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Similarities in the circadian clock and photoperiodism in plants

Young Hun Song^{*}, Shogo Ito^{*}, and Takato Imaizumi

Department of Biology, University of Washington, 24 Kincaid Hall, Box 351800, Seattle, WA, 98195-1800, USA

Summary of recent advances

Plants utilize circadian clocks to synchronize their physiological and developmental events with daily and yearly changes in the environment. Recent advances in *Arabidopsis* research have provided a better understanding of the molecular mechanisms of the circadian clock and photoperiodism. One of the most important questions is whether the mechanisms studied in *Arabidopsis* are conserved in other plants. Homologs of many *Arabidopsis* clock genes have been identified in various plants and some gene functions have been characterized. It seems that the circadian clocks in plants are similar. Recent success in molecular genetics has also revealed the mechanisms of photoperiodic flowering in cereals. The day-length sensing mechanisms appear to have diverged more between long-day plants and short-day plants than the circadian clock.

Introduction

Many organisms, including plants, possess an endogenous timekeeper known as a circadian clock that synchronizes biological events with daily environmental changes. Plants also use internal clocks to anticipate upcoming seasonal changes and adjust their physiology and development accordingly. There has been a tremendous amount of progress made recently in our understanding of the molecular mechanisms of circadian clocks, particularly in *Arabidopsis thaliana*. Recent studies of photoperiodic flowering regulation in *Arabidopsis* and other model plants have increased our knowledge of the mechanisms of photoperiodism in a wide variety of plant species at the molecular levels. Here we summarize recent advances in our studies of the clock and photoperiodism in various plants, and discuss to what degree the circadian clock mechanism and photoperiodism pathways are conserved among these plants.

The circadian clock in *Arabidopsis*

As we have the best understanding of the molecular regulation of the clock in *Arabidopsis*, our knowledge of *Arabidopsis* serves as a reference for other plant species. Here we introduce the major clock components and summarize the overall structure of the clock (see more details in recent reviews [1–3]).

The *Arabidopsis* circadian clock comprises multiple feedback regulations centered on two MYB transcription factors, CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) [1–3]. CCA1 and LHY, both of which are most

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Corresponding author: takato@u.washington.edu.

^{*}These authors contributed equally.

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abundant at dawn, directly activate the transcription of *PSUEDO-RESPONSE REGULATOR9* (*PRR9*) and *PRR7* in the morning [4]. *CCA1* and *LHY* concomitantly suppress the expression of the other clock genes with afternoon to evening peaks, such as *PRR5*, *TIMING OF CAB EXPRESSION1* (*TOC1*), *CCA1 HIKING EXPEDITION* (*CHE*), *GIGANTEA* (*GI*), *LUX ARRHYTHMO* (*LUX*) and *EARLY FLOWERING4* (*ELF4*) [5–10]. *PRR9*, *PRR7*, and *PRR5* proteins are expressed throughout the day. They physically associate with the *CCA1* and *LHY* promoters and repress their transcription [11]. As *CCA1* and *LHY* protein levels decrease, *CCA1/LHY*-dependent repression fades away, facilitating accumulation of the evening clock gene transcripts. *TOC1* (also known as *PRR1*) physically associates with the *CCA1* and *LHY* promoters and participates in the transcriptional activation of both genes by partially interfering with the function of *CCA1*-specific transcriptional repressor *CHE* [10]. No *PRR* proteins possess DNA binding domains, yet they associate with the promoters of *CCA1* and *LHY* and regulate their expression [10,11]. It is of great interest to understand the precise molecular mechanisms by which *PRR* proteins induce *CCA1* and *LHY* transcription. The GARP domain transcription factor *LUX* (also known as *PHYTOCLOCK 1* (*PCL1*)) and the small nuclear protein *ELF4* are also involved in *CCA1* and *LHY* induction [6,7]. In addition to transcriptional feedback regulation, posttranslational regulation is built into the molecular circuits of the circadian clock. Three related photoreceptor F-box proteins, *ZEITLUPE* (*ZTL*), *LOV KELCH PROTEIN2* (*LKP2*) and *FLAVIN-BINDING KELCH REPEAT F-BOX 1* (*FKF1*), regulate the stability of both *TOC1* and *PRR5* to fine tune the pace and robustness of circadian clock oscillation [12]. *GI* protein also plays a role in this regulation by directly binding these F-box proteins [13,14]. Considering that *Arabidopsis* is a model organism for plants, an important question is how much the *Arabidopsis* clock system is conserved in other plants. In recent years, homologs of *Arabidopsis* clock genes have been identified in various plant species, enabling us to systematically compare the plant molecular clocks (Table 1).

