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Circadian Biology: A 2.5 Billion Year Old Clock

A recent study suggests that circadian clocks may have evolved at the time of the Great Oxidation Event 2.5 billion years ago in order to drive detoxification of reactive oxygen species.

Andrew S.I. Loudon

The regular 24 hourly rotation of the earth has led to the evolution of circadian oscillators in virtually all life forms, from prokaryotes to eukaryotes. Synchronized circadian rhythms provide an organism with a predictive mechanism to tune its internal physiology to the external world, and several studies have shown that a robust internal clock offers a significant competitive advantage [1,2]. Despite widely divergent origins, a common design principle applies to the molecular clockwork of all organisms in which the timing mechanisms have been investigated, from bacteria to man. Here, a rhythmic transcriptional–translational feedback loop (TTFL) model has been proposed in which a few core genetic elements drive output pathways and rhythmic physiology. However, the problem is that there appears to be little conservation of the TTFL across evolutionary time. In cyanobacteria, for instance, three proteins (KaiA, B and C) are involved [3] but these are not conserved in eukaryotes. Here, apparently different mechanisms have appeared, with different elements driving the clockwork of the fungus *Neurospora* (FREQUENCY and WHITE COLLAR) and plants (TOC1 and CCA1), while in animal clocks (both invertebrate and vertebrate) another set of transcription factors (BMAL1 and PERIOD proteins) is used. The implication, therefore, is that clocks have appeared many times to achieve a common purpose, and evolved independently in different lineages.

Now a recent paper challenges that assumption [4]. The story starts with the discovery by O'Neill and Reddy in 2011 of circadian timekeeping in human red blood cells [5]. These cells lack a nucleus, and also mitochondria, so according to the TTFL model should not oscillate. Remarkably, however, these cells exhibited a robust oscillation in the oxidation state of peroxiredoxin (PRX) proteins, and when these authors studied cells from humans, mice and algae, they observed similar oscillations [6]. PRX proteins are important in the inactivation of reactive oxygen species (ROS), and in particular hydrogen peroxide. The '2-Cys' PRX enzymes contain a cysteine amino acid residue in a region that is strongly conserved and which is oxidized when ROS accumulate in the cell. This results in a transition from a monomeric to dimeric state. Because the protein is so strongly conserved, it is possible to use a common antibody, which recognizes the oxidized state of PRX in widely divergent organisms: in bacteria, archaea and eukaryotes.

Starting with a range of eukaryotes, the authors of the current study first showed robust oxidized PRX rhythms in mice, testing the central brain pacemaker structure (suprachiasmatic nucleus) and liver. Intriguingly, the PRX rhythms in these two structures peaked at different times of day, but in phase with the local TTFL system, suggesting a possible link. They then extended this to show similar PRX oxidation rhythms in flies (*Drosophila*), plants (*Arabidopsis*) and fungi (*Neurospora*). Is this also seen for the prokaryotic clocks, in both bacteria and archaea? Turning to the well-studied cyanobacterium (*Synechococcus elongatus*), the authors defined robust circadian cycles of PRX oxidation,

