



Review Article

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The evolution and function of the *PSEUDO RESPONSE REGULATOR* gene family in the plant circadian clock

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Abstract

PSEUDO-RESPONSE PROTEINS (PRRs) are a gene family vital for the generation of rhythms by the circadian clock. Plants have circadian clocks, or circadian oscillators, to adapt to a rhythmic environment. The circadian clock system can be divided into three parts: the core oscillator, the input pathways, and the output pathways. The PRRs have a role in all three parts. These nuclear proteins have an N-terminal pseudo receiver domain and a C-terminal CONSTANS, CONSTANS-LIKE, and TOC1 (CCT) domain. The PRRs can be identified from green algae to monocots, ranging from one to >5 genes per species. *Arabidopsis thaliana*, for example, has five genes: *PRR9*, *PRR7*, *PRR5*, *PRR3* and *TOC1/PRR1*. The *PRR* genes can be divided into three clades using protein homology: *TOC1/PRR1*, *PRR7/3*, and *PRR9/5* expanded independently in eudicots and monocots. The PRRs can make protein complexes and bind to DNA, and the wide variety of protein-protein interactions are essential for the multiple roles in the circadian clock. In this review, the history of PRR research is briefly recapitulated, and the diversity of PRR genes in green and recent works about their role in the circadian clock are discussed.

Keywords: Circadian clock, circadian rhythms, pseudo-response regulators, core oscillator, gene evolution.

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Introduction

Plants have an internal timekeeping mechanism that allows them to anticipate periodical events, such as dawn and dusk, track seasons' passage, and modulate internal and external signals (Farré and Liu, 2013; McClung, 2021). This timekeeping mechanism is called the circadian clock or circadian oscillator. The circadian clock system is usually divided into input pathways, core oscillator, and output pathways. The core oscillator is a regulatory network that generates sustainable rhythms at the cellular level. Even though the core oscillator can run under constant environmental conditions, it can be continually regulated or reset by the input pathways to stay synchronised with environmental rhythms (Webb *et al.*, 2019). Plants with internal rhythms that are not synchronised with external rhythms are less productive and have lower fitness (Dodd *et al.*, 2005). Input pathways bring external cues to the core oscillator, such as light and temperature, or internal, such as sugar levels. The output pathways take the temporal information generated between the core oscillator and input pathways to the rest of the plant.

The core oscillator generates rhythms through a series of interlocked transcriptional-translational feedback loops. The main components of the plant core oscillator are the LATE ELONGATED HYPOCOTYL/ CIRCADIAN CLOCK ASSOCIATED 1 (LHY/CCA1), GIGANTEA (GI), the EVENING COMPLEX (EC), composed of LUX ARRHYTHMO (LUX), EARLY FLOWERING 3 (ELF3)

and ELF4, and the PSEUDO-RESPONSE REGULATOR (PRR) family. The PRR gene family comprises five genes in *Arabidopsis thaliana* (L.) Heynh (Brassicaceae): *AtPRR1*, also known as *TIME OF CAB EXPRESSION 1* (*AtTOC1*), *AtPRR3*, *AtPRR5*, *AtPRR7* and *AtPRR9*. These nuclear proteins have an N-terminal pseudo receiver domain (PR) and a C-terminal CONSTANS, CONSTANS-LIKE, and TOC1 (CCT) domain. The PR domain is similar to the receiver domain of a two-component response regulator, but they lack the characteristic phospho-accepting aspartate site in the receiver domain. However, the PR domain is still necessary for the PRRs to make homo- and heterodimers. The CCT domain is found in 45 *Arabidopsis* proteins, shares similarities with some histones motifs, and can bind DNA and proteins (Wenkel *et al.*, 2006; Tiwari *et al.*, 2010). The *Arabidopsis* proteins *AtPRR9*, *AtPRR7* and *AtPRR5* also have a motif involved in transcriptional repression in the intermediate region (IR) between their PR and CCT domains (Nakamichi *et al.*, 2010; Wang *et al.*, 2013). The PRRs are essential for the proper function of the plant circadian clock, but the details of their function are still unknown. These genes are frequently targets for selection during breeding, changing the plant perception of the photoperiod (Turner *et al.*, 2005; Beales *et al.*, 2007; Murphy *et al.*, 2011). Here, the early history of PRR research in *Arabidopsis*, the evolution of this gene family in green plants, and our current understanding of their function in the circadian clock are reviewed.

