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Wheels within wheels: the plant circadian system

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Abstract

Circadian clocks integrate environmental signals with internal cues to coordinate diverse physiological outputs so that they occur at the most appropriate season or time of day. Recent studies using systems approaches, primarily in *Arabidopsis*, have expanded our understanding of the molecular regulation of the central circadian oscillator and its connections to input and output pathways. Similar approaches have also begun to reveal the importance of the clock for key agricultural traits in crop species. In this review, we discuss recent developments in the field, including: a new understanding of the molecular architecture underlying the plant clock; mechanistic links between clock components and input and output pathways; and our growing understanding of the importance of clock genes for agronomically important traits.

Keywords

Circadian clock; *Arabidopsis*; systems biology; input; output; agricultural traits

Complexity of the circadian clock

Circadian clocks enhance growth and fitness by coordinating numerous biological events with the environment [1]. Conceptually, the circadian system is composed of three major parts: a self-sustaining central oscillator (or clock), input pathways that integrate oscillator function with environmental timing cues, and output pathways that control diverse processes. The influence of the clock on plant life is inescapable because the abundance of thousands of transcripts and the timing of multiple processes that occur throughout the plant life cycle are clock-regulated [2]. Both the molecular mechanisms underlying the circadian clock and its interactions with environmental sensing and physiological output pathways involve sophisticated and complex networks and, thus, represent appealing problems for systems biology approaches.

Recent large-scale approaches have considerably advanced our understanding of the plant clock. Genomic, transcriptomic, and proteomic approaches have led to the identification of novel clock components and downstream targets [3–11], helping to reveal the network structure of the clock system. In addition to experimental approaches, mathematical modeling and computational techniques have helped elucidate how known components function both within the oscillator network and as links to other physiological pathways and have in some cases led to the prediction of unknown players [12–16]. These developments

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have not only improved our insight into how the plant clock mechanism works but also how it is connected to input and output pathways. In this review, we focus on new findings that provide a revised view of the architecture of the clock, increase our understanding of the connections between clock components and input and output pathways, and demonstrate the importance of clock genes for yield-related traits in diverse crop species.

Clock mechanism

To date, more than 20 clock or clock-associated components have been identified in *Arabidopsis* (*Arabidopsis thaliana*) (Table 1), and homologs of these genes in other plant species have begun to be characterized. In plants, as in other eukaryotes, the clock is composed of interlocking transcription–translation feedback loops. However, the architecture of the *Arabidopsis* circadian oscillator has been found to be significantly more complex than that of other model eukaryotes [3, 17]. Different clock proteins act at distinct times throughout the day and night to reciprocally regulate expression of other clock genes at both the transcriptional and post-transcriptional levels.

Transcriptional regulation

Many clock components in *Arabidopsis* regulate the transcription of other, differentially phased, clock genes. As detailed below, recent work has led to the identification of new clock components and to the reassignment of function of previously known proteins. A simplified schematic of the network structure is shown in Figure 1A and a more complete diagram is shown in Figure 1B. The clock components described below are also listed in Table 1.

Morning-phased components (CCA1 and LHY)

CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) are two MYB-like transcription factors. Their transcript and protein levels are highly abundant in the morning and these proteins physically interact with each other [18, 19]. They have long been known to repress expression of an evening-phased component, *TIMING OF CAB EXPRESSION 1* (*TOC1*), also known as *PSEUDO-RESPONSE REGULATOR 1* (*PRR1*), via binding to a motif in the *TOC1* promoter called the evening element (EE) [20, 21]. *TOC1* in turn directly regulates expression of *CCA1* and *LHY*, forming the first described plant clock transcriptional feedback loop (see below).

Recently, CCA1 and LHY have also been shown to repress several other evening genes, including *GIGANTEA* (*GI*), *LUX ARRHYTHMO* (*LUX*), *BROTHER OF LUX ARRHYTHMO* (*BOA*, also known as *NOX*, meaning ‘night’ in Latin), *EARLY FLOWERING 3* (*ELF3*) and *ELF4* [22–24]. The repression of evening genes by CCA1 and LHY is dependent on *DEETIOLATED1* (*DET1*), a key repressor in photomorphogenesis [25]. Besides functioning as transcriptional repressors, genetic data suggest that CCA1 and LHY may also function as activators for the day-phased clock genes *PRR9* and *PRR7* [24].

Day-phased components (PRR9, PRR7, LNK1 and LNK2)

PRR9 and *PRR7*, together with their homologs *PRR5* and *PRR1* (*TOC1*), play key roles in the clock. *PRR9* is expressed earliest, just after dawn, followed by *PRR7*, *PRR5*, *PRR3* and finally *TOC1* in the evening [26]. *PRR9* and *PRR7*, together with *PRR5*, play partially redundant roles in repressing expression of the morning genes *CCA1* and *LHY* [27]. Recently, the PRRs have been shown to also repress expression of *REVEILLE8* (*RVE8*, also known as *LHY* and *CCA1-like 5*, *LCL5*) (see below) [6, 9]. The importance of the PRRs to clock function is underscored by the arrhythmic phenotype of *prp9 prr7 prr5* triple mutants [26].