Circadian clocks in other eudicots

Can a system resembling the *Arabidopsis* circadian clock be identified in other eudicots? As shown in Table 1, homologs of most *Arabidopsis* clock genes exist in legumes, soybeans (*Glycine max*), peas (*Pisum sativum*), common beans (*Phaseolus vulgaris*), and *Medicago truncatula* [15–18]. The expression patterns of many legume clock homologs resemble those of *Arabidopsis* clock genes, suggesting that the legume plant clock is similar to the *Arabidopsis* clock. Recently, the function of two pea clock genes was characterized. The *LATE BLOOMER1* (*LATE1*) and the *DIE NEUTRALIS* (*DNE*) gene are the pea orthologs of *Arabidopsis GI* and *ELF4*. Pea plants flower earlier in long day (LD) conditions. Like *Arabidopsis gi* and *elf4* mutants, *late1* and *dne* mutants are both photoperiod insensitive and always flower late and early, respectively [19,20]. In the *late1* mutant, the expression of pea *TOC1*, *ELF4* and *MYB1* (*LHY* homolog) dampens and becomes arrhythmic under continuous weak light and dark conditions [19]. This clock phenotype is stronger than that of the *Arabidopsis gi* mutants, indicating that *GI* is essential for the rhythmic expression of the clock genes in peas, although we do not know how the molecular function of pea *GI* differs from *Arabidopsis GI*. Interestingly, circadian oscillation stops under continuous intense light in the wild type pea cultivar examined. The common spring-sown pea cultivars, including the one used in these studies, contain a mutation in the *HIGH RESPONSE* (*HR*) locus, which causes this conditional arrhythmicity [17,20]. Therefore, the mutant phenotype observed could be a combinational effect with the *hr* mutation. Unlike the *Arabidopsis elf4* mutant that shows the arrhythmic clock phenotype, the *dne* mutant exhibits a subtle effect on the rhythmic expression of the clock genes examined, although it shows a short period phenotype [20]. Overexpression of *DNE* in the *elf4* mutant complements flowering and hypocotyl growth phenotypes, indicating that *DNE* and *ELF4* share a similar function at least for regulating these responses. The different contributions of *LATE1* and *DNE* to the circadian clock could be pea specific.

Further analysis deciphering the more precise role of LATE1 and DNE possibly with HR in the clock is awaited.

Homologs of *Arabidopsis* clock genes were also identified in perennial (woody) eudicots such as poplar (*Populus trichocarpa* and *Populus nigra*) [21] and chestnut (*Castanea sativa*) [22, 23](Table 1); however, the molecular function of these genes has not been characterized yet. In the chestnut, *CsLHY*, *CsTOC1*, *CsPRR9*, *CsPRR7* and *CsPRR5* mRNA levels oscillate similarly to *Arabidopsis* clock gene mRNAs during growth seasons; however, during winter dormancy their mRNA levels become constitutive. This is caused by exposure to low temperature (4 °C), but not by the dormant status of tissues, since endodormant tissues transferred to 22 °C regain daily oscillation without breaking dormancy [22,23]. The *Arabidopsis* clock still sustains oscillation even at lower temperatures, although it is weakened [24]. Having similar expression patterns to many clock genes that peak at different times of the day indicates that similar feedback regulation among clock genes might exist in chestnuts. It is possible that some components in the network are more sensitive to lower temperatures, thus pausing their clock at some point. Stopping circadian-regulated physiology and development may help perennial trees cope with severe winter conditions. Although there is some species specific modification in the clock, overall expression patterns appear to be conserved within eudicots.

Circadian clocks in monocots

The divergence between eudicots and monocots is estimated to have occurred 200 million years ago [25]. We wondered whether monocots have evolved clock systems similar to eudicots. Recent studies have revealed the function of some clock genes in distant species of monocots, such as rice and duckweed, providing us with clues to help answer this question.

Homologs of most *Arabidopsis* clock genes exist in rice, although the rice genome only contains one *CCA1/LHY* homolog (Table 1). The circadian expression profiles of the putative rice clock genes highly overlap those of their *Arabidopsis* counterparts. In addition, ectopic overexpression of both *OsPRR1* and *OsZTL* in *Arabidopsis* caused a similar arrhythmic clock phenotype to the *TOC1* and *ZTL* overexpressors [26]. Overexpression of *OsLHY* in the rice callus also repressed rhythmic expression of the *OsPRR1:LUC* circadian reporter [27]. Moreover, *OsPRR1* and *OsPRR37* rescue the clock phenotypes of *toc1* and *prr7* mutants, respectively [26]. Therefore, these rice genes are orthologs of *Arabidopsis* clock genes. Even the posttranslational regulation of LHY is likely conserved in rice, as OsLHY protein also accumulates under light [27].