Early PRR research in *Arabidopsis*

The first core oscillator mutant in plants was described in 1995 (Millar *et al.*, 1995). The short-period *toc1-1*, identified in a mutant screening looking for *Arabidopsis* with defects

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in the luminescence rhythms generated by LUCIFERASE expression under the control of a *CHLOROPHYLL A/B BINDING PROTEIN 2 (AtCAB2)* promoter (Millar *et al.*, 1995). In 2000, *AtTOC1* was cloned and identified as a PRR, and the *toc1-1* phenotype resulted from a point mutation in the CCT domain (Strayer *et al.*, 2000). Four other *PRRs* were identified and associated with the core oscillator (Strayer *et al.*, 2000). Later, the *PRRs* were shown to have transcription rhythms during the daytime, with peaks 2 h to 3 h apart, forming “waves of expression”: *AtPRR9* is the first to peak near dawn, then *AtPRR7*, *AtPRR5*, *AtPRR3* and *AtTOC1*, near dusk (Matsushika *et al.*, 2000). In 2001, the first model of a plant core oscillator was proposed as a feedback loop between *AtTOC1* and *AtLHY/CCA1* (Alabadí *et al.*, 2001). In this early model, *AtLHY/CCA1* repressed *AtTOC1* by binding to its promoter, while *AtTOC1* would activate *AtLHY/CCA1* expression. At that moment, no DNA binding motif was known in *AtTOC1*. In 2003, ZEITLUPE (*AtZTL*) was shown to interact with *AtTOC1*, targeting it for degradation and changing the core oscillator’s period, the first description of protein-level regulation of the core oscillator (Más *et al.*, 2003). In 2005, *AtPRR7* and *AtPRR9* were suggested to form an additional feedback loop with *AtLHY/CCA1* (Farré *et al.*, 2005).

In 2007, *AtPRR3* was found to be expressed only in the vasculature, forming protein-protein complexes with *AtTOC1* in competition with *AtZTL* (Para *et al.*, 2007). In 2009, CCA1 HIKING EXPEDITION (*AtCHE*) was shown to interact with *AtTOC1* while binding to the *AtCCA1* promoter. Thus, *AtCHE* was suggested to be the molecular link between *AtTOC1* and *AtCCA1* (Pruneda-Paz *et al.*, 2009). However, *AtCHE* does not bind to the *AtLHY* promoter, leaving the model incomplete.

In 2010, *AtPRR9*, *AtPRR7*, and *AtPRR5* were shown to be transcriptional repressors of *AtLHY/CCA1*, despite lacking a typical DNA binding domain (Nakamichi *et al.*, 2010). In these proteins, but not *AtTOC1*, the IR contained a motif essential for repressing *AtLHY/CCA1* expression (Nakamichi *et al.*,

2010). In the same year, the CCT domain of CONSTANS (*AtCO*), which was thought as a protein-protein interaction domain, was also shown to bind to DNA (Tiwari *et al.*, 2010). In 2012, *AtTOC1* was also described as a transcription factor, acting mainly as a transcriptional repressor (Gendron *et al.*, 2012; Huang *et al.*, 2012).

In 2013, sugars from photosynthesis were shown to regulate the circadian oscillator through *PRR7*, in a process called “metabolic dawn” (Haydon *et al.*, 2013). Later, this regulation was shown to be mediated by the transcription factor bZIP63, trehalose-6-phosphate metabolism, and SnRK1/KIN10 (Frank *et al.*, 2018).

The evolution of *PRRs* in plants

PRRs can be found in all green plants (Viridiplantae) (Table 1). This review analysed the protein sequence of *PRR* genes from fourteen species to show how this gene family expanded within the green plants (Figure 1). The *PRRs* can be divided into three clades based on their identity: *TOC1/PRR1*, *PRR7/3*, *PRR9/5* (Figure 1B) (Murakami *et al.*, 2003; Takata *et al.*, 2010; Satbhai *et al.*, 2011; Farré and Liu, 2013; Linde *et al.*, 2017).

In green algae, such as *Ostreococcus tauri* C.Courties & M.-J.Chrétiennot-Dinet, 1995 (Chlorophyta), only one *PRR* can be found. These algae are believed to have a simple core oscillator: a *TOC1/PRR1* ortholog forming a simple feedback loop with an *LHY/CCA1* ortholog (Corellou *et al.*, 2009; Thommen *et al.*, 2010). Bryophytes have genes from the *TOC1/PRR1* and the *PRR7/3* clades. *Marchantia polymorpha* L. (liverwort, Marchantiales) has one gene from the *TOC1/PRR1* clade (*MpTOC1*) and one from the *PRR7/3* clade (*MpPRR*). Some circadian oscillator genes have expanded in bryophytes, but some were also lost (Linde *et al.*, 2017). In *Physcomitrium patens* (Hedw.) Mitt. (synonym: *Physcomitrella patens*, Funariales), four genes from the *PRR7/3* clade (*PpPRR1*, *PpPRR2*, *PpPRR3*, *PpPRR4*) resulted from a recent expansion, but no *TOC1/PRR1* ortholog was found (Holm *et al.*, 2010; Satbhai *et al.*, 2011).

Table 1 – Number of *PRR* members of each clade in fourteen different species. Numbers in parenthesis correspond to pseudogenes that have sequences similarities. The complete sequence list can be found in Table S1.