A small family of genes with clock-regulated and light-induced expression has recently been implicated in clock function. *NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED GENE 1 (LNK1)* and *LNK2* transcripts peak near the middle of the subjective day and loss of these genes causes a long period phenotype and downregulation of many clock-regulated genes with afternoon and evening phases [28]. Transcript levels of *PRR5* and *ELF4* are significantly reduced in *lnk1 lnk2* double mutants, suggesting that *LNK1* and *LNK2* may act as transcriptional activators for these genes in the afternoon [28].

Afternoon-phased components (RVE8, and probably RVE4 and RVE6)

Several genes homologous to *CCA1* and *LHY* have recently been identified as important clock components [3, 10]. Several of these *RVE* transcription factors have dawn-phased clock-regulated gene expression, which suggests that they might provide an important signal to plants at dawn (hence their name ‘REVEILLE’, which evokes the bugle call starting the military day). Like *CCA1* and *LHY*, *RVE8* binds to the EE [9], a motif overrepresented among promoters of clock-regulated genes that have an evening phase [20]. However, unlike *CCA1* and *LHY*, which act as repressors of the EE, experiments with plants expressing an inducible form of *RVE8* demonstrated that *RVE8* induces hundreds of evening genes that contain EEs in their promoters. The direct nature of this regulation was shown for many evening-phased EE-containing clock genes, including *PRR5*, *TOC1*, *GI*, *LUX* and *ELF4*, since their expression increased upon *RVE8* induction even in the presence of an inhibitor of translation [3, 10]. In addition, *RVE8* has been shown to associate with the *PRR5* and *TOC1* promoters [3, 10]. Two close homologs of *RVE8*, *RVE4* and *RVE6*, also play partially redundant roles with *RVE8* within the circadian system. Plants mutant for all three of these genes exhibit a more extreme long-period phenotype than the *rve8* single mutant and the *rve4 rve6 rve8* triple mutant has lost the predominant afternoon-phased EE-binding activity [3]. This finding, along with the observation that *RVE8* protein levels peak in the subjective afternoon [9], indicates that at least some *RVEs* are afternoon-phased clock components and suggests that a better name for them might have been ‘*SUNSET*’ (a bugle call signaling the end of the official day).

Evening-phased components (PRR5, TOC1, CHE, LUX, BOA (NOX), ELF3 and ELF4)

As mentioned above, *PRR5* forms a negative feedback loop with *RVE8* [9] and plays a partially redundant role with *PRR9* and *PRR7* to repress the morning genes *CCA1* and *LHY* [27]. The role of *TOC1* in the regulation of *CCA1* and *LHY* was ambiguous until recently. Experimental and computational modeling work now indicate that *TOC1* represses *CCA1* and *LHY* expression, similar to the roles of the *TOC1* homologs *PRR9*, *PRR7* and *PRR5* [4, 11, 14]. Together, these four *PRR* proteins ensure that *CCA1* and *LHY* are only expressed during a small fraction of each day. In addition, *CCA1* HIKING EXPEDITION (*CHE*) interacts with *TOC1* to help repress *CCA1* expression in an as-yet undefined manner [8]. Finally, *LUX* (a MYB-like transcription factor), *ELF3* and *ELF4* (two unrelated novel nuclear proteins) interact to form the ‘evening complex’ that represses expression of the day-phased clock gene *PRR9* [7, 29–31]. Mutation of any member of the evening complex causes plants to become arrhythmic [24]. A homolog of *LUX*, alternatively called *BOA* or *NOX*, has been reported to form a complex with *ELF3* and *ELF4* (like *LUX*) and also directly promotes *CCA1* expression [7, 22].

The EE has emerged as an essential regulatory nexus for central clock oscillation. Most clock components either regulate the EE (*CCA1* and *LHY* as repressors and *RVE4*, *RVE6*, and *RVE8* as activators) [3, 4, 9, 21] or are regulated by other clock components through EE in their promoters (*PRR5*, *TOC1*, *LUX*, *ELF4*, *GI* and *PRR9*) (Figure 1) [3, 9, 24]. This reciprocal regulation between clock components ensures the ordered expression of clock genes and forms a complex interconnected network comprising the plant circadian

oscillator. Emerging data indicate that regulation of histone modifications also plays an important role in this transcriptional network [32]. As if this picture were not complicated enough already, there are also cell- and tissue-specific variations of the oscillator network described above [33–35]. For example, *PRR3* is a modulator of *TOC1* stability that acts primarily in the vasculature [33] and different cell types in leaves have slightly different free-running periods and expression levels of some clock genes [34].

Post-transcriptional regulation

In addition to transcriptional regulation, many post-transcriptional regulatory mechanisms are key to the plant circadian oscillator (Figure 2).

