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Metabolic and Nontranscriptional Circadian Clocks: Eukaryotes

Akhilesh B. Reddy and **Guillaume Rey**
Department of Clinical Neurosciences, University of Cambridge Metabolic Research Laboratories, National Institute for Health Research Biomedical Research Center, Wellcome Trust--Medical Research Council Institute of Metabolic Science, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 0QQ, United Kingdom

Abstract

Circadian clocks are cellular timekeeping mechanisms that coordinate behavior and physiology around the 24-h day in most living organisms. Misalignment of an organism's clock with its environment is associated with long-term adverse fitness consequences, as exemplified by the link between circadian disruption and various age-related diseases in humans. Current eukaryotic models of the circadian oscillator rely on transcription/translation feedback loop mechanisms, supplemented with accessory cytosolic loops that connect them to cellular physiology. However, there is mounting evidence questioning the absolute necessity of transcription-based oscillators for circadian rhythmicity, supported by the recent discovery of oxidation-reduction cycles of peroxiredoxin proteins, which persist even in the absence of transcription. A more fundamental mechanism based on metabolic cycles could thus underlie circadian transcriptional and cytosolic rhythms, thereby promoting circadian oscillations to integral properties of cellular metabolism.

Keywords

peroxiredoxin; oxidation-reduction; redox; oscillator; posttranslational; posttranscriptional

INTRODUCTION

Biological clocks are ubiquitous timekeeping mechanisms that provide living organisms with temporal organization over timescales ranging from seconds to years. Organisms have evolved such rhythms to adapt to repetitive environmental and geophysical events, such as solar cycles, tides, the rotation of the Moon, and the recurring seasons. Among biological oscillators, the circadian clock holds a preeminent role because it allows organisms from bacteria to humans to anticipate the alternation of our days and nights.

The Latin etymology of the word circadian---*circa*, about; *diem*, a day---reflects that the intrinsic period of these rhythms is not exactly 24 h but, for example in humans, closer to 25 h. These clocks do not, however, need to be extremely accurate, because in their natural

areddy@cantab.net

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setting they are reset daily by external cues, such as light-dark cycles, that ensure their synchrony with environmental cycles (a process sometimes termed entrainment). When kept in constant conditions (that is, when the cycle is removed, such as in constant darkness), an organism relies only on its intrinsic clock to keep time and thus exhibits a so-called free-run rhythm because it is no longer directly constrained by the external driving cycle.

These observations are exemplified by an experiment performed in 1962 by the French cave-explorer Michel Siffre (1), when he revealed the existence of such an intrinsic timekeeping mechanism in humans by isolating himself for two months in a 130-m-deep cave in the French Alps. He recorded his sleep-wake activity during the study and found that, even though he had completely lost conscious perception of the passing of time, his activity pattern clearly demonstrated a persistent 24.5-h rhythm for the duration of the experiment. A few years later, Jürgen Aschoff (2) confirmed Siffre's discovery by conducting experiments in more controlled laboratory conditions and observed the influence of the circadian clock on the daily pattern of numerous physiological functions, including body temperature, blood pressure, cognitive performance, and hormone plasma concentration. Since then, intensive research in humans and in a wide variety of prokaryotic and eukaryotic model organisms have established the pervasive role of the circadian clock in regulating physiological and behavioral processes (3--5). Moreover, studies in cyanobacteria and plants have revealed the clear adaptive significance of circadian clocks, given that strains with a period matched to the external cycle---that is, in resonance with their environment---outcompeted the others (6--8). Similarly, in humans, there is mounting evidence that circadian misalignment, a mismatch between external and internal cycles, as it occurs in shift work and jet lag, has adverse health consequences and can increase the risk of cancer, metabolic, and mental diseases (9).

In the quest for the origin of circadian rhythms in higher species, researchers have attempted to locate anatomical loci that would be responsible for the generation of behavioral rhythms. In mammals, such approaches led to the identification of a paired structure in the brain's hypothalamus called the **suprachiasmatic nuclei (SCN)** and subsequently revealed a hierarchical organization of the mammalian circadian system (10). Working as a central pacemaker, the SCN are entrained by the environment through photic input from the retina and, in turn, synchronize peripheral organs using a variety of systemic signals (4). However, peripheral organs are not merely forced to oscillate by the master SCN clock because most cells (and, indeed, tissues) in our body harbor self-sustained circadian oscillators in their own right. Thus, in the body, there is a pervasive network of cellular oscillators, all kept in synchrony by the central clock in the SCN (11).

> Suprachiasmatic nuclei (SCN): in mammals, a paired structure in the brain's hypothalamus working as a master pacemaker

In the past 20 years, our understanding of circadian timekeeping has experienced tremendous advances stemming from the successful identification of such cell-autonomous mechanisms. The current model for circadian timekeeping in all species is thought to incorporate similar **transcription/translation feedback loop (TTFL)** mechanisms at their core. However, each species' clock employs a different set of "gears" in its timing

mechanism, given that so-called clock genes are not phylogenetically conserved among vertebrates, fungi, plants, and bacteria (12).

Transcription/translation feedback loop (TTFL): designates current models of transcription-based circadian oscillators

Transcription-based models, however, were only one of the several models proposed before the successful identification of circadian genes. Notably, molecular (in vitro), biochemical network, and membrane models were developed before the advances of molecular genetics (13). These models were thereafter discarded, probably not because of their inaccuracy, but rather because of the difficulty in identifying bona fide clock components and therefore in constructing detailed molecular models.

Recently, posttranscriptional and posttranslational mechanisms have gained considerable attention owing not only to their importance in transcription-based feedback models (14--16), but also to the discovery of nontranscriptional clocks in various species. The most notable evidence of nontranscriptional circadian oscillations was published in 2005, when Kondo and colleagues reconstituted a molecular clock in a test tube, composed of only the three Kai proteins from the cyanobacteria *Synechococcus elongatus*, supplemented with ATP as an energy source (17). A few years later, rhythmic oxidation-reduction of **peroxiredoxins (PRDXs)**, antioxidant proteins involved in scavenging intracellular hydrogen peroxide, was demonstrated in human red blood cells and also in conditions when transcription is arrested in the unicellular alga *Ostreococcus tauri* (18, 19). Furthermore, a subsequent study revealed the deep phylogenetic conservation of PRDX redox oscillations from *Archaea* to humans (20), suggesting that nontranscriptional oscillators may be common to all circadian systems.

Peroxiredoxins (PRDXs): a family of thiol-specific antioxidant enzymes involved in scavenging hydrogen peroxide inside the cell

In this review, we discuss the importance of metabolic and nontranscriptional rhythms for circadian timekeeping in eukaryotes. To this end, we first recapitulate the main results and concepts underlying current transcription-based models and highlight the numerous challenges encountered in attempts to reconcile these models with experimental observations. We then summarize current knowledge about the two most prominent examples of nontranscriptional rhythms: the KaiC oscillator in cyanobacteria and PRDX oxidation-reduction rhythms. Finally, we discuss how metabolic and circadian cycles may be coupled, the potential role of PRDXs and other metabolic oscillations in circadian timekeeping, and perspectives on the evolution of 24-h rhythms in living organisms.

THE PRINCIPLE OF TRANSCRIPTION/TRANSLATION FEEDBACK MODELS

The Core Transcriptional Oscillator

The first step in constructing current transcriptional models was the identification of a genomic locus involved in behavioral rhythms in *Drosophila* in the early 1970s. Konopka & Benzer (21) performed a mutagenesis screen for abnormal eclosion rhythms in these fruit flies and found three mutations that all mapped to the same genomic locus, which they termed “period.” The three mutant alleles had dramatic effects on behavioral rhythms,

because they caused arrhythmic, short- (19 h) or long-period (28 h) phenotypes. The cloning of *period* (*per*) independently by the Young and Rosbash laboratories in the 1980s allowed its subsequent molecular characterization (22, 23), which revealed that both *per* mRNA and its protein are expressed in a circadian fashion (24, 25), with a delay between the production of RNA and protein. These results had already suggested that *per* would be part of a negative-feedback-loop mechanism in which PER protein represses its own expression (24).

