FORUM REVIEW ARTICLE

Transcriptional Control of Antioxidant Defense by the Circadian Clock

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Abstract

Significance: The circadian clock, an internal timekeeping system, is implicated in the regulation of metabolism and physiology, and circadian dysfunctions are associated with pathological changes in model organisms and increased risk of some diseases in humans. **Recent Advances:** Data obtained in different organisms, including humans, have established a tight connection between the clock and cellular redox signaling making it among the major candidates for a link between the circadian system and physiological processes. **Critical Issues:** In spite of the recent progress in understanding the importance of the circadian clock in the regulation of reactive oxygen species homeostasis, molecular mechanisms and key regulators are mostly unknown. **Future Directions:** Here we review, with an emphasis on transcriptional control, the circadian-clock-dependent control of oxidative stress response system as a potential mechanism in age-associated diseases. We will discuss the roles of the core clock components such as brain and muscle ARNT-like 1, Circadian Locomotor Output Cycles Kaput, the circadian-clock-controlled transcriptional factors such as nuclear factor erythroid-2-related factor, and peroxisome proliferator-activated receptor and circadian clock control chromatin modifying enzymes from sirtuin family in the regulation of cellular and organism antioxidant defense. *Antioxid. Redox Signal.* 20, 2997–3006.

Introduction

THE PHYSIOLOGICAL IMPORTANCE of free radicals, such as reactive oxygen and nitrogen species (ROS/RNS), is well documented (20); the activities of the cardiovascular, respiratory, and immune systems depend on ROS/RNS (*i.e.*, nitric oxide and hydrogen peroxide [H_2O_2]) levels. ROS/RNS regulate metabolism, secretion, and proliferation of cells. Excessive levels of ROS/RNS lead to oxidation of DNA, proteins, and lipids, affecting the normal functions of these biological macromolecules and cellular structures. Acute and chronic oxidative stress is implicated in the development of age-associated diseases, such as cancer, pathologies of the cardiovascular system, and metabolic syndromes (20).

Production of ROS/RNS is important for cellular signaling and is regulated by enzymatic systems, such as nitric oxide synthases or nicotinamide dinucleotide (NAD) peroxidases, but the majority of cellular ROS are generated in the form of superoxide as a by-product of mitochondrial oxidative phosphorylation (20). Most likely, mitochondria-generated ROS are responsible for oxidative-stress-induced damage.

Detoxification of ROS is a function of the antioxidant defense system, which is formed by antioxidant enzymes, such as superoxide dismutases (SODs), catalase, glutathione peroxidase (GPx), and acceptors of free radicals, such as peroxiredoxins (PRDXs), glutathione (GSH), or thioredoxin (TRX) (20). The activity of the antioxidant system is regulated on different levels by multiple mechanisms. The circadian clock was proposed as a system that orchestrates the antioxidant defense (31, 47), and recent data generated through experiments in different organisms support the hypothesis about this evolutionarily conserved role of the circadian system.

The circadian clock generates near 24-h internal rhythms in different biological processes, such as behavior, growth, hormone secretion, and gene expression (58). The circadian clock functions in different organisms, and it is believed that circadian rhythms increase an organism's fitness through synchronization of physiological processes in the organism with periodical changes of the environment (5, 67). The importance of circadian rhythms in humans is supported by the growing list of diseases associated with circadian misalignments (5, 27, 51). Data in model organisms also demonstrate

the involvement of the circadian system in the regulation of diverse physiological process, as well as development of diseases upon genetic disruption of the clock. The molecular mechanisms of clock-dependent control of physiology are not well understood. Circadian-clock-dependent regulation of redox status, ROS homeostasis, and antioxidant defense is a natural candidate for such a mechanism. It is unclear whether the circadian clock is involved in the regulation of ROS/RNS generation and in ROS signaling, but increasing data are accumulating on the role of the clock as the oxidative stress response system. Connection between the clock and redox state of the cell is further strengthened by recently observed circadian rhythms in oxidation/reduction of PRDXs (61, 62); it is intriguing that these rhythms are evolutionarily conserved (22) and transcriptionally independent (61, 62). Here

we will discuss the orchestration of antioxidant defense and management of oxidative stress by the circadian clock with a special emphasis on transcriptional regulation and the implications in aging, cancer, and metabolic syndromes.

Molecular Mechanisms of the Circadian Clock

The transcriptional translation feedback loop (TTFL) is the core of molecular circadian clock mechanisms in different organisms (58). For the purpose of the review we will describe the organization of the loop in mammals; detailed reviews on the molecular clockwork in plants and invertebrate animals are available elsewhere. Figure 1 represents the schematic organization of TTFL in mammalian cells. Period circadian protein (PER)1/PER2 and cryptochrome (CRY)1/CRY2 proteins

FIG. 1. TTFL formed by circadian clock proteins. Transcriptional factors BMAL1 and CLOCK, acting in the form of a heterodimer, positively regulate transcription of *Per*, *Cry*, *Rev-Erb*, and *Ror* genes. PERs and CRYs interact with the BMAL1:CLOCK complex and suppress BMAL1:CLOCK-mediated transcription inhibiting the transcription of their own genes. REV-ERB and ROR proteins form another feedback loop that regulates negatively and positively transcription of *Bmal1*. Elements of circadian clock TTFL also regulate expression of genes that are not core components of the circadian clock; these genes are known as clock-control genes. Transcription of some of these genes is directly regulated by the BMAL1:CLOCK or REV ERB and RORs, while others can be regulated by indirect mechanisms such as through the control of transcriptional factors. BMAL1, brain and muscle ARNT-like 1; CLOCK, Circadian Locomotor Output Cycles Kaput; CRY, cryptochrome; PER, period circadian protein; REV-ERB, NR1D1 (nuclear receptor subfamily 1, group D, member 1); ROR, retinoid orphan receptor; TTFL, transcriptional translation feedback loop.

