Introduction

Circadian rhythms are endogenous rhythms with a period that closely approximates the 24-hour period of the rotation of the earth. Crucial defining properties of circadian rhythms include an endogenous, self-sustaining period of 24 hours, entrainment to the environmental period (often by light or temperature cues), and temperature compensation [1]. An underlying premise to the study of circadian rhythms has been that the circadian clock allows an organism to coordinate its biology with its temporal environment subjects and thus enhances evolutionary fitness. This premise has now been experimentally verified in several organisms, including Arabidopsis, where it has been shown that net photosynthesis is greatest when the endogenous circadian period matches the environmental period, even in mutants where the circadian period diverges substantially from 24 hours [2].

There was a time when a review of the current research in plant circadian rhythms for Current Opinions could be comprehensive. Comes a time when the volume of research simply exceeds the word limit of this type of review. Plant circadian research has soared past this threshold, which is tremendously exciting but necessitates an apology to those whose work is omitted (but certainly not overlooked). Comes a time also when the old ways of gene-at-a-time analysis must be augmented with systems-level analysis. I have attempted to highlight this transition and to emphasize some of the most exciting areas in plant clocks research, but there is much more worthy of your attention. Comprehensive reviews of the plant circadian system are available [3–5].

The Arabidopsis oscillator

Oscillations arise from negative feedback loops that include a time delay. All eukaryotic circadian oscillators studied to date are based on multiple interlocked negative feedback loops [1]. Mathematical analysis suggests that the increased complexity associated with multiple interlocked loops increases flexibility, which enhances robust entrainment and temperature compensation [6]. Current models of the Arabidopsis circadian clock (Figure 1) postulate multiple interlocked feedback loops [7**,8**]. A pair of single Myb-domain transcription factors A and B (Myb) that control the expression of the clock gene C15. C15 encodes a transcription factor that binds to the promoter of another clock gene C15. C15 encodes a transcription factor that binds to the promoter of another clock gene C15 and is required for the maintenance of circadian rhythms. C15 encodes a transcription factor that binds to the promoter of another clock gene C15 and is required for the maintenance of circadian rhythms.
Simplified cartoon outlining the architecture of the Arabidopsis clock. Yellow arrows indicate light regulation and indicate sites of entrainment by light. An activated form of TOC1, indicated as TOC1*, is one possible component of X. Casein kinase 2 (CK2) phosphorylates CCA1 and may also be a component of X.
response of clock phase to light pulses [27]. The clock governs an oscillation in cytosolic free Ca$^{2+}$ that is uncoupled from the oscillation in LIGHT HARVESTING CHLOROPHYLL A/B BINDING PROTEIN (LHCb) expression by the toc1-1 but not by the toc1-2 mutation, providing new evidence in support of multiple oscillators [28].

Circadian regulation of the transcriptome

It has long been known that the circadian clock controls the transcription of many genes, including both output genes, genes not required to generate the oscillation itself, and some, but not all, clock genes, genes required for wild-type oscillator function. Initial microarray analyses indicated that 10–15% of the Arabidopsis transcriptome shows circadian oscillation in abundance during free run in constant conditions following entrainment to photocycles [17,29,30,31**]. Clock-regulated transcripts were enriched in a set of transcripts with short half-lives, suggesting that transcript stability might obscure transcriptional oscillations [32]. Moreover, enhancer trapping [33] suggested that clock control of transcription was more widespread than was captured in the initial transcriptome studies. Recently, a comprehensive investigation of plants grown under a variety of thermocycles, photocycles, and free-run conditions has shown that ~90% of the Arabidopsis transcriptome cycles in at least one condition [34**].