A few years later, studies in the fungus *Neurospora crassa* offered proof of the principle that a transcriptional feedback loop could indeed be the molecular basis of behavioral rhythms, because the protein product of *frequency* negatively regulates its own transcription (26). The loop was later closed similarly in flies in the late 1990s, when the heterodimeric basic-helix--loop--helix transcription factors CLOCK and CYCLE were shown to activate transcription of *per* and *timeless* (*tim*), and their expression was found to be repressed by their protein products (27--29). The potential involvement of *Clock* in circadian timekeeping had been previously suggested in mice, because a mutant allele in this gene caused a lengthening of the circadian period (30). Interestingly, however, deletion of this gene does not have appreciable consequences for behavioral and molecular rhythmicity (31), thus causing researchers to question its central importance in the core transcriptional clockwork.

Extensive research has further dissected the genetic basis of circadian rhythmicity and has permitted the construction of detailed TTFL models in all species that exhibit overt circadian rhythms (32). The fundamental principle relies on a transcriptional activator inducing the transcription of a repressor, which results in the accumulation of the latter over time, until it reaches a sufficient level to repress its own activation (Figure 1). In mammals, the “core” transcriptional machinery is composed of two interlocked feedback loops, in which the transcription factors CLOCK/BMAL1 (or NPAS2/BMAL1) occupy a central role, acting as heterodimers. These activate *Period* (*Per*) and *Cryptochrome* (*Cry*) gene families through interaction with E-box regulatory sequences (33). As a result, the PER and CRY protein products form heterotypic complexes that accumulate over time in the cytoplasm. The level of PER/CRY complexes increases until it reaches a threshold, marking the beginning of the repressive phase, in which PER/CRY complexes translocate back to the nucleus and repress CLOCK/BMAL1 transactivation.

In a second loop, CLOCK/BMAL1 positively regulate the transcription of the heme-sensing nuclear hormone receptors REV-ERBα (NR1D1) and REV-ERBβ (NR1D2), which in turn repress the transcription of *Bmal1* (34). The REV-ERB transcriptional repressors are also involved in transcriptional control of *Per* and *Cry*, likely modulating the phase of expression of the latter (35, 36). This simplistic description exemplifies the principle of interlocked negative-feedback loops, but CLOCK/BMAL1 and REV-ERBs are also directly involved in global regulation of circadian transcription (35--38), which constitutes a significant proportion of the total transcriptome. Approximately 5--10% of the transcriptome in mouse and 1--5% in flies is estimated to be under clock control (39). Moreover, virtually every step from transcription factor binding to protein degradation may have a circadian component in its orchestration, given that epigenetic, posttranscriptional, and posttranslational regulatory mechanisms have important roles in current transcriptional models (14--16, 40, 41).

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Posttranscriptional and Posttranslational Mechanisms in the Clockwork

Posttranslational regulation is an essential mechanism within the circadian clockwork, because it modulates a myriad of processes, including protein turnover, intracellular localization, and DNA-binding affinity. As such, it contributes to setting the pace of the oscillator (14, 15, 42). Phosphorylation is the most well-documented posttranslational modification in the clockwork. In particular, regulation of protein stability by phosphorylation has emerged as a strong determinant of circadian period in the different taxa (15), but many other modifications such as acetylation (43, 44), methylation (45, 46), sumoylation (47), and glycosylation (48--51) have been implicated in regulating circadian processes. The phosphorylation status of circadian repressors, such as the mammalian PER and CRY proteins, increases daily owing to the concerted action of kinases and phosphatases, which subsequently leads to degradation of repressors via F-box proteins. Moreover, enzymes such as casein kinase 1 and 2 (CK1 and CK2, respectively) and protein phosphatase 2A (PP2A) serve phylogenetically conserved roles in species-specific **TTFL** models (14), despite clear differences in their clock gene components.

In mice, the nuclear F-box protein FBXL3 controls the half-lives of CRY proteins by targeting phosphorylated substrates for proteasomal degradation (52--54), and its action is counteracted by the cytoplasmic F-box protein FBXL21 (55, 56). In accordance with a model in which an increase in the stability of the repressors would lead to longer periods, *Fbxl3*-mutant mice have a behavioral period of ~27 h (52, 54), whereas *Fbxl21*-mutant mice exhibit a period shortening of more than 1 h (56). Similarly, the F-box protein beta-transducin repeat containing protein 1 (β-TrCP1) regulates the ubiquitylation-mediated degradation of PERIOD2 (57). The importance of F-box protein--mediated degradation is also observed in other organisms, as *Drosophila* PER and TIM proteins are regulated by SLIMB (58) and JETLAG (59), respectively. Similar mechanisms have also been proposed in *Neurospora* (60) and in plants (61). The importance of the proteasomal degradation machinery alone is supported by the fact that *Ostreococcus tauri* requires a functional proteasome for the generation of circadian rhythms (62). That F-box proteins, which are so intimately involved in protein turnover in general within cells, may impact mechanisms that require protein degradation to oscillate is perhaps not surprising.

The purely posttranslational oscillator described in cyanobacteria also strongly suggests that posttranslational mechanisms may be fundamental to the clockwork in higher species (see next section for details). This is implied by the results of global approaches that reveal posttranslational control as a key mechanism not only in the core transcriptional oscillator, but also in the sculpting of the circadian proteome. An examination of cycling proteins in mice showed that only half of the circadian proteome had a corresponding oscillation at the transcriptional level in the liver (40) and that this proportion went down even further down (11--38%) in the SCN (63). In addition, reversible lysine acetylation shapes the hepatic proteome in a circadian fashion, targeting cytosolic and mitochondrial metabolic enzymes preferentially (64).

Additionally, a growing momentum implicates posttranscriptional mechanisms in the circadian clockwork, including microRNA-mediated degradation and RNA-binding proteins

involved in transcript stability and translational regulation (16). Moreover, recent studies highlight the role of nuclear export (65), splicing (66), and polyadenylation (67) in the clockwork. The general importance of circadian posttranscriptional regulation is further supported by recent genome-wide studies showing that correlation between rhythmic transcription and mRNA accumulation is much lower than expected (38, 68--70).

Secondary Loops Connect the Core Oscillator to Cellular Physiology

In addition to the posttranscriptional and posttranslational mechanisms described above, there is strong evidence that cytosolic processes have important roles in broadcasting transcriptional oscillations to downstream cellular machinery to control cellular physiology (42, 71). This picture has recently emerged in response to observations that a number of clock-controlled cytosolic processes can feed back on the transcriptional oscillator. Accordingly, such processes form accessory loops that tightly connect the clockwork to cellular metabolism (Figure 2).

An exemplar of this metabolic feedback is **nicotinamide adenine dinucleotide (NAD^+)** biosynthesis and its salvage pathway. The rate-limiting enzyme in NAD^+ biosynthesis, nicotinamide phosphoribosyltransferase (NAMPT), is transcriptionally controlled by CLOCK/BMAL1 in mice, and NAD^+ levels exhibit circadian oscillation in the cytoplasm (72, 73). NAD^+/NADH redox poise may also directly regulate NPAS2/BMAL1 DNA-binding affinity (74), and thus close this accessory loop. NAD^+ levels can also be indirectly transduced back to the clockwork through the NAD^+-dependent deacetylase SIRT1, which deacetylates PER2 (44) and balances CLOCK-mediated acetylation (43). Although its activity is driven by feeding cycles, poly(ADP-ribose) polymerase 1 (PARP-1), an NAD^+-dependent ADP-ribosyltransferase, is also involved in transmitting NAD^+ rhythms because it mediates ADP-ribosylation of CLOCK in a daily manner, thus modulating CLOCK/BMAL1 DNA-binding affinity (75). Similarly, other cytosolic pathways that may loop back into the transcriptional oscillator include **cyclic adenosine monophosphate (cAMP)** and Ca^{+2} signaling (76), AMP kinase (77), and glucose levels (78).

Cyclic adenosine monophosphate (cAMP): a second messenger important for regulation of cellular metabolism in eukaryotes

Nicotinamide adenine dinucleotide (NAD^+): a coenzyme working as an electron carrier that is involved in redox reactions

The fact that all these accessory loops are involved in maintaining energy homeostasis inside the cell further strengthens the existing links between circadian clocks and metabolic pathways and shows that the boundaries between cellular timekeeping and metabolism are becoming blurred as new knowledge is acquired. Importantly, all these observations have been made in the presence of a transcriptional oscillator. Thus, it is difficult to tease apart the relative dependence of so-called accessory loops and the timekeeping mechanism, if this distinction is actually meaningful in view of the current evidence. Indeed, cellular metabolic cycles may be coupled to 24-h rhythms within the cells, and they may still continue to oscillate, perhaps with a different period, even in absence of clock genes (79). Accordingly, NAD(P)H levels can exhibit circadian rhythms in the absence of input from the transcriptional oscillator, as exemplified in human red blood cells (18).

