Circadian Time Redoxed

Mino D. C. Belle and Hugh D. Piggins

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PHYSIOLOGY

Circadian Time Redoxed

Oscillations of the molecular clock and redox states control the electrical activity of mammalian clock neurons.

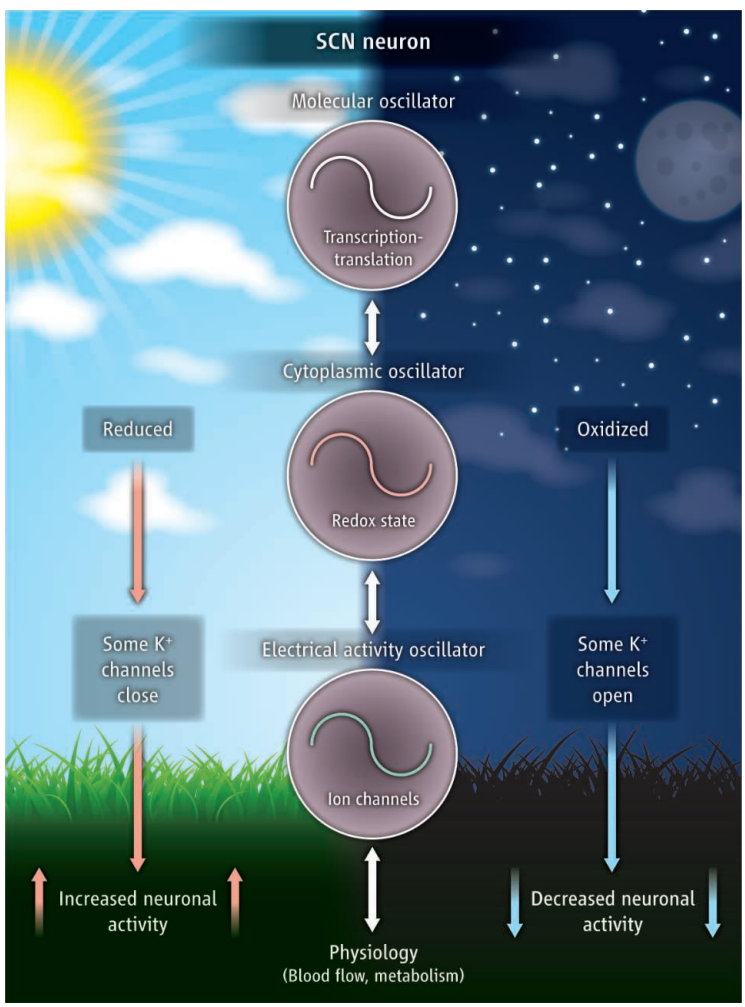
Mino D. C. Belle and Hugh D. Piggins

In all living cells, oxidation and reduction (redox) processes occur as potentially harmful oxidizing or reducing agents (free radicals), such as nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), accumulate as by-products of metabolism. In response, biologically active molecules quickly stabilize the relative concentrations of these redox agents. Although redox processes have been associated with cellular housekeeping and homeostasis, recent research has provided a new twist on cellular redox states, linking them to the intrinsic daily (circadian) clock (*1*–*4*). On page 839 in this issue, T. A. Wang *et al*. (*5*) show that the molecular circadian clock and redox states regulate the electrical activity of the neurons that comprise the mammalian central circadian clock.

Circadian rhythms pervade all aspects of our physiology and behavior. For example, at night we sleep and our metabolic activity is low, while during the day, we are awake and active, and our metabolism is high. Genes and proteins that underpin the molecular timekeeper of these rhythms have been modeled as a transcription-translation feedback loop (TTFL). This TTFL clock is present in cells, tissues, and organs of eurkaryotes, and some of its molecular components are conserved across animal species. In mammals, the master circadian clock is in the brain's suprachiasmatic nuclei (SCN) of the hypothalamus. Individual neurons of the SCN contain the TTFL clock, and the coordinated activity of these cell-autonomous oscillators conveys timekeeping signals to the rest of the brain and body.

Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, UK. E-mail: hugh.d.piggins@manchester.ac.uk

Neurons of the SCN generate daily rhythms in electrical activity, such that the cells are in a more excited state during the day than at night (*6*, *7*). The precise mechanisms by which the TTFL clock drives this electrical rhythm are unknown, but variation in the opening and closing of potassium (K^+) channels is implicated (*8*). For example, opening of K^+ channels at night renders a neuron's resting membrane potential (V_m) more negative, thereby reducing the possibility that it will spontaneously generate and fire action potentials (changes in electrical potential that serves to transmit signals). During the day, some K^+-channel activity is reduced, thus enabling a neuron to become more active. Remarkably, this rhythm of neuronal excitability is maintained ex vivo when the SCN is kept in a brain slice preparation. Although the TTFL clock influences this oscillation (*9*–*11*), impairment of the electrical rhythm can feed back and alter the molecular clock (*12*). So, neuronal membrane activity and excitability are integral to the clock and provide a bidirectional interface between the molecular oscillator and brain signals. Wang *et al*. show that redox state is an important variable in sculpting this 24-hour rhythm in SCN brain cell activity. They demonstrate that this cycling in redox states is driven by the TTFL clock and, startlingly, may form a secondary cytoplasmic oscillator complementary to that of the TTFL clock.



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Influential oscillators. The molecular (TTFL) clock and the non-TTFL circadian oscillators in mammalian SCN neurons are illustrated. In this model, the oscillators influence each other during the day and night. Neuron activity feeds back onto the TTFL clock by unknown mechanisms (not shown).