Alternative splicing

Alternative splicing has been reported for many clock genes (*CCA1*, *LHY*, *PRR9*, *PRR7*, *PRR5*, *PRR3*, *TOC1*, *RVE4* and *RVE8*) [36, 37]. PROTEIN ARGININE METHYLTRANSFERASE 5 (*PRMT5*) regulates the alternative splicing of *PRR9*, presumably via its methylation of splicing factors, and *prmt5* mutants have a long circadian period [38, 39]. SNW/Ski-interacting protein (*SKIP*), a component of the spliceosome, also regulates alternative splicing of *PRR9* and other clock genes including *PRR7*, *CCA1*, *LHY*, and *TOC1* [40]. Similar to *PRMT5*, loss of *SKIP* causes a long period phenotype [40]. A third protein involved in mRNA splicing has also been recently implicated in clock function. Mutation of SPLICEOSOMAL TIMEKEEPER LOCUS1 (*STIPL1*), a homolog of a human spliceosomal proteins involved in spliceosome disassembly, causes a long period phenotype [41]. As reported for *skip* mutants, the accumulation of splicing variants of several clock gene transcripts is altered in *stipl1* mutants, making it difficult to conclude whether altered splicing of one or more genes causes the clock phenotype [41].

Further evidence of a role for alternative splicing in the circadian oscillator comes from work on *CCA1*. A splice variant *CCA1* transcript with a retained fourth intron produces a protein called *CCA1β* that lacks the MYB-like DNA-binding domain. *CCA1β* can interfere with *CCA1* function by interacting with full-length *CCA1* (*CCA1α*) or *LHY* to form nonfunctional heterodimers [42]. *CCA1pro::CCA1α ccal-2* plants that do not express *CCA1β* have a long period, whereas *35S::CCA1β* has a short period similar to *ccal lhy* double mutants [42]. Hence this naturally produced *CCA1β* modulates activity of full-length *CCA1α* protein and fine-tunes the pace of the clock.

Protein–protein interactions and regulation of protein stability

Several clock components are known to interact with other proteins that are essential for their regulatory activity. As mentioned earlier, the repressive activity of *CCA1* and *LHY* on evening genes relies on *DET1* [25]. Similarly, *PRR9*, *PRR7* and *PRR5* physically interact with a transcriptional co-repressor, *TOPELESS*, to inhibit expression of *CCA1* and *LHY* [43]. *GI* has been shown to have separable functions in the cytoplasm and then nucleus [44, 45] and its partitioning between these cellular compartments is regulated by physical interactions with *ELF4* [46].

A common theme in all eukaryotic circadian systems is the regulated degradation of clock proteins. A key enzyme involved in regulated protein degradation in the plant clock is *ZEITLUPE* (*ZTL*), a F-box protein with a blue light photosensing LOV (light, oxygen, and voltage) domain and a *KELCH* protein-protein interaction domain. Along with its two homologs, *FLAVIN BINDING, KELCH REPEAT, F-BOX* (*FKF1*) and *LOV KELCH PROTEIN 2* (*LKP2*), *ZTL* targets *TOC1* and *PRR5* for ubiquitination and subsequent proteasome degradation [47, 48]. *GI* physically interacts with *ZTL* and stabilizes it, while

ZTL also controls GI stability and nucleocytoplasmic partitioning [49]. Finally, the physical interaction between ELF3, the E3-ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and GI promotes GI degradation [50].

Some post-translational modifications also affect clock protein stability and interactions with other proteins. Elevated phosphorylation of all of the PRR proteins is observed before their degradation by the proteasome [51], suggesting this may be a means to control their stability. Indeed, phosphorylation of PRR5 and TOC1 promotes their interactions with ZTL and enhances their subsequent degradation [52]. PRR5 also interacts with TOC1, enhancing its accumulation in the nucleus and preventing it from being targeted for degradation by ZTL, which is exclusively found in the cytoplasm [52].

Interactions between inputs and the clock

The central clock can be synchronized with the environment by input pathways that sense external timing cues. Light and temperature are the two most studied entraining signals. Light influences the clock in many ways, affecting transcription, messenger RNA stability and translation rate of several clock genes [53] and the protein stability of several others [54]. Reciprocally, several clock components are involved in the regulation of light input pathways [55].

Recent studies have provided clues as to how light mediates these effects on the clock. In addition to ZTL, the blue light circadian photoreceptor that regulates TOC1 protein stability (see above), plants also possess phytochromes (reversible red and far-red light receptors) and cryptochromes (blue light receptors). Both phytochromes and cryptochromes are involved in the entrainment of the clock and contribute to fluence rate-dependent shortening of the period [55]. Recent studies on *phyA phyB phyC phyD phyE* quintuple mutant have revealed that phytochromes do not simply increase the pace of the clock in a light-dependent manner [56, 57]. As expected based on the period-shortening effects of light, the *phyA phyB phyC phyD phyE* mutant has a longer period than wild type at relatively high fluence rates of continuous red light. However, at lower fluence rates ($<10 \mu\text{mol m}^{-2} \text{s}^{-1}$) the *phyA phyB phyC phyD phyE* mutant has a shorter period than that of wild type [56]. To explain this fluence-rate dependent effect, it has been proposed that the Pfr (far-red light absorbing) form of phytochromes decreases the pace of the clock whereas the Pr (red-light absorbing) form accelerates clock pace [56].