Challenging Transcriptional Models

Current transcription/translation models are sufficient to explain most of the fundamental features of the circadian clock, such as its persistence in constant conditions, the ability to entrain to external cues, and coordination of output functions such as behavior and physiology. However, an increasing number of results question the necessity of a functional transcriptional oscillator for cellular rhythmicity. For example, circadian cycles are relatively insensitive to cell division in mammalian cells, and mother cells transmit circadian-phase information to their daughters (80). A similar phase coherence between mother and daughter cells has been reported in cyanobacteria (81). Given the stochastic nature of circadian transcription in mammalian cells (82), the perturbation of transcription during cell division is expected to result in greater phase variability. In line with this hypothesis, global inhibition of transcription with actinomycin D and α-amanitin has revealed the robustness of circadian oscillators to such severe perturbations, as single cells continue to exhibit bioluminescence rhythms even when the transcription rate is reduced by up to 70% (83).

Furthermore, studies showing that constitutive expression, or deletion, of clock genes does not abolish circadian rhythms call into question the importance of transcription in current clock models. In flies, expression of both *per* and *tim* under the control of a constitutive promoter can affect circadian rhythms, but approximately half of the flies still exhibit robust behavioral rhythms (84). Moreover, fungi can exhibit conidiation (spore formation) rhythms even in the absence of central components of the feedback loop, leading to questions about the very definition of clock genes (85). Furthermore, in some organisms, the dominant mechanism regulating circadian rhythms seems to be posttranscriptional, as evidenced in circadian control of translation of luciferin binding protein in the unicellular alga *Gonyaulax polyedra* (86, 87).

The situation is somewhat trickier to dissect in mammals because circadian genes often have multiple homologs. Therefore, double-mutant animals are generally needed to observe a behavioral phenotype. *Bmal1* was thought to be the only exception to this, with its suppression leading to clear behavioral arrhythmicity (88). Constitutive brain-specific expression of *Bmal1* in knock-out animals, however, can restore behavioral rhythmicity, casting doubt on the necessity of rhythmic *Bmal1* transcription (89). In addition, brain-specific knockout of *Bmal1* expression produces gross pathology, with a striking abundance of activated microglia in the brains of mice, which gets progressively worse over the first 6 months of life. This makes it extremely difficult to dissociate the effects of these pathological findings with those specifically related to the malfunction of a biological clock (90).

Even more importantly, imaging of SCN slices from arrhythmic $Bmal1^{-/-}$ and $Cry1^{-/-}Cry2^{-/-}$ animals with bioluminescence reporters has revealed the persistence of low-amplitude rhythms in individual neurons (91, 92). Critically, developmental effects may well belie the apparent arrhythmicity that is observed when adult animals are assayed, as is the case in most experimental paradigms (93). Interestingly, the period of such rhythms tends to be shorter, ~20 h instead of 24 h. Such phenomena may stem from a cytoplasmic

oscillator, because cAMP signaling is thought to be linked to such “clockless” oscillations (91). The methamphetamine-sensitive circadian oscillator is another 24-h oscillator that appears to function independently of the known circadian clockwork. When methamphetamine is supplied to rodents in drinking water, it has the peculiar property of restoring behavioral rhythms in arrhythmic animals caused by either lesion of the **SCN** (94, 95) or genetic defects in clock genes (96). Together, such overwhelming evidence indicates that current TTFLs cannot possibly account for the multiple lines of experimental evidence that have revealed circadian oscillations in the presence of inactivated feedback loops, or, indeed, in their absence, as discussed further below.

NONTRANSCRIPTIONAL CLOCK MECHANISMS

Early Evidence and Models

The numerous experimental anomalies surrounding the current transcriptional/translational model of the circadian clock suggest that other mechanisms are required to explain fully the molecular basis of circadian timekeeping. In this respect, it is worth highlighting that transcriptional mechanisms were regarded as only one of several possibilities investigated before the discovery of clock genes (13). Of note, models based on feedback control in biochemical networks were examined in great detail as a mechanistic basis for the generation of self-sustained oscillations. These models successfully explained ultradian (that is, with a period less than 24 h) oscillators such as the glycolytic oscillator in yeast (97, 98) and **cAMP** oscillations in slime molds (99,100). In addition, mitochondrial calcium cycles were proposed to explain the circadian oscillation of NAD^+ and $NADP^+$ in *Euglena* through the opposing effects of the Ca^{2+}-activated calmodulin complex on NAD^+ kinase and $NADP^+$ phosphatase (101). Indeed, rhythms of Ca^{2+} in another plastid, the chloroplast, and in the cytoplasm of plant cells have been observed, suggesting that rhythmic ion fluctuations may be a common feature in organisms in the green lineage (102).

Another class of models postulated that membranes and cellular compartments could be involved in the generation of circadian rhythmicity, given that disruption of ion transport and other membrane properties perturbs overt rhythms (103, 104). Such models proposed that ion gradients influence active ion transport systems in an autoregulatory fashion that provides the nonlinearity required for self-sustained oscillations. The supposedly slow passive diffusion across membrane was thought to be responsible for the relatively long circadian period. Later models incorporated protein synthesis as a component on which ion concentration may impinge to stimulate active ion transport (105, 106).

The enthusiasm for membrane and biochemical models in the 1970s was stimulated by experimental evidence supporting a limited contribution by the cell’s nucleus to normal circadian rhythms. The macroscopic unicellular alga *Acetabularia* can, for example, maintain self-sustained circadian rhythmicity in photosynthetic activity, even when its nucleus is removed by cutting off its nucleus-containing rhizoid process (107). Intriguingly, its nucleus is able to dictate the phase of oscillation but is dispensable for entrainment and phase shifting (108). Moreover, inhibition of transcription with actinomycin D did not suppress rhythms in both nucleated and enucleated *Acetabularia* cells, although the former lost rhythmicity after two weeks under these conditions (109). In addition, studies in

erythrocytes, which lack a nucleus and are therefore unable to perform transcription, revealed daily rhythms in the membrane-bound Mg^{2+}-ATPase (110). Similarly, platelets were used to show that glutathione exhibited circadian oscillations relying on de novo synthesis of this tripeptide (111), again in the absence a nucleus, but in the presence of mitochondria that are normal inhabitants of platelets. The examples above point to the fact that current circadian models are not able to explain many early observations that, in some cases, were made more than 40 years ago and that had already suggested the existence of nontranscriptional rhythms.

To reconcile these seemingly paradoxical points of view---current transcriptional networks and nontranscriptional oscillators---one can envision the following two major possibilities. On the one hand, transcription and translation in the current models may have only a limited role in setting the pace of the oscillator and may be needed mainly for maintaining the levels of clock proteins and for controlling circadian output functions. In this view, posttranslational modifications of known clock proteins could be the fundamental oscillators, but the transcriptional oscillator would be important for robustness and may amplify posttranslational oscillations. A prototype for such a model exists in cyanobacteria, in which the master transcriptional regulator KaiC is also part of its posttranslational oscillator. On the other hand, circadian timekeeping may have evolved more than one clock in the cell to meet the requirements of precision, robustness, and stability. Accordingly, the known transcriptional oscillator may be coupled to an uncharacterized posttranslational oscillator. The recent discovery of circadian oxidation-reduction cycles in PRDX proteins is supportive of such a hypothesis (18, 19) and, moreover, immediately suggests a common phylogenetic origin for circadian timekeeping mechanisms in virtually all species relying on oxygen as the terminal electron acceptor for energy metabolism (20).

The KaiC Oscillator

Although this review focuses mainly on nontranscriptional mechanisms in eukaryotic species, it is worth briefly describing the purely posttranslational oscillator discovered in the cyanobacterium *Synechococcus elongatus*. The finding that phosphorylation of a substrate could exhibit 24-h rhythms on its own offered the first mechanistic basis for nontranscriptional rhythms. It also triggered an intense debate about the roles of transcriptional and nontranscriptional oscillators in both prokaryotic and eukaryotic species.