SCN neurons are metabolically more active during the day than at night (*13*). Using noninvasive methods to assess the relative concentrations of NAD^+ and FAD in the cytoplasm of rodent SCN neurons, Wang *et al*. established that the redox state in the SCN in vitro shows an endogenous circadian rhythm that requires functional molecular clockwork. They further show that this day-night change in redox state directly influences SCN neuronal activity. This provides a decisive pathway through which the TTFL clock influences SCN electrical activity.

Because SCN neurons can sustain their circadian rhythm in neuronal activity and V_m in vitro, Wang *et al*. could measure very fast, low-amplitude electrical events that influence the V_m. The circadian oscillation of redox state aligned with the known day-night variation in the V_m of SCN neurons. Further, the night-time oxidized state in the SCN produced conditions that directly influenced the membrane of the cells,

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increasing the activity of specific K^+ channels, which reduced excitability in the neurons. During the day, the reduced state shuts down some K^+-channel activity while modulating the conductances of others, thereby increasing excitability in these cells.

Because clock gene transcription is itself sensitive to redox state, the study by Wang *et al*. and recent work in other circadian systems provide evidence for a metabolically sensitive, nontranscriptional pathway to temporally sculpt the clock's transcriptional and electrical machinery (see the figure). However, further work is necessary to establish how, for example, clamping the redox state at an intermediate level affects the molecular clockwork and its eventual electrical output. Also, confirming the observations of Wang *et al*. and others in vivo will be challenging. Because SCN cells do not exist in isolation but are part of a network of tightly packed neurons that can influence the activity of one another, the question of whether metabolic state is the cause or the result of neuronal activity remains unclear.

Nevertheless, the results of Wang *et al*. and the ideas they propose are consistent with several findings that challenge an exclusive TTFL model of the molecular clock. Recent research has shown that peroxiredoxins, proteins that are present in virtually all living organisms and that function to buffer the intracellular environment, undergo daily rhythms of oxidation-reduction across a range of species, including those whose cells are enucleated (*2*–*4*). There are similarities here also to the circadian clock in cyanobacteria, which do not employ the TTFL clock, as well as the clock in plants, which has both TTFL and non-TTFL components (*14*, *15*). Thus, it seems that across many life forms, TTFL and non-TTFL timekeeping processes are not mutually exclusive, and in mammals, they cooperate to orchestrate circadian output of clock neurons.

Although several K^+ channels in the SCN show circadian variation in the abundance of their transcripts and in their functional activity, the study by Wang *et al*. illustrates how focusing on a direct link between the activity of these channels and the molecular clockwork may have discouraged the consideration of other important possibilities. Indeed, because neurons and subregions of the SCN are heterogeneous, it will be important to determine if redox state differentially influences SCN neuron activity. Similarly, because other brain regions that influence metabolic physiology may also contain a TTFL molecular clock (*16*), the influence of redox state on cellular activity in these neural structures should be analyzed as well.

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PALEONTOLOGY

Reproduction in Early Amniotes

P. Martin Sander

Recent fossil finds help to explain why the early fossil record is dominated by live-bearing amniotes, although live-bearing amniotes evolved later than egg-laying ones.

The conquest of dry land by vertebrate animals began with the evolution of the first four-legged, amphibious animals ~360 million years ago (*1*, *2*). Amniotes originated ~50 million years later (*1*) and have since become the most diverse clade of land-living vertebrates, including mammals, turtles, lizards, snakes, crocodiles, and birds. Evolutionary changes in reproduction were crucial for the move from the sea via swamps to dry land. However, the reproductive structures and early life stages of amniotes fossilize poorly. Exceptional insights into early amniote reproduction are offered by recent fossil discoveries (*3*–*6*). The fact that these fossils come from ancient seas and lakes and not from dry land helps to explain the paradox that there is an older fossil record for live-bearing amniotes than for egg laying in amniotes.

Steinmann Institute of Geology, Mineralogy, and Paleontology, Division of Paleontology, University of Bonn, Nussallee 8, 53115 Bonn, Germany. E-mail: martin.sander@uni-bonn.de

The key evolutionary innovation that enabled amniotes to colonize habitats away from water was the cleidoic egg. Its complex structure added extraembryonic membranes (the chorion and the amnion) and a shell to the primitive vertebrate egg design with its embryo, yolk, and jelly layers (*2*). These membranes and eggshell enable egg laying and development on dry land. The shell and egg membranes allow gas exchange to and from the developing embryo, letting oxygen in and carbon dioxide out but retaining water. The shell may be either leathery or calcified. Phylogenetic inference shows that leathery shells evolved first; calcified eggshells evolved independently from leathery eggshells at least four times (see the figure).

The evolution of the cleidoic egg had two main effects. One was internal fertilization. The other was that eggs could no longer be laid in water, where the embryo would suffocate. This had major implications for the secondarily marine amniotes that frequently evolved from terrestrial lineages.

Soon after their origin, Amniota split into two major lineages: the mammal-line amniotes (Synapsida) and the bird-line amniotes (Reptilia). Phylogenetic inference from living animals (see the figure) suggests that amniote egg laying evolved no later than in the last common ancestor of mammals and birds, ~310 million years ago (*1*). Both animal groups have a cleidoic egg, although this has been lost in mammals more derived than monotremes. It is highly unlikely that this complex egg structure evolved more than once (*2*). Paleontologists have inferred egg laying on dry land from skeletal indicators of full terrestriality—such as well-developed limb joints—in tetrapods close to the mammal-bird split (*2*).

However, fossils of the cleidoic egg from near the mammal-bird split have been hard to come by. The oldest cleidoic egg fossils postdate amniote origins by 90 million years. At sites in Argentina and South Africa, fossilized egg clutches and embryos of prosauropod dinosaurs have been found that are