Review

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Circadian redox oscillations and metabolism

Nikolay B. Milev and Akhilesh B. Reddy

Department of Clinical Neurosciences, University of Cambridge Metabolic Research Laboratories, National Institute for Health Research (NIHR) Biomedical Research Centre, Wellcome Trust–Medical Research Council (MRC) Institute of Metabolic Science, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK

Circadian rhythms are 24 h oscillations in physiology and behavior which allow organisms to anticipate and adapt to daily demands associated with the day/night cycle. The currently accepted model of the molecular clockwork is described as a transcriptional process composed of negative regulatory feedback loops. However, ample evidence underlines the important contribution of non-transcriptional and metabolic oscillations to cellular timekeeping. We summarize recent evidence pointing to the relationship between the transcriptional oscillator and metabolic redox state, with particular emphasis on the potential nodes of interaction. We highlight the intrinsic difficulty in segregating these two tightly coupled and interdependent processes, in living systems, and how disruption of their synchronicity impacts upon (patho)physiological processes as diverse as cardiovascular and metabolic disorders, aging, and cancer.

Co-evolution of the circadian and redox systems

The circadian clock is an intrinsic timekeeping mechanism that provides organisms from bacteria to humans with the means to temporally organize behavioral, physiological, and molecular events around the 24 h day–night cycle. These clocks likely evolved to enable organisms on Earth to resonate with their environment such that their internal cycles anticipate and match external rhythms. In mammals, many physiological processes are under circadian regulation, including the sleep–wake cycle, core body temperature, feeding behavior, glucose homeostasis, and various endocrine secretions (e.g., cortisol and melatonin), as well as some pathologies, including hypertensive crises, myocardial infarctions, and asthma and allergy attacks, that occur at specific times of the day [1–3].

Circadian clocks only confer an advantage when they beat in harmony with the external environment. Disturbance of circadian timing, as exhibited in rotational shift workers, is linked to significant health issues such as breast and prostate cancer, diabetes, and other metabolic complications [4–6]. In mammals, a central oscillator within the hypothalamic suprachiasmatic nuclei (SCN) integrates light signals from the retina, and orchestrates tissue/organ functions using neuronal efferents and humoral factors. Because almost all cells within tissues exhibit self-sustained oscillations [7–9], a key function of the SCN is to maintain proper phase alignment of peripheral clocks to ensure synchrony in the system [10]. The current model of the molecular clockwork (see Glossary) is based on a transcription/translation feedback loop (TTFL) mechanism, whereby a set of transcriptional activators induce the transcription of repressor genes whose protein products then feed back to inhibit their own transcription (Figure 1). However, mounting evidence suggests that transcription-based oscillators are not the only means by which the cells track time. The discovery of the self-autonomous redox rhythms exhibited by the peroxiredoxin (PRX) proteins provided the first convincing example of such transcription-independent oscillations in eukaryotes [11,12]. These highly conserved oscillations, that are found in organisms from Archaea to man, suggest a strong link between circadian rhythmicity and redox metabolism [13].

Why might redox regulation be tightly linked with circadian biology? The foundations of this coupling may have been laid down around the time of the Great Oxidation Event (GEO) approximately 2.5 billion years ago. The increase in atmospheric oxygen levels as a result of the newly acquired ability of photosynthetic bacteria to use water as the main electron donor are thought to have created a strong selective pressure on anaerobes to evolve defense systems to deal with this harsh and unprecedented

Glossary

Antioxidant defense: defense mechanisms which neutralize cytotoxic oxygen intermediates and maintain redox homeostasis.
Clockwork: the molecular and biochemical mechanisms which drive biological clocks.
Glutathione: a low molecular weight thiol-containing antioxidant, which serves as the main redox buffer in the cell.
Oscillator: a multicomponent system that never reaches equilibrium and is capable of generating self-sustained oscillations in various parameters such as gene expression and metabolite levels.
Peroxiredoxins: small, ubiquitously expressed antioxidant proteins which serve as the main sink for hydrogen peroxide (H_2O_2) in the cell.
Reactive oxygen/nitrogen species (ROS/RNS): a generic term used to describe reactive molecules and free radicals derived from oxygen. Examples include superoxide (O^{2-}), peroxynitrite ($OONO^-$), and H_2O_2.
Redox metabolism: the ensemble of redox reactions involved in core metabolism such as the reactions involved in glucose oxidation to pyruvate.
Redox poise: a term synonymous to redox homeostasis that is used to describe the balance in intracellular redox potential.
Restricted feeding (RF): a specialized feeding regimen wherein mice are allowed access to food for a limited period during the light phase.