To more closely examine how light inputs shape the clock system, plants were entrained with far-red light so that PHYA is expected to be the only active photoreceptor [58]. Surprisingly, clock gene expression is significantly altered under far-red light with elevated expression of evening genes and decreased expression of morning genes [58]. Through mutant analysis, ELF4 was identified as a potential target involved in these far-red effects [58]. In addition, a study has shown that three positive regulators of PHYA signaling pathways, Far-red Elongated Hypocotyl 3 (FHY3), Far-red Impaired Response 1 (FAR1) and LONG HYPOCOTYL 5 (HY5) activate *ELF4* directly during the day [59], thus providing a clue as to how far-red light signaling affects the central oscillator.

Although daily changes in temperature can entrain the circadian clock [1], clock pace is largely invariant when plants are grown across a range of different physiologically relevant temperatures, a phenomenon called temperature compensation. PRR7 and PRR9 have been shown to be essential for this process, preferentially repressing expression of *CCA1* and *LHY* at elevated temperatures [60]. In addition, several other clock genes show altered expression after short or long exposure to cold temperatures [36, 61], perhaps playing a role in temperature compensation. Surprisingly, a recent study has implicated the cryptochrome

blue light photoreceptors in temperature compensation [13]. Using experimental and computational modeling approaches, the authors of this study suggest that light and temperature pathways may converge on common target genes within the circadian oscillator [13].

Temperature also regulates the alternative splicing of clock genes, including *CCA1*, *LHY*, *PRR9*, *PRR7*, *PRR5*, *PRR3*, and *TOC1* [36, 37]. As mentioned above, *CCA1* has at least two physiologically relevant splicing variants: *CCA1 α* and *CCA1 β* , which encode full-length and truncated CCA1 proteins, respectively. Under low temperatures, *CCA1 β* production is suppressed [42]. Intriguingly, elevated *CCA1 α* levels seem to be essential for cold tolerance because *35S::CCA1 α* plants have better freezing tolerance whereas *35S::CCA1 β* plants are hypersensitive to freezing [42]. Thus temperature-regulated splicing of clock genes is implicated not only in oscillator function but also in plant adaptation to temperature extremes. The circadian system is also involved in many other environmental response pathways, with a large fraction of clock-regulated output genes regulated by various stresses (see below) [2].

Interactions between the clock and outputs

Recently, several genome-wide studies have shown that central clock components, including *TOC1*, *PRR5*, *PRR7*, and *RVE8*, directly control groups of genes enriched for specific circadian phases and biological processes [3–6]. Current systems approaches have expanded our understanding of how these processes, including growth, metabolism, and abiotic and biotic stresses, are influenced by the circadian system.

Growth

The clock generates a large number of circadian rhythms that optimize growth and development [62]. One particularly well-studied growth process is the diurnal control of hypocotyl elongation, with daily rhythms in growth that are influenced by external cues and by the internal clock. There are at least four pathways coordinated by the clock that are involved in rhythmic hypocotyl growth, including light, sucrose, hormone signaling pathways, and the direct action of clock components themselves. Together these four pathways converge upon *PHYTOCHROME INTERACTING FACTOR4* (*PIF4*) and/or *PIF5*, basic helix–loop–helix (bHLH) transcription factors that promote hypocotyl growth. First, the evening complex clock proteins (*ELF3*, *ELF4* and *LUX*) directly repress expression of *PIF4* and *PIF5* in the evening, thus generating circadian rhythms in *PIF4* and *PIF5* transcript levels with a peak phase in the late night and early morning [7, 23]. In addition, light and sucrose control PIF protein stability: *PHYB* promotes *PIF4* and *PIF5* degradation under red light [63], whereas high sucrose levels stabilize *PIF5* [64]. Given that the clock influences phytochrome signaling [55, 65] and sugar metabolism (see below), the circadian system influences *PIF4* and *PIF5* abundance at multiple levels.

Another important regulator of daily rhythms in hypocotyl elongation is the hormone gibberellic acid (GA) [66]. The clock-controlled expression of *GA 20ox1* (a GA biosynthetic enzyme) and *GA-INSENSITIVE DWARF1* (*GID1*, which encodes a GA receptor) contribute to higher abundance of a GA–*GID1* complex around dawn, which promotes degradation of *DELLA* transcription factors at this time [66, 67]. *DELLA* proteins are crucial repressors of the GA signaling pathway and block *PIF4* activity by binding to the *PIF4* DNA binding domain [68]. Degradation of *DELLA* proteins in the morning therefore helps fine-tune the daily timing of *PIF4* activity and the expression of *PIF4* targets that promote hypocotyl growth [68].