Species	TOC1/PRR1 clade	PRR7 clade	PRR9 clade	Total
<i>Ostreococcus tauri</i>	1	0	0	1
<i>Marchantia polymorpha</i>	1	1	0	2
<i>Physcomitrium patens</i>	0	4	0	4
<i>Nymphaea colorata</i>	1	1	2	4
<i>Arabidopsis thaliana</i>	1	2	2	5
<i>Carica papaya</i>	1	2	2	5
<i>Populus trichocarpa</i>	1	2	4	7
<i>Vitis vinifera</i>	1 (2)	2	2	7
<i>Solanum lycopersicum</i>	1	2 (1)	2	6
<i>Beta vulgaris</i>	1	2	2	5
<i>Oryza sativa</i>	1	2	2	5
<i>Brachypodium distachyon</i>	1	2	2	5
<i>Setaria viridis</i>	1	2	2	5
<i>Sorghum bicolor</i>	1	2	2	5

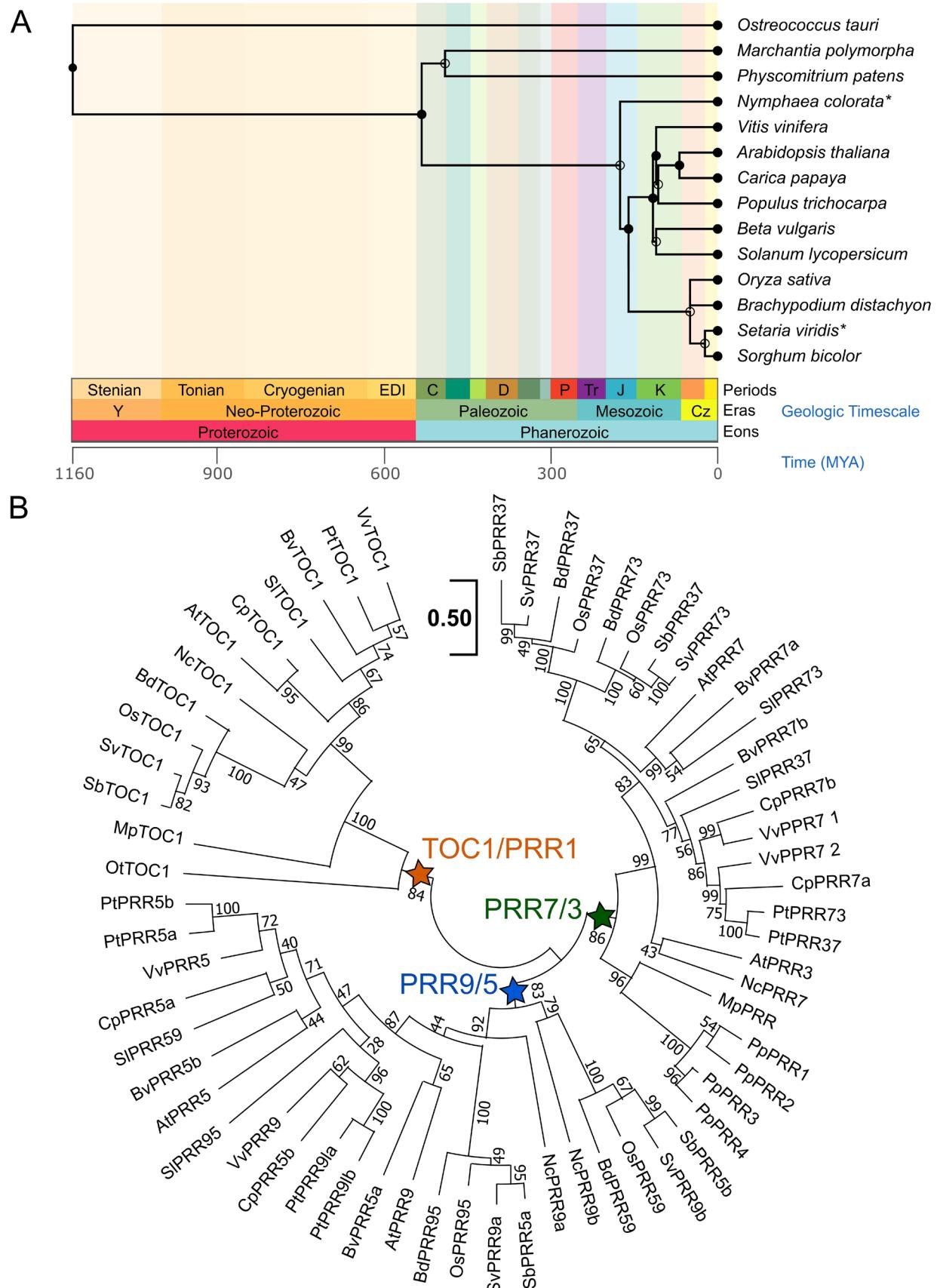


Figure 1 – Phylogenetic relations of PRR proteins. **(A)** Timetree of the fourteen species used for sequence analysis (Kumar *et al.*, 2017). * species that were substituted by the species of the same genera. Some branches were flipped for visualisation purposes. **(B)** The phylogenetic tree was built using Maximum Likelihood Bootstrap (500 replicates) after sixty-three PRR proteins from fourteen species were aligned using MUSCLE (MEGA11). Evolutionary distances were calculated using the JTT+F matrix—scale bar, 0.2 substitutions per site. Values at the nodes represent bootstrap support values. The nodes that define the TOC1/PRR1 (orange), PRR7/3 (green) and PRR9/5 clades (blue) are shown as stars. Sequences ID can be found in Table S1.

