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Circadian timekeeping and output mechanisms in animals

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Daily rhythms in animal behavior, physiology and metabolism are driven by cell-autonomous clocks that are synchronized by environmental cycles, but maintain ~24 hours rhythms even in the absence of environmental cues. These clocks keep time and control overt rhythms via interlocked transcriptional feedback loops, making it imperative to define the mechanisms that drive rhythmic transcription within these loops and on a genome-wide scale. Recent work identifies novel post-transcriptional and post-translational mechanisms that govern progression through these feedback loops to maintain a period of ~24 hours. Likewise, new microarray and deep sequencing studies reveal interplay among clock activators, chromatin remodeling and RNA Pol II binding to set the phase of gene transcription and drive post-transcriptional regulatory systems that may greatly increase the proportion of genes that are under clock control. Despite great progress, gaps in our understanding of how feedback loop transcriptional programs maintain ~24 hours cycles and drive overt rhythms remain.

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Introduction

Organisms exposed to daily environmental cycles display diurnal rhythms in physiology, metabolism and behavior. These rhythms are generated and sustained by cell-autonomous circadian clocks, which help organisms anticipate predictable changes in the environment. They continue to operate in constant environmental conditions (i.e., free-run) with a period of about 24 hours. Genetic and molecular analysis of circadian clocks in Drosophila and mice revealed that the circadian timekeeping mechanism consists of interlocked transcriptional feedback loops, which drive rhythmic transcription of 'clock genes' that encode feedback loop components and 'output genes' that control physiological, metabolic and behavioral rhythms. Most clock genes are well conserved from insects to humans, and with few exceptions, play similar roles in the timekeeping mechanism.

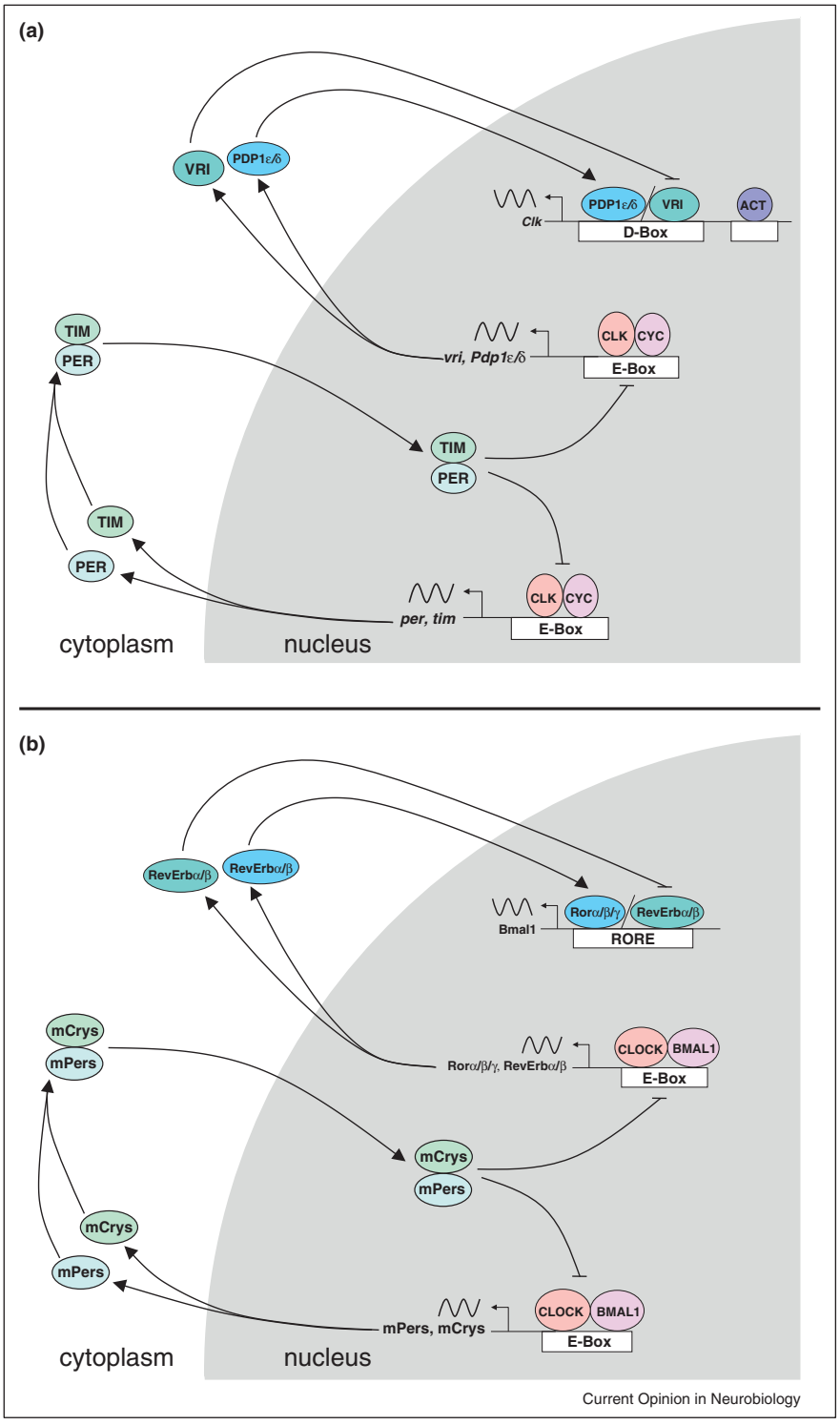
Although transcriptional feedback loops were established as the molecular basis of circadian timekeeping more than 20 years ago [1,2], fundamental questions remain about the mechanisms by which these feedback loops sustain ~24 hours rhythm and drive rhythmic expression of output genes. Here we will review recent studies of clock protein synthesis and modifications that provide significant insight into post-transcriptional mechanisms that control feedback loop progression, and whole genome analysis of transcription, protein–DNA binding and chromatin modifications that shed new light on clock regulation of rhythmic gene expression.