are products of *per1/per2* and *cry1/cry2* genes, respectively, and have partially redundant functions. PERs and CRYs form a complex (formation of this complex is controlled through phosphorylation-dependent degradation of these proteins), which acts as a specific negative regulator of transcription. The mechanism of PER/CRY-dependent transcription control is not well known. The PER:CRY complex interacts with the brain and muscle ARNT-like 1 (BMAL1):Circadian Locomotor Output Cycles Kaput (CLOCK) complex and is recruited to the BMAL1:CLOCK complex at chromatin, where it suppresses BMAL1:CLOCK-mediated transcription (26). Another possible mechanism is through the regulation of BMAL1 and CLOCK post-translational modifications and stability; indeed, posttranslational modifications and transcription-associated degradation of BMAL1 and CLOCK are critical for their functions as transcriptional factors (34). Irrespective to the exact mechanisms, the PER:CRY complex inhibits transcription of their own genes because these genes are under the control of the BMAL1:CLOCK complex. Thus, by inhibiting their own expression, PERs and CRYs form a negative feedback loop. BMAL1 and CLOCK are basic helix-loop-helix PAS-domain-containing transcriptional factors, acting in the form of a heterodimer. This complex positively regulates the expression of the earlier-mentioned *Per* and *Cry* genes and also transcription of the *Rev-Erb* and *Ror* genes. REV-ERB and retinoid orphan receptor (ROR) proteins form another feedback loop that regulates negatively and positively transcription of *Bmal1*, respectively.

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Enzymatic Antioxidant Defense System and the Circadian Clock

In aerobic organisms, ROS are produced as a consequence of respiration and substrate oxidation. Exogenous sources of ROS include UV radiation, chemotherapeutic agents, and various pollutants. ROS include hydroxyl radicals, superoxide anions, and various peroxides that are constantly generated in response to external and internal stimuli. ROS have diverse effects on cells. In small amounts, ROS act as secondary messengers and are indispensable for stimulation of cell proliferation and immune defense against bacterial infections; on the contrary, elevated ROS levels are dangerous for an organism and give rise to oxidative stress, resulting in oxidative damage to biological macromolecules and finally cell death through apoptosis or necrosis (20). As such, ROS have been implicated in various diseases, including cancer, aging, neurodegenerative diseases, and atherosclerosis. The antioxidant defense system's role in balancing free radicals is highly essential for sustaining physiological ROS levels. The GSH antioxidant defense system is a predominant guardian against oxidative stress.

A simplified example of ROS-detoxifying pathway is presented in Figure 2. Superoxide anions are converted to H_2O_2 by SODs. There are three different SODs in mammals with different patterns of intracellular and extracellular distribution. H_2O_2 can be further detoxified through several different pathways. Catalase, found in all the tissues of all species, converts H_2O_2 to water. H_2O_2 can also be reduced by GSH in reaction catalyzed by GPx. Glutathione reductase (GR) uses NADPH as a substrate to reduce oxidized glutathione (GSSG). The GSH-based system is the most powerful antioxidant system that neutralizes free radicals produced by several toxins. PRDXs scavenge H_2O_2 and catalyze their own oxidation into PrxSO₂/3. This oxidized form is then reduced by TRX to regain its antioxidant ability. Six *Prdx* genes have been found

FIG. 2. Detoxification of superoxide anion. Superoxide anion generated by cells is converted to hydrogen peroxide by SODs. Hydrogen peroxide is further reduced by catalase or through peroxiredoxin or glutathione antioxidant system. Antioxidant enzymes are shown as open boxes; proteins which serve as substrate for redox reactions are shown by gray boxes. CAT, catalase; GSH and GSSG, reduced and oxidized glutathione; GSH Px and GSH R, glutathione peroxidase and reductase; Prx, peroxiredoxin; Sesn, sestrin; SOD, superoxide dismutase; Trx-H and Trx-S-S, reduced and oxidized thioredoxin; Trx-R, thioredoxin reductase.

FIG. 3. Clock and cellular ROS homeostasis. Daily rhythms of activity dictate rhythms in metabolism and ROS production, which can lead to oxidative stress. Clock also regulates pathways, which reduces the effects of oxidative damage and help maintain ROS homeostasis (details in text). ROS, reactive oxygen species.

in mammals. The oxidized TRX in turn is reduced by electrons from nicotinamide dinucleotide phosphate (catalyzed by the thioredoxin reductase [TXNRD]) (73a).

The circadian rhythms in an organism's daily activities, such as feeding, locomotion, and the sleep/wake cycle, modulate the intensity of metabolic processes during the day (Fig. 3). In agreement with this, reported daily rhythms in body temperature are a reflection of rhythms in metabolism intensity. Metabolism is tightly connected with the generation of ROS as by-products of mitochondrial functions (20). Additionally, detoxification of food-derived xenobiotics also involves ROS production. Thus, the generation of ROS should oscillate as a reflection of the daily activities of organisms. Indeed, rhythms in ROS and products of oxidation have been reported in different tissues (18, 31, 48, 50). The rhythmic generation of oxidants dictates the symmetric response of organisms—rhythmic induction of the antioxidant defense system to balance different demands for reduction and in agreement with that rhythms in oxidative stress response have been observed in flies (52). As illustrated in Figure 2, ROS detoxification is a chain of redox reactions, where products of one reaction serve as substrates in the next reaction. Thus, for example, upregulation of just one enzyme would be inadequate for efficient detoxification. The expression and activity of multiple components of the antioxidant defense system must be coordinated.

The circadian clock is the most natural candidate for coordination of daily rhythms of antioxidant defense (Fig. 3). In addition to regulating the expression of antioxidant enzymes and levels of reducing agents, the clock can regulate ROS-induced damage repair, cell survival, and production of secreted antioxidants, such as melatonin. All these clock functions together will contribute to the daily control of ROS homeostasis and efficient antioxidant defense.