Corresponding author: Reddy, A.B. (areddy@cantab.net).
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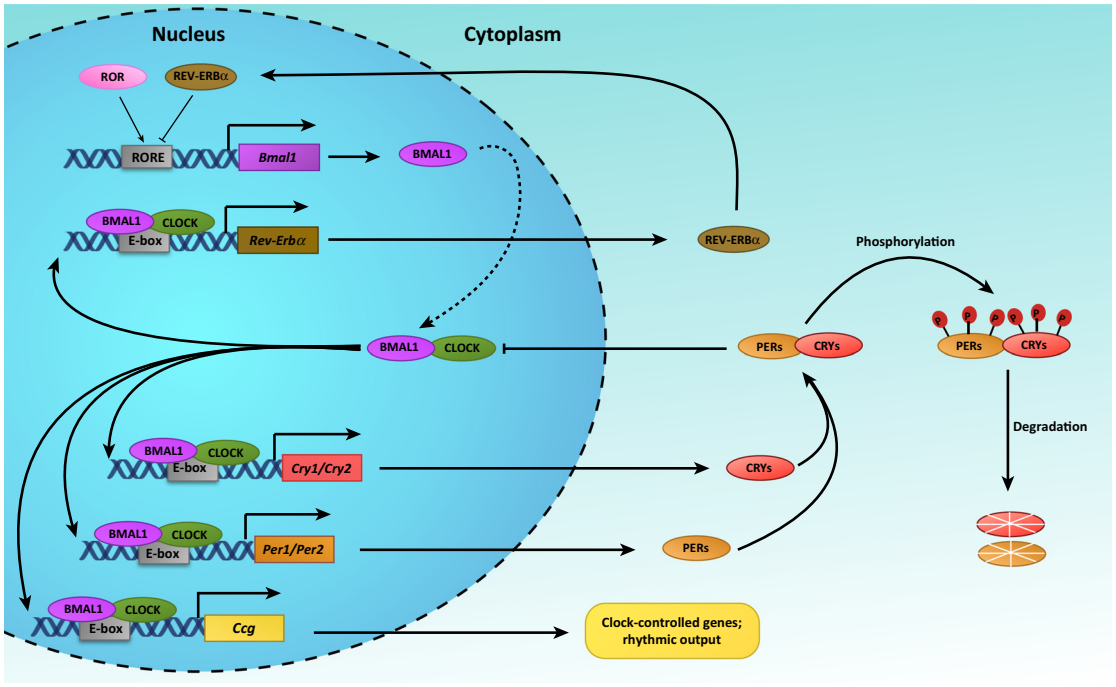

Figure 1. Simplified schematic diagram of the transcription-translation feedback loop (TTFL) in mammals. The basic helix–loop–helix (bHLH) transcription factors CLOCK and NPAS2 (neuronal PAS domain protein 2) associate with the bHLH transcription factor, BMAL1 (brain and muscle ARNT-like 1), to form heterodimeric transcriptional activator complexes. During the day, CLOCK/BMAL1 and BMAL1/NPAS2 bind to E-box enhancer elements and activate transcription of the period (*Per1*/*Per2*) and cryptochrome (*Cry1*/*Cry2*) genes. Upon accumulation in the cytoplasm, PER and CRY proteins assemble into heterotypic complexes that translocate back to the nucleus, where they repress their own transcriptional by inhibiting BMAL1/CLOCK. During the night, the PER and CRY complexes are gradually phosphorylated (P) and are targeted for degradation in the proteasome, thereby lifting the repression on CLOCK/BMAL1, which can then activate a new cycle of transcription. In addition to the primary feedback loop, the system also incorporates an accessory loop composed of the nuclear orphan receptors, retinoid-related orphan receptor (ROR), and nuclear receptor subfamily 1 group D members 1 and 2 (NR1D1/2 or REV-ERBα/β). The latter are under the direct transcriptional control of the BMAL1/CLOCK activator complex and negatively feed back to inhibit *Bmal1* transcription via binding to retinoic acid-related orphan receptor response elements (ROREs) in the *Bmal1* promoter. By contrast, ROR acts as a positive regulator and competes with REV-ERBs for binding to ROREs. The entire cycle takes approximately 24 h. Abbreviation: CCG, clock-controlled gene.

oxidizing environment [11,14]. Rhythmic photosynthesis, and thus oxygen production as a function of the changing day and night, as well as the generation of reactive oxygen species (ROS) by metabolic reactions, or directly by UV radiation, could have forced the coevolution of the circadian and redox systems. Thus, the generation of ROS and those processes sensitive to oxidation were temporally segregated, preventing harmful oxidative stress that would otherwise have led to cell dysfunction and death.

Metabolic redox oscillations in mammals

Temporal separation of cellular metabolism might be an adaptation to prevent the simultaneous occurrence of mutually-antagonistic reactions that would otherwise result in energetically-wasteful futile cycles. Consistent with this, numerous metabolic pathways and metabolite levels are under circadian regulation, and both genetic perturbation (e.g., clock gene mutants) and physiological disturbance (e.g., shift work) of the clock have been shown to increase susceptibility to metabolic stress [4,15]. Reciprocally, growing evidence highlights that core metabolism feeds back to the central oscillator, thereby affecting clock function. For instance, restricted feeding (RF) in mice is capable of entraining the hepatic clock in SCN-lesioned animals [16]. This is further supported by studies showing that RF phase-shifts both locomotor activity rhythms and circadian gene transcription in the periphery, but does not affect rhythms in the SCN [17,18]. Furthermore, in mice, high-fat diet results in alteration of the locomotor activity and the expression of canonical and clock-controlled genes [19], while in humans 3 weeks of circadian dys-synchrony is sufficient to induce a decrease in the resting metabolic rate and raise plasma glucose concentration after a meal, symptoms usually associated with the onset of diabetes mellitus [20].

The fact that metabolic changes can directly impact upon the transcriptional oscillator raises the question – what is the molecular mechanism that mediates this coupling? Of all the parameters that change as a function of cellular metabolism, redox poise represents one of the strongest candidates. This is not only due to its crucial role in cellular metabolism, but also because of the tight interplay between redox state and the circadian system. Accumulating evidence in recent years has pointed to the many potential nodes of interaction between the circadian and redox systems (Figure 2). The following sections detail some of the most important discoveries thus far.

Linking metabolic redox rhythms to the transcriptional oscillator

One of the first and, until now, most convincing examples of coupling between the transcriptional circadian oscillator and the redox systems is the finding that the molecular clock directly regulates the abundance of the redox metabolite Nicotinamide adenine dinucleotide, NAD^+ [21,22]. Binding of the CLOCK (Circadian Locomotor Output