The core cyanobacterial oscillator is composed of three proteins (KaiA, KaiB, and KaiC), and negative-feedback regulation of the expression of the *kaiBC* promoter was initially proposed to be part of a TTFL model similar to those described in eukaryotes (5). However, the fact that the phosphorylation state of KaiC robustly oscillates in the cell with a 24-h period, even under conditions, where neither transcription nor translation was permitted, raised strong doubts about the transcription/translation hypothesis in cyanobacteria (112). Kondo and colleagues subsequently showed that KaiC circadian oscillations could be reconstituted in vitro using only the three purified Kai proteins supplemented with ATP as a source of energy, providing definitive evidence of a purely posttranslational oscillator (17).

KaiC is a phosphoprotein with both autokinase and autophosphatase activities at the serine 431 and threonine 432 residues. It forms an hexamer complex that is regulated by KaiA. The

latter dimerizes to enhance the autophosphorylation of KaiC and drives it toward a hyperphosphorylated state, whereas KaiB associates with KaiC to inhibit KaiA's activity. Detailed dissection of the phosphorylation cycle of KaiC has shown that four forms of KaiC phosphorylation states appear and disappear in an ordered pattern and that the relative abundance of each phosphorylated form determines the phase of the oscillator (113--115). Recent studies have also shed light on the entrainment properties of the cyanobacterial posttranslational oscillator by showing that it can directly sense intracellular metabolic states through the ADP:ATP ratio (116). Moreover, the Kai oscillator can also be entrained by light-driven redox changes, because the redox-sensitive protein LdpA modulates the levels of KaiA and CikA, a key input-pathway component (117). In addition, KaiA binding to a quinone reduces its own stability and diminishes KaiC autophosphorylation activity (118), providing another input pathway.

The relative contribution of transcriptional and posttranslational cycles is currently under intense investigation in the cyanobacterial circadian system. Emerging consensus supports a model in which both the posttranslational and transcriptional oscillators are needed for physiological timing, given that disruption of one or the other leads to defects in timekeeping in the whole organism (119--122). The question of whether the transcriptional components constitute a self-sustained or damped oscillator is still under debate (119, 120), but the extraordinary robustness of the cyanobacterial posttranslational oscillator, even in the absence of a cell, indicates that a biochemical oscillator is at the core of cellular timekeeping. In any case, these results imply that eukaryotic models are also likely to have similar posttranslational components that provide them with their reported resilience to transcriptional perturbations (80, 83, 84, 91, 92). An autonomous posttranslational mechanism may indeed exist in higher species, as an in silico model suggests that an oscillator consisting of a substrate reacting with two counteracting enzymes---for example, a phosphoprotein controlled by the activity of a kinase and a phosphatase---would be sufficient to recapitulate the fundamental properties of circadian oscillators (123).

Peroxiredoxin Rhythms

Posttranslational modifications are an integral feature of current TTFL models, but a definitive posttranslational oscillator has not yet been identified in eukaryotic species, likely owing to the difficulty of teasing apart the contribution of transcriptional and nontranscriptional mechanisms in their complex, multiorganellar cells. In this context, the recent discovery of oxidation cycles in PRDX proteins offers a new perspective on nontranscriptional rhythms in higher organisms (18--20, 124).

PRDXs are an antioxidant protein family involved in hydrogen peroxide metabolism and signaling (125). Their catalytic mechanism involves the oxidation of a catalytic cysteine residue in the enzymes' active site to sulfenic acid (Cys-SOH), which then forms a disulfide bond with another noncatalytic (so-called resolving) cysteine residue. In most organisms, the thioredoxin system completes the cycle by reducing this disulfide bond at the expense of oxidizing a molecule of NADPH (Figure 3). This catalytic loop has rapid turnover and allows the maintenance of low levels of intracellular hydrogen peroxide. The so-called typical 2-Cys PRDXs, a subclass of PRDX whose basic functional unit is a homodimer in

which catalytic and resolving cysteines belong to different PRDX molecules, can undergo further oxidation of their catalytic cysteine to sulfinic and sulfonic acid forms (Cys-$SO_{2/3}H$). Over-oxidized Cys-SO_2H residues can be slowly recycled through ATP-dependent reduction by sulfiredoxin (126), whereas further oxidation to Cys-SO_3H (termed hyperoxidation) is thought to be irreversible. Hyperoxidation of catalytic cysteines is thought to be involved in peroxide signaling through disulfide bond exchange and the chaperone activity of hyperoxidized oligomers (125). Importantly, typical 2-Cys PRDXs have the property of shifting from a homodimer to a doughnut-shaped decameric structure depending on the redox status (127).

In human red blood cells, where transcription is naturally absent, PRDXs exhibit circadian accumulation of their dimeric overoxidized form (Cys-$SO_{2/3}H$) over several days (18). Such rhythms fulfill the criteria of bona fide circadian rhythms: persistence in constant conditions, the ability to entrain (via temperature cycles in this case), and temperature compensation (the clock does not run faster in higher temperatures and vice versa). Moreover, these redox rhythms are accompanied by oscillations in hemoglobin oxidation and metabolic variables including NAD(P)H and ATP levels. Similar rhythms are present in the unicellular alga *Ostreococcus tauri*, even when transcription is inhibited by prolonged darkness (19), lending strong support to nontranscriptional circadian processes in these diverse organisms separated by 1 billion years of evolution. Strikingly, the deep phylogenetic conservation of PRDX redox rhythms extends to include fungal, plant, bacterial, and even archaeal species (20). Critically, such rhythms are not dependent on previously identified clock genes, because mutants lacking circadian components maintain redox oscillations, albeit slightly phase shifted.

The phylogenetic conservation of PRDX rhythms suggests that primordial redox oscillators probably evolved following the Great Oxidation Event 2.5 billion years ago, when photosynthetic bacteria acquired the ability to evolve oxygen from water and caused a dramatic increase in atmospheric oxygen. Rhythmic production of oxygen and **reactive oxygen species (ROS)** by sunlight may have been a key driving force in the coevolution of clock mechanisms and ROS removal systems that could anticipate and resonate with externally driven redox cycles (20).

Reactive oxygen species (ROS): oxygen-containing molecules with oxidant activity, whose accumulation can lead to harmful oxidative stress in cells

REDOX AND METABOLIC CLOCKS IN EUKARYOTES

Linking Metabolic and Redox Rhythms to Transcriptional Clocks

The interplay between circadian and metabolic cycles is a recurring theme in chronobiology, and the reciprocal effects that disruption of one cycle has on the other is clear both at physiological and molecular levels (71, 128). For example, a high-fat diet lengthens the behavioral period of rhythms in mice and changes the expression pattern of clock genes (129). Conversely, a recent long-term laboratory study conducted with healthy patients showed that three weeks of circadian dyssynchrony is sufficient to cause prediabetic symptoms (130). Growing evidence suggesting that circadian rhythms are fundamentally metabolic requires that current transcriptional oscillations be tightly coupled to metabolic

cycles, in particular redox oscillations. This hypothesis is strongly supported by numerous examples of accessory loops embedding the circadian transcriptional clock within cellular metabolism (Figure 2).

The NAD^+/NADH redox pair is likely to play an important role in connecting cytosolic and compartment-specific redox states to transcriptional clock components such as PER2 (44) and CLOCK/BMAL1 (43, 74, 75). In addition, other redox-sensitive mechanisms have been identified in the clockwork, in particular, the heme-sensing transcriptional regulators. For example, heme controls the DNA-binding activity of BMAL1/NPAS2 in vitro by inhibiting DNA binding in response to carbon monoxide (131). The nuclear receptors REV-ERBα and -β are heme-binding transcriptional repressors (132, 133) that, at least in the case of REV-ERBβ, employ reactive cysteine residues in their ligand-binding domain (133). Similarly, PER2 interaction with CRY proteins is inhibited by ferric heme (134).

Regulation of protein function by small metabolites covalently modifying their amino acid residues may represent another class of coupling mechanisms, as the availability of reactive metabolites could feedback on circadian and metabolic proteins via associated posttranslational modifications. In a similar fashion, the metabolic status of the cell is thought to regulate gene expression through the epigenetic modification of histones (135). In particular, nonenzymatic acetylation of lysine residues has been described for the latter (136), suggesting that a local increase in the concentration of acetyl-CoA could favor the acetylation of surrounding proteins (137). Importantly, acetylation has been associated with the regulation of many metabolic enzymes, especially in mitochondria, (138, 139). In this way, metabolic flux producing acetyl-CoA may be controlled by a feedback mechanism using acetylation of metabolic proteins, thereby leading to metabolic oscillations. Such an acetylation system would be readily coupled to the clockwork because acetylation regulates PER2 stability (44) and CLOCK/BMAL1 transactivation, counteracted by the deacetylase activity of SIRT1 (43).