Daily rhythms of antioxidant enzymes

Rhythms in GPX and GR (reductase) activities have been observed in many tissues of different organisms. Interestingly, the GPX peak precedes GSSG and GR peaks,

consistently with the detoxification chain (18). Marked circadian fluctuation in catalase activity occurs in mouse livers and kidneys with a peak in the mid-dark phase (70); this is in line with evidence that shows rhythmic catalase expression on the protein level revealed by proteomic analysis in mice (66). The TRX system is a very important system in plants and has been shown to be under circadian control; however, little is known about its regulation by the circadian clock in mammals (56).

In the rat hippocampus, *CAT* and *Gpx* mRNA expression, protein levels, and activity demonstrated oscillations (24). Additionally, mRNA oscillations of *Cat*, *Sod*, *Gpx*, *GSH-S-transferase*, and γ glutamyl cysteine synthase (glutamyl cysteine ligase, *GCL*) have been reported in mouse livers (75). Various isoforms of glutathione-S-transferase (*GST*) and glutamyl cysteine synthase (*GCS*) displayed rhythmicity with a peak during the light phase. In *Drosophila* heads similar rhythms in mRNA expression and activity of *GCL* and *Gst-D1* have been demonstrated, which was disrupted in clock mutants (6); this further supports the circadian mechanisms of antioxidant enzyme expression.

Rhythmic expression on the mRNA level suggests transcriptional regulation, and in this review we plan to concentrate on transcriptional mechanisms of circadian-clock-dependent control of antioxidant defense. Recently observed evolutionarily conserved non-transcriptional mechanisms of clock regulation of cellular redox balance (61, 62) are beyond the scope of the current review. Next we will discuss the potential molecular mechanisms of the circadian-clock-dependent transcriptional regulation of antioxidant defense.

The transcriptional control of genes involved in antioxidant defense and oxidative stress response by the circadian clock can occur in several ways. Some genes can be under direct regulation by the circadian clock transcriptional factors BMAL1/CLOCK, RORs, and Rev-Erbs. Another set of genes can be regulated by clock-controlled transcriptional factors. Finally, regulation may occur through clock-dependent control of chromatin structure by clock-controlled chromatin-modifying enzymes. *In silico* analysis of the promoter regions of many antioxidant genes, such as *GPx-1*, *Cat*, *Sod-1*, and

TXNRD-1, reveals the presence of E-box elements in rodents and humans, thus indicating that these genes may be under direct transcriptional control of the BMAL1:CLOCK complex (49). A recent study (69) explored potential downstream target genes for BMAL1 in mouse liver, but none of the antioxidant genes were identified as direct targets for BMAL1 in this study.

Nuclear factor erythroid-2-related factor-dependent mechanisms of circadian regulation of antioxidant defense

Xu *et al.* in 2012 investigated the expression patterns for antioxidant genes in mice liver and they found that nuclear factor erythroid-2-related factor 2 (*Nrf2*) expression pattern was highest during daytime and showed a peak at 18:00, similar to D site of albumin promoter (albumin D-box) binding protein hence proving that circadian variations of *Nrf2* could modulate cell response to oxidative stress (75). The leucine zipper transcription factors *Nrf1* and *Nrf2* are present in the cytoplasm in a quiescent state bound to Kelch-like ECH-associated protein 1 (Keap1) (See Fig. 4). Upon activation by oxidative or ER stress, it is released from Keap1 and translocates to the nucleus where along with the small musculoaponeurotic fibrosarcoma protein it binds to the antioxidant response element of various antioxidant enzymes, such as γ -glutamyl cysteine synthetase, *GST*, and *catalase*, hence regulating their transcription (43, 46). *Nrf1* and *Nrf2* have similar binding specificity and expression profiles; hence, they share mutually overlapping target genes and overlapping activities (46). Upregulation of many antioxidant and detoxification genes is severely impaired in the *Nrf2*-null mutant mice (40). *Nrf1*^{-/-} fibroblasts have decreased GSH levels and are hypersensitive to oxidative stress, which correlates with downregulation of γ -glutamyl cysteine synthetase and *glutathione synthetase* (54). Interestingly, *Nrf-2* exhibits circadian patterns in its mRNA expression, similar to *Per1*, *Per2*,

and *Rev-Erb*, with peak late in light phase (75), and *Nrf-1* contains a GC-rich motif found in most of the clock-controlled genes (9), indicating that these may be under direct transcriptional control of the BMAL1:CLOCK heterodimer.

Peroxisome proliferator-activated receptor-dependent mechanisms of circadian regulation of antioxidant defense

Regulation of peroxisome proliferator-activated receptor (PPAR) activity by the circadian clock occurs at several levels (Fig. 5). *Ppar α* mRNA and protein levels exhibit diurnal rhythms in rat liver (57) and in mouse white adipose tissue (WAT) (57), black adipose tissue, and liver (57). PPAR γ exhibits rhythms specifically in WAT and the liver (76). Additionally, PER2 directly interacts with the N-terminal domain of PPAR γ and represses its activity (29). Promoter region of *Ppar α* contains BMAL1 binding site making it a likely candidate for the CLOCK:BMAL1 complex to drive its expression (11, 69). Interestingly, PPAR α is involved in a positive feedback loop with BMAL1 (10). Similarly, PPAR γ induces BMAL1 expression in blood vessels (74).

PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily, and exist as three subtypes: PPAR α , PPAR β (or PPAR δ), and PPAR γ (17). PPAR γ is well distributed in adipose tissue and less so in the liver, while PPAR α is abundantly expressed in the liver, kidney, and heart. PPAR γ and PPAR α are not only well known for their function in fatty acid metabolism and adipocyte differentiation but are also implicated in oxidative stress. Several antioxidant enzymes, including *SODs* and *GPxs*, are under PPAR α transcriptional control (39, 72). PPAR γ heterodimerizes with Retinoid X receptor and interacts with the PPAR binding element in the promoter region of *catalase* and *Gpx3* in a tissue-specific manner (14, 19, 28, 36). Treatment with PPAR γ agonist increases expression of *Sod-2* and *Gpx-3* in cells, while PPAR γ deficiency leads to reduction of the expression (19, 28).