The hormone auxin is also involved in hypocotyl growth regulation. The clock regulates plant sensitivity to many stimuli in a time-of-day-specific manner, a phenomenon called 'gating'. Auxin-induced gene expression is gated by the clock and the time of maximal auxin responsiveness correlates with the time of maximal clock-regulated hypocotyl growth [69]. The reduced auxin-induced growth of *pif4 pif5* double mutants suggests that auxin regulates growth at least partially through PIF4 and PIF5 [70]. There are multiple connections between these bHLH factors and auxin signaling: PIF4 has been shown to influence auxin production [71, 72] and PIF4 and PIF5 both affect auxin signaling downstream of biosynthesis [70, 73, 74]. In addition, a clock-controlled transcription factor, RVE1, also regulates hypocotyl growth by controlling auxin biosynthesis genes in a PIF4 and PIF5-independent manner [75]. It seems likely that the circadian network influences growth in additional ways that have yet to be discovered. Further systems biology studies are needed to fully elucidate how diverse growth-regulatory pathways are coordinated with each other and with the circadian system.

Metabolism

Photosynthesis and carbohydrate metabolism have long been known to be clock-regulated. Many transcripts involved in photosynthesis, including both those encoded by the nuclear and chloroplast genomes, are controlled by the clock [76]. Circadian regulation of these two genomes is coordinated by SIGMA FACTOR 5 (SIG5), a subunit of the plastid RNA polymerase. SIG5 is a nuclear gene expressed with a circadian rhythm; upon import into the chloroplast, it generates circadian expression of several chloroplast genes involved in photosynthesis [77]. Daily rhythms in photosynthetic capacity, light availability, and sugar metabolism together lead to the daily accumulation and mobilization of starch, the major form in which fixed carbon is stored by plants. Plants accumulate transitory starch reserves during the day and break down these reserves during the night to support growth and metabolism. Intriguingly, the rate of starch breakdown is tightly controlled so that 95% of starch is consumed by the anticipated time of dawn, even when the time of lights off is altered in unexpected ways [78]. The authors of a recent study have suggested that this is achieved by biological molecules that carry out a sort of arithmetic division, calculating both the amount of starch that has accumulated by the end of the day and the time until dawn to determine the rate of starch degradation [15].

Many clock-controlled outputs also serve as clock input signals, feeding back on the oscillator mechanism. For example, sucrose can serve as an entrainment signal to reset the clock in constant darkness [12]. Sucrose also affects the amplitude of clock gene expression and modulates period length [79]. More recently, it has been demonstrated that the production of sugar through photosynthesis can reset the clock, via a pathway that requires PRR7 [80]. Similarly, not only is iron homeostasis controlled by the clock but application of iron also feeds back to regulate clock pace [81–83]. Glucosinolates, clock-controlled metabolites involved in plant defense, also fine-tune the pace of the clock [16]. It will be interesting to investigate the mechanisms by which these nutrients and metabolites interact with the central clock.

Abiotic and biotic stresses

Given the predictable nature of daily changes in the physical environment, it is not surprising that the circadian clock helps plants adapt to abiotic stresses. Perhaps more unexpectedly, several recent studies have also reported that the clock has a role in the response to biotic stresses.

It has long been known that the clock influences plant response to cold [84]. The major players in cold acclimation are the C-REPEAT BINDING FACTOR 1 (CBF1), CBF2 and

CBF3 transcription factors that activate downstream cold-regulated (COR) genes. Transcript levels of CBFs are clock-regulated, peaking at midday, and cold-induction of these genes is gated by the clock [84]. Several clock components, including CCA1, PRR5, PRR7, and TOC1, have been reported to bind to promoters of some or all these CBFs [4, 6, 85]. CCA1 activates CBFs and promotes freezing tolerance [85] whereas PRR5 and PRR7 repress the expression of CBFs and inhibit freezing tolerance [86]. In addition to regulation of cold acclimation, CCA1 is also involved in other stress responses. Recently, reactive oxygen species (ROS) homeostasis was reported to be clock-controlled; this is likely to be through regulated expression of ROS genes by CCA1 [87]. Like several metabolites mentioned above, exogenous ROS also act as an input signal that affects clock pace [87].

Many genes implicated in plant defense against pathogens contain an EE in their promoters and are controlled by the clock [88]. Disruption of *CCA1* in *Arabidopsis* results in decreased resistance to downy mildew at dawn (when infection is most likely to occur), whereas overexpression of *CCA1* results in enhanced resistance [88]. Similarly, *Arabidopsis* is most resistant to *Pseudomonas syringae* in the subjective morning, a time when pathogen-associated molecular pattern (PAMP)-triggered immunity genes are highly expressed [89]. The circadian clock also provides enhanced defense against herbivores by promoting accumulation of the defense hormone jasmonate at times of likely herbivore attack [90]. Interestingly, metabolites involved in defense against herbivores continue to show daily rhythms in a variety of fruits and vegetables even after harvest [91], highlighting the importance of the clock throughout the process of food production. Finally, recent work has shown that not only does the clock affect immune responses but that immune responses can also affect clock pace, demonstrating crosstalk between these pathways [92].