The absence of a *TOC1/PRR1* gene is uncommon among vascular plants, but other non-vascular plants share the same loss: *Anthoceros agrestis* Paton (Anthoceratales), *Sphagnum fallax* H. Klinggr. (Sphagnales), *Ceratodon purpureus* (Hedw.) Brid. (Dicranales). It remains to be established how the loss of an essential gene in other species would have on the circadian clock of these species and how this could be compensated. For example, in *M. polymorpha*, loss of the *LHY/CCA1* ortholog is compensated by *DE-ETIOLATED1* (*MpDET1*), arrhythmic in *Arabidopsis* (Lagercrantz *et al.*, 2021).

The *PRR9/5* clade only appears in Angiosperms, which usually have one gene from the *TOC1/PRR1* clade (Figure 2) and 2 or 3 genes of the *PRR7/3* (Figure 3) and *PRR9/5* (Figure 4). While the appearance of the *PRR7/3* and *PRR9/5* clades precedes the Eudicot-Monocot split, their expansion probably happened independently in both groups. Analysis of the eudicot *PRR7/3* and *PRR9/5* gene expansions using chromosomal synteny suggests that it is the result of the γ (gamma) polyploidy event, a whole-genome duplication (WGD) event that occurred early in eudicot divergence (Tang *et al.*, 2008; Takata *et al.*, 2010; Chanderbali *et al.*, 2022). The same analysis suggests that the expansion of the *PRR7/3* clade in monocots resulted from the ρ (rho) polyploidy event, but the *PRR5/9* clade was duplicated before (Takata *et al.*, 2010). However, the *Nymphaea colorata* L. (water lily, Nymphaeales) genome has only one *PRR7/3* but two *PRR9/5*. As Nymphaeales is considered to have diverged from the other plants before the Eudicot-Monocot split (Zhang L *et al.*, 2020), the *PRR5/9* duplication event in eudicots may have happened before the γ polyploidy event. However, the *PRR5/9* genes in water lilies are more similar to the monocots genes by sequence identity and positional orthology (Figures 1 and 3), suggesting that this group's history may be more complicated than expected.

When analysing the *PRR9/5* genes in eudicots using positional orthology (Figure 4), it is possible to notice that a *LATE EMBRYOGENESIS ABUNDANT PROTEIN 2* (*LEA2*)

flanks most *PRR9/5*. A *bHLH57* transcription factor also flanks one group (Figure 4A), and a *30S RIBOSOMAL PROTEIN SUBUNIT* flanks the other (Figure 4B). In monocots, one group is flanked by *LEA2*, and a *PENTATRICOPEPTIDE REPEAT PROTEIN (PPR)* gene or a *PROTEIN STAY GREEN* (Figure 4C), while an *EXOCYTOSIS COMPONENT 70 (EXO70)* gene flanks the other (Figure 4D).

When analysing the *PRR7/3* in eudicots using positional orthology (Figure 3), most genes have a *PROTEIN KINASE* within 1 to 3 genes. In addition, the *PRR7/3* can be divided into two groups: a larger group that is also flanked by the genes for a *NUCLEOTIDE KINASE*, a *GLUTATHIONE S-TRANSFERASE* and/or TBC domain-containing protein (Figure 3A), and a smaller group that is also flanked by the genes for an *ALCOHOL DEHYDROGENASE*, a *HYDROLASE* and/or a *MAPK* (Figure 3B). The genes from the larger group can be found in all the eudicots and duplicated in *Populus trichocarpa* Torr. & A. Gray ex Hook. (Malpighiales) (*PtPRR37* and *PtPRR73*), *Solanum lycopersicum* L. (Solanales) (*SlPRR37* and *SlPRR37-like*) and *Beta vulgaris* L. (beets, Caryophyllales) (*BvPRR7* and *BvBTCl*). *S. lycopersicum* also has one gene that does not fit either group (*SlPRR73*). The genes from the smaller group are restricted to the Rosids, including *Carica papaya* L. (Brassicaceae) and *Vitis vinifera* L. (Vitales) (Figure 3B), and *Citrus clementina* Hort. ex Tan. (Sapindales), *Medicago truncatula* Gaertn. (Fabales) and *Theobroma cacao* L. (Malvales) (not shown). Non-rosid eudicots with two genes, such as beets, have duplications in the larger group (*BvPRR7* and *BvBTCl*) and none in the smaller group (Pin *et al.*, 2012). AtPRR3 does not fit either group, even though it is usually associated with the smaller group. A *PROTEIN KINASE* also flanks *PRR7/3* genes in monocots. They can be divided into two groups of similar size: one usually called *PRR37*, which is flanked by a gene for *ASCORBATE PEROXIDASE* (Figure 3C), and one called *PRR73*, flanked by the genes for a TBC domain-containing protein and a Ribosomal protein (Figure 3D).

