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# Stem cells and the circadian clock

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ABSTRACT

The circadian timing system is a complex biological network of interacting circadian clocks that regulates 24 h rhythms of behavioral and physiological processes. One intriguing observation is that stem cell homeostasis is subject to circadian clock regulation. Rhythmic oscillations have been observed in a variety of embryonic and adult stem cell dependent processes, such as hematopoietic progenitor cell migration, the hair follicle cycle, bone remodeling, regenerative myogenesis and neurogenesis. This review aims to discuss the nature of the circadian clock in embryonic stem cells and how it changes during differentiation. Furthermore, it will examine how the circadian clock contributes to adult stem cell function in different tissues of the body with an emphasis on the brain and adult neurogenesis.

## 1. The circadian clock

### 1.1. The hallmarks of the circadian timing system

Organisms face regular changes in their environment linked to day and night cycles, including, for example, variations in the availability of food or the activity of predators. In order to adapt to these cyclical daily changes, organisms possess an internal timing system, proactively orchestrating their behavior and physiology. In modern societies, humans are no longer subjected to variations in prey and predator presence, but many aspects of human behavior (*e.g.*, sleep/wake cycle) and physiology (*e.g.*, hormone secretion, body temperature, metabolism) are still regulated by the same timing system. This system consists of biological clocks that can be found in almost every cell of the body. Via regulatory mechanisms including rhythmic transcriptional, post-transcriptional and post-translational modulation of gene expression and function such clocks produce rhythmic changes in behavior and physiology (Atger et al., 2017; Lim and Allada, 2013; Reddy et al., 2006a, 2006b). These clocks have particular hallmarks: they conduct rhythms with a periodicity of approximately 24 h and, thus, are called “circadian” clocks (coined from Latin: circa-diem = around a day). Circadian clocks are endogenous and self-sustained, leading to rhythms that persist in constant conditions such as sustained darkness. However, to remain synchronized with their environment, they are “entrainable” or “resettable” by external time cues, the most prevailing one being light (Roenneberg et al., 2013). In chronobiology, these cues are called *Zeitgeber* (German, literally: “time giver”). This property of the clock becomes obvious during "jet-lag", which causes a temporary disruption of the sleep/wake cycle that soon adapts to the new environmental light conditions.

### 1.2. The organization of circadian clocks in vertebrates

The first experiments aiming to locate the clock that drives circadian rhythms in mammals pointed to the suprachiasmatic nucleus (SCN). This small region of the brain is a paired neuronal structure located in the anteroventral hypothalamus above the optic chiasm (Brancaccio et al., 2014). Ablating the SCN in rodents resulted in abolished circadian locomotor and endocrine rhythms (Moore and Eichler, 1972), as well as in circadian feeding (Nagai et al., 1978) and drinking behavior (Stephan and Zucker, 1972). A transplantation of SCN tissue can restore these rhythms (Lehman et al., 1987). Moreover, the donor tissue dictates its period length to the restored rhythms of the recipient (Ralph et al., 1990). The discovery of the first circadian clock genes led to the observation that their self-sustained oscillatory expression is not restricted to neural structures such as the SCN, but can also be found in virtually all cells of the body (Dibner et al., 2010). In this network of oscillating cells, the mammalian SCN fulfils the role

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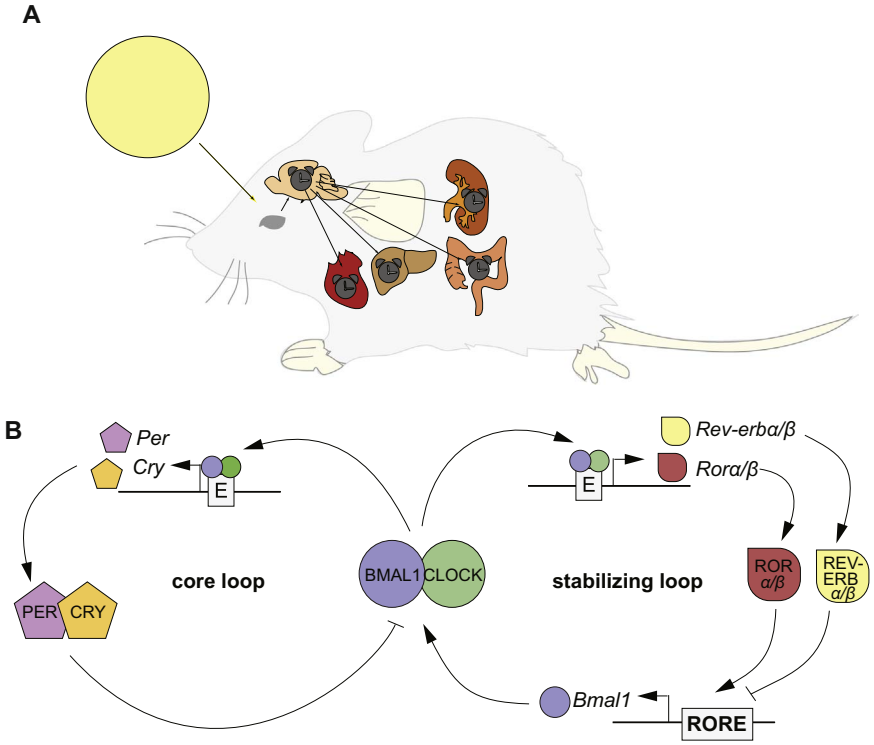