Although very little is known about the clock regulation of PPARs in the control of oxidative stress, it would be highly compelling to study this potential mechanism (Fig. 6) that can aid in control of aging and age-related disorders.

FIG. 4. NRF. NRF is a master regulator of antioxidant defense. NRF forms an inactive complex with KEAP-1, which is destroyed by oxidative stress. NRF translocates to the nucleus where it drives the expression of several antioxidant enzymes. The BMAL1:CLOCK complex directly regulates the expression of NRF through E box element in the promoter. KEAP-1, Kelch-like ECH-associated protein 1; NRF, nuclear factor erythroid-2-related factor.

FIG. 5. PPAR. PPAR α expression is positively regulated by the BMAL1:CLOCK complex and is negatively regulated by PER1. The PPAR complex controls the expression of several antioxidant enzymes. PPAR, peroxisome proliferator-activated receptor.

FIG. 6. SIRT1. (A) CLOCK acetylates its transcriptional partner BMAL1 following BMAL1:CLOCK-dependent regulation of the expression of NAMPT—a rate-limiting enzyme in NAD⁺ production pathway. NAD⁺ regulates activity of SIRT1 deacetylase that deacetylates BMAL1, thus forming a feedback loop. (B) SIRT1 deacetylates and regulates the activities of transcriptional factors, such as PGC1α, FOXO3a, and p53, and these transcriptional factors in turn regulate the expression of several antioxidant enzymes. Additionally, oxidative stress leads to a change in chromatin localization of SIRT1 and hence a change in the global transcriptional program. FOXO, forkhead box; NAD, nicotinamide dinucleotide; NAMPT, nicotinamide phosphor-ribosyltransferase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; SIRT, sirtuin.

Antioxidant activity of circadian-clock-controlled hormone melatonin

Melatonin is a hormone produced and released by the pineal glands in a circadian manner; melatonin plays a role in many physiological processes including regulation of circadian rhythms and sleep homeostasis (32, 64). Melatonin is one of the most efficient antioxidants; as a free radical scavenger, melatonin has the following advantages: it is able to cross the cellular plasmatic membrane, it can reduce a broad spectrum of ROS/RNS, and it can facilitate the action of other scavengers. Finally, melatonin is a terminal scavenger, so it cannot be reduced back to its original state and cannot initiate oxidation of other substrates (30, 64, 71). This activity is evolutionarily conserved in contrast to many other melatonin activities, including hormone activity and regulation of cell death and proliferation, which is reported mostly in vertebrates. The clinical efficiency of melatonin has been tested in animal models of diseases associated with increased oxidative stress, such as neurodegeneration, cancer, and cardiovascular diseases. According to several reports, melatonin efficiently prevents the development or reduces the severity of these diseases (3, 42, 73). Melatonin was proposed as a potential therapeutic agent; a detailed discussion of melatonin clinical applications can be found in recent reviews (30, 68).

In vertebrates, melatonin production is controlled by the circadian clock. Arylalkylamine *N*-acetyltransferase (AANAT) is the rate-limiting enzyme in melatonin biosynthesis. AANAT expression is regulated on multiple levels, and these regulations are species specific; different mechanisms operate in nocturnal and diurnal organisms. The mRNA level is regulated on both the transcriptional and post-transcriptional levels. Protein production is also regulated through internal ribosome entry site-mediated control of translation and post-translational regulation of degradation (45). Thus, circadian-clock-generated rhythms in melatonin may contribute to daily rhythms of antioxidant defense.

Clock and chromatin modifications

Recently, a strong correlation has been established between circadian clock regulation and Sirtuin 1 (SIRT1) (Fig. 6A). CLOCK is a histone acetyl transferase protein (19a) that acetylates BMAL1 at lysine 537 (37). This acetylation is counterbalanced by SIRT1, which binds to the BMAL1:CLOCK complex and deacetylates BMAL1 (59). In turn, the BMAL1:CLOCK complex regulates SIRT1 activity. SIRT1 is NAD⁺-dependent deacetylase, and NAD⁺ level exhibits circadian rhythms in nicotinamide phosphor-ribosyltransferase (NAMPT)-dependent manner. NAMPT is a rate-limiting enzyme in the NAD⁺ salvage pathway. The *nampt* gene promoter contains E-boxes to which the BMAL1:CLOCK complex can bind in a circadian manner resulting in rhythmic expression of NAMPT (60, 65).

SIRT1s are ubiquitously expressed in various tissues (the brain, heart, liver, pancreas, skeletal muscle, spleen, and adipose tissues); they have been implicated in a number of diverse functions, such as DNA repair, stress response, chromatin silencing, aging, metabolism, and cell proliferation (38). Their expression patterns are influenced by both external (e.g., caloric restriction and exercise) and internal stimuli (oxidative stress in cells) (13).

A link between SIRT1 and oxidative stress has been established by numerous studies in a variety of tissues. For example, SIRT1 expression enhances tolerance to oxidative stress in neuronal cells (12) and in the hearts of transgenic mice (1), and negates the harmful effects of ROS in renal tubular cells (35). Another homolog of SIRT1, SIRT3, has also been found to provide resistance to oxidative stress (7). SIRT1 can provide protection from oxidative damage in a number of ways as illustrated in Figure 6B.

Studies conducted by Oberdoerffer *et al.* showed that under oxidative stress, redistribution of SIRT1 occurs at the chromatin level such that upon DNA damage, SIRT1 redistributes to chromatin at DNA break sites and promotes DNA repair; thus,

SIRT1 contributes to genomic stability and prevents age-related transcriptional changes (63). SIRT1 has also been found to regulate the activity of peroxisome proliferator-activated receptor gamma coactivator 1-alpha, FOXO3a, and p53 transcriptional factors known to be involved in the regulation of antioxidant enzyme expression (38). The expression of different *Sods*, *Cat*, *Prdxs*, and *Trxs* and *TrxRs* is regulated by these transcriptional factors in a tissue- and stress-dependent manner.

