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Review

Circadian Clocks and Metabolism: Implications for Microbiome and Aging

Georgios K. Paschos[1] and Garret A. FitzGerald[1,*]

The circadian clock directs many aspects of metabolism, to separate in time opposing metabolic pathways and optimize metabolic efficiency. The master circadian clock of the suprachiasmatic nucleus synchronizes to light, while environmental cues such as temperature and feeding, out of phase with the light schedule, may synchronize peripheral clocks. This misalignment of central and peripheral clocks may be involved in the development of disease and the acceleration of aging, possibly in a gender-specific manner. Here we discuss the interplay between the circadian clock and metabolism, the importance of the microbiome, and how they relate to aging.

Trends

The circadian clock directs rhythms of cellular energy metabolism, while cellular energy status regulates circadian clock-driven transcription.

Advances in metabolomics and metagenomics allow quantification of the enteral microbiota and permit the characterization of diurnal rhythms in microbiota abundance.

Changes in cellular energy metabolism associated with aging may be driven by reduced amplitude of circadian rhythms in older age.

The Circadian Clock

Life has adapted to energetic cycles, governed by the Earth's rotation, by evolving molecular mechanisms that anticipate the most advantageous time of day for biological processes. As a result, the majority of biological functions exhibit daily rhythms. In mammals, these **diurnal** (Glossary) oscillations are evoked by autoregulatory transcriptional and translational feedback loops known as circadian clocks [1]. The circadian clock of the **suprachiasmatic nucleus (SCN)** in the hypothalamus of mammals serves as the central pacemaker at the level of the organism. This has been shown directly in hamsters, where lesioning of the SCN rendered the animals arrhythmic while implantation of brain grafts containing fetal SCN restored circadian rhythms [2]. SCN neurons receive light information from specialized melanopsin-expressing intrinsically photosensitive ganglion cells in the retina via the retinohypothalamic tract and synchronize the phase of their circadian clock to the phase of the light [3]. Explanted SCN is capable of maintaining robust circadian rhythmicity for many days *in vitro*, while peripheral tissues, although rhythmic when explanted, show much less robust rhythms that do not persist as long [4]. This suggests that the circadian clocks in peripheral tissues require continuous **entrainment** to remain synchronized. The SCN transmits its rhythmic information to other brain regions and peripheral organs via neuronal connections, endocrine signals, body temperature rhythms, and indirect cues, provoked by oscillating behavior such as feeding rhythms (Figure 1, Key Figure) [3]. At the molecular level, the transcription factors brain muscle Arnt-like protein 1 (BMAL1) and clock locomotor output kaput (CLOCK) or neuronal PAS domain protein 2 (NPAS2) heterodimerize during the early circadian day, bind to E-box-containing elements of gene promoters, and induce transcription of downstream genes. Among these genes are Period (*Per*) and Cryptochrome (*Cry*), which encode repressors of BMAL1:CLOCK/NPAS2. During the early circadian night, PER and CRY translocate to the nucleus and form large complexes [5] that repress the transcriptional activity of BMAL1:CLOCK/NPAS2, thus down-regulating their own expression. Degradation of PER and CRY during the night ends this repression and allows the start of a new transcriptional cycle with a period of approximately 24 h. In an additional feedback loop, BMAL1:CLOCK/NPAS2 activates the transcription of

[1]Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, 3400 Civic Center Boulevard, Smilow – 10th Floor, Room 122, Building 421, Philadelphia, PA 19104, USA

*Correspondence: garret@exchange.upenn.edu (G.A. FitzGerald).

 http://dx.doi.org/10.1016/j.tig.2017.07.010

Rev-erb [also known as nuclear receptor subfamily 1, group D (*Nr1d*)] and RAR-related orphan receptor (*Ror*), which encode the nuclear receptors REV-ERB and ROR. REV-ERBs inhibit and RORs activate the transcription of *Bmal1* [6,7]. This second feedback loop generates oscillations of *Bmal1* mRNA. The rhythmic binding of BMAL1:CLOCK/NPAS2 and REV-ERB/ROR respectively on E-box and REV-ERB/ROR sequences in regulatory elements drive the rhythmic expression of a substantial fraction of genes in any particular cell or tissue involved in many different functions [8]. The function and timing of the transcriptional feedback loops depend on post-translational modifications regulating the stability and degradation of the transcription factors [9] and epigenomic regulation of their transcriptional activity [10]. This molecular oscillator exists in almost all cells/tissues throughout mammals and the rhythmic expression of genes ultimately generates rhythms of physiological relevance [3].

