Ticking over

Circadian systems across the kingdoms of life

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The ability to anticipate the day–night cycle and direct physiology accordingly has proven to be a general phenomenon across all kingdoms of life. Considerable fitness benefits are conferred by an internal 24-hour clock, which is known as a circadian clock. Extensive multi-disciplinary studies in a range of model organisms have elucidated many of the components involved in generating and sustaining daily rhythms. When comparing the circadian systems across the kingdoms, it is fascinating to observe the commonalities and differences in their molecular architecture, and the many adaptations which have evolved to deal with organism-specific requirements of biological timing.

Key words: *Arabidopsis thaliana, cellular oscillator, circadian clock, suprachiasmatic nucleus, temperature compensation*

General properties of circadian systems

It is apparent that circadian clocks comprise negative-feedback loops of gene expression that generate oscillations of approximately 24-hour periods. These molecular clocks then relay timing information to physiology. Circadian clocks remain synchronized with the external environment through entrainment, the resetting of the phase of the clock in response to signals which might arrive from a light-, nutrient- or temperature-sensitive pathway, often activated by environmental stimuli such as dawn. Despite the sensitivity of phase to light and temperature, the period of circadian clocks is often very robust to prolonged changes in light and temperature. The response of circadian clocks to light is time-dependent, or 'gated', to prevent transient light pulses from effecting entrainment at night, while remaining sensitive to entraining signals around dawn. In some organisms, this is very strong, restricting the range of T cycles (environment period) to which they can entrain. For instance, most rodents cannot entrain outside the range 23–25 hours, whereas plants and fungi can have rather larger deviations from T=24 hours. Finally, despite the Q_{10} law, which states that chemical reactions approximately double their rate with every 10°C increase in temperature, the free-running period of most circadian clocks is relatively stable in the physiological range, a phenomenon known as temperature compensation.

These general properties enable robust 24-hour periodic oscillations to persist in a range of environmental conditions, including daily, seasonal or even longer timescale variations.

The circadian network in *Arabidopsis thaliana*

In eukaryotes, cell-autonomous oscillations are generated by networks of interlocking positive- and negative-feedback loops. In the model higher plant, *A. thaliana*, a transcriptional negative-feedback loop involving the transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) and the pseudo-response regulator TIMING OF CAB EXPRESSION 1 (TOC1) lies at the heart of the rhythm-generating central oscillator mechanism, which results in recurring cycles of mRNA and protein accumulation and degradation (Figure 1). Additional transcriptional loops have also been identified, and have been formalized in a series of mathematical models of the *Arabidopsis* central oscillator[1]. In addition to transcriptional loops, a cADP-ribose (cADPR)-based signalling pathway has been proposed to form a loop with the central oscillator[2], suggesting that the cell-autonomous oscillators are responsive to changes in physiology. Conversely, binding of CCA1–LHY dimers to a conserved promoter element ('evening element', EE) found in the promoting regions of approximately 5% of the genome (including *TOC1*) confers widespread rhythmic control of gene expression and therefore physiological processes[3]. Rhythmic control is also modulated by direct light-sensing pathways, with combined regulation necessary for seasonal ad-

aptation in the timing of the output rhythm[4].

The cells of aerial tissue are thought to sustain light-entrainable oscillations independently, as it is possible to entrain two halves of the same leaf to opposite phases by antiphasing light input to each half of the leaf[5]. This suggests that the circadian oscillators of each leaf cell can be autonomous. Roots also have circadian rhythms, despite being in the dark of the soil and presumably receiving no light signals, which implicates intercellular coupling to be an important facet of circadian signalling in plants[6] (Figure 1). Root rhythms synchronize with aerial tissue in light–dark cycles, although synchrony is disrupted following treatment with sucrose or the photosynthesis inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), indicating an important role for metabolism in inter-organ signalling within the circadian network[6]. Sucrose also decreases the period of *Arabidopsis* rhythms and increases clock gene expression in aerial tissue, although not in *sensitive to freezing*6 (*sfr6*) mutants[7]. Understanding the metabolic inputs to the clock and circadian coupling between plant cells will undoubtedly improve our knowledge of daily timing at the systems level.