Circadian clock and diseases

Disruption of circadian rhythms through shift work or frequent time zone traveling significantly increases the risk of several types of cancers in humans (27). Experiments in model organisms also suggest a connection between the circadian clock and cancer (4, 55); however, interpretation of these data is complicated by the fact that different laboratories used different circadian clock mutants and different models of tumorigenesis (77). Circadian-clock-dependent control of cell cycle, DNA damage checkpoints, DNA repair, and cell death is considered among possible molecular mechanisms and has been recently reviewed (41, 44). Oxidative stress is an important contributor to tumorigenesis; in animal models, increased oxidative stress resulted in increased risk of cancer, while reduction of oxidative damage could delay or prevent cancer development (20).

Cardiovascular diseases, metabolic syndromes, and obesity are interrelated; risks of these diseases also increase in rotating workers (5, 15, 23). Data on the pathological changes observed in animal models of circadian disruption are generally in agreement with epidemiologic data in humans. Desynchronization of the circadian rhythms in metabolism, misalignment of hormone secretion, and changes in feeding behavior were proposed as underlying mechanisms. All of the just-mentioned diseases are also associated with elevated oxidative stress, and antioxidant treatment can reduce the severity of these diseases in experimental models (2).

The free radical theory of aging is one of the most widely investigated hypotheses of aging and still a subject of debate (8, 33). According to this theory, free radicals that are generated predominantly as a result of metabolic activity damage macromolecular components of cells. With time, damage accumulates, resulting in reduced functional activity of the cell and the entire organism. Detoxification of ROS by the antioxidant defense system attenuates oxidative-stress-induced damage and delays aging and the development of age-associated diseases; accordingly, deterioration of antioxidant defense results in an accelerated rate of aging. While the free radical theory of aging (along with its numerous modifications) has been challenged (8), there is a significant amount of evidence in support of this theory (78).

A reciprocal regulation of the aging process by the circadian clock has been demonstrated in mammals and flies (53). Disruption of the circadian clock genes through genetic mechanisms leads to accelerated aging and reduced lifespan in mice (4, 21, 25, 48). Forced circadian desynchronization through environmental intervention (forced phase shift of the circadian cycle) increases mortality in aged rodents (16). The most severe phenotype of accelerated aging was observed in *Bmal1*-deficient mice, suggesting that *Bmal1* may also have clock-independent functions (48). Increased levels of ROS were reported in different tissues of *Bmal1*-deficient mice.

Treatment of *Bmal1*-deficient mice with antioxidants delayed aging and increased their lifespan, supporting the importance of the circadian clock as an antioxidant defense system (49). Thus, the circadian clock acts as a gerosuppressor system, at least partially through the regulation of antioxidant defense.

Figure 7 illustrates connections between the clock, antioxidant defense, oxidative stress, and diseases. The circadian clock orchestrates the activities of the antioxidant defense and oxidative stress response systems through the earlier-discussed mechanisms. Circadian misalignment caused by mutations, aging, or environmental interventions compromises the antioxidant defense, which leads to oxidative stress. Oxidation of DNA may result in accumulation of mutations, which can initiate cancer. Oxidized proteins and lipids, if not degraded, can form extra- and intracellular deposits. Severe damage can also lead to cell death. These degenerative processes are major contributors to heart attack, stroke, atherosclerosis, and diabetes. Oxidative damage can provoke cell and tissue senescence and accelerate the aging process. Degeneration induced by chronic or acute oxidative stress can also provoke inflammatory response, and inflammation will further contribute to the development of aging and age-associated pathologies.

Conclusions

We have reviewed the recent progress in transcriptional mechanisms of the circadian-clock-orchestrated antioxidant defense. The circadian clock is also involved in transcription-independent regulation of the redox system: indeed, rhythms in the PRDX redox state have been reported. While the mechanism is still unknown, it is clear that these rhythms do

FIG. 7. Connection between the circadian clock, oxidative stress, and diseases. Circadian-clock-dependent orchestration of antioxidant defense contributes to the organism's response to oxidative stress. Circadian disruption results in oxidative damage of biological macromolecules, which leads to mutations, affects cell physiological functions, death and proliferation, which contributes to the pathology of cardiovascular diseases, cancer, metabolic syndromes, and aging.

not depend on the circadian transcriptional–translational feedback loop. Circadian orchestration of antioxidant defense is species specific and most likely tissue specific, mirroring rhythms of metabolic activities of the organism and tissue. The next important question is how to apply this knowledge to medical practice? Oxidative stress without any doubt is a significant contributor to the pathologies of various organs and systems, and potentially the major cause of aging. Multiple attempts using a variety of antioxidants and antioxidative strategies to treat or prevent pathologies have been ventured with a sundry rate of success. One of the reasons why some of these attempts fail is that the activities of different components of the antioxidant defense system must be coordinated. The circadian clock must be considered as a potential target for intervention as a natural coordinator of the antioxidant defense. However, more studies on the mechanisms, with an emphasis on tissue specificity, are necessary to guarantee the optimal control and regulation of the antioxidant defense through manipulations of the circadian clock.