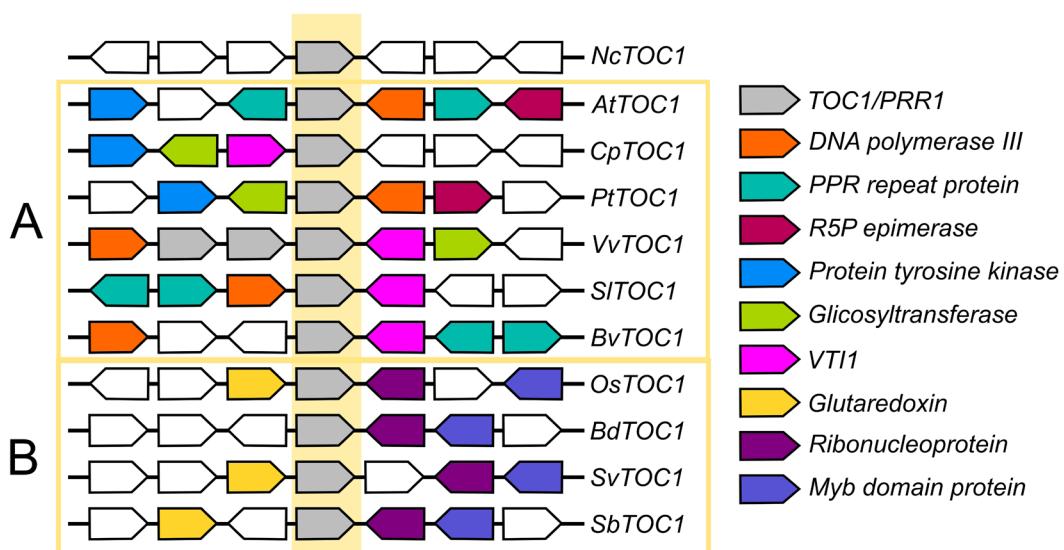


Figure 2 - Positional orthology of members of the *TOC1/PRR1* clade. The flanking genes of the *TOC1/PRR1* orthologs (grey polygons in the yellow centre) of eleven vascular plant clades were identified and colour-coded according to their identity—the polygons point toward the annotated direction of the gene. Two groups of orthologs can be identified through similarities: one for eudicots (A) and one for monocots (B). Sequences ID can be found in Table S1.