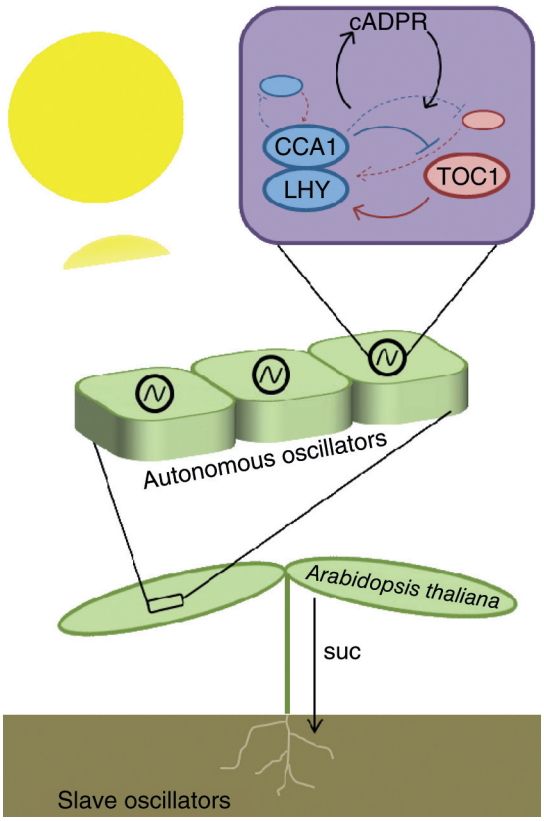

Figure 1. The circadian system in *A. thaliana* incorporates multiple feedback loops. CCA1, CIRCADIAN CLOCK-ASSOCIATED1; LHY, LATE ELONGATED HYPOCOTYL; TOC1, TIMING OF CAB EXPRESSION1. Additional feedback loops are indicated by blank ovals, and represent loops involving GIGANTEA (GI), EARLY FLOWERING 4 (ELF4), ELF3, ZEITLUPE (ZTL) and PSEUDO-RESPONSE REGULATORS 3/5/7/9 (PRR3/5/7/9) and other components. See ref 1 for details.

Master and slave oscillators in mammals and fruitflies

Unlike plants, in which the majority of cellular oscillators can perceive light directly, mammalian clocks receive light stimuli from the retina, via the retino-hypothalamic tract (RHT), into a small cluster of neurons known as the suprachiasmatic nucleus (SCN; Figure 2). The neurons of the SCN are thought to comprise a master circadian oscillator which in turn drives peripheral or slave oscillators in the rest of the body, such as found in the heart, liver, kidneys and other areas of the brain (Figure 2). Direct light inputs enable circadian oscillators to maintain tight synchrony with the environment, although when unavailable must be compensated for by intercellular signalling. In plants, such intercellular signalling is probably required only for root clocks, whereas in mammals, limited photic input and distributed physiological systems require a more connective and hierarchical arrangement for cellular circadian oscillators.

At the cellular level, mammals also rely on transcriptional negative feedback to generate and sustain autonomous oscillations (see Dibner et al.[8] for a review). Genes with an E-box promoter element (morning element) are transcriptionally activated by heterodimeric binding of basic helix–loop–helix (bHLH)-PAS (Period/Arnt/Single-minded)-containing transcription factors, CLOCK and BMAL1 (brain and muscle Arnt-like protein-1) (Figure 2). Among these are three *period* genes, two *cryptochrome* genes and Rev-Erbα. Following translation, PER and CRY proteins form complexes with protein kinase CK1, are phosphorylated, then translocate back into the nucleus and inhibit transcription by direct interaction with CLOCK–BMAL1, forming a negative-feedback loop. Cytosolic signalling and metabolism also interlink with transcriptional regulation in mammals, with cAMP[(9)] and NAD^{+}[(10)] forming feedback loops within the central clock architecture.

In *Drosophila melanogaster*, the common fruitfly, the central oscillator mechanism exhibits remarkable similarities to the mammalian clock. CLOCK (CLK) and CYCLE (CYC) form heterodimers which bind E-box regulatory elements, activating transcription of *per* and *tim*. In this way, CLK–CYC is equivalent

to CLOCK–BMAL1 in mammals, although this is only an example of the many orthologues and/or functional equivalents between fruitfly and mammal clocks (reviewed in Yu and Hardin[11]). Furthermore, the structure of the circadian system in *Drosophila* is very similar to mammals, with a hierarchical arrangement of master pacemaking neurons (functionally equivalent to SCN) entraining a series of peripheral oscillators which mediate temporal regulation of physiology.

The slave oscillators in mammals and insects receive entraining signals directly from SCN/pacemaker neurons via multiple intercellular pathways, which can largely be categorized as either neuronal or hormonal[8,12]. In addition, however, the SCN can co-ordinate peripheral oscillators via more indirect routes, by regulating core body temperature or behavioural rhythms such as feeding. The peripheral oscillators in rat livers can be entrained out of phase from the SCN, by imposing conflicting feeding regimes[13]. In contrast with previous thinking, peripheral oscillators can maintain robust yet asynchronous circadian rhythms in the absence of a functional SCN or imposed feeding regimes[8]. Temperature resetting of peripheral oscillators is strongly gated by the SCN, which possesses an intrinsic robustness to temperature fluctuations. In contrast, peripheral oscillators do entrain to temperature cycles, both *in vivo* and *in vitro*[14].

