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MINI-REVIEW

Circadian adaptation to cell injury stresses: a crucial interplay of BMAL1 and HSF1

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Abstract The circadian clock system confers daily anticipatory physiological processes with the ability to be reset by environmental cues. This "circadian adaptation system" (CAS), driven by cell-autonomous molecular clocks, orchestrates various rhythmic physiological processes in the entire body. Hence, the dysfunction of these clocks exacerbates various diseases, which may partially be due to the impairment of protective pathways. If this is the case, how does the CAS respond to cell injury stresses that are critical in maintaining health and life by evoking protective pathways? To address this question, here we review and discuss recent evidence revealing life-protective (pro-survival) molecular networks between clock (e.g., BMAL1, CLOCK, and PER2) and adaptation (e.g., HSF1, Nrf2, NF-κB, and p53) pathways, which are evoked by various cell injury stresses (e.g., heat, reactive oxygen species, and UV). The CK2 protein kinase-integrated interplay of the BMAL1 (clock) and HSF1 (heat-shock response) pathways is one of the crucial events in CAS.

Keywords Circadian clock · Heat shock · Oxidative stress · Adaptation · Protein kinase

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Why did circadian clock systems evolve? We assume that daily changes in certain stressors on ancient earth, such as strong reactive oxygen species (ROS)-producing solar radiation during the daytime, caused lethal damage and various diseases by ROS-induced genotoxic/proteotoxic stresses, compelling the evolution of such clocks as a daily-regulated protection system. The circadian system, with the ability to undergo adaptive synchronization (resetting) by environmental cues, orchestrates a wide variety of physiological rhythms via global rhythmic gene expression. In mammals, the molecular core of the circadian system consists of clock proteins (BMAL1, CLOCK, CRY1/2, and PER1–3) that operate in transcriptional–translational negative feedback loops [1–6]. The functions of clock proteins are modulated by protein modification enzymes (e.g., CK1, CK2, CLOCK, and SIRT1) [7–15]. In this review, we summarize advances in the study of the circadian adaptation system (CAS) in which clock and life-protective (pro-survival; survival-promoting) adaptation (e.g., anti-oxidant, anti-apoptotic, DNA repair, and heat-shock response; HSR) pathways form molecular networks that act against critical environmental stresses (Fig. 1). We also discuss whether circadian clocks are indispensable for life.

Responses of circadian clocks to critical stresses

For the maintenance of health and survival in response to critical stresses, such as heat, cold, ROS-producing stimuli, low/high pH, and UV, circadian clocks are synchronized (reset; i.e., each cellular clock phase is shifted), changing the period or amplitude of circadian oscillators. Circadian *Per2* rhythms of fibroblasts are synchronized in response to an appropriate level of acute heat shock (43 °C for approximately 30 min) [16]. Longer exposure to heat

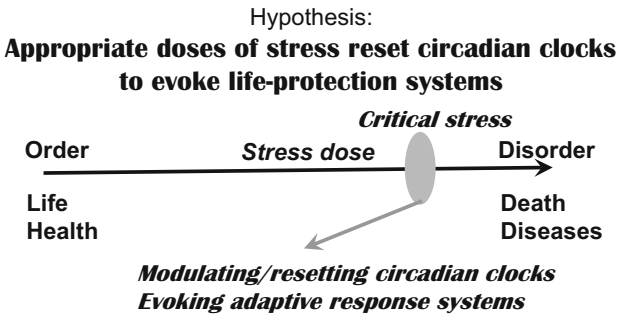

Fig. 1 Critical stress reset circadian clocks to evoke life-protection systems. See the text for more details

