Slaves to the rhythm

Oscillations, cycling and the pace of life

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Plants, as sessile organisms, are forced to take advantage of the limited availability of sunlight, their most important resource. Not surprisingly, many aspects of physiology and development are therefore organized by an endogenous chronometer in plants. This so-called 'circadian' clock imposes a 24-hour rhythm on metabolic reactions and physiological processes to optimally align them with the environmental light–dark cycle[1].

The molecular clockwork underlying these endogenous rhythms in flies, mammals and *Neurospora crassa* comprises proteins that generate a self-sustained 24-hour oscillation through negative autoregulation of their transcription. Orthologues of these clock genes have not been found in the *Arabidopsis* genome, but the basic layout of interdependent feedback loops at the core of the clockwork turned out to be the same, suggesting that the plant clock mechanism has evolved separately.

At present, three cycling elements that reciprocally regulate each other are thought to build the molecular framework of the *Arabidopsis* oscillator; two MYB-type transcription factors, LHY (late elongated hypocotyl) and CCA1 (circadian clock associated 1), which peak around dawn and TOC1 (timing of *CAB* expression), which peaks around dusk[2]. Increasing CCA1 and LHY levels at the beginning of the day lead to *TOC1* repression. The successive decline in CCA1 and LHY levels releases this block, and rising TOC1 levels subsequently activate *CCA1* and *LHY* expression through an, as yet undefined, mechanism, presumably involving additional components (expertly reviewed by McWatters[3]).

Cycling transcripts are not limited to the oscillator. Systematic searches for rhythmic transcripts provided a comprehensive view on clock-output transcripts and shed some light on signalling downstream of the oscillator. In this review, we discuss some recent clues as to how the output is linked to the oscillator.

Keywords: *Arabidopsis thaliana*, *Arabidopsis thaliana* glycine-rich RNA-binding protein (*At*GRP7), circadian clock, early-phytochrome-responsive 1 (EPR1), slave oscillator.

Ups and downs: the cycling *Arabidopsis* transcriptome

In the era of systems biology, cDNA microarrays and oligonucleotide gene chips allow the simultaneous quantification of entire genomes. This high-throughput technique combined with statistical tools is particularly useful in the circadian analysis that requires a large sample pool. To classify transcripts as rhythmic, the expression level of samples taken around the clock should match a cycling mathematical function with a certain probability[4,5].

Transcript profiling of seedlings sampled at 4-hour intervals revealed that approximately 6% of the *Arabidopsis* genome is cycling[5]. A large number of transcripts, which were not known previously to be clock-controlled, were identified with phases that were, more or less, evenly distributed around the clock (Figure 1).

The application of bioinformatics also allows genes to be clustered into functional groups, and this revealed that entire pathways are under clock control. Most notably, 23 phenylpropanoid biosynthetic genes are co-ordinately activated before dawn, which may prepare the plant for upcoming sunlight through the production of UV-protective compounds[5]. Moreover, alignment of the 5′ region of genes in groups with the same phase revealed a common motif, which confers clock control upon target genes[5].

Interestingly, apart from clock-output transcripts, cycling transcription factors have been identified, including the key regulator PAP1 (production of anthocyanin pigment 1), which cycles in phase with the phenylpropanoid biosynthetic genes and thus may cause their synchronous activation prior to sunrise (Figure 2A)[5]. Thus the analysis of clock-output genes aids in tracing the regulatory cascade back to the central oscillator.