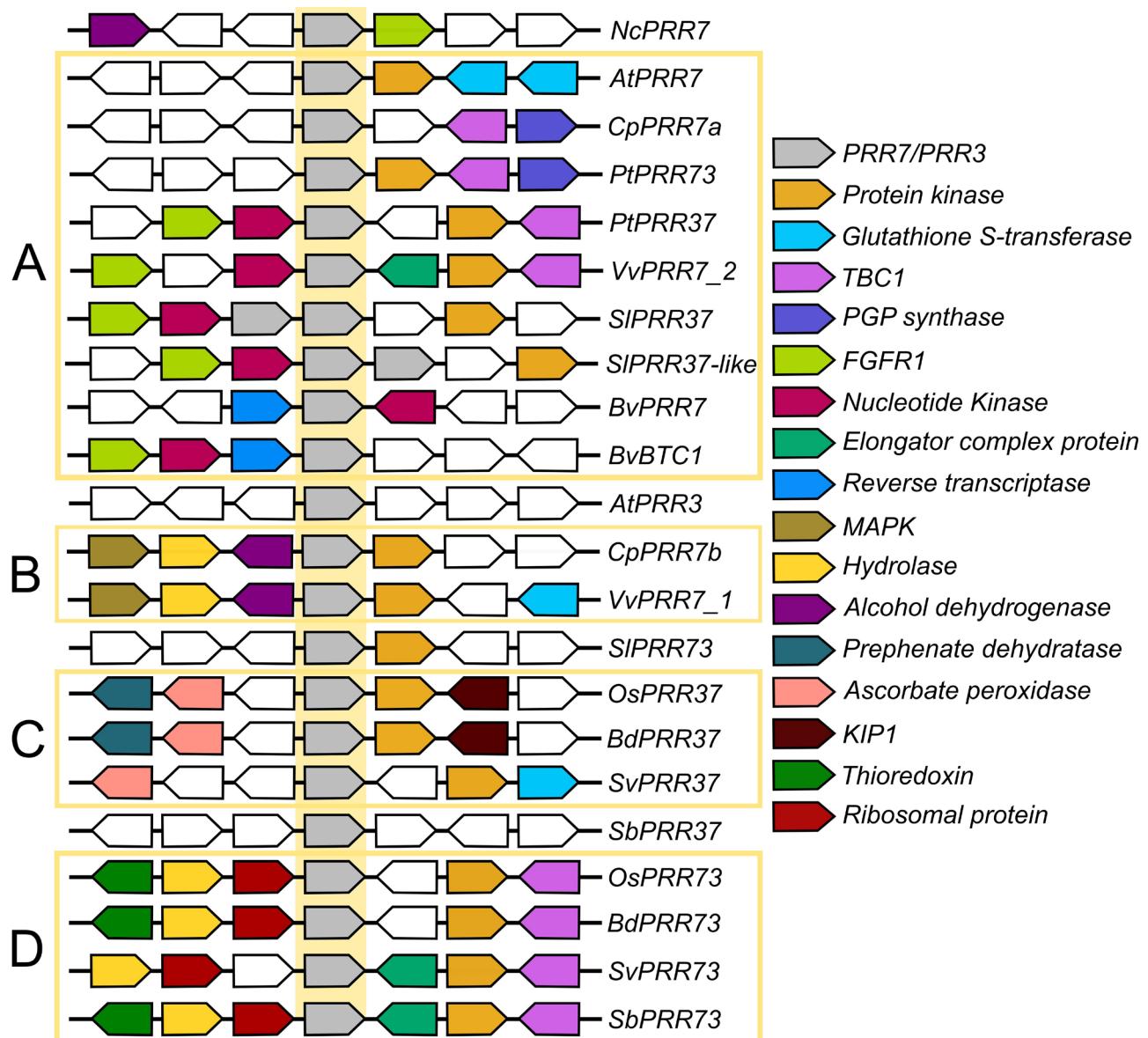


Figure 3 - Positional orthology of members of the PRR7/3 clade. The flanking genes of the PRR7/3 orthologs (grey polygons in the yellow centre) of eleven vascular plant clades were identified and colour-coded according to their identity—the polygons point toward the annotated direction of the gene. Four groups of orthologs can be identified through similarities: two for eudicots (**A and B**) and two for monocots (**C and D**). Sequences ID can be found in Table S1.

PRRs in crops

Circadian rhythms affect plant productivity (Dodd *et al.*, 2005); thus, it is not surprising that they may have a role in Agriculture (Steed *et al.*, 2021; Hotta, 2021). Crop domestication frequently leads to the selection of mutants in the circadian oscillator due to their effects on photoperiodic responses, such as flowering (Bendix *et al.*, 2015; McClung, 2021). In *Hordeum vulgare* L. (barley, Poales), a cultivar with reduced response to photoperiod allowed the use of this crop in northern parts of Europe. These changes were associated with a mutation in the *Photoperiod-H1* (*Ppd-H1*) locus. Cloning this locus showed that the *ppd-H1* mutation is a single nucleotide change in the CCT domain of a PRR7/3, *HvPRR37* (Turner *et al.*, 2005). This mutation changes the flowering time on long days but has no apparent effect on the circadian

oscillator (Campoli *et al.*, 2012). *Ppd-H1* is collinear with the *Ppd-D1* allele in *Triticum aestivum* L. (wheat, Poales), a Green Revolution mutation that turns wheat into a photoperiod insensitive plant (Beales *et al.*, 2007). Mutations in the PRR37 orthologs selected by breeding can also be found in *Sorghum bicolor* (L.) Moench (sorghum, Poales) (Murphy *et al.*, 2011) and *Oryza sativa* (rice, Poales) (Koo *et al.*, 2013).

Mutations in genes belonging to the PRR7/3 clade were also selected in eudicot crops. The domestication of beets selected a rare allele of *BvBTC1*, an ortholog from the PRR7/3 clade, that reduces the sensitivity to photoperiod (Pin *et al.*, 2012). As this sensitivity reduction is reverted by vernalisation, beets with a mutated *Bvbtc1* allele turn from an annual to a biannual crop (Pin *et al.*, 2012). During the domestication of *Glycine max* (L.) Merr. (soybeans, Fabales),

changes in a pair of *PRR7/3* orthologs (*GmPRR3A* and *GmPRR3B*) led to the loss of their CCT domain, resulting in the earlier flowering and reduction of the growth period (Li *et al.*, 2019; Li and Lam, 2020).