Acknowledgments

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References

- Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, Tian B, Wagner T, Vatner SF, and Sadoshima J. Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circ Res* 100: 1512–1521, 2007.
- Anea CB, Cheng B, Sharma S, Kumar S, Caldwell RW, Yao L, Ali MI, Merloiu AM, Stepp DW, Black SM, Fulton DJ, and Rudic RD. Increased superoxide and endothelial NO synthase uncoupling in blood vessels of Bmal1-knockout mice. *Circ Res* 111: 1157–1165, 2012.
- Anisimov VN, Alimova IN, Baturin DA, Popovich IG, Zabezhinski MA, Rosenfeld SV, Manton KG, Semchenko AV, and Yashin AI. Dose-dependent effect of melatonin on life span and spontaneous tumor incidence in female SHR mice. *Exp Gerontol* 38: 449–461, 2003.
- Antoch MP, Gorbacheva VY, Vykhovanets O, Toshkov IA, Kondratov RV, Kondratova AA, Lee C, and Nikitin AY. Disruption of the circadian clock due to the Clock mutation has discrete effects on aging and carcinogenesis. *Cell Cycle* 7: 1197–1204, 2008.
- Bass J and Takahashi JS. Circadian integration of metabolism and energetics. *Science* 330: 1349–1354, 2011.
- Beaver LM, Klichko VI, Chow ES, Kotwica-Rolinska J, Williamson M, Orr WC, Radyuk SN, and Giebultowicz JM. Circadian regulation of glutathione levels and biosynthesis in *Drosophila melanogaster*. *PLoS One* 7: e50454, 2012.
- Bell EL and Guarente L. The Sirt3 divining rod points to oxidative stress. *Mol Cell* 42: 561–568, 2011.
- Blagosklonny MV. Aging: ROS or TOR. *Cell Cycle* 7: 3344–3354, 2008.
- Bozek K, Relogio A, Kielbasa SM, Heine M, Dame C, Kramer A, and Herzel H. Regulation of clock-controlled genes in mammals. *PLoS One* 4: e4882, 2009.
- Canaple L, Rambaud J, Dkhissi-Benyahya O, Rayet B, Tan NS, Michalik L, Delaunay F, Wahli W, and Laudet V. Reciprocal regulation of brain and muscle Arnt-like protein 1 and peroxisome proliferator-activated receptor alpha defines a novel positive feedback loop in the rodent liver circadian clock. *Mol Endocrinol* 20: 1715–1727, 2006.
- Charoensuksai P and Xu W. PPARs in rhythmic metabolic regulation and implications in health and disease. *PPAR Res* 2010, pii: 243643, 2010.
- Chong ZZ and Maiese K. Enhanced tolerance against early and late apoptotic oxidative stress in mammalian neurons through nicotinamide and sirtuin mediated pathways. *Curr Neurovasc Res* 5: 159–170, 2008.
- Chong ZZ, Shang YC, Wang S, and Maiese K. SIRT1: new avenues of discovery for disorders of oxidative stress. *Expert Opin Ther Targets* 16: 167–178, 2012.
- Chung SS, Kim M, Youn BS, Lee NS, Park JW, Lee IK, Lee YS, Kim JB, Cho YM, Lee HK, and Park KS. Glutathione peroxidase 3 mediates the antioxidant effect of peroxisome proliferator-activated receptor gamma in human skeletal muscle cells. *Mol Cell Biol* 29: 20–30, 2009.
- Curtis AM and Fitzgerald GA. Central and peripheral clocks in cardiovascular and metabolic function. *Ann Med* 38: 552–559, 2006.
- Davidson AJ, Sellix MT, Daniel J, Yamazaki S, Menaker M, and Block GD. Chronic jet-lag increases mortality in aged mice. *Curr Biol* 16: R914–R916, 2006.
- Desvergne B and Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 20: 649–688, 1999.
- Diaz-Munoz M, Hernandez-Munoz R, Suarez J, and Chagoya de Sanchez V. Day-night cycle of lipid peroxidation in rat cerebral cortex and their relationship to the glutathione cycle and superoxide dismutase activity. *Neuroscience* 16: 859–863, 1985.
- Ding G, Fu M, Qin Q, Lewis W, Kim HW, Fukui T, Bacanamwo M, Chen YE, Schneider MD, Mangelsdorf DJ, Evans RM, and Yang Q. Cardiac peroxisome proliferator-activated receptor gamma is essential in protecting cardiomyocytes from oxidative damage. *Cardiovasc Res* 76: 269–279, 2007.
- Doi M, Hirayama J, and Sassone-Corsi P. Circadian regulator CLOCK is a histone acetyltransferase. *Cell* 125: 497–508, 2006.
- Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2002.
- Dubrovsky YV, Samsa WE, and Kondratov RV. Deficiency of circadian protein CLOCK reduces lifespan and increases age-related cataract development in mice. *Aging (Albany NY)* 2: 936–944, 2010.
- Edgar RS, Green EW, Zhao Y, van Ooijen G, Olmedo M, Qin X, Xu Y, Pan M, Valekunja UK, Feeney KA, Maywood ES, Hastings MH, Baliga NS, Mellow M, Millar AJ, Johnson CH, Kyriacou CP, O'Neill JS, and Reddy AB. Peroxiredoxins are conserved markers of circadian rhythms. *Nature* 485: 459–464, 2012.
- Esser KA and Young ME. The role of clock genes in cardiometabolic disease. *J Appl Physiol* 107: 1316–1317, 2009.
- Fonzo LS, Golini RS, Delgado SM, Ponce IT, Bonomi MR, Rezza IG, Gimenez MS, and Anzulovich AC. Temporal patterns of lipoperoxidation and antioxidant enzymes are modified in the hippocampus of vitamin A-deficient rats. *Hippocampus* 19: 869–880, 2009.
- Fu L, Pelicano H, Liu J, Huang P, and Lee C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response *in vivo*. *Cell* 111: 41–50, 2002.
- Gachon F, Nagoshi E, Brown SA, Ripperger J, and Schibler U. The mammalian circadian timing system: from gene expression to physiology. *Chromosoma* 113: 103–112, 2004.