Clock genes and agricultural traits

Although the plant clock has been best characterized in *Arabidopsis*, its importance in plants of agronomic importance is becoming clear [93]. Many species of crops have undergone whole genome duplication followed by considerable gene loss and diploidization. Studies in the crop *Brassica rapa* have shown that clock genes, including *PRR* and *RVE* family genes, were preferentially retained during this process [94], perhaps because of gene dosage constraints.

Numerous studies have shown that the circadian clock plays an essential role in agricultural traits. For example, photoperiodic control of flowering time is a key factor that determines crop performance. The *Photoperiod-1* gene, a homolog of *PRR7*, is involved in flowering regulation in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) [95, 96]. Recently rice (*Oryza sativa*) *ELF3* (*OsELF3*) has been reported to control heading date under long days [97]. Two studies have also reported that a commercial barley variety bred for short-growing seasons carries a mutation in a homolog of *ELF3* [98, 99]. The accelerated transition from vegetative to reproductive growth enables this barley variety to be cultivated in regions with short growing seasons. Early maturity in another barley variety is likely to be due to variation in a homolog of the *Arabidopsis* clock gene *LUX* [100]. Further, studies in wheat and sorghum (*Sorghum bicolor*) have also identified key photoperiodic flowering genes that are controlled by the clock [95, 101]. In addition to breeding, transgenic approaches toward yield improvement via manipulation of clock genes have also been successful. Constitutive expression of an *Arabidopsis* clock-regulated B-box domain gene (*AtBBX32*) in soybean (*Glycine max*) altered expression of clock gene homologs and significantly enhanced yield, perhaps by altering the timing of crucial phases of reproductive development [102].

Concluding remarks

The circadian clock is a complicated system that coordinates external stimuli and an internal timing mechanism to optimize growth and development. Further investigation into the functions of known clock components and the elucidation of interactions between the clock and input and output pathways will help us to understand how plants thrive in a predictably changing environment. As illustrated by recent studies in crop plants, these insights are likely to have important implications for agricultural improvement.

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Highlights

- The circadian system is a complex regulatory network
- Multiple links exist between the clock and other cellular pathways and signaling networks
- Recent systems approaches have advanced our understanding of the clock system
- Insights into the Arabidopsis clock have been applied to several crop species