In a similar fashion, intracellular nutrient levels can be sensed through *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) glycosylation, which uses uridine diphosphate (UDP)-GlcNAc as a donor, the end product of the hexosamine biosynthesis pathway. The latter integrates input from glucose, amino acid, and fatty acid metabolism pathways; therefore, UDP-GlcNAc concentration is indicative of general nutrient availability. Such nutrient-responsive modification has been implicated in regulating a large range of cellular processes including signaling, transcription, and the stress response (140). A number of core oscillator proteins are glycosylated, both in flies and in mammals, underlining the importance of *O*-GlcNAc glycosylation to the clockwork (48--51). For instance, both BMAL1 and CLOCK are rhythmically modified by *O*-GlcNAc, and this moiety stabilizes CLOCK/BMAL1 by inhibiting their ubiquitylation (51). Moreover, disruption of *O*-linked GlcNAc transferase perturbs glucose homeostasis in mice, probably via improper control of gluconeogenic genes by the liver clock. Thus, these results emphasize that metabolite and nutrient levels can be sensed and retroactively act on the clockwork and metabolic enzymes, exploiting metabolites as donors for posttranslational modifications.

Circadian Redox Rhythms

Even in early molecular studies of the circadian clock, before clock genes had been discovered in any model organism, rhythms in redox had been reported. In plants, the $NADP^+$-to-NADPH ratio exhibits circadian cycles in seedlings kept in constant darkness (141). In rodents, several studies showed that key redox parameters including pyridine and glutathione redox ratios were diurnally regulated in the liver, with the proviso that these oscillations may have been partially driven by food intake (142--146). Nevertheless, human platelets kept in vitro showed circadian rhythms in glutathione content (111), suggesting that the aforementioned feeding cycles may resonate with these cell-autonomous biochemical rhythms. This may also have effects on platelet function, which has been reported recently in humans (147).

The hypothesis that metabolic cycles may be a fundamental mechanism underlying biological clocks has been proposed on the basis of both theoretical and experimental observations (148). Potential evidence for this in mammals came from McKnight and colleagues when they discovered that BMAL1/CLOCK DNA-binding activity can be modulated in vitro by the redox poise of $NAD(P)^+$/NAD(P)H coenzymes (74, 149). Moreover, the concerted action of BMAL1/CLOCK on the expression of genes encoding **NAD^+**-producing enzymes including lactate dehydrogenase and NAMPT could potentially feed back onto intracellular redox balance (72--74). These results, however, still lack in vivo confirmation, given the relatively high concentration (millimolar range) of the coenzymes used in previous in vitro assays. Nevertheless, the recent discovery of PRDX oscillations in nontranscriptional systems offers supportive evidence that redox cycles can function as circadian oscillators in their own right.

This line of thinking is substantiated by several recent studies illustrating the connection of redox balance, as well as the regulation of intracellular peroxide levels, with cellular timekeeping. For instance, hydrogen peroxide is able to induce circadian gene expression in zebrafish cells through the mitogen-activated protein kinase pathway (150). In *Drosophila*, both glutathione biogenesis and the response of flies subjected to hydrogen peroxide are regulated over the circadian cycle (151, 152). In mice, redox cycles in the SCN probably play a role in the daily variation of neuronal firing (153). Such rhythms may be driven by BMAL1, given that slices from *Bmal1*-deficient animals, which exhibit low-amplitude circadian oscillations (91), fail to exhibit redox oscillations.

Redox systems clearly affect downstream cyclical physiology as well as influence circadian molecular oscillations. In mammals, for example, the mitochondrial isoform of the PRDX family, PRDX3, has a physiological role in the generation of rhythms of corticosterone production in the mouse adrenal cortex, as its circadian inactivation is required for normal hormone secretion (154). Surprisingly, under normal laboratory conditions, the rhythmic transition of PRDX3 to its over/hyperoxidized state was apparent only in the adrenal cortex and not in other tissues, suggesting that other compartment-specific PRDX isoforms likely participate in PRDX oxidation cycles observed in mouse liver and the SCN (20) (Figure 4). In the fungus *N. crassa*, ROS homeostasis also seems to be required for normal output rhythms, as the use of mutants for superoxide dismutase or NADPH oxidase to manipulate

genetically **ROS** levels results in enhanced oscillations or arrhythmicity, respectively (155). In addition, superoxide dismutase suppression leads to slightly shorter conidiation (spore formation) rhythms (156), whereas its overexpression has the opposite effect (157). Even if the in vitro DNA-binding activity of the fungal transcriptional activator complex is sensitive to H_2O_2 levels, and may be involved in transducing peroxide levels (155), it is equally likely that intracellular H_2O_2 levels are a key variable of a putative redox oscillator, whose dynamic properties would also be affected by the manipulation of peroxide levels.

Peroxiredoxins: A Biochemical Clock or a Conserved Biomarker?

Although evidence supporting a metabolic oscillator is increasingly convincing, an underlying mechanism has yet to be described. The PRDX system may well be part of this oscillator, given the striking phylogenetic conservation of its circadian oxidation rhythm, extending to all domains of life. In such a model, sulfiredoxin, which recycles the sulfinic form (Cys-SO_2H) of PRDX to its sulfenic form (Cys-SOH), is likely to play an important role (Figures 3 and 4). This ATP-dependent enzyme has the peculiar property of being poorly efficient, with a turnover rate in the range of only 6 to 11 molecules per hour (158). Interestingly, the converse and spontaneous reaction, PRDX oxidation to its sulfinic form, also proceeds relatively slowly: Only approximately 1 in every 1,000 PRDX1 molecules per turnover undergoes inactivation when H_2O_2 is maintained at low steady-state levels (159). By comparison, reaction rates of phosphorylation in the KaiC oscillator are even slower, in the range of 0.1 to 0.5 per hour (113). Therefore, PRDX hyperoxidation kinetics may be compatible with the generation of redox cycles but may require interactions with other redox or metabolic systems to achieve 24-h periodicity. However, it is not clear which multimeric form is measured when performing in vitro activity assays using purified sulfinic acid PRDX (158). Therefore, the transition between dimeric and decameric conformations of 2-Cys PRDXs (and, indeed, any intermediate forms) may have an important effect on kinetic parameters. In this respect, there may be parallels with the KaiC molecule, because it also forms multimers (hexamers) during its catalytic cycling, which has important biophysical effects that contribute to its oscillatory properties (160).

An alternative hypothesis is that PRDX are a conserved biomarker of underlying biochemical oscillations. Accordingly, deletion of PRDX in cyanobacteria and plants does not abolish rhythmicity (20). However, a clear argument for the maintenance of rhythms is that a putative PRDX oscillator and existing (known) clockwork could be two complementary timekeeping mechanisms that can exist independently of each other. The hypothesis of independent metabolic and transcriptional oscillators is supported by recent experiments showing that circadian glucose uptake in undifferentiated stem cells precedes the rhythmic expression of clock genes appearing in differentiated cells (161). In this context, note that the unicellular alga *Gonyaulax polyedra* can exhibit independent rhythms of aggregation and bioluminescence and that the phasing of two putative oscillators can be teased apart under special conditions (79). This also seems to be the case in other single-celled species, including *Neurospora* (162), but the molecular basis of these oscillators remains obscure.

In organisms in which metabolic oscillations have been found, but conventional TTFLs have not been discovered (such as in the worm *Caenorhabditis elegans*), insights into such oscillatory mechanisms may be more forthcoming. However, a search for a role for a *period* homolog in this species was not fruitful, and, ultimately, the *lin-42* gene was found to be a developmentally regulated gene, but not a clock gene (163). Thus, it is eminently plausible that more fundamental metabolic oscillations could drive PRDX oscillations in the absence of known transcriptional feedback oscillators (124). So-called accessory loops including NAD^+/NADH and cAMP cycles are potential candidates for self-sustained metabolic oscillators, but further studies of their oscillatory properties in clock mutant backgrounds will be of great interest to researchers attempting to identify bona fide components of metabolic oscillators.