27. Gery S and Koeffler HP. Circadian rhythms and cancer. *Cell Cycle* 9: 1097–1103, 2010.
28. Girmun GD, Domann FE, Moore SA, and Robbins ME. Identification of a functional peroxisome proliferator-activated receptor response element in the rat catalase promoter. *Mol Endocrinol* 16: 2793–2801, 2002.
29. Grimaldi B, Bellet MM, Katada S, Astarita G, Hirayama J, Amin RH, Granneman JG, Piomelli D, Leff T, and Sassone-Corsi P. PER2 controls lipid metabolism by direct regulation of PPARgamma. *Cell Metab* 12: 509–520, 2010.
30. Hardeland R. Melatonin in aging and disease-multiple consequences of reduced secretion, options and limits of treatment. *Aging Dis* 3: 194–225, 2012.
31. Hardeland R, Coto-Montes A, and Poeggeler B. Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. *Chronobiol Int* 20: 921–962, 2003.
32. Hardeland R, Madrid JA, Tan DX, and Reiter RJ. Melatonin, the circadian multioscillator system and health: the need for detailed analyses of peripheral melatonin signaling. *J Pineal Res* 52: 139–166, 2012.
33. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 11: 298–300, 1956.
34. Harms E, Kivimae S, Young MW, and Saez L. Post-transcriptional and posttranslational regulation of clock genes. *J Biol Rhythms* 19: 361–373, 2004.
35. Hasegawa K, Wakino S, Yoshioka K, Tatematsu S, Hara Y, Minakuchi H, Washida N, Tokuyama H, Hayashi K, and Itoh H. Sirt1 protects against oxidative stress-induced renal tubular cell apoptosis by the bidirectional regulation of catalase expression. *Biochem Biophys Res Commun* 372: 51–56, 2008.
36. Heo KS, Kim DU, Ryoo S, Nam M, Baek ST, Kim L, Park SK, Myung CS, and Hoe KL. PPARgamma activation abolishes LDL-induced proliferation of human aortic smooth muscle cells via SOD-mediated down-regulation of superoxide. *Biochem Biophys Res Commun* 359: 1017–1023, 2007.
37. Hirayama J, Sahar S, Grimaldi B, Tamaru T, Takamatsu K, Nakahata Y, and Sassone-Corsi P. CLOCK-mediated acetylation of BMAL1 controls circadian function. *Nature* 450: 1086–1090, 2007.
38. Imai S and Guarente L. Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. *Trends Pharmacol Sci* 31: 212–220, 2011.
39. Inoue I, Noji S, Awata T, Takahashi K, Nakajima T, Sonoda M, Komoda T, and Katayama S. Bezafibrate has an antioxidant effect: peroxisome proliferator-activated receptor alpha is associated with Cu²⁺, Zn²⁺-superoxide dismutase in the liver. *Life Sci* 63: 135–144, 1998.
40. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, and Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun* 236: 313–322, 1997.
41. Kang TH and Sancar A. Circadian regulation of DNA excision repair: implications for chrono-chemotherapy. *Cell Cycle* 8: 1665–1667, 2009.
42. Karbownik M, Reiter RJ, Cabrera J, and Garcia JJ. Comparison of the protective effect of melatonin with other antioxidants in the hamster kidney model of estradiol-induced DNA damage. *Mutat Res* 474: 87–92, 2001.
43. Kensler TW, Wakabayashi N, and Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol* 47: 89–116, 2007.
44. Khapre RV, Samsa WE, and Kondratov RV. Circadian regulation of cell cycle: molecular connections between aging and the circadian clock. *Ann Med* 42: 404–415, 2010.
45. Kim TD, Woo KC, Cho S, Ha DC, Jang SK, and Kim KT. Rhythmic control of AANAT translation by hnRNP Q in circadian melatonin production. *Genes Dev* 21: 797–810, 2007.
46. Klaassen CD and Reisman SA. Nrf2 the rescue: effects of the antioxidative/electrophilic response on the liver. *Toxicol Appl Pharmacol* 244: 57–65, 2010.
47. Kondratov RV. A role of the circadian system and circadian proteins in aging. *Ageing Res Rev* 6: 12–27, 2007.
48. Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, and Antoch MP. Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. *Genes Dev* 20: 1868–1873, 2006.
49. Kondratov RV, Vykhovanets O, Kondratova AA, and Antoch MP. Antioxidant N-acetyl-L-cysteine ameliorates symptoms of premature aging associated with the deficiency of the circadian protein BMAL1. *Aging (Albany NY)* 1: 979–987, 2009.
50. Kondratova AA, Dubrovsky YV, Antoch MP, and Kondratov RV. Circadian clock proteins control adaptation to novel environment and memory formation. *Aging (Albany NY)* 2: 285–297, 2010.
51. Kondratova AA and Kondratov RV. The circadian clock and pathology of the ageing brain. *Nat Rev Neurosci* 13: 325–335, 2012.
52. Krishnan N, Davis AJ, and Giebultowicz JM. Circadian regulation of response to oxidative stress in *Drosophila melanogaster*. *Biochem Biophys Res Commun* 374: 299–303, 2008.
53. Krishnan N, Kretzschmar D, Rakshit K, Chow E, and Giebultowicz JM. The circadian clock gene period extends healthspan in aging *Drosophila melanogaster*. *Aging (Albany NY)* 1: 937–948, 2009.
54. Kwong M, Kan YW, and Chan JY. The CNC basic leucine zipper factor, Nrf1, is essential for cell survival in response to oxidative stress-inducing agents. Role for Nrf1 in gamma-gcs(l) and gss expression in mouse fibroblasts. *J Biol Chem* 274: 37491–37498, 1999.
55. Lee S, Donehower LA, Herron AJ, Moore DD, and Fu L. Disrupting circadian homeostasis of sympathetic signaling promotes tumor development in mice. *PLoS One* 5: e10995, 2010.
56. Lemaire SD, Miginiac-Maslow M, and Jacquot JP. Plant thioredoxin gene expression: control by light, circadian clock, and heavy metals. *Methods Enzymol* 347: 412–421, 2002.
57. Lemberger T, Saladin R, Vazquez M, Assimacopoulos F, Staels B, Desvergne B, Wahli W, and Auwerx J. Expression of the peroxisome proliferator-activated receptor alpha gene is stimulated by stress and follows a diurnal rhythm. *J Biol Chem* 271: 1764–1769, 1996.
58. Lowrey PL and Takahashi JS. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. *Annu Rev Genomics Human Genet* 5: 407–441, 2004.
59. Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, and Sassone-Corsi P. The NAD⁺-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134: 329–340, 2008.
60. Nakahata Y, Sahar S, Astarita G, Kaluzova M, and Sassone-Corsi P. Circadian control of the NAD⁺ salvage pathway by CLOCK-SIRT1. *Science* 324: 654–657, 2009.
61. O'Neill JS and Reddy AB. Circadian clocks in human red blood cells. *Nature* 469: 498–503, 2011.
62. O'Neill JS, van Ooijen G, Dixon LE, Troein C, Corellou F, Bouget FY, Reddy AB, and Millar AJ. Circadian rhythms

