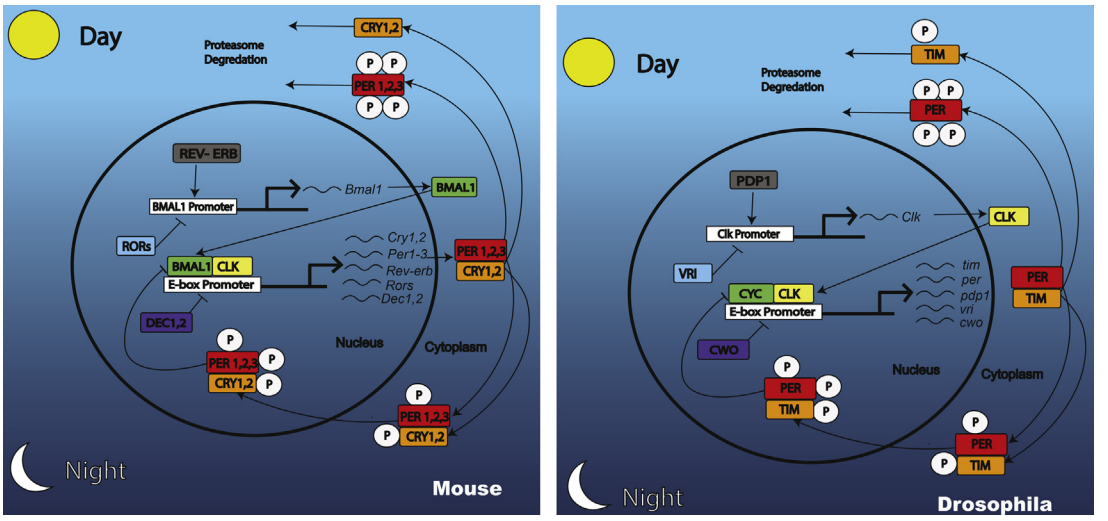

Fig. 1. Canonical molecular clocks in flies and mammals. Simplified *Drosophila* and mammalian circadian circuits. Arrows indicate activation and bars denote inhibition. Wavy lines represent rhythmic transcription. P marks phosphorylation. See text for more detail. BMAL1, brain and muscle Arnt-like protein-1; CRY, cryptochrome; CWO, clockwork orange; DEC, deleted in esophageal cancer; PDP, PAR-domain protein; PER, period; REV-ERB, reverse orientation c-erbA; ROR, retinoic acid related orphan receptor; TIM, timeless; VRI, vrille.