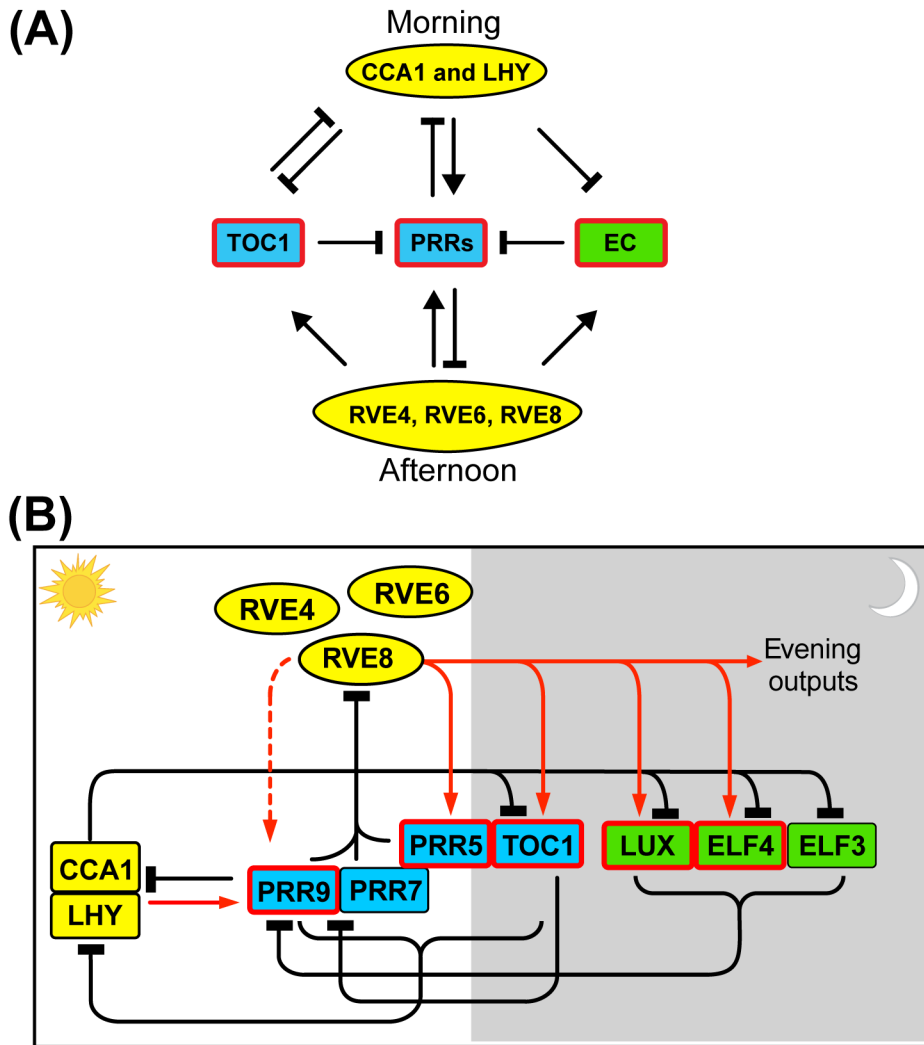


Figure 1. Transcriptional regulation of the clock in *Arabidopsis*. **(A)** A simplified model for transcriptional regulation among classes of clock components. CCA1, LHY and RVE components are shown in yellow; pseudo response regulator (PRR) components are shown in blue; evening complex (EC) components are shown in green. Members of each group that contain the evening element(s) in their promoter regions are marked with red boxes. Arrows indicate activation; perpendicular bars indicate repression. In the morning, CCA1 and LHY repress most EE-regulated clock genes; whereas in the afternoon, RVE4, RVE6, and RVE8 activate EE-regulated clock genes. These EE-controlled components also feed back to regulate expression of CCA1, LHY, RVE4, RVE6, RVE8 and each other. **(B)** A more detailed model for transcriptional regulation among the clock components. The relative timing of action for each component during a day–night cycle is shown from left to right. White area indicates subjective day; gray area indicates subjective night. Yellow, blue and green colors indicate members of each family described above. Genes with EE(s) in their promoter regions are marked with red boxes. Solid red arrows indicate activation; broken red arrow indicates conditional activation; perpendicular bars indicate repression.

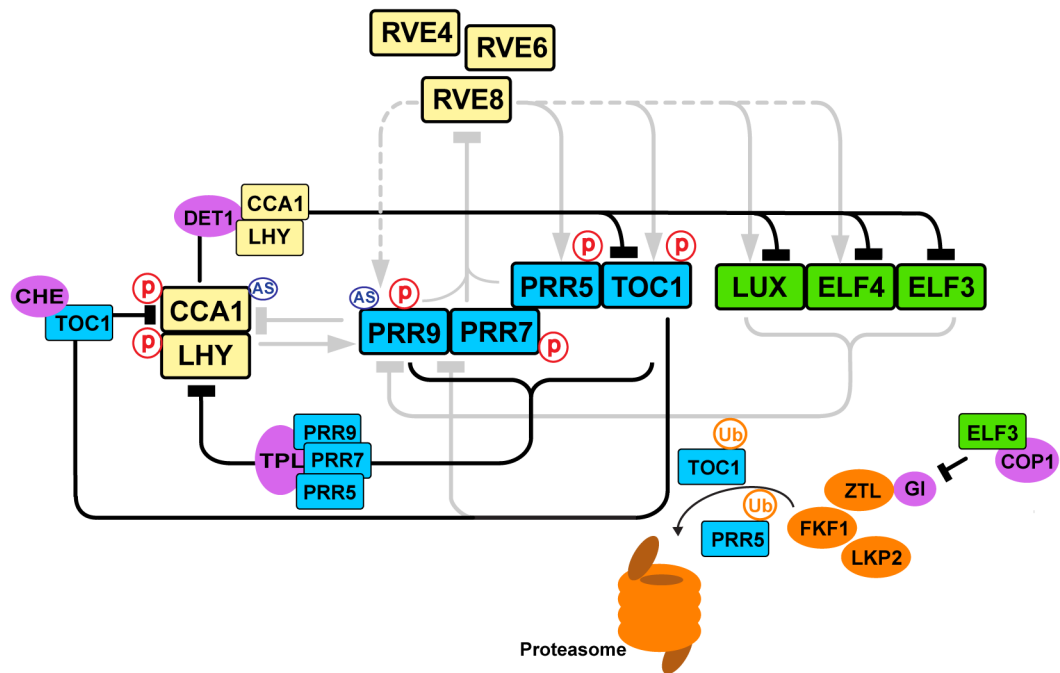


Figure 2. Post-transcriptional regulation of the clock in *Arabidopsis*. CCA1, LHY and RVE components are shown in yellow; pseudo response regulator components are shown in blue; evening complex components are shown in green. F-box proteins that are a part of E3 ubiquitin ligase complex are shown in orange. Other interacting proteins are shown in magenta. Abbreviations: AS, alternative splicing; P, protein phosphorylation; Ub, ubiquitination.