Mechanistically, how are rhythmic induction and repression of transcription mediated? The details of circadian transcription in plants are not yet fully described. cis-Regulatory elements associated with phase-specific expression have been defined [34**], but in very few cases have the cognate DNA-binding proteins been identified and characterized. The best—though still incompletely—characterized example is TOC1. Recently it has been established that rhythmic transcription of TOC1 is correlated with binding of chromatin remodeling factors to the TOC1 promoter and histone H3 acetylation, associated with open chromatin structure [35*]. Rhythmic binding of CCA1 (and presumably LHY) to the evening element (EE) antagonizes histone H3 acetylation at the TOC1 promoter [35*]. Pharmacological inhibition of histone deacetylation alters the waveform of TOC1 mRNA abundance [35*], but the responsible histone deacetylase(s) is/are not known. In mammals, CLOCK has been established to have histone deacetylase activity [36].

Post-transcriptional regulation

Post-transcriptional and post-translational regulation plays crucial roles in clocks of plants, as in other taxa [1]. Oscillations in transcript abundance can originate through transcriptional regulation, but the clock regulates the degradation of a subset of transcripts via the downstream instability determinant (DST) pathway [37]. Recently it has been shown that light regulates the stability of the CCA1 transcript, offering a new route for light input to set clock phase [38]. Alternative splicing has been implicated in the slave oscillator involving AtGRP7, which autoregulates its expression by influencing alternative splicing of its own pre-mRNA. Mutation of the AtGRP7 RNA recognition motif abolishes auto-regulation as well as regulation of downstream targets, including AtGRP8 [39].

Post-translational regulation

The temporally regulated proteasomal degradation of specific clock proteins is necessary for progression through the oscillation. The stability of a number of plant clock proteins, including GI [40], LHY [41], CASEIN KINASE 2 BETA 4 ($\text{CkB}4$) [42], PRR7 [43*], and PRR9 [44] is clock-regulated. Most is known about the TOC1 protein, which peaks in abundance at dusk and must be turned over for the cycle to proceed. What TOC1 does at a molecular level remains enigmatic. TOC1 is a positive regulator of CCA1 and LHY transcription, though TOC1 lacks demonstrated DNA-binding activity and so must act indirectly, possibly through interactions with transcription factors such as ELF4 [9,10] and LUX/PCL1 [11,12]. Period is sensitive to TOC1 abundance; reduced TOC1 shortens and elevated TOC1 lengthens period. An E3 ubiquitin ligase SCF complex including the F-box protein ZEITLUPE (ZTL) [45] is crucial for clock-regulated proteasomal degradation of TOC1 [46]. Consistent with its role in TOC1 degradation, the effects of reduction or increase in ZTL abundance are period lengthening and shortening, respectively [47]. ZTL also targets PRR5 for proteasomal degradation through direct interaction with the P3f domain of PRR5 [48*]. ZTL is a large protein with two recognized functional motifs in addition to the F box. The Kelch repeats of ZTL are necessary for interaction with TOC1. PRR3 binds directly to TOC1, which perturbs the interaction of TOC1 with ZTL and, hence, stabilizes TOC1 [49*]. As PRR3 expression is limited to the vasculature, this emphasizes the potential for spatially restricted modulation of clock function.

ZTL possesses a LOV (light, oxygen, voltage) domain capable of flavin binding and implicated in blue-light photochemistry. The LOV domain is responsible for the interaction of ZTL with GI, which stabilizes ZTL [50**]. Because GI abundance cycles, driven by rhythmic GI transcription, this interaction provides a molecular explanation for the rhythm in ZTL protein abundance despite a conspicuous lack of cycling in ZTL transcript abundance. The ZTL–GI interaction is dramatically enhanced by blue light and this enhancement is abolished by mutational disruption of LOV domain photochemistry; thus, ZTL is a blue-light photoreceptor that mediates direct light input into the clock [50**].
Circadian regulation of growth and reproduction

Circadian regulation of plant physiology and development is widespread [5]. One mechanism by which the clock attains such broad influence is through modulation of signaling pathways. One dramatic example of this is the regulation of auxin signal transduction; the clock controls sensitivity to auxin and thereby modulates both transcriptional and growth responses to this hormone [31**]. This observation offers a mechanism to effect circadian regulation of multiple aspects of plant growth and development, potentially including tropisms and organ formation. There is accumulating evidence for crosstalk among multiple hormone signaling pathways in growth and development, and clock function is modulated by several phytohormones, including abscisic acid, brassinosteroids, and cytokinin [51,52], though not by auxin [31].