Duckweed is a free-floating aquatic monocot that provides an additional example of how circadian clocks in monocots are similar to *Arabidopsis*. Two species (*Lemna gibba* and *L. paucicostata*) have two *LHY/CCA1* (*LHYH1* and *LHYH2*), three *PRRs* (*PRR95*, *PRR59* and *PRR37*), *GI* (*GIH1*) and *ELF3* (*ELF3H1*) genes that show circadian expression patterns [28]. Transient transfection analysis with overexpression and RNA interference (RNAi) constructs of *LHYH1*, *LHYH2*, *GIH1* and *ELF3H1* revealed that these genes have similar functions to their *Arabidopsis* counterparts. (Table 1) [29]. Like the pea *GI* mutant, *GIH1* RNAi induces arrhythmic expression of clock markers, suggesting that *GIH1* plays an important role in the circadian clock in duckweed. Thus, the results obtained from several monocots suggest that both eudicots and monocots possess similar molecular circadian clocks.

Circadian clocks in moss and alga

Currently there are some reports regarding the function of circadian clock proteins in plants other than angiosperms, such as a moss and a unicellular alga. The moss *Physcomitrella patens* possesses *PpCCA1a*, *PpCCA1b* and *PpPRRa* genes [30]. The expression patterns of

these genes show daily oscillation with morning peaks (*PpCCA1a* and *PpCCA1b*) and a mid-day peak (*PpPRRa*) under light-dark cycles and continuous dark conditions. Interestingly, their expression profiles become arrhythmic under continuous light conditions [30]. Gene disruption is feasible in this moss by homologous recombination. The *PpCCA1a PpCCA1b* double disruptant presents shorter and dampened rhythms in clock-regulated gene expression, which resembles the *Arabidopsis cca1 lhy* double mutant phenotype [30]. The double disruptant colony grows faster than the wild-type colony in LD [31], indicating the circadian clock regulates growth rates in this moss. Similar phenotypes of both the double mutants in *Arabidopsis* and *Physcomitrella* suggest that not only the functions of counterpart genes but also the structure of the clock gene networks are at least partly conserved between the two species. Since bryophytes are one of the earliest diverging groups of land plants, the basic structure of the current angiosperm clock formed long ago.

Even in the green unicellular alga *Ostreococcus tauri*, *TOC1* and *CCA1* appear to be conserved, although it does not have other *Arabidopsis* clock homologs [32]. *Ostreococcus TOC1* (and also moss *PpPRRa*) may function as a response regulator, because it conserves the aspartic acid residue required for phosphotransfer. *OtTOC1* and *OtCCA1* expression patterns are anti-phasic and *OtCCA1* likely binds to the *OtTOC1* promoter and regulates rhythmic expression. *OtCCA1* and *OtTOC1* overexpressors and the *OtTOC1*-antisense line all lead to arrhythmia of promoter *LUC* reporter activities [32]. The *Ostreococcus* clock system seems to be similar to but relatively simpler than that of higher plants. Therefore, this type of the circadian clock may potentially be an ancestor of the land plant clocks.

Are photoperiodic pathways conserved in plants, like the circadian clocks?

In most plant species in which clock genes have been identified, the molecular characterization of clock proteins has just begun. However, it seems that the clock components and their function are likely conserved in land plants. In angiosperms, circadian clocks play important roles in seasonal flowering time regulation. Through circadian clock-regulated function, many plants measure changes in day length to precisely control flowering time to maximize reproductive success. Some plants respond to LD, and some respond to short days (SD), while others do not respond to any day length changes. Are the molecular mechanisms of day-length measurement conserved among plants that have different preferences? Among the plants that respond to the same day length, do they utilize similar mechanisms?

Photoperiodic flowering mechanism in *Arabidopsis*

The long-day photoperiod promotes flowering in *Arabidopsis* mainly through the function of CONSTANS (CO) and FLOWERING LOCUS T (FT). CO is a transcription activator of *FT* [33]. The circadian clock regulates the timing of *CO* gene expression and light and CO induces *FT* expression. Since daytime *CO* expression occurs only in LD, *FT* is induced under those conditions [33,34]. To accurately measure the difference in day length, clock-dependent timing regulation of *CO* is a crucial mechanism. During the day, CCA1 and LHY regulate the expression timing of *PRR9*, *PRR7*, *PRR5*, *GI* and *FKF1*, all of which regulate *CO* expression [4,8,35,36]. These three PRRs subsequently regulate the expression of the *CO* repressor, *CYCLING DOF FACTOR1* (*CDF1*) [36]. CDF1 and other CDFs repress *CO* expression during the morning, and in the afternoon the FKF1-GI complex degrades CDFs to facilitate the expression of *CO* [14,37]. Once *CO* is expressed under light, CO protein is stabilized by light signals and induces *FT* expression [38]. FT protein is a florigen and it is synthesized in the leaf vasculature [39]. It is then translocated to the shoot apical meristem to initiate the expression of floral identity genes by binding to bZIP transcription factor FD [39,40]. *FT* expression is also regulated by many other transcription factors with redundant function [2]. Winter annual accessions require exposure to prolonged cold temperature (vernalization) to gain