The role of PRRs in green plants

Apart from *TOC1/PRR1*, the role of the *PRRs* in the circadian oscillator is not fully understood. In *Arabidopsis*, the *PRRs* are considered part of the three interlocked loops of the core oscillator (Pokhilko *et al.*, 2012). *AtTOC1* is part of the core loop with *AtHY/AtCCA1* (Alabadí *et al.*, 2001) and the evening loop with the EC (Pokhilko *et al.*, 2012). *AtPRR7*, *AtPRR9* and *AtPRR5* are part of the morning loop with *LHY/CCA1* (Farré *et al.*, 2005; Nakamichi *et al.*, 2010) while also interacting with the EC (Chow *et al.*, 2012; Pokhilko *et al.*, 2012). Mutation in *AtTOC11* or *AtPRR5* leads to a short period (Millar *et al.*, 1995; Yamamoto *et al.*, 2003), while a mutation in *AtPRR9* or *AtPRR7* leads to an

extended period (Eriksson *et al.*, 2003; Michael *et al.*, 2003; Yamamoto *et al.*, 2003). Arrhythmia is only observed in the triple mutant *Atprr5 Atprr7 Atprr9* in constant conditions (Nakamichi *et al.*, 2005). The triple mutant also shows less photoperiodic and photomorphogenic responses (Nakamichi *et al.*, 2005). The *PRRs* act as transcriptional inhibitors by binding to the DNA through their CCT domains (Nakamichi *et al.*, 2010; Gendron *et al.*, 2012; Nakamichi *et al.*, 2012). Thus, the waves of expression of *PRRs* regulate the transcription of genes throughout the day. For example, *AtPRR5* targets are repressed from noon until midnight (Nakamichi *et al.*, 2012). However, in monocots, no changes in the circadian oscillator were observed when some genes from the *PRR3/7* clade were mutated to change flowering, suggesting subfunctionalisation. For example, changes in *OsPRR73* did not lead to changes in flowering, nor did changes in *OsPRR37* lead to changes in the circadian oscillator (Murakami *et al.*, 2003; Higgins *et al.*, 2010).