Metabolic and Redox Oscillators as Prototypes of a Circadian Metabolic Clock

Since the 1960s, biochemical clocks have been investigated as potential underlying mechanisms of behavioral rhythms. The well-known example of the glycolytic oscillator, first described in yeast (164, 165), has been extensively studied, and detailed mathematical models have been successfully built to recapitulate its dynamic properties (166). Notably, such ultradian glycolytic oscillations are not restricted to yeast, because many mammalian cells including muscle (167), pancreatic β (168), and heart cells (169) can generate such short-period oscillations. These models were considered prototypes for the circadian clock, and several theoretical studies proposed mechanisms to generate 24-h rhythms from these short-period oscillations, including frequency demultiplication (170), interaction with a large storage pool (171), and coupling between a collection of oscillators (172, 173; for reviews, see References 13,166).

Glycolytic oscillations, however, lack the feature of temperature compensation, which is normally expected of circadian clocks. On the contrary, the yeast metabolic cycle, which generates a metabolic oscillation with periods ranging from dozens of minutes to a few hours, depending on the growth conditions, exhibits this peculiar property of circadian systems (174, 175). Under nutrient-limiting conditions, the budding yeast *Saccharomyces cerevisiae* exhibits ultradian respiratory rhythms that drive gene expression of approximately half of the yeast transcriptome (174). These metabolic rhythms are characterized by a striking temporal organization that separates reductive and oxidative processes in successive phases. Such a metabolic program is visible at both the transcriptome and metabolome levels (174, 176, 177), with a long reducing phase, where nonrespiratory modes predominate, followed by a short period of intense respiration in the oxidative phase.

Similar metabolic rhythms have recently been described in the nitrogen-fixing cyanobacteria *Cyanothece* (178). However, in this system, the period is circadian under nutrient-limiting conditions (low light and low CO_2), whereas ultradian oscillations prevail under saturating conditions. Importantly, the short-period rhythms were not temperature compensated, possibly suggesting different underlying mechanisms.

A final interesting example is the phenomenon of periodic increases in body temperature during hibernation. Animals oscillate between periods of torpor, when body temperature is

low and metabolism is depressed, and interbout arousal, when internal temperature is maintained and metabolic processes are more active. Such a phenomenon may result from long-period (from days to weeks) metabolic cycles similar to those described in yeast and cyanobacteria (179, 180). Accordingly, it has been proposed that interbout arousals could consist of a respiration burst following a long reductive phase (torpor). The different examples of metabolic oscillators described in this section span period lengths from minutes to weeks and suggest that circadian metabolic oscillators may be only one example among many others of oscillators that are specifically adapted to the 24-h day (Figure 5).

Evolution of Metabolic Clocks

The results and ideas presented here propose that the requirements of robust timekeeping selected a mechanism based on several clocks. It is likely that the identified TTFLs are mainly dedicated to controlling the output of the clock, that is, coordinating behavior and physiology around the circadian day. By contrast, actual timekeeping would come from an underlying biochemical oscillator.

The fact that PRDX oxidation cycles are conserved among all domains of life, whereas TTFL models are generally restricted to a given phylum, supports a model wherein rhythmic transcriptional processes have evolved in addition to an underlying metabolic oscillator to fulfill the particular needs of different species in their particular environmental niche. A long-standing hypothesis about the origin of circadian clocks is the “escape from light” hypothesis, which proposes that primordial organisms have evolved timekeeping mechanisms to segregate UV-sensitive processes, such as DNA replication, into the dark period of the day (181). However, circadian gating of cell division during the day in modern cyanobacteria contradicts such a hypothesis (182) and suggests that other selective agents may have predominated. One such agent could be another source of cellular stress, such as ROS-induced damage, as intimated by the role PRDXs play in maintaining levels of ROS homeostatically. The existence of metabolic rhythms ranging from minutes to weeks would indicate that temporal organization may be the fundamental feature of such biochemical oscillations rather than a by-product of diverse transcriptional oscillators. In this view, circadian metabolic oscillations may have principally evolved to anticipate and separate processes needed during the energy-harvesting (day) and utilization (night) phases (183), thereby alleviating energy-consuming futile cycles in metabolic pathways (9, 180). The soundness of such a hypothesis seems clear for unicellular photosynthetic organisms growing in conditions of limited resources, which would have crucially needed to manage their energetic reserves optimally. However, for multicellular mammals, the complex organization of physiology, which guarantees an almost-constant supply of energy to nonmetabolic tissues, makes the interpretation of such a hypothesis more challenging.

CONCLUSION

Perhaps one of the most exciting features of circadian clocks is the possibility of linking overt behavioral and physiological rhythms in health and disease states to specific molecular processes and their perturbations. Current TTFL networks, together with their cytosolic accessory loops, offer detailed models that explain how pervasive orchestration of cellular

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physiology is achieved. All cytosolic loops identified so far are involved in energy and redox homeostasis, suggesting an important role for metabolic cycles. The recent discovery of metabolic nontranscriptional cycles further extends this line of thinking and supports the hypothesis of a circadian metabolic oscillator underlying transcriptional and cytosolic rhythms (Figure 2). The PRDX system may be part of this uncharacterized metabolic oscillator, given its broad phylogenetic conservation and its slow kinetics, which could be compatible with 24-h rhythmicity. It may, as well, be a reporter of more fundamental oscillations. Nevertheless, redox cycles seem to be an important component of circadian timekeeping.

The various examples of metabolic clocks spanning the oscillatory period range from minutes to months offer supportive evidence that temporal separation of reductive and oxidative phases may be a key principle of a circadian metabolic oscillator (Figure 5). Given the ubiquitous influence of redox poise on metabolic reactions, dissection of precise molecular components may be a challenging problem, as already experienced by those investigating the yeast metabolic oscillator.

Taken together, a wealth of experimental evidence points toward the view that living organisms probably evolved multiple coupled oscillators to fulfill the many requirements of a clock, including mechanisms to “keep” time and to “tell” time. The deep phylogenetic conservation of the circadian redox oscillations suggests that metabolic oscillators may be more dedicated to keep track of time and organize underlying biochemical networks, whereas the transcription-based oscillators may have evolved in addition to this biochemical clock, with a predominant role in dictating temporal programs specific to the physiology of each species. Therefore, given that time-telling mechanisms may have been primarily uncovered in eukaryotes, a fundamental understanding of a circadian timekeeping mechanism is within our grasp.

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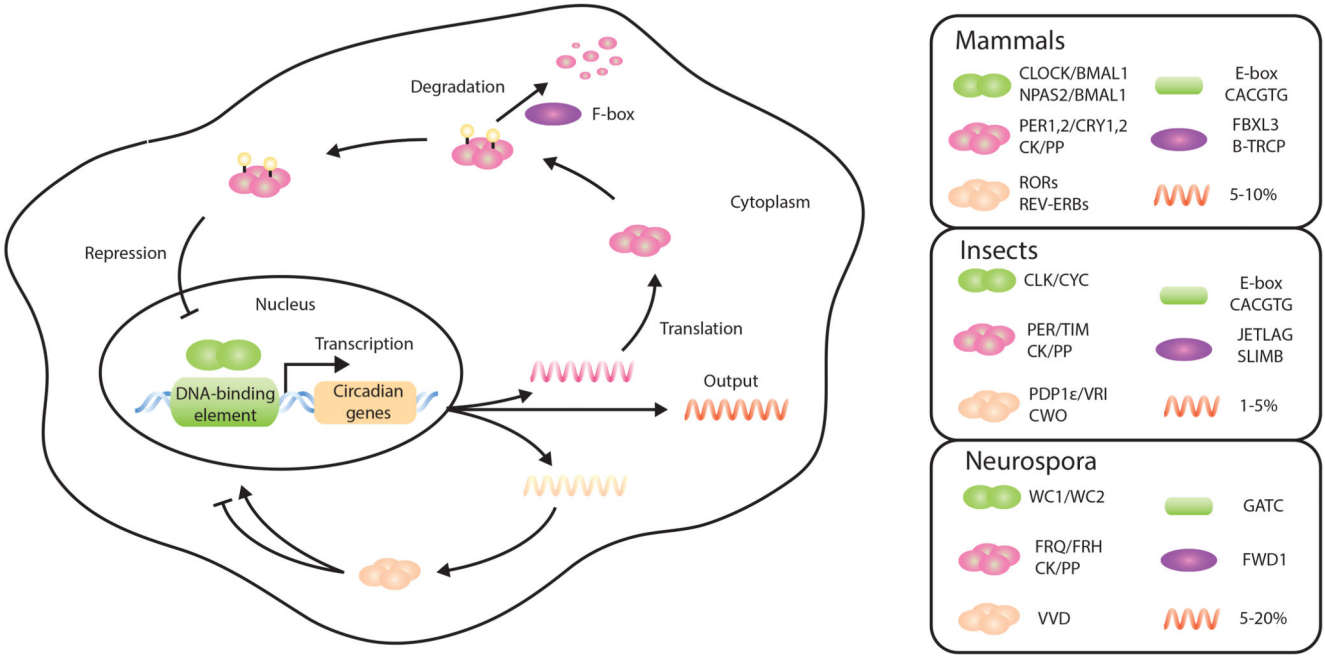

Figure 1.