photoperiodic sensitivity. Without vernalization, FLC accumulates and directly represses the expression of *FT*, *SUPPRESSOR OF OVER EXPRESSION OF CONSTANS 1 (SOC1)* (another floral inducer) and *FD* [41]. Vernalization represses the expression of FLC by changing the chromatin status of the *FLC* locus, thus enabling plants to express *FT* in a day-length dependent manner [42].

Photoperiodic flowering mechanism in long-day plants

Temperate cereals such as barley and wheat are also long-day plants like *Arabidopsis*. Do these monocots have similar mechanisms to induce flowering? In barley and wheat, natural variations in photoperiod sensitivity are mainly controlled by *Photoperiod (Ppd)* loci, which regulate flowering time (Fig. 1) [43]. The *Ppd-H1* gene has been determined to be a barley homolog of *PRR7* [44]. The *ppd-H1* mutation alters the circadian timing of barley *CO* (*HvCO*) expression and represses barley *FT* (*HvFT1*) expression, resulting in late flowering (Fig. 1 and Fig 2) [44]. Conversely, some varieties of wheat carry a deletion in the promoter of the *Ppd-D1* gene (wheat *Ppd-H1*), leading to high expression of *Ppd* genes. This causes up-regulation of wheat *FT* (*TaFT*) expression, leading to early flowering even in SD [45]. *Arabidopsis* *prp7 prr5*, *prp9 prr7*, and *prp9 prr7 prr5* mutants also show an extreme late flowering phenotype (*PRR9*, *PRR7* and *PRR5* have overlapping function) and this phenotype is at least due to the alteration of *CO* expression [36]. Thus, the flowering function of *PRRs* in barley, wheat, and *Arabidopsis* may be similar.

Winter varieties of barley, wheat, oat (*Avena sativa*) and rye (*Secale cereale*) require vernalization to induce flowering in LD [43]. The vernalization response in barley and wheat is mediated by a feedback regulatory loop formed by *VERNALIZATION 1 (VRN1)*, *VRN2*, and *VRN3* (these *VRN* genes are different from *Arabidopsis VRN* genes) [46–48]. None of the *VRN* genes encode *FLC* homologs. *VRN1* is an *APETALA1*-like *MADS*-box transcription factor that is induced by vernalization [48]. *VRN2* encodes a protein containing zinc-finger and CCT domains [47]. *VRN2* inhibits *FT* induction and vernalization represses *VRN2* expression. *VRN3* is an *FT* gene [46]. Activation of *VRN1* expression by vernalization decreases *VRN2* expression to allow the long-day induction of *FT* (Fig 1). Thus, vernalization pathways in *Arabidopsis* and barley/wheat repress photoperiodic sensitivity by suppressing *FT* by utilizing different molecules.

In the poplar, a eudicot tree, LD induces flowering, while SD induces growth cessation and bud set. Poplar *FT* (*PtFT1*) regulates these two important developmental transitions [49]. As in *Arabidopsis*, the circadian clock regulates the timing of Poplar *CO* (*PtCO2*) and a high level of *CO* expression during the day seems to induce *PtFT* expression. (Fig. 2) Overexpression of *PtFT1* induces precocious flowering and represses growth cessation and bud set. [49]. In the Norway spruce (*Picea abies*), a gymnosperm tree, the expression pattern of a *FT* homolog (*PaFT4*) correlates with the timing of bud set, indicating that *FT* plays an important role in developmental transition regulation in both angiosperms and gymnosperms [50].

The long-day plants examined use the *CO-FT* modules, and the expression patterns of both *CO* and *FT* in those plants are very similar (Fig. 2), suggesting that circadian clock-regulated *CO* expression mechanisms may also be conserved.

Photoperiodic flowering mechanism in short-day plants

Can the photoperiod measurement mechanism in long-day plants help us understand the mechanism in short-day plants? Success in QTL mapping on the heading date (flowering date) trait in rice enabled the identification of the genes involved in photoperiodic flowering regulation in rice. Some of the components identified are indeed orthologs of the *Arabidopsis* photoperiodic pathway components, but others are likely rice specific.

