

LIFE TIME—CIRCADIAN CLOCKS, MITOCHONDRIA AND METABOLISM

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A functional circadian clock has long been considered a selective advantage. Accumulating evidence shows that the clock coordinates a variety of physiological processes in order to schedule them to the optimal time of day and thus to synchronize metabolism to changes in external conditions. In mitochondria, both metabolic and cellular defense mechanisms are carefully regulated. Abnormal clock function, might influence mitochondrial function, resulting in decreased fitness of an organism.

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EVIDENCE FOR SELECTIVE ADVANTAGES OF THE CIRCADIAN CLOCK

The Earth's rotation around its axis causes characteristic changes in environmental conditions over the period of 1 day, the most prominent of which is the alternation of light and dark periods. Since these changes occur predictably with a period of about 24 h, an organism able to keep track of time can anticipate them. This allows the coordination of physiological pathways and behavioral processes so as to ensure the organism's activity during the optimal phase of the daily cycle.

A circadian clock capable of conferring a selective advantage is expected to meet certain criteria. It has to generate a rhythm with a period length of about 24 h (circa diem = about a day) which persists even in the absence of external time cues; it must compensate for temperature changes; and its phase must have the capacity to be reset by appropriate stimuli. Such a clock can be found in almost all organisms, from cyanobacteria to mammals. Although key proteins are not conserved, all clocks known to date involve transcriptional-translational feedback loops (Bell-Pedersen et al., 2005; Lakin-Thomas, 2000). It appears that

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circadian clocks have emerged several times during evolution, as a result of convergence to meet a common need.

The selective advantage of clocks is not necessarily obvious, because throughout all species clock mutants are usually viable and do not show any gross abnormalities when compared to wild-type organisms. However, increased fitness of cyanobacterial strains whose internal period length matches the external lighting conditions has been demonstrated (Ouyang et al., 1998). Also, in a multicellular eukaryote, *Arabidopsis thaliana*, investigators have shown that resonance of internal and external period lengths enhances carbon fixation, chlorophyll content, growth, and survival (Dodd et al., 2005).

CIRCADIAN CLOCKS SAVE ENERGY

In prokaryotic organisms like cyanobacteria, all metabolic processes occur in one single cell that is not compartmentalized. Consequently, it is crucial to fine-tune the expression of gene clusters whose products might interfere with the activity of other pathways. For example, genes encoding oxygen-sensitive proteins must not be expressed when oxygen is generated as one of the final products of cyanobacterial photosynthesis. Transcription and translation are processes that consume considerable amounts of energy; thus, the synthesis of a protein that will immediately be rendered inactive by oxygen should be avoided.

For some genes, it is possible to imagine a direct transcriptional regulation by light, such as repression of genes whose products are sensitive to oxygen to prevent unnecessary protein synthesis. However, the presence of a clock allows an organism to anticipate changes in environmental conditions and to be prepared before they occur, instead of merely reacting to acute stimuli. This results in an optimization of metabolism. In plants, the transcription of genes encoding for proteins involved in photosynthesis, such as members of the light-harvesting chlorophyll-binding family, is actually light-inducible, but an additional circadian regulation causes an increase in photosynthetic components already before dawn (Millar and Kay, 1996). The initiation of the synthesis of components of the photosynthetic apparatus before they are needed minimizes the loss of time during which no photosynthesis can occur, since photosynthesis can immediately start with the beginning of the light phase. Interestingly, diurnal expression profiles can also be observed for proteins whose abundance does not oscillate in a circadian manner (Prombona and Argyroudi-Akoyunoglou, 2004). Most of these proteins are subject to light-induced degradation during the day, like certain components of the light-harvesting complex (LHC). In this case, the clock maintains steady-state protein levels. Regulation is thus optimized to ensure optimal photosynthetic performance during the light phase, which in turn guarantees maximal energy gain.