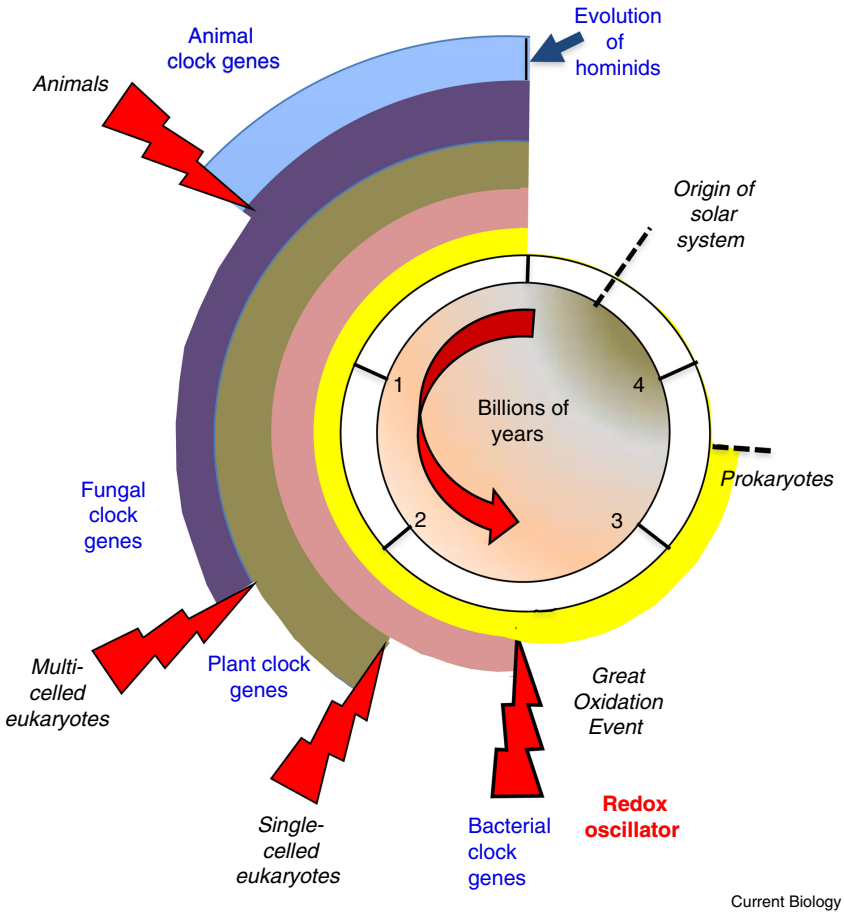

Figure 1. The evolution of a redox oscillator.

Life on earth began ca. 3.5 billion years ago, and the Great Oxidation Event 2.5 billion years ago may have led to the evolution of circadian oscillations in reactive oxygen species and detoxification mechanisms.

but since the Kai proteins of the cyanobacterial TTFL clockwork are not conserved in most archaea, a critical test of the potential universality of the PRX oscillator in prokaryotes was to check a representative archaeon, *Halobacterium*. Here, they also observed strong rhythmic PRX oscillations, providing compelling evidence that a PRX-oxidation oscillation is a conserved circadian feature across all phylogenetic domains.

The next step was to check whether known mutations of the TTFL system would affect the PRX oscillator. Fortunately, a considerable amount is known of these systems and appropriate models are available. Using mutations of the TTFL system, which disables the conventional circadian clock, the authors nonetheless observed oscillations of PRX oxidation in flies, plants, algae and cyanobacteria. This might suggest that the two systems run independently. Clock-disrupting mutations of the TTFL system did, however, alter phasing of the PRX oscillator, and when they checked more subtle TTFL mutants, which just changed period length (i.e., slowed down the clock), they also observed lengthening of the PRX cycle. So, it appears that the TTFL and PRX oscillators may in some way be coupled. Disabling the PRX oxidation rhythms (using mutations of 2-Cys PRX) in plants and bacteria revealed that the core TTFL clockwork continued to tick, but with different phases and amplitudes. Thus, a clock can run without either the TTFL or the PRX system, but for normal physiology, both need to operate.

The evolution of ~24-hour cycles of PRX oxidation–reduction in all domains of life now suggests that cellular rhythms may employ common molecular elements. Critically, PRX proteins are involved in the removal of toxic metabolic byproducts (ROS), and so their appearance may have contributed a selective advantage at the beginning of aerobic life on earth (Figure 1). This is thought to have occurred around 2.5 billion years ago, with the development of photosynthetic bacteria and photo-dissociation of water. This in turn led to the extremely rapid accumulation of (toxic) atmospheric oxygen during what is termed the Great Oxidation Event (GOE). The GOE led to a catastrophic change in earth ecology, with the loss of many anaerobic life forms, while intriguingly the most ancient TTFL clockwork mechanism, found in cyanobacteria, is thought to have evolved at around this time. Thus, during the GOE, rhythms of oxygen consumption/generation and ROS production would be driven by the solar cycle, leading to the evolution of a metabolic clock, which persists in the absence of a conventional TTFL cycle.

The discovery of the PRX oscillation has now opened new avenues for research. It is likely that the PRX system is representative of the 'arms' of an inner rhythmic process, the most likely of which is an internal cycle within the cell of production of hydrogen peroxide, and investigations are already under way to explore this circuit. Importantly, these new discoveries help to explain what up till now has seemed a paradoxical feature of the 'conventional' TTFL molecular clockwork. Here, we see conserved oxygen-sensing PAS-domain proteins as a common feature in many eukaryotic TTFL clocks [7,8]. In animal clocks, the core TTFL drives a great complexity of outputs but included are many members of the nuclear hormone receptor family, key regulators of intermediary metabolism. One member, REVERB-alpha, in particular is of current interest as this hormone receptor has been shown to act as a key redox sensor for the cell and to drive rhythmic metabolic and immune responses [9,10]. Much of our own physiology therefore may reflect an ancient oxygen-sensitive clockwork.

Finally, a fascinating speculation is that the clock as we know it may have appeared at the time of the GOE. A prediction therefore is that methanogenic organisms should lack circadian oscillators of any sort. Perhaps someone can be persuaded to mount an expedition to explore the biology of hyperthermophilic archaea in deep-sea vents of the oceans?

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

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Visual System: Prostriata — A Visual Area Off the Beaten Path

Recent work establishes that Prostriata, a little-studied area of the visual cortex neighboring V1, has distinct but hybrid visual properties which are suggestive of an unsuspected role in the rapid analysis and integration of peripheral visual stimuli.

Kathleen S. Rockland

The cortical visual system in primates consists of a highly specialized primary area (area V1 or 17) and an extensive network of visual association areas. The primary area is unambiguously identified by multiple criteria, such