Cellular Energy Is Circadian

Early efforts at identifying genes with rhythmic expression under constant conditions that are under the control of the circadian clock revealed many that encoded proteins involved in metabolic processes [11,12]. Around the same time, the idea that the circadian clock and the metabolic state of the cell are interconnected was proposed [13]. Since then, many different groups have contributed to our current understanding of the crosstalk between the circadian clock and the energy state within the cell.

The circadian clock regulates mitochondrial activity through temporal regulation of mitochondrial fission, mitophagy, and biogenesis to maintain respiration at times of increased bioenergetic demand [14]. In addition, the circadian clock controls the NAD salvage pathway by imposing rhythms on nicotinamide phosphoribosyltransferase (NAMPT), the enzyme that catalyzes the rate-limiting step in the synthesis of NAD [15]. The circadian clock-directed rhythms in NAD biosynthesis drive oscillations in the activity of the NAD^+-dependent deacetylases, the sirtuins (SIRTs). As a result, the circadian clock orchestrates SIRT-driven cell physiology by dictating oscillations in the energy status of the cell. The rhythmic activity of mitochondrial SIRT3 generates rhythms of mitochondrial oxidative phosphorylation [16]. SIRT3 drives the rhythmic acetylation of mitochondrial proteins to generate rhythms in mitochondrial activity [16]. In turn, the NAD^+/NADH redox state of the cell influences the transcriptional activity of BMAL1:CLOCK [13]. SIRT1 binds BMAL1:CLOCK in a rhythmic manner and promotes the deacetylation of clock proteins and histones [17,18] to create a feedback loop between the redox state and the circadian clock. SIRT6 interacts with the chromatin recruitment of BMAL1:CLOCK to regulate the expression of a set of clock-controlled genes distinct from those influenced by SIRT1 [19]. SIRT6 also regulates chromatin recruitment of the metabolic transcription factor sterol response element-binding protein (SREBP1) to control circadian fatty acid metabolism [19]. Besides mitochondrial activity, ATP cellular levels are directly involved in the crosstalk between the circadian clock and energy state. ATP levels exhibit circadian rhythms in several brain regions including the SCN [20] and the ratio of ATP to AMP regulates the activity of AMP-activated protein kinase (AMPK) [21]. In turn, AMPK phosphorylates and destabilizes CRY1 to promote its degradation [22]. AMPK also phosphorylates casein kinase I epsilon (CKIε) resulting in increased CKIε activity and degradation of PER2 [23]. AMPK controls the expression of *Nampt* to increase cellular NAD^+ levels, feeding into the feedback regulation of the circadian clock through the NAD^+/NADH redox state and the activity of SIRTs [24] (Figure 2). Over- and hyperperoxidation rhythms of peroxiredoxins have been found in human red blood cells [25], which have no nucleus, have been found to be driven by hemoglobin autoxidation rhythms, and are associated with the degradation of hyperoxidized peroxiredoxins [26]. Inhibiting the pentose phosphate pathway, a critical source of NADPH, was found to influence circadian rhythms in cells across species [27], with a recent study showing an effect on the amplitude and phase of the clock [28] This work further supports feedback from cellular energy metabolism to the transcriptional/translational circadian clock.

Glossary

Diurnal: a rhythm recurring every 24 h under rhythmic environmental conditions. If it characterizes an organism, it refers to organisms that are active during the day (in contrast to nocturnal organisms that are active during the night).
Enteral microbiota: the population of microorganisms that resides in the enteral lumen of the digestive system.
Entrainment: the process of synchronization of the circadian clock to a specific phase.
Lipogenesis: biosynthesis of lipids; an anabolic metabolic process that requires energy to be completed.
Suprachiasmatic nucleus (SCN): located in the hypothalamus of the brain and positioned directly above the optic chiasm. In mammals the SCN contains the master circadian clock that maintains the synchrony of circadian clocks throughout the organism with the phase of environmental light.