The architecture of transcriptional feedback loops in animals

Transcriptional feedback loops that keep circadian time in animals have been largely derived from studies in Drosophila and mice. These feedback loops have recently been reviewed [3–5]; thus, we will present a sketch of their essential working parts (Figure 1). In both of these model systems, a pair of orthologous basic helix–loop–helix PER-ARNT-SIM (bHLH-PAS) transcription factors called CLOCK and BMAL1 (or its homologue NPAS2) in mammals and CLOCK (CLK) and CYCLE (CYC) in Drosophila form heterodimers that bind E-box regulatory elements to activate transcription of genes encoding their repressors, CRYPTOCHROME 1 and CRYPTOCHROME 2 (mCRYs) and PERIOD 1 and PERIOD 2 (mPERs) in mammals and PERIOD (PER) and TIMELESS (TIM) in Drosophila [6–10]. mPER–mCRY complexes in mammals and PER–TIM complexes in Drosophila accumulate in the cytoplasm, move into the nucleus, and then bind to and inactivate the CLOCK–BMAL1 and CLK–CYC activators, respectively, to repress transcription [11,12]. mPER–mCRY and PER–TIM are then degraded, which permits the activators to bind E-boxes and initiate the next cycle of transcription. The primary function of this 'core' feedback loop is to determine circadian period.

CLOCK–BMAL1 and CLK–CYC also activate a second 'interlocked' feedback loop that controls rhythmic expression of activator genes (e.g., Bmal1 and *Clk*), which are transcribed in the opposite circadian phase as repressor genes (e.g., mPers/mCrys and *per/tim*) [13,14]. In mammals, this feedback loop is controlled by the nuclear hormone receptors Ror α/β/γ and RevErb α/β, which bind RevErbA/Ror-binding elements (RREs) to activate and repress Bmal1 transcription, respectively [15,16]. In

Figure 1



Interlocked feedback loops that keep circadian time. Genetic architecture of the core and interlocked feedback loops of Drosophila **(a)** and mice **(b)**. Gene, protein and regulatory element names are as defined in the text. Sinusoidal lines represent rhythmic mRNAs; arrows depict the synthesis, assembly and/or localization of clock proteins; blocked line denotes repression; gray background indicates events in the nucleus; white background indicates events in the cytoplasm.