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Review article

Redox regulation of circadian molecular clock in chronic airway diseases☆

Isaac K. Sundar[a], Michael T. Sellix[b], Irfan Rahman[a,*]

[a] Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY, USA
[b] Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Rochester Medical Center, Rochester, NY, USA

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ABSTRACT

At the cellular level, circadian timing is maintained by the molecular clock, a family of interacting clock gene transcription factors, nuclear receptors and kinases called clock genes. Daily rhythms in pulmonary function are dictated by the circadian timing system, including rhythmic susceptibility to the harmful effects of airborne pollutants, exacerbations in patients with chronic airway disease and the immune-inflammatory response to infection. Further, evidence strongly suggests that the circadian molecular clock has a robust reciprocal interaction with redox signaling and plays a considerable role in the response to oxidative/carbonyl stress. Disruption of the circadian timing system, particularly in airway cells, impairs pulmonary rhythms and lung function, enhances oxidative stress due to airway inhaled pollutants like cigarette smoke and airborne particulate matter and leads to enhanced inflammosenescence, inflammasome activation, DNA damage and fibrosis. Herein, we briefly review recent evidence supporting the role of the lung molecular clock and redox signaling in regulating inflammation, oxidative stress, and DNA damage responses in lung diseases and their exacerbations. We further describe the potential for clock genes as novel biomarkers and therapeutic targets for the treatment of chronic lung diseases.

1. Introduction

Circadian rhythms are innate biological rhythms with a frequency or period close to 24 h. In mammals, circadian rhythms of gene expression, cellular/organismal physiology and behavior are regulated by the Circadian Timing System. At the cellular level, circadian rhythms are driven by an autoregulatory feedback loop oscillator of interacting transcription factors, kinases and nuclear receptors commonly referred to as clock genes [1]. The molecular clock has been described in nearly every mammalian cell and is tightly linked to variations in cellular metabolism, redox signaling and oxidative stress responses [2]. This system includes a central pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus that receives and responds to photic cues from the retina and uses an array of neural and neuroendocrine cues to disseminate these timing cues to targets in both the brain and periphery [3]. The SCN synchronizes the activity of downstream central and peripheral clocks in order to maximize the temporal cohesion between variables in the environment (e.g. food availability) and physiology (e.g. insulin release) [4]. The Circadian Timing System makes a substantial contribution to nearly every aspects of mammalian physiology, including pulmonary function, have been reviewed recently by us [5,6]. Herein, we briefly review recent evidence supporting the role of the lung circadian molecular clock and redox signaling lungs based on regulating inflammation, oxidative stress, and DNA damage responses in lung diseases. We further describe the potential for clock genes as novel biomarkers and therapeutic targets for the treatment of chronic lung disease.

2. The mammalian circadian clock and its role in pulmonary physiology

It is widely accepted that circadian rhythms in mammals are generated by a cell based and inheritable molecular clock [1]. At its core, the clock is regulated by the heterodimeric BMAL1:CLOCK activator complex and its targets. Though Bmal1 expression oscillates, Clock tends to be stably expressed in most tissues. Though both proteins have DNA binding domains, Clock has innate histone acetyltransferase activity and thus plays the most critical role in transactivation [7].

Abbreviations: BMAL1, brain and muscle ARNT-like 1; CCG, clock-controlled genes; CLOCK, circadian locomoter output cycles protein kaput; CRY, Cryptochrome; KO, knock-out; NAD^+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); NADPH, nicotinamide adenine dinucleotide phosphate; Nrf2, nuclear factor erythroid 2-related factor 2; Rev-Erbα (Nr1d1), nuclear receptor subfamily 1 group D member 1; PER, period; ROR, Retinoid-Related Orphan Receptor; ROS, reactive oxygen species; SCN, suprachiasmatic nucleus; SIRT, sirtuin

☆ Akhilesh B. Reddy, David M. Pollock and Martin E. Young.

* Correspondence to: Department of Environmental Medicine, University of Rochester Medical Center, Box 850, 601 Elmwood Avenue, Rochester 14642, NY, USA.
E-mail address: irfan_rahman@urmc.rochester.edu (I. Rahman).