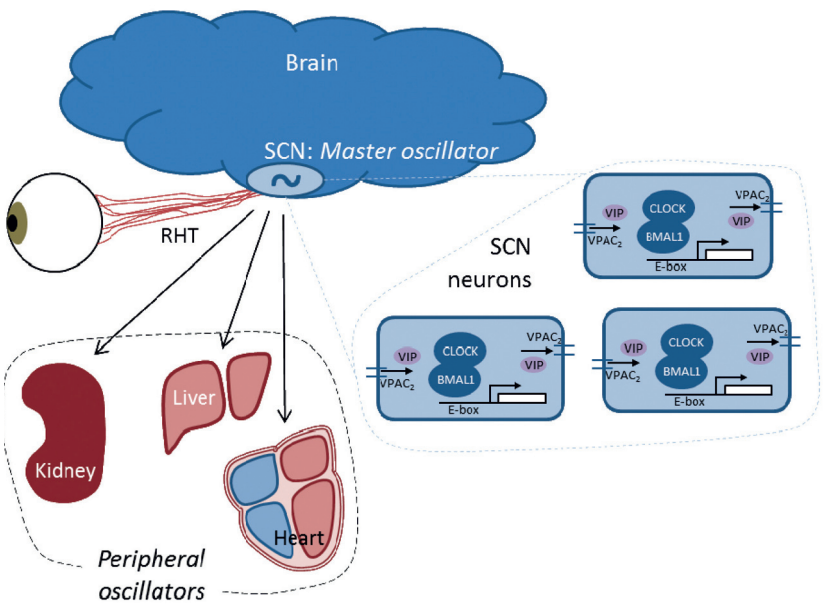

Figure 2. The circadian system in mammals

Intercellular coupling, loop structure and light input confer robust oscillations

Intercellular coupling goes far deeper in mammals than the communication between master and peripheral oscillator regions. SCN neurons are able to synchronize their timing as a population, via intercellular coupling mediated by neuropeptides such as vasoactive intestinal peptide (VIP) and its receptor $VPAC_2$[15] (Figure 2). Loss of VIP or $VPAC_2$ severely disrupts rhythmic behaviours, halving the number of neurons firing with a circadian rhythm, and desynchronizing those that are rhythmic. The intercellular communication between SCN neurons has also been shown to enhance robustness to mutations in the circadian network[16]. Despite this desirable property, it is not shared by peripheral oscillators, which rely more heavily on cell-autonomous robustness[8].

A series of mathematical analyses are helping to uncover how and why multiple feedback loop structures are so pervasive in circadian systems. The robustness of cell-autonomous oscillators to environmental fluctuations in light availability and temperature, and to internal fluctuations in gene expression and signalling cascades, is thought to derive from multiple feedback loops[17]. By incorporating multiple feedback loops, circadian clocks maintain greater evolutionary flexibility, being able to uncouple the phase relationships of specific components with respect to changing photoperiod. Coincident to these mathematical studies of robustness in multi-loop architectures of complex organisms, interesting complementary work in simpler organisms is illustrating the proposed benefits of multiple loops. For instance, the moss *Physcomitrella patens* expresses homologues of the *Arabidopsis* circadian clock genes *CCA1*, *EARLY FLOWERING 3*, *EARLY FLOWERING 4* and *LUX ARRHYTHMO*, but is lacking a loop active in the evening formed by *TOC1* and *GIGANTEA* and possibly, as a consequence, has reduced temperature compensation[18].

The post-transcriptional oscillators in cyanobacteria

Cyanobacteria, such as *Synechococcus elongatus*, are among the oldest and simplest known organisms to have circadian oscillations[19]. Interactions between three proteins, KaiA, KaiB and KaiC, are sufficient for circadian rhythmicity; reconstitution of these proteins with ATP *in vitro* results in robust temperature-compensated circadian rhythms of KaiC phosphorylation[19]. This remarkable finding defies the general assumption that circadian

clocks rely on transcriptional feedback, suggesting that post-translational mechanisms are also important. One possibility is that circadian clocks based on post-translational feedback mechanisms might pre-date transcriptional clocks evolutionarily. Alternatively, the circadian oscillations generated by transcriptional loops might be amplified by post-translational loops, or vice versa. Post-translational mechanisms also exist in other circadian systems. For example, PER–CRY–CK1 complexes are phosphorylated in mammals and TOC1 undergoes light-dependent proteolysis in plants. However, it is not clear whether these mechanisms are sufficient for circadian oscillations in the same way as the kaiA/B/C loop in cyanobacteria.

Conclusions

The generation and sustaining of robust circadian rhythms at the cellular level is achieved by an intriguing network of transcription, translation, post-translational modification and intra- and inter-cellular signalling. The model organisms studied thus far have shown that the co-ordination of circadian rhythms at a systems level is species-specific, often being an evolutionary response to the topology of the organism and the way in which they perceive external cues. Experimental and mathematical analyses in both simple and complex organisms are making great progress in uncovering the principles that enable robust timekeeping across all kingdoms of life. ■

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