Review

Molecular architecture of the mammalian circadian clock

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Circadian clocks coordinate physiology and behavior with the 24 h solar day to provide temporal homeostasis with the external environment. The molecular clocks that drive these intrinsic rhythmic changes are based on interlocked transcription/translation feedback loops that integrate with diverse environmental and metabolic stimuli to generate internal 24 h timing. In this review we highlight recent advances in our understanding of the core molecular clock and how it utilizes diverse transcriptional and post-transcriptional mechanisms to impart temporal control onto mammalian physiology. Understanding the way in which biological rhythms are generated throughout the body may provide avenues for temporally directed therapeutics to improve health and prevent disease.

A clockwork physiology

Mammalian physiology and behavior are coordinated by an intrinsic molecular clock into rhythms that are synchronized with the 24 h solar day. Circadian (Latin *circa diem*, meaning 'about a day') synchronization allows anticipation of regular environmental changes to influence molecular and behavioral decisions that impact fitness and survival, including food intake and metabolism, predator/prey interactions, and the evasion of DNA damage from environmental insults, amongst others [1]. Circadian rhythms therefore allow an animal to achieve temporal homeostasis (see Glossary) with its environment at the molecular level by regulating gene expression to create a peak of protein expression once every 24 h to control when a particular physiological process is most active with respect to the solar day. For example, DNA damage induced by solar irradiation is preferentially repaired by the nucleotide excision repair pathway in the late afternoon and early evening, whereas the ability to repair such damage is at its lowest before dawn [2]. Temporal regulation of this pathway, which plays a critical role in maintaining genomic integrity, is conferred by the circadian clock through control of xeroderma pigmentosum A (XPA) protein expression, a rate-limiting factor in excision repair of UV-induced dipyrimidine photoproducts [3]. To understand better the powerful role of the circadian clock in coordinating physiology and behavior we highlight recent advances in our understanding of the molecular mechanisms used to generate circadian rhythms of protein expression. Exciting new studies point towards an unanticipated integration of diverse transcriptional and post-transcriptional mechanisms to generate circadian rhythms of protein expression on a tissue-specific basis, demonstrating that the molecular clock utilizes many strategies to regulate circadian output and temporal homeostasis with the external environment.

Clocks throughout the body

A hierarchical timing system

Circadian rhythms are genetically encoded by a molecular clock located in nearly every cell that generates internal timing of approximately 24 h in the absence of external cues (Box 1). Molecular clocks located throughout the body in peripheral tissues are organized into a coherent, hierarchical system by a 'master' clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus [4]. The SCN is comprised of approximately 20 000 neurons that form a highly unified circadian network [5]. This master clock is the only molecular clock to receive light input from the retina that synchronizes internal clock timing to the external solar day, which it passes on to peripheral clocks via endocrine and systemic cues [6,7]. Molecular clocks located in neurons of the SCN and throughout peripheral tissues share the same molecular architecture and capacity to generate sustained circadian rhythms [8], although one key difference between master and peripheral clocks lies in the degree of their intercellular coupling [9]. A high degree of intercellular coupling among neurons of the SCN forms a neuronal network that is resistant to phase perturbations from internal cues [6], whereas the phase of

Glossary

Circadian time (CT): a standard of time based on the internal free-running period of a circadian clock. By convention, the onset of activity in diurnal organisms defines circadian time zero (CT 0; usually 6 am), whereas the onset of activity in nocturnal organisms defines circadian time 12 (CT 12).
Entrainment: synchronization of an internal circadian oscillator to an environmental stimulus that occurs at regular intervals (usually with ~24 h periodicity).
Free-running: the state of a self-sustaining molecular rhythm (oscillation) in the absence of any external cues that may affect the period of the oscillator.
Homeostasis: the tendency to maintain internal equilibrium by adjusting physiological processes.

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Box 1. The molecular clock in mammals

The cell-autonomous molecular clock in mammals is generated by two interlocking transcription/translation feedback loops (TTFL) that function together to produce robust 24 h rhythms of gene expression. The core TTFL is driven by four integral clock proteins: two activators (CLOCK and BMAL1) and two repressors (PER and CRY), as well as by kinases and phosphatases that regulate the phosphorylation (P) and thereby localization and stability of these integral clock proteins (kinases: CKIα, CKIδ, and CKIε; phosphatases PP1, PP5). CLOCK and BMAL1 are subunits of the heterodimeric basic helix-loop-helix-PAS (PER-ARNT-SIM) transcription factor CLOCK:BMAL1 [59], which activates transcription of the repressor *Per* and *Cry* genes, as well as other clock-controlled output genes. PER and CRY proteins heterodimerize in the cytoplasm and translocate to the nucleus to interact with CLOCK:BMAL1, inhibiting further transcriptional activation. As PER and CRY proteins are degraded through ubiquitin (Ub)-dependent pathways [72,73,109–111], repression on CLOCK:BMAL1 is relieved and the cycle begins again with ~24 h periodicity (Figure I). The casein kinases CKIδ and CKIε play an important role in determining the intrinsic period of the clock by controlling the rate at which the PER:CRY complexes are either degraded or enter the nucleus, and their activity is either counteracted or regulated by the phosphatases PP1 and PP5, respectively [48,112]. Notably, familial mutations resulting in the loss of a single phospho-acceptor site on PER2 (S662G) [113] or a loss-of-function mutation in CKIδ (T44A) [114] shorten the intrinsic period of the clock in mice and give rise to sleep phase disorders in humans. A key role for the casein kinases in establishing period length has also been demonstrated pharmacologically via modulation of the kinases with small-molecule inhibitors, which dramatically lengthen the period by modulating PER localization and stability [104,107]. A second TTFL is generated through transcriptional activation by the retinoid-related orphan receptors (RORa, b, c) [115] and repression by REV-ERBα/REV-ERBβ [32]. TTFL drives rhythmic changes in *Bmal1* transcription and introduces a delay in *Cry1* mRNA expression that offsets it from genes regulated strictly by CLOCK:BMAL1 [55]. Although rhythmic changes in BMAL1 abundance are not required to drive the core TTFL loop [17], the ROR/REV TTFL-induced delay in *Cry1* expression is crucial for proper circadian timing [55]. The presence of cooperative, interlocking feedback loops provides robustness against noise and environmental perturbations to help maintain accurate circadian timing, and also helps to generate phase delays in circadian transcriptional output that optimally time gene expression for local physiology [44]. Abbreviations: BMAL1, brain and muscle ARNT-like 1; CLOCK, circadian locomotor output cycles kaput; CKI, casein kinase I; CRY, cryptochrome; PER, period; PP, protein phosphatase.

