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Circadian proteins in the regulation of cell cycle and genotoxic stress responses

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The mammalian circadian system has been implicated in the regulation of the genotoxic stress response of an organism; however, the underlying molecular mechanisms are not well understood. Recent data suggest that, in addition to circadian variations in the expression of genes involved in genotoxic stress responses, core circadian proteins PERIOD1 (PER1) and TIMELESS (TIM) interact with components of the cell cycle checkpoint system, such as ataxia telangiectasia mutated (ATM)-checkpoint kinase 2 (Chk2) and ataxia telangiectasia and Rad3-related (ATR)-Chk1, and are necessary for activation of Chk1 and Chk2 by DNA damage. Moreover, in complex with its recently identified partner, TIM-interacting protein (TIPIN), TIM interacts with components of the DNA replication system to regulate DNA replication processes under both normal and stress conditions. These discoveries shed new light on the role of core circadian proteins in various cellular and physiological processes.

Introduction

Multiple physiological and behavioral processes in a broad variety of organisms demonstrate 24-hour periodicities that are driven by an intrinsic time-keeping system called the circadian system. It is thought that the circadian system evolved to enable adaptation of organisms to periodically changing light in their environment. In the past decade, genes encoding the core components of the molecular clock have been identified, and mice with targeted disruption of individual clock genes have been generated. The spectrum of phenotypes observed in circadian mutant mice underscores the functional importance of the circadian proteins and the circadian system in the general fitness of an organism [1,2]. Here, we discuss recent data revealing roles for core clock proteins in cell cycle regulation and genotoxic stress responses; these data might aid our understanding and treatment of cancer.

The organization of the mammalian circadian system and molecular circadian oscillator

The mammalian circadian clock is organized in a hierarchical way, in which the central pacemaker is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. Individual SCN neurons generate and sustain 24-hour periodicities in electrical and metabolic activities, and produce rhythmic neuronal and humoral output signals that synchronize multiple peripheral clocks located in other organs and tissues. These peripheral oscillators, in turn, govern rhythms in the gene expression or enzymatic activities that underlie the overt rhythms in physiology, behavior and metabolism [3].

At the molecular level, the circadian clock is organized as transcription–translation-based networks of positive and negative feedback loops, schematically shown in Figure 1. The positive components of the loops are the transcription factors CLOCK (or its closest homolog, neuronal PAS-domain 2 (NPAS2)) and brain and muscle aryl hydrocarbon receptor nuclear translocator-like (BMAL1), which form a complex that regulates the expression of target genes harboring E-box regulatory elements in their promoter regions. Among the genes directly induced by CLOCK-BMAL1 are those encoding their own repressors, PERIODs (PERs) and CRYPTOCHROMEs (CRYs), which represent negative components of the major feedback loop, in addition to the nuclear receptors REV-ERB-α and retinoid-related orphan receptor-α (ROR-α), which are responsible, respectively, for inhibition or activation of *Bmal1* transcription, thus forming an interlocked regulatory loop. In addition to transcriptional regulation of core components of molecular circadian machinery, the CLOCK-BMAL1 complex regulates the expression of multiple clock-controlled genes (CCGs) either directly or indirectly through the activity of other transcription factors harboring E-box elements in their promoter regions [4]. It is believed that rhythmic expression of first- and second-order CCGs underlies circadian output in physiology and metabolism [5].

Various post-translational modifications of the core circadian proteins provide an additional level of regulation of the molecular oscillator. All of them are subject to modifications such as phosphorylation and/or sumoylation, and the pattern of these modifications also displays circadian rhythmicity [6,7]. As would be expected, post-translational modifications affect the functional activity of circadian proteins. Thus, the phosphorylation status of the CLOCK-BMAL1 complex is associated with its stability, nuclear or cytoplasmic distribution, and transcriptional activity [8]. CLOCK-dependent sumoylation of BMAL1 alters its