Table 1

Molecular functions of clock genes in *Arabidopsis*

Gene	AGI	Time of protein activity	Clock phenotypes of loss-of-function mutant	Molecular function	Refs.
Genes believed to function primarily within the clock system					
<i>CCA1</i>	AT2G46830	Morning	Short period (partially redundant with <i>LHY</i>)	Transcription factor; primarily represses genes with EE	[1]
<i>LHY</i>	AT1G01060	Morning	Short period (partially redundant with <i>CCA1</i>)	Transcription factor; primarily represses genes with EE	[1]
<i>PRR9</i>	AT2G46790	After dawn	Long period (partially redundant with <i>PRR7</i> and <i>PRR5</i>)	Transcription factor; represses expression of <i>CCA1</i> and <i>LHY</i>	[27, 103]
<i>PRR7</i>	AT5G02810	After dawn	Long period (partially redundant with <i>PRR9</i> and <i>PRR5</i>)	Transcription factor; represses expression of <i>CCA1</i> and <i>LHY</i>	[27, 103]
<i>RVE8 (LCL5)</i>	AT3G09600	Afternoon	Long period (partially redundant with <i>RVE4</i> and <i>RVE6</i>)	Transcription factor; promotes expression of genes with EE	[3, 9, 10]
<i>RVE4</i>	AT5G02840	Presumably afternoon	No obvious period or phase change (partially redundant with <i>RVE6</i> and <i>RVE8</i>)	Transcription factor; promotes expression of genes with EE	[3]
<i>RVE6</i>	AT5G52660	Presumably afternoon	No obvious period or phase change (partially redundant with <i>RVE4</i> and <i>RVE8</i>)	Transcription factor; promotes expression of genes with EE	[3]
<i>PRR5</i>	AT5G24470	Afternoon	Short period (partially redundant with <i>PRR9</i> and <i>PRR7</i>)	Transcription factor; represses expression of <i>CCA1</i> and <i>LHY</i> ; regulates TOC1 nuclear localization	[6, 27, 52]
<i>PRR3</i>	AT5G60100	Evening	Short period; tissue specific function	Putative transcription factor; modulates TOC1 stability	[33]
<i>TOC1 (PRR1)</i>	AT5G61380	Evening	Short period	Transcription factor; represses expression of <i>CCA1</i> and <i>LHY</i>	[4, 11, 14]
<i>CHE</i>	AT5G08330	Evening	No obvious period or phase change	Transcription factor; modulates <i>CCA1</i> expression	[8]
<i>LUX</i>	AT3G46640	Evening	Arrhythmic under constant light	Transcription factor; represses expression of <i>PRR9</i> and output genes	[7, 30]
<i>BOA (NOX)</i>	AT5G59570	Evening	Both no phenotype and short period phenotypes have been reported	Transcription factor; activates expression of <i>CCA1</i> and regulates output genes	[7, 22]
<i>ELF3</i>	AT2G25930	Evening	Arrhythmic under constant light	Transcriptional regulator; represses expression of <i>PRR9</i> and output genes	[7, 29]
<i>ELF4</i>	AT2G40080	Evening	Arrhythmic under constant light	Transcriptional regulator; represses expression of <i>PRR9</i> and output genes	[7, 31]
<i>ZTL</i>	AT5G57360	Evening	Long period (partially redundant with <i>FKF1</i> and <i>LKP2</i>)	F-box protein, blue-light receptor; regulates TOC1 protein stability	[47, 48]
Genes with multiple functions in addition to circadian regulation					
<i>CKB4</i>	AT2G44680	-	Not reported	Regulatory β subunit of CK2 kinase	[104]

Gene	AGI	Time of protein activity	Clock phenotypes of loss-of-function mutant	Molecular function	Refs.
<i>FKF1</i>	AT1G68050	–	Not obvious (partially redundant with <i>ZTL</i> and <i>LKP2</i>)	F-box protein, blue-light receptor; regulates CO expression	[47]
<i>GI</i>	AT1G22770	–	Short period under most conditions	Nuclear protein, physically interacts with diverse proteins	[12, 44, 46, 50, 105, 106]
<i>LKP2</i>	AT2G18915	–	Not obvious (partially redundant with <i>ZTL</i> and <i>FKF1</i>)	F-box protein, blue-light receptor; regulates CO expression	[47]
<i>PRMT5</i>	AT4G31120	–	Long period	Methyl transferase that demethylates a splicing factor	[38, 39]
<i>SKIP</i>	AT1G77180	–	Long period	SNW/Ski-interacting protein, a component of the spliceosome	[40]
<i>STP1L1</i>	AT1G17070	–	Long period	Splicing factor	[41]
Genes with unknown roles within the circadian system					
<i>LNK1</i>	AT5G64170	–	Not obvious (redundant with <i>LNK2</i>)	Nuclear protein with unknown function	[28]
<i>LNK2</i>	AT3G54500	–	Long period (redundant with <i>LNK1</i>)	Presumably nuclear protein	[28]
<i>FIO1</i>	AT2G21070	–	Long period	Nuclear protein with unknown function	[107]
<i>JMJ5</i>	AT3G20810	–	Short period	Putative histone demethylase	[108, 109]
<i>LIP1</i>	AT5T64813	–	Short period	Small GTPase	[110]
<i>LWD1</i>	AT1G12910	–	Not obvious (redundant with <i>LWD2</i>)	Nuclear protein; transcription factor?	[111, 112]
<i>LWD2</i>	AT3G26640	–	Not obvious (redundant with <i>LWD1</i>)	Nuclear protein; transcription factor?	[111, 112]
<i>TEJ</i>	AT2G31870	–	Long period	Poly (ADP-ribose) glycohydrolase	[113]
<i>TTC</i>	AT3G22380	–	Short period	Nuclear protein with unknown function	[114, 115]
<i>XCT</i>	AT2G21150	–	Short period	Nuclear protein with unknown function	[116]