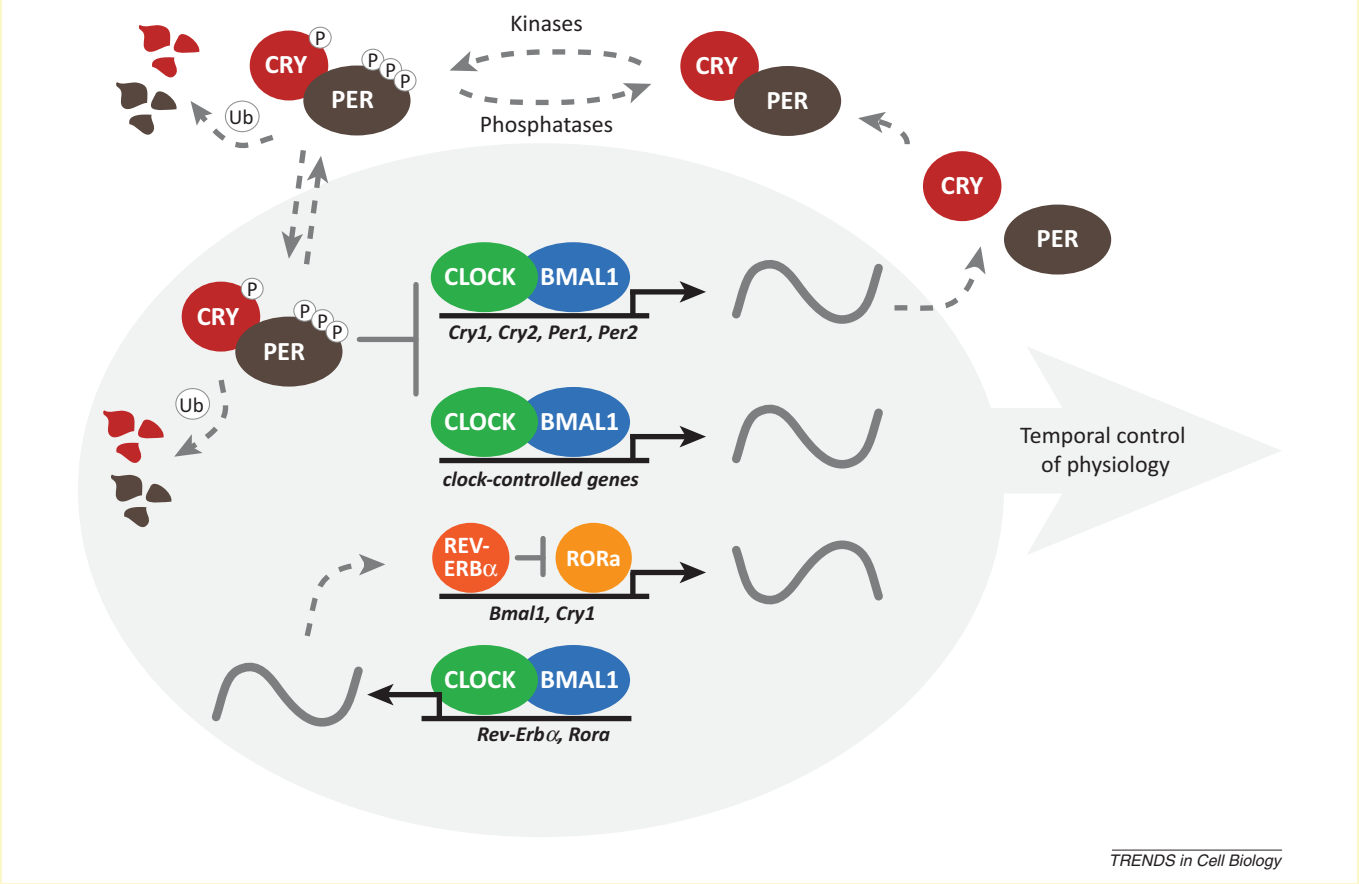

Figure I. Temporal control of physiology via four integral clock proteins: two activators (CLOCK, circadian locomotor output cycles kaput; and BMAL1, brain and muscle ARNT-like 1) and two repressors (period, PER; and cryptochrome, CRY).

peripheral clocks is susceptible to adjustment from the SCN clock via circulating hormones and other metabolic cues [10,11], as well as via systemic changes such as body temperature [12,13]. This network logic ensures that the master clock faithfully keeps intrinsic ~24 h timing to maintain temporal coordination with the external solar cycle, whereas peripheral clocks adapt to reflect the local metabolic status of the tissues in which they function [14].

Circadian regulation of physiology is locally controlled

Recent studies utilizing genetic tools have explored how master and peripheral clocks are integrated with circadian control of physiology. Single knockouts of most integral