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Through binding and activation at E-box promoter regions, BMAL1:-CLOCK regulates the expression of both the *period* (Per1-3) and *cryptochrome* (Cry1-2) gene families. Once translated, PER and CRY form heterodimers, are phosphorylated by Casein Kinase and move to the nucleus where they repress their own transcription by blocking the activity of the BMAL1:CLOCK complex [1]. In addition to PER/CRY, the activator complex also drives the expression on the nuclear receptors REV-ERBα/β and RORα/γ. REV-ERB and ROR stabilize the oscillator by regulating the timing and amplitude of Bmal1 expression. Clock proteins are also heavily influenced by posttranslational modifications that affect both their activity and stability [8,9]. In addition to the primary clock proteins, the molecular oscillator also dictates the timing of tissue and cell specific genes, referred to as clock-controlled genes or CCGs [1]. These tissue/cell specific clock-regulated genetic programs are the hands of the clock, facilitating its temporal program on systems physiology. Thus, disruption of clock gene expression inevitably disturbs the timing and amplitude of these CCGs and the physiological processes which they control and has been implicated in chronic diseases [10,11].

In healthy individuals, overall lung function exhibits a robust diurnal rhythm, with a daytime peak (12:00 h) and early morning trough (04:00 h). Diurnal variation in peak expiratory flow (PEF), peak expiratory volume (PEV) and respiration (V_T and V_E) have also been reported and follow the same daily patterns [12,13]. The well-defined early morning decline in lung function coincides with increased risk of exacerbations among patients with chronic obstructive pulmonary disease (COPD) and asthma [14,15], suggesting that the lung clock plays a considerable role in the pathophysiology of chronic lung disease [5]. We have also recently reported that lung function varies significantly across the day in mice and that these rhythms are drastically altered by inflammatory mediators and viral infection [16–23] (see Fig. 1). As in many tissues, the timing of lung function likely depends on a complex interplay between both SCN-dependent cues and localized molecular clock function in lung cells [3]. Outside of these SCN-driven cues, circadian rhythms of clock gene expression have also been reported in lung tissue [24–26]. Studies suggest that the molecular clock in the lung plays a critical role in optimizing the organization of cellular function and responses to environmental stimuli [27,28]. The timing and amplitude of clock genes and CCG expression in rodent lungs is altered by oxidative stress/redox changes mediated by environmental tobacco smoke, air pollution (airborne particulate matters), hypoxia/hyperoxia, jet-lag/shift work, pro-inflammatory mediators, bacterial endotoxin, bacterial/viral infections and other stressors [16–18,20–23,29–36] (Fig. 1). Moreover, substantial evidence suggests that clock disruption, either global or targeted, has a profound influence on pulmonary function and lung pathophysiology, particularly in lung epithelium [5]. The implication being that disturbance of the clock is not only a critical biomarker of the response to oxidative stress and inflammatory mediators in the lungs, but may contribute to the etiology of disease, making targeting of clock function an exciting potential avenue for novel therapeutics.