The rice ortholog of *GI* (*OsGI*) regulates diurnal expression of a rice *CO* gene, *Heading date 1* (*Hd1*) [51]. In contrast to *Arabidopsis* *CO*, *Hd1* possesses two opposite functions in the regulation of the rice *FT* gene, *Heading date 3a* (*Hd3a*). *Hd1* inhibits *Hd3a* expression in LD but promotes its expression and subsequent flowering in SD (Fig. 1) [52,53]. The functional conversion of *Hd1* on *Hd3a* repression and promotion in response to photoperiod changes is unclear, but phytochrome signaling may differently affect *Hd1* activity in LD and SD [52, 54].

Hd3a expression is also up-regulated by another strong rice-specific floral activator gene, *Early Heading date 1* (*Ehd1*), which encodes a B-type response regulator [55]. Although *Ehd1* expression is low in LD, *Ehd1* antagonizes *Hd1* function to enable rice to flower under these conditions (Fig. 1). The expression of *RICE FLOWERING LOCUS T1* (*RFT1*), the closest *Hd3a* homolog, induces flowering in LD, although *RFT1* also promotes flowering in the absence of *Hd3a* in SD [56·]. *Ehd1* expression is positively regulated by *OsMADS50* (a rice homolog of *SOC1*) in LD, and by *OsMADS51* in SD [57,58]. A rice ortholog of the maize *Indeterminate1* (*ld1*) gene *OsId1* (also known as *Ehd2* and *RID1*) also induces *Ehd1* expression in both LD and SD (Fig. 1) [59–61]. As a negative regulator of flowering, *Ghd7*, which contains a CCT domain related to *VRN2* in barley and wheat, inhibits flowering through the repression of the *Ehd1-Hd3a* pathway [62·]. Phytochrome signaling through *PhyB* also negatively regulates *Ehd1-RFT1* expression and down-regulates *Hd3a* expression through *Hd1* activity in LD [56·]. In addition, the mutation in *PHOTOPERIOD SENSITIVITY 5* (*SE5*), which encodes a heme oxygenase implicated in phytochrome chromophore biosynthesis, has a similar effect on rice *FT* expression regulation [54]. Cultivated rice plants grow throughout a wide area, spanning large latitudinal changes with diverse day-length changes [63]. Having two discrete floral regulators (*Hd1* and *Edh1*) independently regulated by day-length conditions enables rice plants to adjust to environments with different day-length changes throughout the year.

A different photoperiodic response than those in *Arabidopsis* and in rice is found in the short-day eudicot *Pharbitis* (*Ipomoea nil*). Clock-regulated *Pharbitis* *FT* (*PnFT*) expression (Fig. 2) promotes flowering specifically in SD. In addition, the pattern of *PnFT* mRNA abundance is not highly correlated with the expression of *PnCO*, indicating that the photoperiod response in *Pharbitis* is controlled by a different mechanism for day-length sensing than that described for *Arabidopsis* and rice [64].

Although regulating *FT* expression seems to be the common final outcome of photoperiodic regulation in short-day plants, both rice and *Pharbitis* evolved their own regulatory mechanisms in addition to or separate from the *CO-FT* module, which is likely conserved in long-day plants.

Concluding remarks

Arabidopsis plays an important role as the model plant for increasing our understanding of the circadian clock and photoperiodism at molecular levels. The knowledge gained from *Arabidopsis* research has helped us to identify components and analyze the function of the circadian clock and photoperiodism in other plant species. Based on our current understanding, the circadian clock seems to be conserved in many plant species. However, our knowledge regarding the function of clock genes and the molecular architecture of clocks in plants other than *Arabidopsis* is still quite limited. More time is needed to truly understand how conserved the molecular clock systems are in plants.

In contrast, we have rapidly accumulated knowledge regarding the molecular mechanisms of photoperiodic flowering pathways in several plants, such as *Arabidopsis*, rice, and barley/wheat. The molecular mechanisms of photoperiod measurement are more diverse between long-day plants and short-day plants than between eudicots and monocots. Even in the short-

day plant rice, the CO-FT module still exists and thus it could be a prototype of day-length sensing mechanisms. It would be interesting to find out whether other long-day plants use the CO-FT module and also to find out how other short-day plants measure changes in day length for flowering regulation.

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Figure 1.