The transcriptional feedback loop model in eukaryotes, showing the principle of the TTFL model common to mammals, insects, and fungi. Only key components are represented to highlight the similarity of the feedback mechanism, despite the divergence of molecular components in the different phyla (12). Legend shows the core clock components for the three different model organisms together with the estimated fraction of the transcriptome under circadian control (*bottom right of each panel*) (39, 184). Abbreviations: CK, casein kinases; CWO, clockwork orange; FRQ, frequency; FRH, FRQ-interacting RNA helicase; FWD1, F-box and WD40 repeat-containing protein 1; PDP1ε, PAR-domain protein 1 epsilon; PP, protein phosphatases; RORs, RAR-related orphan receptors; TTFL, transcription/translation feedback loop; VVD, vivid; VRI, vrille; WC1/2, white collar 1,2.

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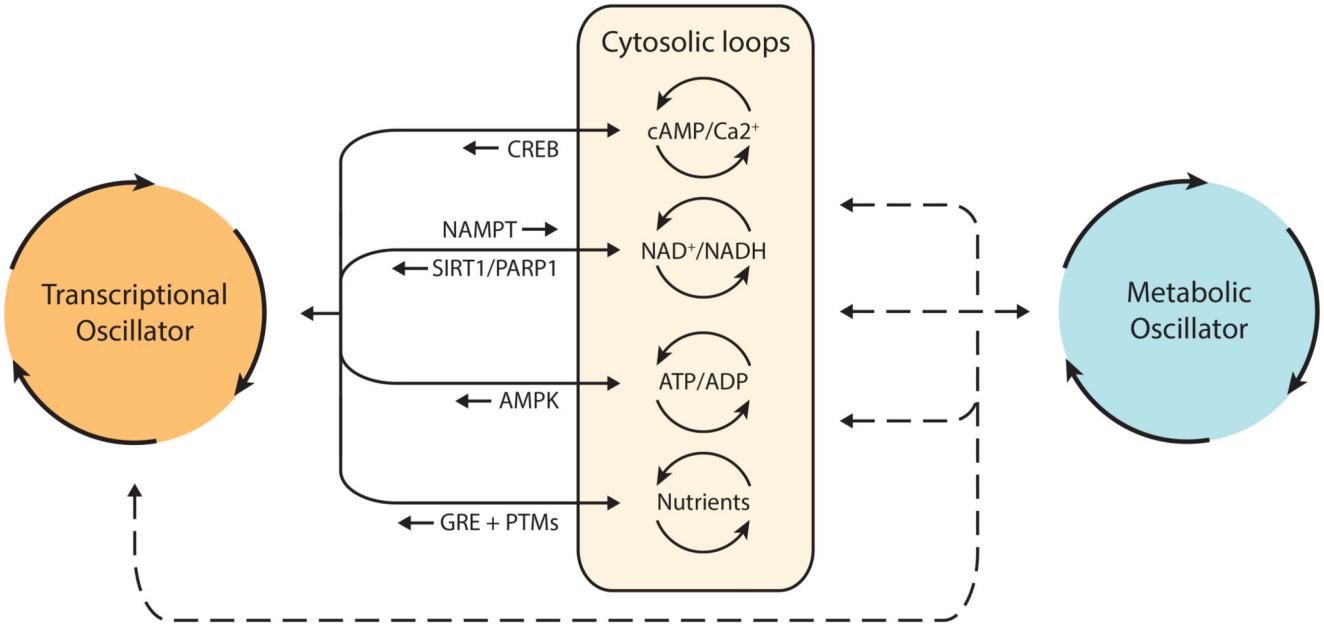

Figure 2.

Transcriptional, cytosolic, and metabolic cycles. The discovery of cytosolic processes as integral to the TTFL models extended the concept of genetic timekeeping to include cytosolic components (42, 71). The latter are mainly involved in redox and energy metabolism and form accessory loops that are controlled by the TTFL oscillator and in turn feedback to it. The reported resilience of current TTFL models to transcriptional perturbations (80, 81, 83, 91, 92) together with the discovery of nontranscriptional rhythms both in prokaryotic (17) and eukaryotic species (20, 107, 111) suggest that a nontranscriptional oscillator underlies transcriptional and cytosolic rhythms. The molecular identity of the latter has not yet been resolved, but the deep phylogenetic conservation of peroxiredoxins' oxidation cycles suggests that it is of metabolic origin (20). Abbreviations: CREB, cAMP response element--binding protein; GRE, glucose response element; PTMs, posttranslational modifications; TTFL, transcription/translation feedback loop.

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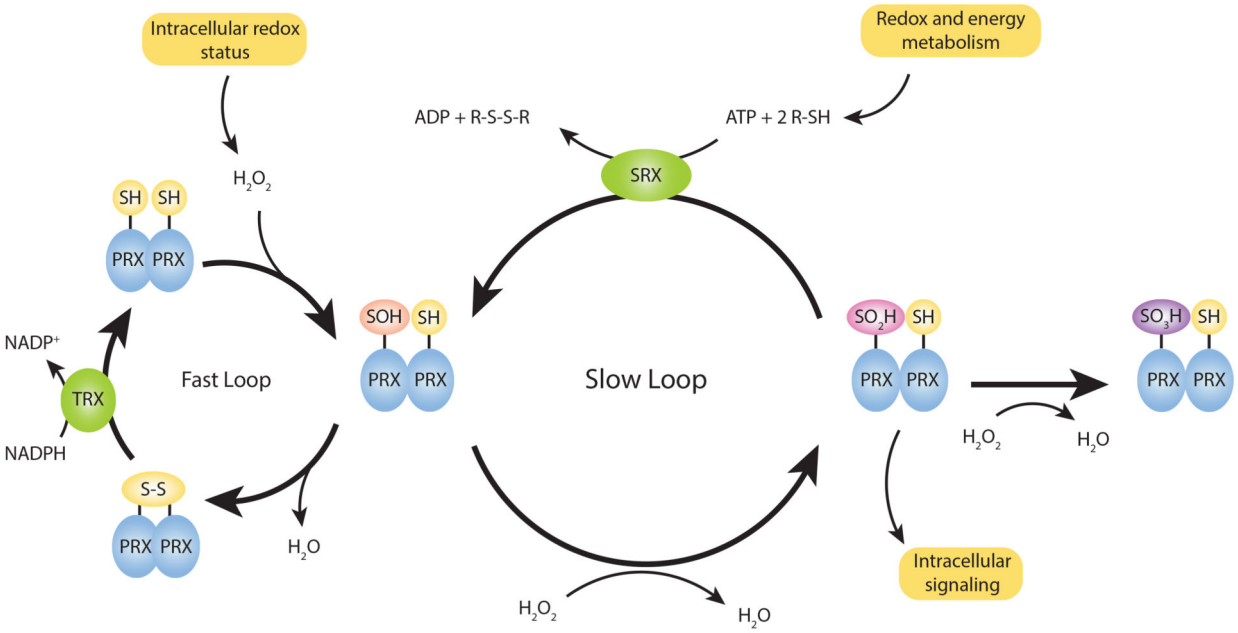

Figure 3.

The typical 2-Cys PRDX system. The catalytic mechanism of typical 2-Cys PRDXs is composed of two interlocked cycles. In the first cycle, PRDXs undergo peroxidation of their catalytic cysteine to sulfenic acid form (Cys-SOH), followed by the disulfide bond formation with the resolving cysteine (resolution) and the recycling step catalyzed by TRX. The sulfenic moiety of PRDXs (Cys-SOH) can be further oxidized to sulfinic and sulfonic acid forms (Cys-$SO_{2/3}H$), thereby entering the second cycle in which overoxidized Cys-SO_2H residues can be slowly recycled through ATP-dependent reduction by sulfiredoxin (126). Further oxidation to Cys-SO_3H (termed hyperoxidation) is thought to be irreversible. In vitro measurement of the turnover rate of each cycle shows that the peroxidatic cycle is fast (185), whereas the overperoxidatic cycle is much slower (158). Abbreviations: PRDX, peroxiredoxin; SH, sulfhydryl group; SOH, sulfenic group; SO_2H, sulfinic group; SO_3H, sulfonic group; SRX, sulfiredoxin system; S-S, disulfide bond; TRX, thioredoxin system.