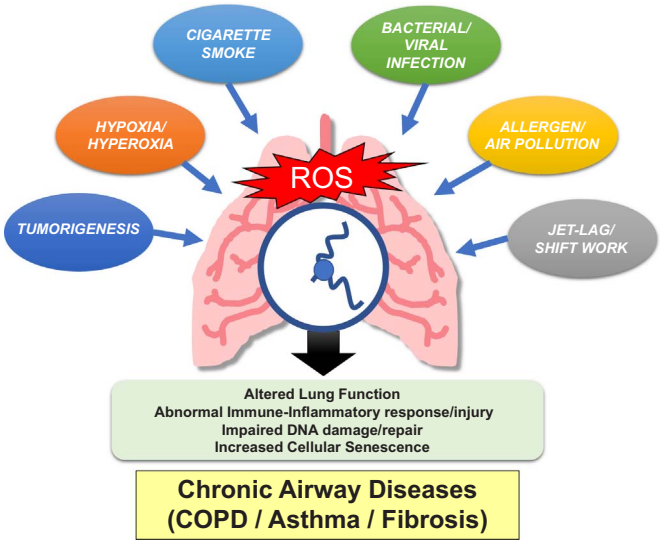

Fig. 1. Factors that contribute to molecular clock dysfunction in the lungs. The lungs are exposed to different kinds of stressors such as environmental tobacco smoke, bacterial/viral infections, allergens, air pollutants, jet-lag/shift work, etc. that can increase the reactive oxygen species (ROS) burden and thereby alter the rhythmic expression of clock genes. Circadian disruption in the lung affects immune-inflammatory signaling (target genes/canonical pathways) resulting in altered lung function, abnormal inflammatory/immune response, impaired DNA damage/repair responses, and ultimately cause chronic lung pathologies (airway disease: COPD, asthma, and fibrosis). Thus, the reciprocal interaction between redox signaling and the clock is a major target of the pulmonary exposome, contributing markedly to the impact of these factors on lung function.

3. Redox regulation of circadian molecular clock

Evidence demonstrates that the circadian clock regulates cellular redox state. For example, Wang et al. reported that the redox state in the SCN in vitro and ex vivo is heavily influenced by the rhythmic expression and activity of two cofactors of cellular metabolism, both flavin adenine nucleotide (FAD) and nicotinamide adenine dinucleotide phosphate (NADPH) [37]. Moreover, the rhythmic levels of these cofactors in SCN neurons depends on a functional molecular clockwork in rodent SCN [37]. Additional evidence on the oscillations of NAD/NADH in the epidermis of mice [38]; total NAD levels in mouse liver and cultured myoblasts [39] and total NAD(P)H in human erythrocytes [40] demonstrates the ubiquity of the reciprocal interaction between the clock and cellular redox state. NAD^+ biosynthesis depends on the synthetic enzyme nicotinamide phosphoribosyltransferase (*Nampt*) which is rhythmically expressed under the control of the molecular clock [41]. It has been shown that the level of NAD^+ feeds back onto the molecular clock and globally affects the proteome [8]. Additionally, NAD^+ oscillation can modulate the activities of NAD-dependent protein modifying enzymes, such as SIRT1 and PARP1 that in turn can deacetylate and poly (ADP)-ribosylate clock proteins [42,43]. In another study, using a transient/stable cell line containing REX::VP16 (REX, a bacterial NADH binding protein, fused to the VP16 activator) and the REX binding operator (ROP), NADH oscillations was demonstrated in vitro. Here, REX was used as a NADH sensor to report intracellular dynamics of redox homeostasis in mammalian cells in real-time [44]. Nuclear FAD levels also oscillate in the liver of mice exposed to a 12:12L:D cycle, suggesting that this rhythm depends on light input. However, the nuclear level of riboflavin kinase was cycling in the liver even when mice were housed in constant darkness [45]. Intriguingly, Hirano et al., showed that the redox cofactor FAD stabilizes cryptochrome (CRY), modifying clock function [45]. Together, these data suggest that FAD levels oscillate and also feed-back to modulate the activity of the clock.

Peroxiredoxins (PRDXs) make up a phylogenetically ancient family of proteins that potentially evolved in direct response to the Great Oxidation Event and whose primary role is associated with H_2O_2 detoxification [46]. Peroxiredoxin enzymes work by reducing H_2O_2 to water. In this catalytic cycle, oxidized PRDX is normally re-reduced in a manner that ultimately consumes a reducing equivalent supplied by nicotinamide adenine dinucleotide phosphate (NADPH). Prior reports have established that the redox state of PRDXs oscillates in cells from humans, mice and algae, reflecting the endogenous rhythm in the generation of reactive oxygen species (ROS) [40,47–51]. In erythrocytes, these redox rhythms are accompanied by several other metabolic oscillations such as oxidation of hemoglobin, and cycling of NAD(P)H and ATP levels. The rhythmic hyper-oxidation state of PRDXs can be interpreted as a memory of the influx via the catalytic system and likely reflect underlying changes in the redox environment on a circadian scale, rather than essentially being a timekeeping mechanism