Photoperiodic flowering pathways in long-day and short-day plants. Long-day plants (*Arabidopsis*, barley and wheat) sense day-length increases and promote flowering in late spring and/or early summer. In these plants, photoperiodic flowering responses are regulated by the CO-FT modules. The circadian clocks regulate *CO* expression through the function of PRRs, FKF1, GI, and CDFs in *Arabidopsis*, and Ppd1 (a homolog of PRR7) in barley and wheat. Vernalization represses *FLC* expression in *Arabidopsis*, while it induces *VRN1* expression in barley and wheat. *VRN1* depresses the expression of *VRN2*, which represses *FT* expression. Therefore, vernalization releases the strong repression of *FT* and enables these plants to promote flowering in LD. In the short-day plant rice, the circadian clock regulates *Hd1* (a homolog of *CO*) expression through OsGI function. *Hd1* represses the expression of a rice *FT* (*Hd3a*) in LD, while it promotes the expression of *Hd3a* and *RFT1* (another rice *FT* homolog) in SD. Phytochromes and *SE5* signaling promotes *Hd1*-dependent repression of *Hd3a* in LD. The expression of a rice-specific floral inducer *Ehd1* is regulated by activators (*OsMADS50*, *OsMADS51*, *OsGI*, and *Ehd2/OsId1/RID*) and repressors (*Ghd7*, *SE5*, and *phys*). To promote flowering *Ehd1* induces *RFT1* expression in LD and both *Hd3a* and *RFT1* expression in SD. Cultivated rice plants grow in a wider range of latitudes with different changes in day-length conditions. Depending on where they grow, either the long-day or the short-day pathways control flowering. Grey dotted lines indicate potential interaction.

Figure 2.

The expression patterns of *CO* and *FT* in various plant species. *CO* expression profiles are similar in long-day and short-day plants under both long day (LD) and short day (SD) conditions. Even in the day neutral plant maize, *conz1* (a homolog of rice *Hd1*) expression showed similar patterns [77]. These results suggest that the molecular mechanisms of *CO* transcriptional regulation may also be highly conserved among plants. In long-day plants, *FT* mRNA levels are high at the end of the day in LD and extremely low throughout the day in SD. In contrast, in short-day plants, *FT* mRNA levels are high in the morning in SD. White and black boxes designate day and night, respectively.

Table 1

Homologs of *Arabidopsis* clock genes in plants

Gene names in <i>A. thaliana</i> Protein motifs and expression peak(ZT) ^a [65]	Homologs in other species ^b	Species	Characteristic remarks, gene expression peak (ZT) ^a	Refs
CCA1 (ZTO) LHY (ZTO) Single MYB DNA binding domain	<i>OsCCA1/OsLHY</i>	<i>O. sativa</i>	<i>OsCCA1-OX^c</i> shows late flowering and long hypocotyl phenotypes (ZT0~4)	[26,52]
	<i>PnLHY1, PnLHY2</i>	<i>P. nigra</i>	(ZTO~3)	[21]
	<i>CsLHY</i>	<i>C. sativa</i>	(ZT4)	[23]
	<i>MYB1/LHY</i>	<i>P. sativum</i>	(ZT-4~4)	[19,20..]
	<i>GmLCL1, GmLCL2</i>	<i>G. max</i>	(ZT1~3)	[15]
	<i>PvLHY</i>	<i>P. vulgaris</i>	Induced by light. Putative <i>Lhcb</i> activator, (ZTO~6)	[16]
	<i>LgLHYH1, LgLHYH2</i>	<i>L. gibba</i>	<i>LgLHYH1-OX^d</i> possesses low and advanced circadian rhythms. <i>LgLHYH2-OX^d</i> shows arrhythmic phenotype. <i>LgLHYH1 RNAi^d</i> shows short period phenotype. <i>LgLHYH1</i> (ZT-2~0), <i>LgLHYH1</i> (ZT2~6)	[28,29]
	<i>LpLHYH1, LpLHYH2</i>	<i>L. paucicostata</i>	<i>LpLHYH1</i> (ZT-4~2), <i>LpLHYH2</i> (ZT4~4)	[28]
	<i>OrCCA1</i>	<i>O. tauri</i>	<i>OrCCA1-OX</i> shows arrhythmic phenotype, (ZT12~24)	[32..]
	<i>McCCA1/LHY</i>	<i>M. crystallinum</i>	(ZTO)	[66]
TOC1/PRR1 (ZT10~12) Pseudo-receiver and CCT domains	<i>PpCCA1a, PpCCA1b</i>	<i>P. patens</i>	<i>PpCCA1a</i> (ZTO), <i>PpCCA1b</i> (ZT-4~0)	[30,31]
	<i>OsTOC1/OsPRR1</i>	<i>O. sativa</i>	<i>OsPRR1-OX</i> in <i>Arabidopsis</i> shows arrhythmic, small and short hypocotyl phenotypes, (ZT7~13)	[26,67,68]
	<i>CsTOC1</i>	<i>C. sativa</i>	(ZT15~18)	[22.,23]
	<i>TOC1</i>	<i>P. sativum</i>	(ZT8~16)	[19,20..]
	<i>GmTOC1</i>	<i>G. max</i>	(ZT13~17)	[15]
	<i>BrPRR1</i>	<i>B. rapa</i>	(ZT9~18)	[69]
	<i>McTOC1</i>	<i>M. crystallinum</i>	(ZT7)	[66]
	<i>OrTOC1</i>	<i>O. tauri</i>	True response regulator, (ZT9~12)	[32..]