In animals, the circadian oscillation of many behavioral processes and physiological parameters is paralleled on the organ level. In the liver, the onset of the expression of genes involved in gluconeogenesis and energy metabolism precedes the activity onset to prepare for energy storage. Similarly, the expression of glucose transporters and rate-limiting enzymes in hexose catabolism starts to rise before, and peaks precisely at, the time when most feeding occurs (Panda et al., 2002).

MASTER CLOCK VERSUS ENVIRONMENT—UNCOUPLING

One of the most convincing pieces of evidence for the importance of the correct timing of physiological processes comes from restricted-feeding experiments. In mammals, the master clock, which is entrained to the external photoperiod by light cues, resides in the suprachiasmatic nuclei (SCN) of the hypothalamus. Clocks in peripheral organs are normally synchronized to the phase of the master clock via humoral and neural signals. However, when mice, which are nocturnal animals that feed during the night, are only allowed access to food during the day, the phase of peripheral oscillators completely inverts. In contrast, rhythms in the SCN remain unaffected (Damiola et al., 2000). For peripheral clocks, food availability appears to be a potent zeitgeber, uncoupling them from the central pacemaker. Obviously, it is more important for the expression of metabolically relevant genes to be in phase with the feeding schedule rather than with the SCN clock.

CIRCADIAN CLOCKS AS GUARDS

Synchronization of gene expression to external time can also help minimize the damage caused by external stressors. In many microorganisms with a 24 h cell cycle, cell division occurs exclusively during the night, probably to avoid UV-mediated DNA damage during DNA replication. In general, UV-sensitive processes appear to be scheduled to the night. The survival of *C. reinhardtii* after irradiation by UV light displays a circadian rhythmicity, with maximal survival during the day and minimal survival during the night (Nikaido and Johnson, 2000). In accordance with this finding, the expression of a protein conferring UVB-resistance has recently been described to be regulated in a circadian fashion, with a peak just before the beginning of the day (Kucho et al., 2005).

Protection from the deleterious effects of sunlight is also an issue for plants. In *Arabidopsis*, the expression of 23 genes encoding enzymes of the phenylpropanoid biosynthetic pathway, whose products act as photoprotectors, was found to peak before dawn (Harmer et al., 2000), ensuring sufficient photoprotection already at the very beginning of the day. For animals, defenses against stressors other than light are more important.

In mouse liver, the expression of many plasma proteins conferring innate immunity peaks at the end of the subjective day. The immune system is thus able to react efficiently in case of pathogen encounters in the early hours of the activity phase (Panda et al., 2002).

MITOCHONDRIA—WHERE METABOLISM AND CELLULAR DEFENSE MECHANISMS MEET

For heterotrophic organisms, the mitochondrial respiratory chain that mediates oxidative phosphorylation is the primary site of ATP production. A synchronization with metabolic demands is therefore desirable. The SCN neurons of mice display circadian variations in their firing rate, with peaks during the first half of the day (Aujard et al., 2001). Consistent with this finding, the expression of more than 20 components of the respiratory chain is regulated in coordination and reaches its maximum shortly before dawn in SCN neurons (Panda et al., 2002), assuring maximal metabolic capacity during the phase of neuronal activity. In *C. reinhardtii*, several genes encoding factors of the respiratory chain were identified in a screen for transcripts with rhythmic expression patterns (Kucho et al., 2005). Contrary to the findings in SCN neurons, their expression peaks at the end of the subjective day, which, however, is to be expected in a phototrophic organism that relies on its oxidative phosphorylation system primarily at night.

Reactive oxygen species (ROS) are a by-product of respiratory chain activity that are generated due to electron leakage, especially from complexes I and III (Turrens, 2003). ROS can cause substantial cellular damage by the oxidation of proteins, lipids, and DNA, which needs to be minimized for the survival of cells. Since ROS production occurs in a circadian manner, circadian variations are found in many ROS defense systems. In *Arabidopsis*, the activity of the peptide methionine sulfoxide reductase, a ubiquitous enzyme repairing oxidatively damaged proteins, displays a circadian profile with a peak toward the end of the night (Bechtold et al., 2004). This corresponds to the time of maximal respiration and oxidative damage.