**Fig. 1.** The circadian timing system in mammals. (A) Schematic overview of the circadian clock system in mammals. The suprachiasmatic nucleus (SCN), a small region in the brain, is hierarchically at the top of all body clocks. After receiving light (yellow circle representing the sun) entrainment information from the eyes, the SCN acts as a central pacemaker to synchronize the circadian clocks outside the SCN, including the circadian clock of, for example, the liver and the heart via systemic cues. (B) Schematic of the molecular mechanism of the circadian clock oscillator. On a molecular level, circadian clocks consist of a core loop and accessory loops, such as the stabilizing loop. In the core loop, a heterodimer of bHLH/PAS transcription factors, namely CLOCK (green) and BMAL1 (blue), bind to E-box enhancer elements (E; gray) of the *Per* (purple) and *Cry* (orange) genes in order to initiate transcription. The PER and CRY proteins are translated and accumulate in the cytosol. Here, they heterodimerize and are translocated into the nucleus to repress CLOCK/BMAL1 activity, causing the repression of their own transcription. This mechanism is regulated by several posttranslational modifications that cause delays in the process such that a cycle takes about 24 h to complete. In the stabilizing loop, CLOCK/BMAL1 activity leads to the expression of REV-ERBα/β (yellow) and RORα/β (red), which regulate the rhythmic expression of *Bmal1* by binding to the RORE (gray).

of a "central" or "master" pacemaker orchestrating the tissue clocks in peripheral organs (Fig. 1A). Environmental light sensed by the retina leads to entrainment of the central pacemaker clocks in the SCN. This timing information is then forwarded via neuronal and humoral signals, to other areas of the brain, such as the pineal gland responsible for melatonin release, and to the peripheral organ clocks (Dibner et al., 2010; Hastings et al., 2007). Interestingly, under certain conditions, conflicting systemic signals can lead to a decoupling of peripheral clocks from synchronization with the central pacemaker. For example, when mice are fed only during daytime (their rest phase) their liver clocks show a phase shift of up to 12 h compared to the SCN, which remains locked to the light phase (Damiola et al., 2000). This indicates that the peripheral clocks are able to integrate various physiological signals in order to mount appropriate rhythms in their tissues.

It has been proposed that mammals possess a more centralized organization of their circadian clocks than non-mammalian vertebrates (Cahill, 2002; Falcon et al., 2010; Menaker et al., 1997). In fish, amphibians, reptiles and birds, the retina and the pineal gland serve as central pacemaker structures, acting together with or even dominating the SCN or other brain clocks. In zebrafish, peripheral tissue clocks are directly light sensitive, reflecting expression of photoreceptors such as opsins in a wide variety of tissues (Cavallari et al., 2011; Whitmore et al., 2000). In contrast to the SCN-centered mammalian brain (Wilsbacher et al., 2002), the anatomically defined SCN equivalent in zebrafish is only one of many brain nuclei showing a high clock gene expression and activity (Moore and Whitmore, 2014; Weger et al., 2013). However, under some conditions, the SCN seem to be dispensable for systemic rhythm generation also in mammals, and other ill-defined oscillators take over. For example, a food-entrainable oscillator drives food anticipatory activity rhythms (Guilding and Piggins, 2007; Mistlberger, 2011; Patton and Mistlberger, 2013). The precise relationship of this oscillator mechanism to the SCN still needs to be defined. It has recently been suggested that a larger neural network, that comprises the SCN, generates food anticipatory activity (Acosta-Galvan et al., 2011). In this view, it is tempting to speculate that both mammalian and non-mammalian circadian systems possess decentralized oscillator networks. Several modes of centralization may have evolved in the different vertebrate lineages starting from a highly decentralized system in fish to a centralized system with dominance of the SCN pacemaker being a unique innovation of mammals.

### 1.3. The molecular clockwork

The molecular mechanism underlying circadian clock rhythms consists of a transcriptional-translational feedback loop that takes approximately 24 h to complete. The circadian clock genes themselves are not conserved between the different groups of organisms, but a common principle in all organisms is the generation of circadian rhythms by such a transcriptional-translational feedback loop (Bell-Pedersen et al., 2005; Mohawk et al., 2012). In vertebrates, the molecular "clockwork" can be subdivided into the so-called core loop and the stabilizing loop (Fig. 1B). In the core loop, a heterodimer of the "positive" factors of the circadian clock, CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 (Brain and Muscle Arnt-Like protein), binds to E-box enhancer elements to activate transcription of their target genes. Among these target genes are the "negative" factors Cryptochrome (*Cry*) and Period (*Per*), acting as inhibitors of their own expression. After the translation and dimerization of the PER and CRY proteins, the PER/CRY complex translocates into the nucleus, where it inhibits the transcriptional activity of the CLOCK/BMAL1