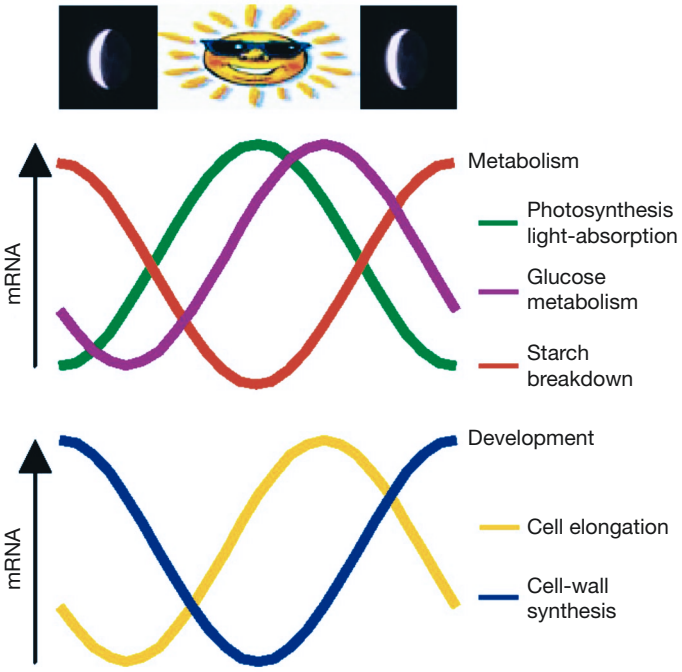

Figure 1. Distribution of gene expression thoughout the day in *Arabidopsis*. The expression of transcripts associated with photosynthetic light-absorption, e.g. *LHCB*, peaks early in the day; transcripts encoding enzymes that convert glucose into metabolites peak in the afternoon and those associated with starch breakdown including β-amylase peak at night when photosynthesis is shut off[4,5]. Growth of a plant cell occurs by cell-wall loosening, water uptake and subsequent reinforcement of the cell wall. Genes involved in cell-wall loosening and auxin relocalization were found to peak at the end of the light phase. Expression of cellulose-synthase-like genes, involved in the deposition of cell-wall material, peaks about 12 hours later, indicating a strict temporal control of this developmental process[5].

Slave oscillators in the circadian system

Another promising model of how the rhythm of the core oscillator is transduced to the different clock outputs envisages regulatory factors downstream of the core oscillator, which themselves undergo negative autoregulation[6,7]. These so-called 'slave' oscillators have several characteristics. First, they are part of a negative-feedback loop that regulates oscillations of their own transcripts, implying that keeping their level constitutively elevated through overexpression represses the accumulation of the endogenous transcript. Secondly, the phase and amplitude of their rhythm are dependent on the core oscillator (the 'master'), while the slave, generally, does not feed back on its master. Disruption of the slave thus should leave the master unaffected.

This is what was observed for a novel MYB protein, EPR1 (early-phytochrome-responsive 1), which closely resembles CCA1 and LHY, and whose expression peaks a few hours later than *CCA1*[7]. *EPR1* rhythms were abolished in plants that constitutively overexpress CCA1 and LHY. In addition, constitutive overexpression of EPR1 decreased the levels of the endogenous *EPR1* transcript. Based on the predicted DNA-binding capability, this negative feedback may occur by interference with transcriptional activation. In addition to the *EPR1* transcript itself, *LHCB* oscillations were perturbed in plants that overexpress EPR1. These characteristics led to the idea that the EPR1 feedback loop operates downstream of the CCA1–LHY–TOC1 oscillator and confers its 24-hour rhythm upon output genes such as *LHCB*. Therefore, in addition to direct interaction of CCA1 (and presumably LHY) with *LHCB* promoters, *LHCB* oscillations seem to be fine-tuned by the EPR1 feedback loop (Figures 2B and 2C)[7,8].

Another slave oscillator that is controlled by the CCA1–LHY–TOC1 oscillator, but that also undergoes negative autoregulation, relies on an evening-specific RNA-binding protein, *At*GRP7 (*Arabidopsis thaliana* glycine-rich RNA-binding protein)[6]. In contrast with the transcription-based loops, down-regulation of the *AtGRP7* steady-state levels in transgenic plants that overexpress the *At*GRP7 protein is not mediated by the promoter and thus occurs at a post-transcriptional level[9]. Residual oscillation of the endogenous *AtGRP7* transcript in *At*GRP7-overexpressors is due to an alternatively spliced *AtGRP7* transcript that appears at the expense of the mature mRNA. Its much shorter half-life may account for the low steady-state abundance, which implies a mechanism by which *At*GRP7 contributes to its own oscillations[10]. In response to *At*GRP7 protein accumulation during the circadian cycle, a shift in splice-site selection favours the production of the alternatively spliced transcript, which decays rapidly. Moreover, a premature stop codon prevents its translation into a functional protein so that the levels of *At*GRP7 protein decline. Because recombinant *At*GRP7 specifically interacts with its transcript *in vitro*, the shift in splice-site selection and down-regulation *in vivo* may be initiated by binding of *At*GRP7 to its pre-mRNA. In addition, *At*GRP7 depresses the oscillations of *AtGRP8* encoding a related RNA-binding protein, indicating that *At*GRP7 regulates other clock-controlled transcripts apart from its own (Figure 2D)[6].