Gene names in <i>A. thaliana</i> Protein motifs and expression peak(ZT) ^a [65]	Homologs in other species ^b	Species	Characteristic remarks, gene expression peak (ZT) ^a	Refs
<i>PRR</i> s Pseudo-receiver and CCT domains <i>PRR9</i> (ZT2~4) <i>PRR7</i> (ZT6~8) <i>PRR5</i> (ZT8~9) <i>PRR3</i> (ZT10)	<i>OsPRR73</i> , <i>OsPRR37</i> , <i>OsPRR95</i> , <i>OsPRR59</i>	<i>O. sativa</i>	<i>OsPRR37</i> partially rescue <i>prp7-11</i> mutation. <i>OsPRR73</i> (ZT4~10), <i>OsPRR37</i> (ZT7~10), <i>OsPRR95</i> (ZT10), <i>OsPRR59</i> (ZT7~13)	[67,68,70]
	<i>PPD</i> (<i>PRR</i>)	<i>H. vulgare</i>	Flowering activator	[44]
	<i>PPD</i> (<i>PRR</i>)	<i>T. aestivum</i>	Flowering activator	[45]
	<i>PPD</i> (<i>PRR</i>)	<i>T. durum</i>	Flowering activator	[71]
	<i>CsPRR9</i> , <i>CsPRR7</i> , <i>CsPRR5</i>	<i>C. sativa</i>	<i>CsPRR9</i> (ZT4~9), <i>CsPRR7</i> (ZT7~12), <i>CsPRR5</i> (ZT9~12)	[22]
	<i>PRR37</i> , <i>PRR59</i>	<i>P. sativum</i>	<i>PRR37</i> (ZT8), <i>PRR59</i> (ZT8)	[20-]
	<i>BrPRR9</i> , <i>BrPRR7</i> , <i>BrPRR5</i> , <i>BrPRR3</i>	<i>B. rapa</i>	<i>BrPRR9</i> (ZT3~12), <i>BrPRR7</i> (ZT12), <i>BrPRR5</i> (ZT6~12), <i>BrPRR3</i> (ZT12~15)	[69]
	<i>LgPRRH95</i> <i>LgPRRH59</i> <i>LgPRRH37</i>	<i>L. gibba</i>	<i>LgPRRH95</i> (ZT6~12), <i>LgPRRH59</i> (ZT8~16), <i>LgPRRH37</i> (ZT6~12)	[28]
<i>LUX</i> (ZTU) GARP DNA binding domain	<i>PpPRRa</i>	<i>P. patens</i>	True response regulator, (ZT4~12)	[30-]
	<i>OsLUX</i>	<i>O. sativa</i>	(ZT10~13)	[26]
<i>ELF3</i> Unknown (ZT16)	<i>OsEF3</i>	<i>O. sativa</i>	Positive flowering regulator in both LD and SD. The loss of function mutant has short roots, but does not have clock phenotype.	[72]
	<i>LgELF3H1</i>	<i>L. gibba</i>	<i>LgELF3H1-OX^d</i> shows weak rhythmicity and long period. <i>LgELF3H1</i> RNAi ^d shows high expression with normal periodicity. <i>LgELF3H1</i> (ZT16 in LD, ZT10 in SD)	[28,29]
	<i>McELF3</i>	<i>M. crystallinum</i>	(ZT9~21)	[66]
<i>ELF4</i> (ZT12) Unknown	<i>PsELF4/DNE</i>	<i>P. sativum</i>	<i>dne</i> mutation affects clock gene expression and causes early flowering, (ZT12~16)	[19,20-]
	<i>McELF4</i>	<i>M. crystallinum</i>	(ZT9~15)	[66]
<i>ZTL</i> (constant) <i>LKP2</i> (constant) <i>FKF1</i> (ZT7~10) LOV, F-box and Kelch repeat domains [13]	<i>OsZTL1</i> , <i>OsZTL2</i> , <i>OsFKF1</i>	<i>O. sativa</i>	<i>OsZTL1-OX^c</i> shows arrhythmic, late flowering, and long hypocotyl phenotypes. <i>OsZTL1</i> (constant), <i>OsZTL2</i> (constant), and <i>OsFKF1</i> (ZT7~13)	[26]