decreases cultured cell viability. Interestingly, exposure to a warm water bath (41 °C for 2 days) stimulates phase shifts of the peripheral (liver and kidney) circadian clocks in PER2::LUCIFERASE mice [17], with a core body temperature maintained at 40–41 °C. Longer exposure to this high core body temperature (1–3 °C higher than normal) would be critical to the health and survival of mice. From these findings on clock synchronization in response to stress, we hypothesized that appropriate (critical) doses of stresses modulate clock function and synergistically evoke stress-resistant adaptation systems (Fig. 1). In particular, critical oxidative stress (cOS) at the threshold of life and death induced by near-lethal doses of ROS (>1 mM H_2O_2 for 10 min) resets circadian *Per2* rhythms in fibroblasts and U2OS cells [18]. In addition, synergistically, this clock-resetting response evokes pro-survival pathways, such as HSF1 (HSR), Nrf2 (anti-oxidant) and NF-κB, (anti-apoptotic) pathways [18]. In UV-irradiated cells, we observed similar responses (paper in preparation). Extracellular pH levels affect circadian *Bmal1* rhythms in Rat-1 fibroblasts. Upon decreasing the pH from 7.5 to 6.2, the period of the rhythm shortens, and the oscillation dampens more rapidly [19]. Regardless of the timing of treatment, alkalinization by changing the pH from 7.0 to 7.4 resets the rhythm, with the trough level being reached at 27.6 ± 0.5 h (examined at CT4, 10, 16, and 22, where CT12 is defined as the trough time points of the rhythm) after the pH shift. In contrast, acidification by changing the pH from 7.0 to 6.6 at the CTs resets the rhythm, with the peak level being reached at 28.6 ± 0.4 h after the treatment. In addition, at low pH (6.7), circadian *Bmal1* rhythms in human primary fibroblasts are shortened and show lower amplitude without affecting cell viability [20]. This suggests that pro-survival programs work at pH 6.7 but not at a lower pH. Thus, our hypothesis may be applicable to cases with low pH. However, even several days of hypoxia in free-running rats did not cause any significant phase shift in the circadian pattern [21, 22], indicating that our hypothesis may not be applicable to cases with hypoxia. However, hypoxia affects the amplitude of circadian rhythms via HIF1α-mediated signaling [23].

To address the issue of whether CAS response is dependent on the circadian phase, we first need to show the phase response curve (PRC) of the clock toward each type of stress. However, thus far, only limited information on this has been reported. PRC of clocks in response to UV has been reported [24]. Wild-type mice show a robust response to UV, and the complete PRC to UV light has been described. Phase delays during the early subjective night (CT15 ± 1.5 h) were −127 ± 11 min, and small but significant advances occurred in the late subjective night (CT21 ± 1.5 h = 29 ± 8 min). No effect of UV was found during the subjective day (CT0–CT12) [24]. Regarding critical ROS, heat shock, and UV stresses that can affect cellular clock gene expression rhythms, we have already obtained PRC data suggesting the circadian-phase-dependent responses of CAS (paper in preparation).

Interplay of circadian and adaptive response systems

Next, we focus on the mechanism underlying the above-mentioned responses and the involvement of circadian clocks in adaptation to critical stresses. Adaptive response systems are directly activated by these stresses, mostly via intracellular signal transduction pathways (e.g., via activation/modulation by protein modification pathways) [25–29]. However, recent findings have revealed that the circadian system modulates (strengthens) the adaptive responsive pathways (Fig. 2) [30–34]. In addition, in response to stress, modifying enzymes for clock proteins, such as CK2 protein kinases and SIRT1 deacetylase, may modulate circadian core oscillators [12–15].