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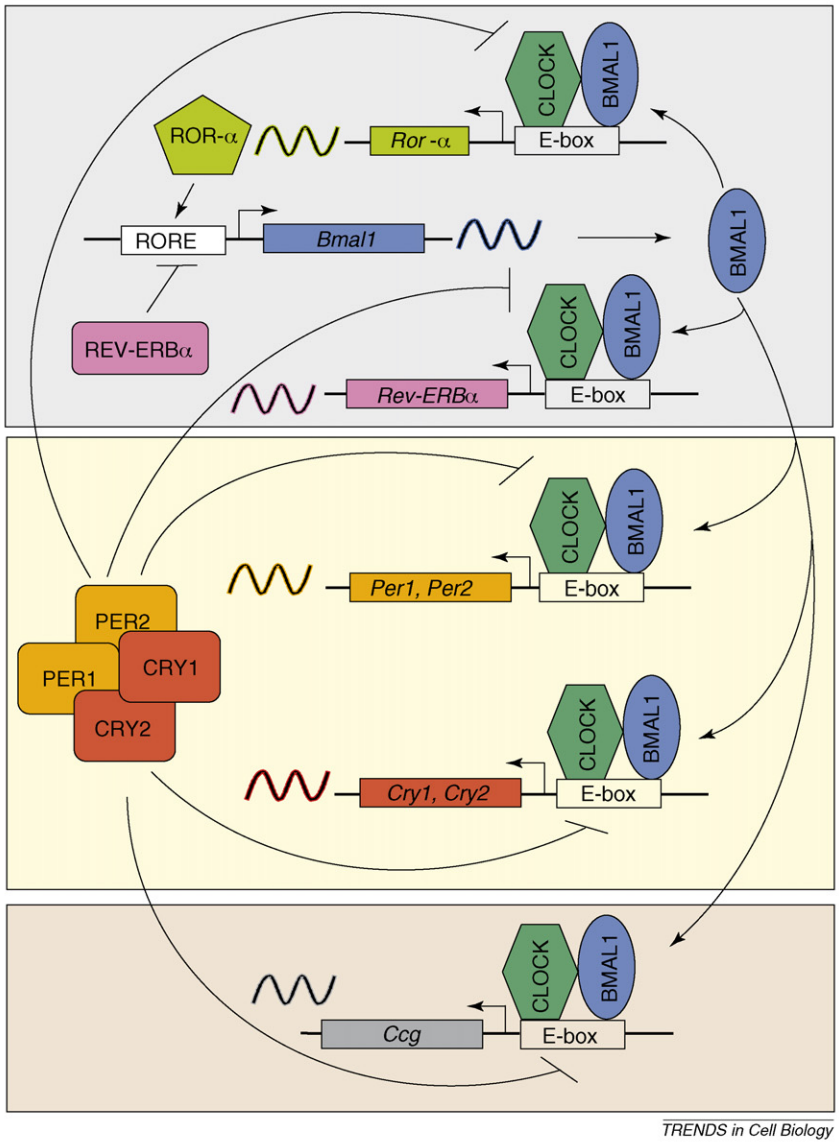

Figure 1. Model of the mammalian circadian oscillator. The basic helix–loop–helix PAS (Per-Arnt-Sim)-domain containing transcription factors BMAL1 and CLOCK (or, in some tissues, its closest homolog, NPAS2) function as a heterodimer to activate transcription of a group of core clock genes (*Pers* and *Crys*) and two nuclear receptors (*Rev-Erbα* and *Rorα*) through a specific E-box element in their promoters. Proteins encoded by *Cry1* and *Cry2* function as potent repressors of CLOCK-BMAL1 transcriptional activity, thus inhibiting their own expression and the expression of other CLOCK–BMAL1 targets. PER proteins interact with CRYs and are important for nuclear to cytoplasmic shuttling of the complex. PERs and CRYs represent negative components of the major circadian transcriptional feedback loop (i) REV-ERB-α and ROR-α are two nuclear receptors that compete for the ROR response element (RORE) in the promoter of the *Bmal1* gene and repress or activate its transcription, respectively. In this way, REV-ERB-α and ROR-α regulate the expression of the positive component of the circadian feedback loop [57] (ii). CLOCK-BMAL1 also regulates the expression of multiple CCGs. This regulation can occur directly through E-box elements in the promoters of target genes (first-order CCGs) or indirectly through the activity of other transcription factors that are controlled by CLOCK-BMAL1 (second-order CCGs) (iii). It is believed that the rhythmic expression of numerous CCGs provides the basis for the rhythmic circadian output in physiology and metabolism.

stability [6]. Phosphorylation of PERs and CRYs affects their stability and intracellular distribution [9,10]. Detailed analysis of post-translational regulation of the circadian proteins has been recently reviewed [11].

The circadian clock regulates genotoxic stress response pathways

A growing amount of experimental data suggests that the circadian system is involved in the regulation of diverse physiological and biochemical processes, involving those that determine the response to genotoxic stress induced by anticancer treatment. This identifies clock proteins as potential targets for pharmacological intervention (Box 1). The initial insight into possible molecular mechanisms underlying the circadian control of response to genotoxic stress came from a series of microarray studies. Temporal gene expression profiling in several tissues indicated that between 2% and 10% of all genes are transcribed in a circadian manner [5,12–14], resulting in 24-hour oscillations in their mRNA levels. In the liver, it includes transcripts encoding many drug-metabolizing enzymes and drug transporters. These proteins are responsible for detoxification and removal of various toxic compounds, including those formed in cells and tissues as a result of genotoxic cancer treatment [15].

In addition to drug-metabolizing and -transporting enzymes, several key regulators of cell cycle progression and genotoxic stress responses display circadian patterns