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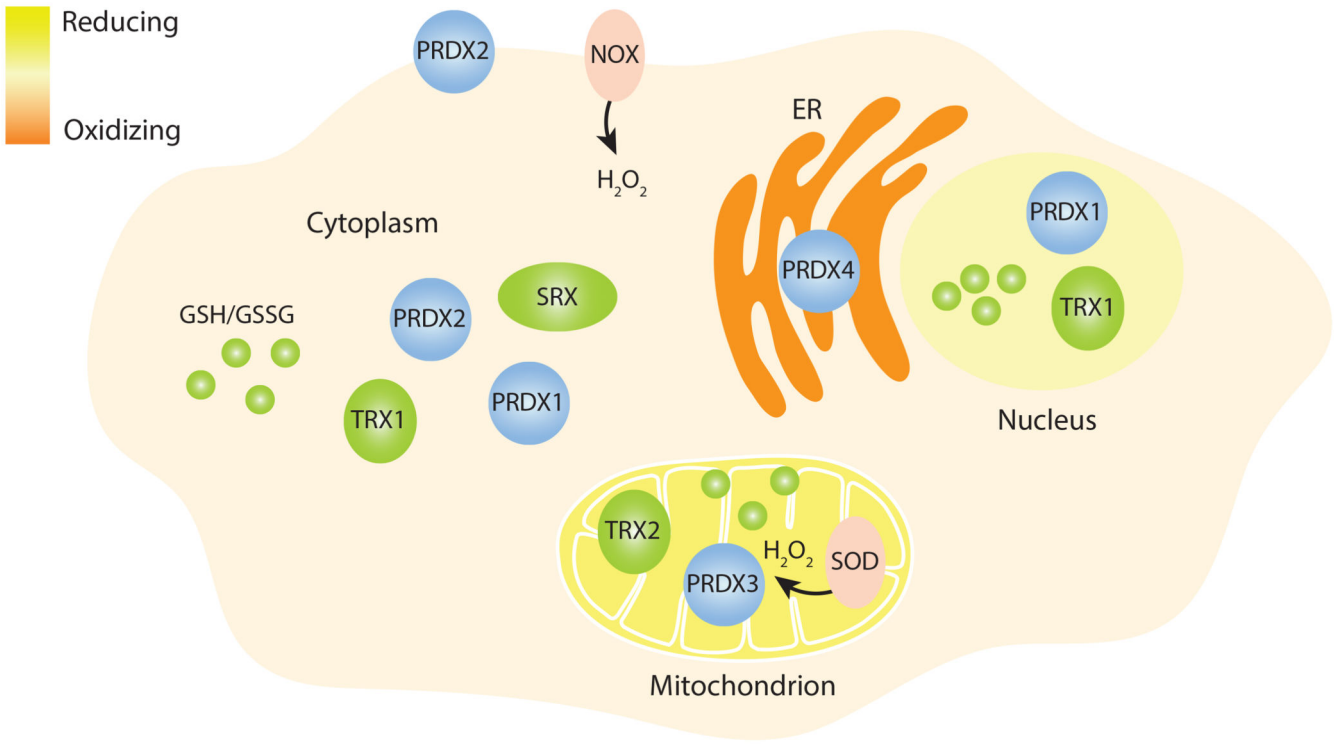

Figure 4.

PRDXs and redox compartmentalization. Given the compartmentalization of redox balances in eukaryotic cells (186), a putative redox oscillator may be localized in specific cellular organelles. Accordingly, the various isoforms of mammalian typical 2-Cys PRDXs have different subcellular localizations, as shown in this schematic representation of a cell with its nucleus, ER, mitochondrion, cytoplasm, and membrane. The SRX is mainly localized in the cytoplasm (158), whereas the TRX has two isoforms found mainly in the cytoplasm and nucleus (TRX1) and in the mitochondrion (TRX2). Compartment-specific pools of reduced and oxidized glutathione are also represented (GSH/GSSG). Two notable examples of ROS-producing enzymes, NOX and SOD, are shown. Each organelle is represented with a color corresponding to its relative reductive or oxidative environment as shown by the scale (*top left*). Abbreviations: ER, endoplasmic reticulum; NOX, NADPH oxidase; PRDXs, peroxiredoxins; ROS, reactive oxygen species; SOD, superoxide dismutase; SRX, sulfiredoxin system; TRX, thioredoxin system.

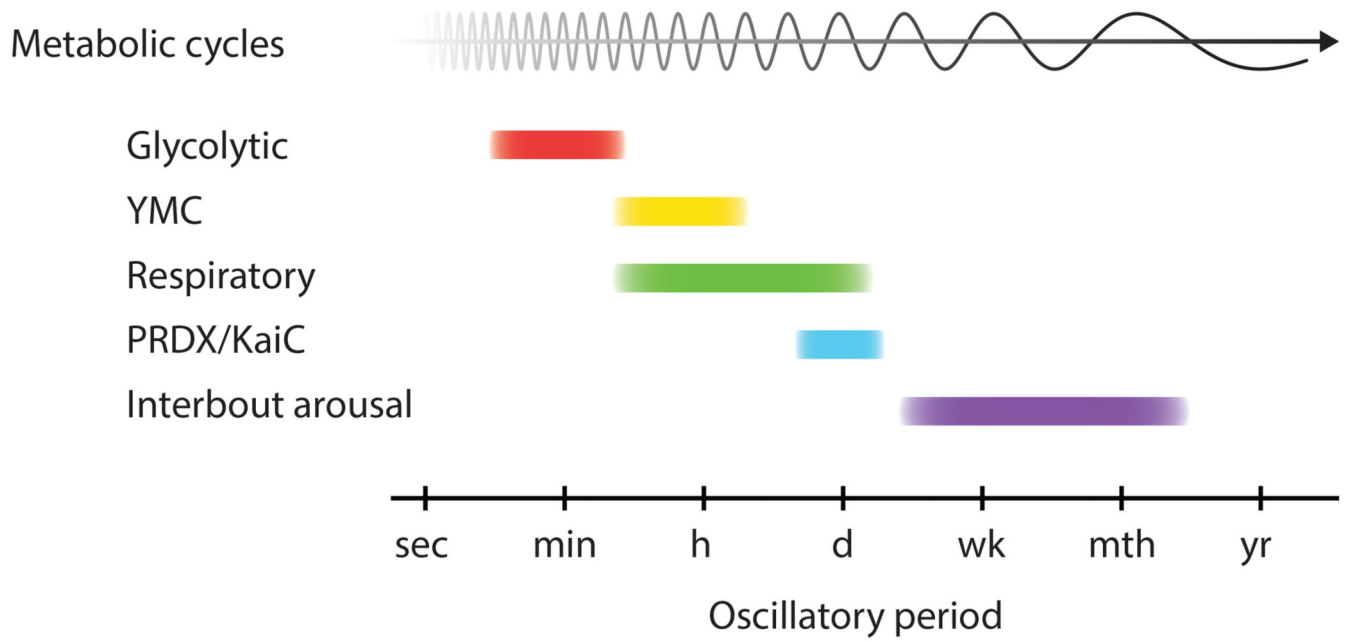

Figure 5.

Metabolic oscillators over timescales ranging from minutes to months. Metabolic oscillators spanning the oscillatory period range from minutes to months have been described in various species. Glycolytic oscillations have been reported in yeast (164) as well as mammalian cells (166); they have a short period in the order of minutes. The YMC is a metabolic oscillator with a period ranging from dozens of minutes to a few hours that is composed of temporally separated reductive and oxidative phases characterized, respectively, by low and high oxygen consumption (174, 175). Similar respiratory cycles have recently been reported in cyanobacteria with a period from 10 h to 24 h, depending on the conditions (178). KaiC phosphorylation and peroxiredoxin oxidation cycles are two of the most preeminent examples of nontranscriptional rhythms in the circadian range. Periodic increase in body temperature during hibernation (interbout arousal) is an example of metabolic oscillation with a period longer than 24 h (from few days to several weeks) (179, 180). Abbreviations: PRDX, peroxiredoxin; YMC, yeast metabolic cycle.