We hypothesize that the triangular interplay of the three categorized components (pathways or systems) forms the core of CAS (Fig. 2). Circadian–heat-shock response (HSR) crosstalk commonly occurs in response to genotoxic/proteotoxic stresses, such as ROS [18], heat shock [16], and UV (paper in preparation). Therefore, among adaptive response systems as the mediator of a core circadian oscillator, the HSR system is probably crucial for CAS operation. In circadian–HSR crosstalk, HSR triggers clock synchronization, whereas the clock activates HSR systems. Strikingly, CK2 phosphorylates HSF1-Thr142 and BMAL1-Ser90 in response to cOS, which induces interaction between BMAL1 and HSF1 to trigger clock synchronization and synergistic activation of pro-survival signaling pathways [18]. This CK2–BMAL1–HSF1 crosstalk exemplifies the core of the triangular interplay in CAS. CLOCK, EP100, and SIRT1 may also be integrators for

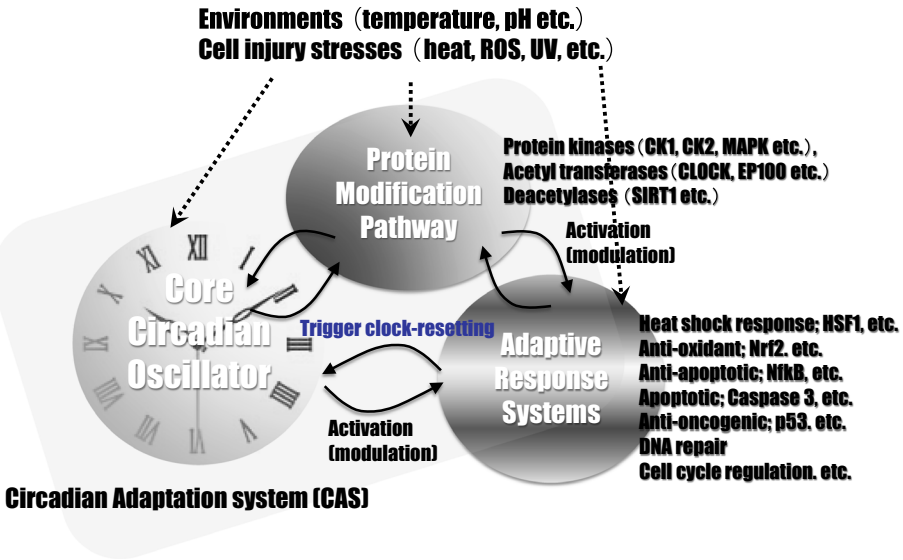

Fig. 2 The triangle network hypothesis of circadian adaptation systems (CAS). See the text for more details

CAS [33, 35] by the CLOCK-mediated acetylation of BMAL1-Lys537 [14], EP100-mediated acetylation of HSF1 [36], and SIRT1-mediated deacetylation of BMAL1 [15] and HSF1 [37].

Circadian–HSR crosstalk then evokes various adaptive response pathways. The circadian pathway activates Nrf2-mediated anti-oxidant [18, 38] and NF-κB,-mediated anti-apoptotic and anti-inflammatory [18, 39] pathways, while down-regulating the caspase-3-mediated apoptotic pathway [18]. The HSR pathway up-regulates the p53-mediated anti-oncogenic pathway via the genotoxic stress-induced HSF1–p53 interaction and the subsequent nuclear entry of p53 [40, 41]. In contrast, p53 suppresses the circadian pathway through the p53-mediated blocking of BMAL1–CLOCK binding to the *Per2* promoter to suppress *Per2* expression [42].

Conclusion and perspective: is the circadian clock indispensable for survival?

In this review, we have summarized advances in the study on the responses of CAS against cell injury stresses and propose a hypothesis explaining how CAS responds to such stresses via the interplay of circadian, adaptive response, and protein modification systems (Fig. 2). Upon exposure to ROS-induced cOS, the circadian system is indispensable for cellular survival. However, the protective role of the clock in multicellular systems, such as tissues/organs and the entire body, remains elusive. If a small population of damaged cells survives without repair through circadian-pathway-evoked pro-survival signals, the propagation of these surviving damaged cells may cause various diseases, such as cancer. In contrast, circadian–HSR crosstalk can evoke repair pathways, e.g., via p53. Therefore, to access the survival/anti-disease role of the circadian system at the entire body level, we would have to investigate the stress response using the disease models of animals.

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