- persist without transcription in a eukaryote. *Nature* 469: 554–558, 2011.
63. Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J, Park SK, Hartlerode A, Stegmuller J, Hafner A, Loerch P, Wright SM, Mills KD, Bonni A, Yankner BA, Scully R, Prolla TA, Alt FW, and Sinclair DA. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell* 135: 907–918, 2008.
 64. Pandi-Perumal SR, BaHammam AS, Brown GM, Spence DW, Bharti VK, Kaur C, Hardeland R, and Cardinali DP. Melatonin antioxidative defense: therapeutical implications for aging and neurodegenerative processes. *Neurotox Res* 23: 267–300, 2013.
 65. Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C, Takahashi JS, Imai S, and Bass J. Circadian clock feedback cycle through NAMPT-mediated NAD⁺ biosynthesis. *Science* 324: 651–654, 2009.
 66. Reddy AB, Karp NA, Maywood ES, Sage EA, Deery M, O'Neill JS, Wong GK, Chesham J, Odell M, Lilley KS, Kyriacou CP, and Hastings MH. Circadian orchestration of the hepatic proteome. *Curr Biol* 16: 1107–1115, 2006.
 67. Reddy AB and O'Neill JS. Healthy clocks, healthy body, healthy mind. *Trends Cell Biol* 20: 36–44, 2010.
 68. Reiter RJ, Coto-Montes A, Boga JA, Fuentes-Broto L, Rosales-Corral S, and Tan DX. Melatonin: new applications in clinical and veterinary medicine, plant physiology and industry. *Neuro Endocrinol Lett* 32: 575–587, 2011.
 69. Rey G, Cesbron F, Rougemont J, Reinke H, Brunner M, and Naef F. Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver. *PLoS Biol* 9: e1000595, 2011.
 70. Sani M, Sebai H, Gadacha W, Boughattas NA, Reinberg A, and Mossadok BA. Catalase activity and rhythmic patterns in mouse brain, kidney and liver. *Comp Biochem Physiol B Biochem Mol Biol* 145: 331–337, 2006.
 71. Tan DX, Manchester LC, Terron MP, Flores LJ, and Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J Pineal Res* 42: 28–42, 2007.
 72. Toyama T, Nakamura H, Harano Y, Yamauchi N, Morita A, Kirishima T, Minami M, Itoh Y, and Okanoue T. PPAR α ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats. *Biochem Biophys Res Commun* 324: 697–704, 2004.
 73. Tutunculer F, Eskiocak S, Basaran UN, Ekuklu G, Ayvaz S, and Vatansever U. The protective role of melatonin in experimental hypoxic brain damage. *Pediatr Int* 47: 434–439, 2005.
 - 73a. Valko M, Rhodes CJ, Moncol J, Izakovic M, and Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160: 1–40, 2006.
 74. Wang N, Yang G, Jia Z, Zhang H, Aoyagi T, Soodvilai S, Symons JD, Schnermann JB, Gonzalez FJ, Litwin SE, and Yang T. Vascular PPAR γ controls circadian variation in blood pressure and heart rate through Bmal1. *Cell Metab* 8: 482–491, 2008.
 75. Xu YQ, Zhang D, Jin T, Cai DJ, Wu Q, Lu Y, Liu J, and Klaassen CD. Diurnal variation of hepatic antioxidant gene expression in mice. *PLoS One* 7: e44237, 2012.
 76. Yang X, Downes M, Yu RT, Bookout AL, He W, Straume M, Mangelsdorf DJ, and Evans RM. Nuclear receptor expression links the circadian clock to metabolism. *Cell* 126: 801–810, 2006.
 77. Yu EA and Weaver DR. Disrupting the circadian clock: gene-specific effects on aging, cancer, and other phenotypes. *Aging (Albany NY)* 3: 479–493, 2010.
 78. Zimniak P. What is the proximal cause of aging? *Front Genet* 3: 189, 2012.

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Abbreviations Used

AANAT	= arylalkylamine N-acetyltransferase
ARE	= antioxidant response element
BMAL1	= brain and muscle ARNT-like 1
Cat	= catalase
CLOCK	= Circadian Locomotor Output Cycles
Kaput	
CRY	= cryptochrome
Dbp	= D site of albumin promoter (albumin
D-box)	binding protein
FOXO	= forkhead box
GCL	= glutamyl cysteine ligase
GCS	= glutamyl cysteine synthase
GPx	= glutathione peroxidase
GR	= glutathione reductase
GSH Px, GSH R	= glutathione peroxidase and reductase
GSH	= glutathione
GSSG	= oxidized glutathione
GST	= glutathione-S-transferase
H ₂ O ₂	= hydrogen peroxide
Keap1	= Kelch-like ECH-associated protein 1
MAF	= musculo-aponeurotic fibrosarcoma
NAD	= nicotinamide dinucleotide
NADPH	= nicotinamide dinucleotide phosphate
NAMPT	= Nicotinamide phosphor-
ribosyltransferase	
NRF	= nuclear factor erythroid-2-related factor
PER	= period circadian protein
PGC-1 α	= peroxisome proliferator-activated
receptor gamma coactivator 1-alpha	
PPAR	= peroxisome proliferator-activated receptor
PPRE	= PPAR binding element
PRDX	= peroxiredoxin
REV-ERB	= NR1D1 (nuclear receptor subfamily 1,
group D, member 1)	
ROR	= retinoid orphan receptor
ROS/RNS	= reactive oxygen species/reactive
nitrogen species	
Sesn	= sestrin
SIRT	= sirtuin
SOD	= superoxide dismutase
TRX	= thioredoxin
Trx-H, Trx-S-S	= reduced and oxidized thioredoxin
TTFL	= transcriptional translation feedback loop
TXNRD, Trx-R	= thioredoxin reductase
WAT	= white adipose tissue