ROS can be scavenged by glutathione peroxidase (GPx), which uses reduced glutathione as the electron acceptor. In various species, GPx is induced by melatonin, and its activity shows a circadian oscillation following the melatonin pattern. Melatonin levels begin to increase toward the middle of the night, and GPx activity reaches its maximum shortly after the melatonin peak. In rats, the lipid peroxidation level in the brain and liver exhibits a pronounced increase at the beginning of the night due to enhanced metabolic activity; however, starting from the middle of the night, this trend is counteracted by the rise in GPx activity (Baydas et al., 2002).

Superoxide dismutase (SOD) also forms part of the cellular ROS defense. In *C. reinhardtii*, two SOD isoforms with circadian oscillations of transcript levels have been identified, one of which peaks in coordination with the components of the mitochondrial respiratory chain (Kucho et al., 2005). These findings are preliminary, but they might represent a way to ensure functional ROS defense during the peak time of respiratory activity. Another example of SOD acting as a defense against ROS produced by electron leakage via photosynthetic transport chains comes from the dinoflagellate *Lingulodinium polyedrum*. In this organism, the chloroplastic Fe-SOD displays a circadian activity profile with a peak during the light phase (Okamoto et al., 2001). This correlates with the photosynthetic maximum and increased chloroplastic ROS production due to enhanced intrachloroplastic oxygen levels and to the high electron flow rate, offering maximal protection against ROS in due time.

RESPIRATORY CHAIN DEFECTS AS RISK FACTORS

ROS production (as an inevitable consequence of metabolism) and ROS defense are two processes controlled at least in part by the circadian clock. However, the system involves many components, and even minor imbalances can have dramatic effects. Mitochondria are the site of major ROS production, and there is a high risk of oxidative damage to mitochondrial (mt) structures such as mtDNA or proteins of the respiratory chain. Oxidation of mtDNA can cause mutations in genes encoding factors of the electron transport chain, and this, or alternatively direct oxidative modification of proteins, eventually interferes with effective electron transport by partial or complete inactivation of respiratory chain complexes. This in turn will further increase ROS production, aggravating the damage and starting a vicious circle.

POSSIBLE INVOLVEMENT OF THE CLOCK

Elevated levels of ROS and, as a result, increased oxidative stress are considered to be the cause of aging and disorders such as Alzheimer's and Parkinson's disease (Huang and Manton, 2004). Complex I appears to be an especially critical factor in this context. First, it contains 7 of the 13 subunits that are encoded by the mtDNA; therefore, mutations in mtDNA caused by oxidative stress will greatly affect this complex. Second, even in an intact system, it is one of the main sites of ROS production, and complex I-derived ROS have been identified as the most prominent ROS species in aging and neurodegeneration (Nicholls, 2002). This crucial role of complex I is supported by the finding that a mutation in a subunit of complex I reduces lifespan and increases ROS damage in *C. elegans* (Kayser et al., 2004).

According to the database of circadian gene expression (<http://expression.gnf.org/circadian>), several respiratory chain components, among them subunits of complex I, seem to display a circadian expression pattern in mice, and a number of others that have yet to be analyzed contain E-box motifs in their promoters. Since E-boxes can serve as binding sites for clock transcription factor complexes like BMAL1-CLOCK or BMAL1-NPAS2 (Hogenesch et al., 1998), this opens the possibility of potential direct clock control on the expression of these subunits. Abnormal clock function might thus interfere with the coordinated expression of respiratory chain components, lead to loss of tight coupling between complexes, and augment electron leakage and ROS production. Additionally, it might desynchronize ROS defense mechanisms, leaving the organism unprotected at peak times of ROS production.

It is tempting to speculate that deficiencies in the circadian clock can accelerate aging or can be a predisposing factor in neurodegenerative diseases. If this were the case, a functional circadian clock would increase an organism's fitness not only by the optimization of metabolism but also by enhancing life span or preventing disease, which might prove to be another very important way for it to confer a selective advantage.

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