Gene names in <i>A. thaliana</i> Protein motifs and expression peak(ZT) ^a [65]	Homologs in other species ^b	Species	Characteristic remarks, gene expression peak (ZT) ^a	Refs
GI (ZT8~10) Nuclear protein that interacts with ZTL family proteins	<i>McZTL, McFKF1</i>	<i>M. crystallinum</i>	<i>McZTL</i> expression oscillates, (ZT9), <i>McFKF1</i> (ZT9)	[66]
	<i>OsGI</i>	<i>O. sativa</i>	<i>OsGI</i> -OX plant is late flowering, (ZT8~12)	[51,73]
	<i>HvGI</i>	<i>H. vulgare</i>	(ZT15 in LD, ZT6 in SD)	[74]
	<i>TaGI</i>	<i>T. aestivum</i>	<i>TaGI</i> -OX ^c shows early flowering and complements <i>gi-2</i> mutant ^c , (ZT12 in LD, ZT8 in SD)	[75]
	<i>BdGI</i>	<i>B. distachyon</i>	<i>BdGI</i> Interacts with <i>AtZTL</i> and <i>AtCOP1</i> in yeast and rescues <i>gi</i> mutant ^c , (ZT12 in LD, ZT8 in SD)	[76]
	<i>LATE1</i>	<i>P. sativum</i>	<i>late1</i> mutant shows short period and low amplitude rhythms and late flowering (ZT12 in LD and ZT8 in SD)	[19,20-]
	<i>MtGI</i>	<i>M. truncatula</i>	(ZT8 in LD)	[18]
	<i>LgGIH1</i>	<i>L. gibba</i>	<i>LgGIH1</i> -OX ^d shows very low amplitude, <i>LgGIH1</i> RNAi ^d shows arrhythmic, (ZT8 10)	[28,29]
	<i>LpGIH1</i>	<i>L. paucicostata</i>	(ZT10~16)	[28]
	<i>McGI</i>	<i>M. crystallinum</i>	(ZT9)	[66]

Gene name abbreviations: *CCA1*, *CIRCADIAN CLOCK-ASSOCIATED 1*; *LHY*, *LATE ELONGATED HYPOCOTYL*; *TOC1*, *TIMING OF CAB EXPRESSION 1*; *PRRs*, *PSEUDO RESPONSE REGULATORS*; *LUX/PCL1*, *LUX ARRHYTHMO/ PHYTOCLOCK 1*; *ELF*, *EARLYFLOWERING*; *ZTL*, *ZEITLUPE*; *LKP2*, *LOV KELCH PROTEIN2*; *FKF1*, *FLAVIN-BINDING KELCH REPEAT F-BOX 1*; *GI*, *GIGANTEA*; *LCL*, *LHY AND CCA1 LIKE*; *PPD*, *PHOTOPERIOD*; *DNE*, *DIE NEUTRALIS*; and *LATE1*, *LATE BLOOMER1*. Species abbreviations: *A. thaliana*, *Arabidopsis thaliana*; *O. sativa*, *Oriza sativa*; *P. nigra*, *Populus nigra*; *C. sativa*, *Castanea sativa*; *P. sativum*, *Pisum sativum*; *G. max*, *Glycine max*; *P. vulgaris*, *Phaseolus vulgaris*; *L. gibba*, *Lemna gibba*; *L. paucicostata*, *Lemna paucicostata*; *O. tauri*, *Ostreococcus tauri*; *M. crystallinum*, *Mesembryanthemum crystallinum*; *P. patens*, *Physcomitrella patens*; *H. vulgare*, *Hordeum vulgare*; *T. aestivum*, *Triticum aestivum*; *B. rapa*, *Brassica rapa*; and *B. distachyon*, *Brachypodium distachyon*.

Monocots are shown in bold.

Note: In *Lotus japonicus*, *Carica papaya*, and *Vitis vinifera*, many *Arabidopsis* clock homolog candidate genes are identified from DNA databases, however there is no other information; therefore these genes are excluded from this table.

^aPeak gene expression phases for RNA expression in the table are in Zeitgeber time (ZT). Light on is at ZT0 and light off is usually at ZT12. In LD conditions, light is turned off at ZT16, in SD conditions, light is turned off from ZT8.

^bThe names of homologs are quoted from the original articles.

^cThese experiments were conducted in *Arabidopsis*.

^dThese results were obtained from transient assays.