Many environmental responses are temporally modulated (gated) by the circadian clock (reviewed by [53]). The clock gates responses to a number of abiotic stresses, such as cold temperature [54], light-quality modulation of cold acclimation is also gated by the clock [55]. The clock may also regulate responses, including stresses mediated through abscisic acid and methyl jasmonate [56*].

Does the pervasive nature of clock regulation of growth, physiology, and environmental responsiveness extend throughout the life of the plant? Circadian oscillation in gene expression is detected in both light-grown and etiolated seedlings within a day or so of seed hydration, which provides an important signal to synchronize the clocks both within a seedling and among a population of seedlings [57]. It will be interesting to explore clock function both very late in life (is the clock important during senescence?) and at the very beginning, during fertilization, embryogenesis, and seed maturation.

Circadian studies have focused on rhythms in constant conditions to emphasize the endogenous nature of the clock, yet plants are normally exposed to diurnal cycles and considerable insight can be gained by studying these more biologically relevant diurnal conditions. An emerging theme is that the coincidence of clock-controlled internal cycles with external environmental cycles allows coordination of plant processes with the environment. For example, hypocotyl elongation has been known for some time to be clock-regulated [3–5], but recent work in diurnal conditions has revealed the underlying mechanism [58**]. Two basic helix-loop–helix transcription factors, PHYTOCHROME INTERACTING FACTOR4 (PIF4) and PIF5, are positive regulators of hypocotyl elongation. Transcript abundance of PIF4 and PIF5 is regulated by the clock, accumulating before dawn, and protein stability is negatively regulated by light. The coincidence of high-transcript levels (internal cycle) and protein stabilization in the dark (external cycle) allows growth promotion at the end of the night [58**].

The photoperiodic pathway of flower induction offers a second example that illustrates this theme of coincidence of external and internal cycles. FLAVIN-BINDING, KELCH REPEATS F-BOX1 (FKF1), a close relative of ZTL, regulates the accumulation of CONSTANS (CO), a crucial inducer of flowering. Blue light perceived via the FKF1-LOV domain stimulates the interaction of FKF1 with GI, analogous to the ZTL–GI interaction described above. The FKF1–GI complex forms on the CO promoter and binds to and mediates the degradation of CYCLING DOF FACTOR1 (CDF1), a transcriptional repressor of CO [59], to allow daytime CO transcription [60**]. Interestingly, CDF1 expression is markedly derepressed in the tac1-2 prr5-11 double mutant, consistent with the late-flowering phenotype of the double mutant and suggestive that these two clock genes encode repressors of CDF1 [25*]. Similar genetic analysis of double and triple mutants suggests that PRR5, PRR7, and PRR9 stimulate flowering through repression of CDF1 [61]. CO protein is stabilized in the light and thus accumulates in long but not in short days [62]. SUPPRESSOR of PHYA-105 (SPA1) interacts with CO and is implicated in its degradation [63,64].

Systems biology

As described above, circadian control of the transcriptome is widespread and influences many metabolic pathways [17,29,30,31**,34**]. In both carbon and nitrogen metabolism, many metabolite–transcript correlations are detected, though changes in enzyme activities and metabolite levels are less dramatic than might be predicted from the large observed changes in transcript abundance [65*]. These observations suggest important feedback by metabolite levels on clock-regulated gene expression [65*]. Retrograde signaling from the chloroplast (see review by Fernández and Strand in this issue) is implicated in the modulation of circadian function by mutations in CHLOROPLAST RNA BINDING (CRB), which alter amplitude and waveform, but not period length, of CCA1 and LHY expression [66]. CCA1, in addition to its role in the clock, is a key regulator of a subnetwork of organic nitrogen responsive genes (see review by Vidal and Gutiérrez in this issue), including key nitrogen assimilatory genes [67*]. CCA1 expression responds to nitrogen status and pulses of either inorganic or organic forms of nitrogen shifts clock phase [67*]. This is consistent with an emerging view of the clock as a crucial integrator of metabolic inputs, allowing temporal coordination of metabolism.