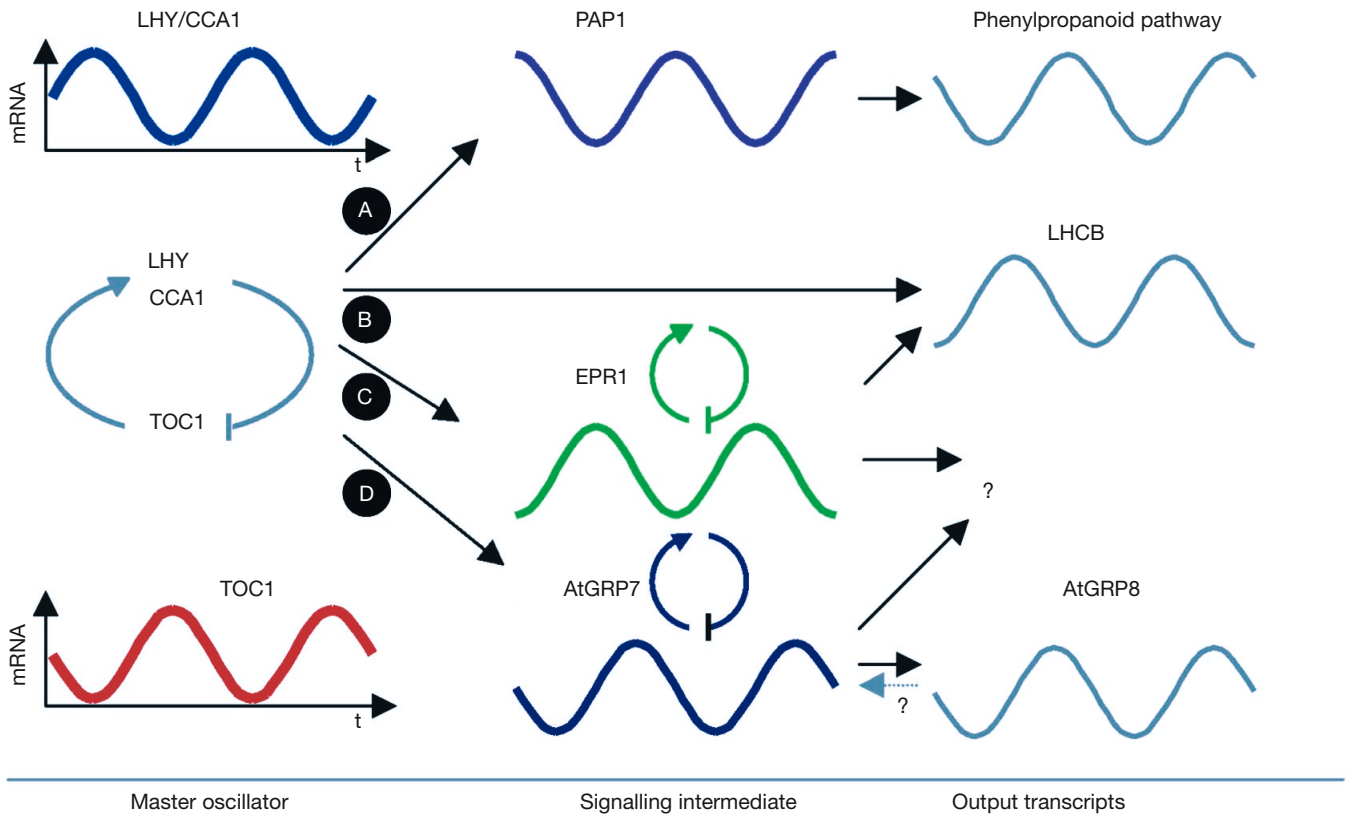

Figure 2. A roadmap of clock output. The 24-hour rhythm of the CCA1–LHY–TOC1 oscillator is transmitted either through a rhythmic key transcription factor (**A**) or direct interaction with promoters (**B**) or intermediate slave oscillators that act either transcriptionally (**C**) or post-transcriptionally (**D**) upon clock-output genes[5–8].

Molecules that themselves oscillate with a high amplitude, partly through autoregulation, may be good signalling components. The negative feedback warrants shut-off of the transducer's activity in time for the next incoming signal and thus prevents damping. Furthermore, depending on the half-lives of their components, such slave oscillators may also convey different phases upon output rhythms.

In conclusion, a comprehensive determination of targets of the individual clock proteins and slave oscillators in plants that either overexpress or underexpress the respective players will help to close the gap between oscillators and output. The degree of overlap will define the function of each loop and their hierarchical organization will be established.

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