Conclusions

Simple models cannot adequately describe the complex network of circadian control of plant physiology and metabolism. Clock control is pervasive. In addition, the
emerging view is that the clock is sensitive to a wide variety of internal metabolic and hormonal signals, as well as to environmental signals. Systems biology and mathematical modeling are increasingly important in capturing the subtle modulation of clock function necessary to coordinate and optimize metabolism, growth, and development in an oscillating environment.

Acknowledgements

I thank Neil Young (Reprise Records, 1978) for the title. My work on circadian rhythms is supported by grants from the National Science Foundation (MCB-034887 and IOB-0517111) and from the United States-Israel Binational Science Foundation (#2005223).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


6. Kiss EA, Khanna R, Quail PH: ELF4 is a phytochrome-regulated component of a negative-feedback loop involving the central oscillator components CCA1 and LHY. Plant J 2005, 44:300-313.


Axin plays a pervasive role in plant growth and development. The authors use transcriptome analysis to show that the circadian clock regulates auxin signal transduction by modulating sensitivity to auxin. The clock gates both transcription and growth responses to exogenous auxin.


The authors systematically analyze the Arabidopsis transcriptome in a comprehensive set of time courses testing seedlings exposed to a variety of thermocycles and photocycles, as well as free-run conditions and show that almost 90% of the transcriptome oscillates under at least one condition. They identify at least three distinct sets of cis-elements that define phase-specific transcription and show that these modules are conserved across Arabidopsis, poplar, and rice.


This study establishes that PRR7 is phosphorylated in a circadian clock-regulated manner and that PRR7 stability is regulated by light and the clock.


The authors show that the F-Box protein ZTL interacts with 434 the PRR5 domain of PRR5 and targets it for proteasomal degradation. Blue light stabilizes PRR5, consistent with the hypothesis that the photoactive LOV domain of ZTL is a photoreceptor and blue light negatively regulates its ability to target PRR5 for degradation.


The authors show that PRR13 interacts physically with TOC1 to decrease the ability of TOC1 to bind to ZTL. Thus PRR13 stabilizes TOC1. This provide the first description of a biochemical function for one of the PRRs.


The authors show that blue light enhances the interaction of ZTL with GI, which stabilizes ZTL. The abundance of GI cycles, which imposes a circadian oscillation on ZTL abundance. Mutations that disrupt ZTL-LOV domain photochemistry block the ZTL-GI interaction, demonstrating that ZTL is a circadian photoreceptor that defines a novel means for light entrainment of the Arabidopsis circadian clock.


This transcriptome level work implicates the clock in gating multiple responses to environmental stresses mediated by abscisic and methyl jasmonate.
The authors show clock and environmental regulation of two positive regulators of hypocotyl growth, PIF4 and PIF5. The coincidence of the internal cycle of high transcription before dawn with protein stability in the dark allows these two transcription factors to accumulate late at night to promote growth during the predawn hours.
CDF1 is a transcriptional repressor of CO, a crucial promoter of flowering. To induce daytime CO transcription, necessary for light-mediated stabilization and subsequent accumulation of CO protein, a complex of the F-Box protein FKF1 and GI assemblies on the CO promoter and targets CDF1 for proteasomal degradation. Blue light stabilizes the FKF1–GI interaction, consistent with FKF1 function as a blue-light photoreceptor.
This work combines genome-wide transcript profiling, metabolite profiling, and enzyme activity measurements. Transcript abundances show dramatic oscillations under diurnal conditions and following transfer to the dark, whereas changes in enzyme activities are smaller. The authors suggest that metabolite levels are buffered against changes in gene expression and conclude that metabolites are more potent regulators of gene expression than vice versa.
The authors characterize genome-wide responses to organic nitrogen and identify the crucial clock component CCA1 as a key transcriptional regulator of a subnetwork of organic nitrogen responsive genes. Regulation of CCA1 by glutamate (or a glutamate-derived metabolite) regulates the expression of key nitrogen assimilatory genes.