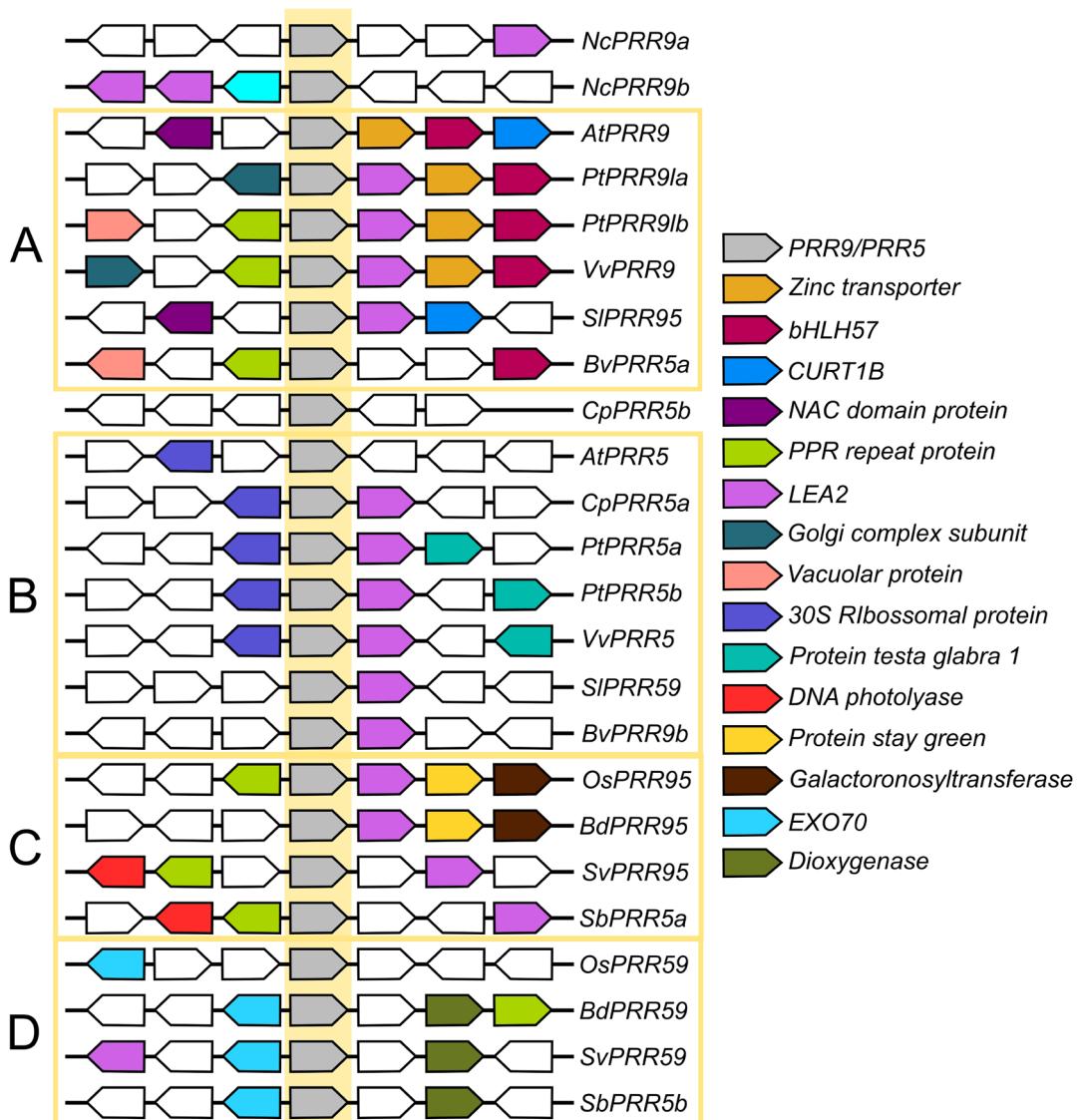


Figure 4 - Positional orthology of members of the *PRR9/5* clade. The flanking genes of the *PRR9/5* orthologs (grey polygons in the yellow centre) of eleven vascular plant clades were identified and colour-coded according to their identity—the polygons point toward the annotated direction of the gene. Four groups of orthologs can be identified through similarities: two for eudicots (**A and B**) and two for monocots (**C and D**). Sequences ID can be found in Table S1.

There is increasing evidence that PRRs act by forming protein complexes to regulate gene expression (Figure 5). In the core oscillator, during the night, AtTOC1 interacts with the TCP transcription factor AtCHE to inhibit *AtCCA1* expression by binding to its promoter (Pruneda-Paz *et al.*, 2009). Other PRR-protein complexes also inhibit *AtCCA1* expression: at dawn, the Groucho/Tup1 corepressors TOPLESS (AtTPL) and TOPLESS-RELATED (AtTPR) form protein complexes with HISTONE DEACETYLASE 6 (AtHDA6), and AtPRR9, AtPRR7 or AtPRR5. The TPL-PRR-HDC complex bind inhibits *AtCCA1* and *AtLHY* expression by directly binding to their promoter (Wang *et al.*, 2013). Later in the day, the B-box zinc-finger transcription factor AtBBX19 forms protein complexes with AtPRR9, AtPRR7 and AtPRR5 to regulate the period of the core oscillator, also by inhibiting *AtCCA1* expression (Yuan *et al.*, 2021). The concerted action of the PRRs and their binding partners restrict *CCA1/LHY* expression to the first hours of the day. As *CCA1/LHY* regulates the expression of several *Arabidopsis* genes, PRR-protein complexes are essential to regulate the phase of transcriptional rhythms during the day. AtPRR9, AtPRR7 and AtPRR5 sequentially interact with PHYTOCHROME INTERACTING FACTORS (PIFs) to repress their induction of growth-related genes, such as the transcription factor *CYCLING DOF FACTOR 5* (*AtCDF5*). *AtCDF5* transcription is induced by PIFs before dawn, inducing cell elongation (Martín *et al.*, 2018). In addition, AtTOC1 and AtPRR5 suppress thermomorphogenesis by interacting with AtPIF4 (Zhu *et al.*, 2016). Thus, PRRs can be a gating mechanism that regulates plant growth. Gating is the regulatory mechanism that changes plant responses to signals due to the time of the day (Hotta *et al.*, 2007). Shade-avoided responses

are gated by PRRs, as AtPRR5 and AtPRR7 directly interact with other PIF proteins, and AtTOC1 directly interacts with PIF3-LIKE 1 (PIL1) (Salter *et al.*, 2003; Zhang Y *et al.*, 2020). Consequently, the maximum response is observed at dusk, when TOC1 levels are highest (Salter *et al.*, 2003).

The PRR-protein interactions also regulate flowering in *Arabidopsis*. The accumulation of AtCO at the end of the day triggers flowering by promoting *FLOWERING LOCUS T* (*AtFT*) expression (Valverde *et al.*, 2004). The circadian oscillator regulates *AtCO* transcription, but protein levels of AtCO are independently stabilised by photoreceptors and PRRs (Valverde *et al.*, 2004; Hayama *et al.*, 2017). The binding of the PRRs to AtCO also increases its binding to the *AtFT* promoter (Hayama *et al.*, 2017). In monocots, PRR7/3 orthologs are associated with flowering initiation or repression (Turner *et al.*, 2005; Beales *et al.*, 2007; Murphy *et al.*, 2011). In barley, HvCO1 activates *HvFT*, triggering flowering under long days (LD). This activation is made stronger by HvPRR37 (Ppd-H1), even though it does not regulate *HvCO1* transcription levels (Campoli *et al.*, 2012). In contrast, SbPRR37 inhibits *SbCO* under LD in sorghum, a short-day plant (Yang *et al.*, 2014). Similarly, OsPRR37 inhibits *OsFT* (*H3a*) expression under LD in rice (Koo *et al.*, 2013).

Other outputs directly regulated by PRRs are the inhibition of photomorphogenic responses to red light, mediated by the interaction between AtTOC1 and AtPIL6 (Fujimori *et al.*, 2004), and abscisic acid (ABA) signalling during germination, mediated by AtPRR5 and AtPRR7 and AtABI5 (Yang *et al.*, 2021). ABA signalling also forms a feedback loop with AtTOC1 (Legnaioli *et al.*, 2009; Lee *et al.*, 2016).

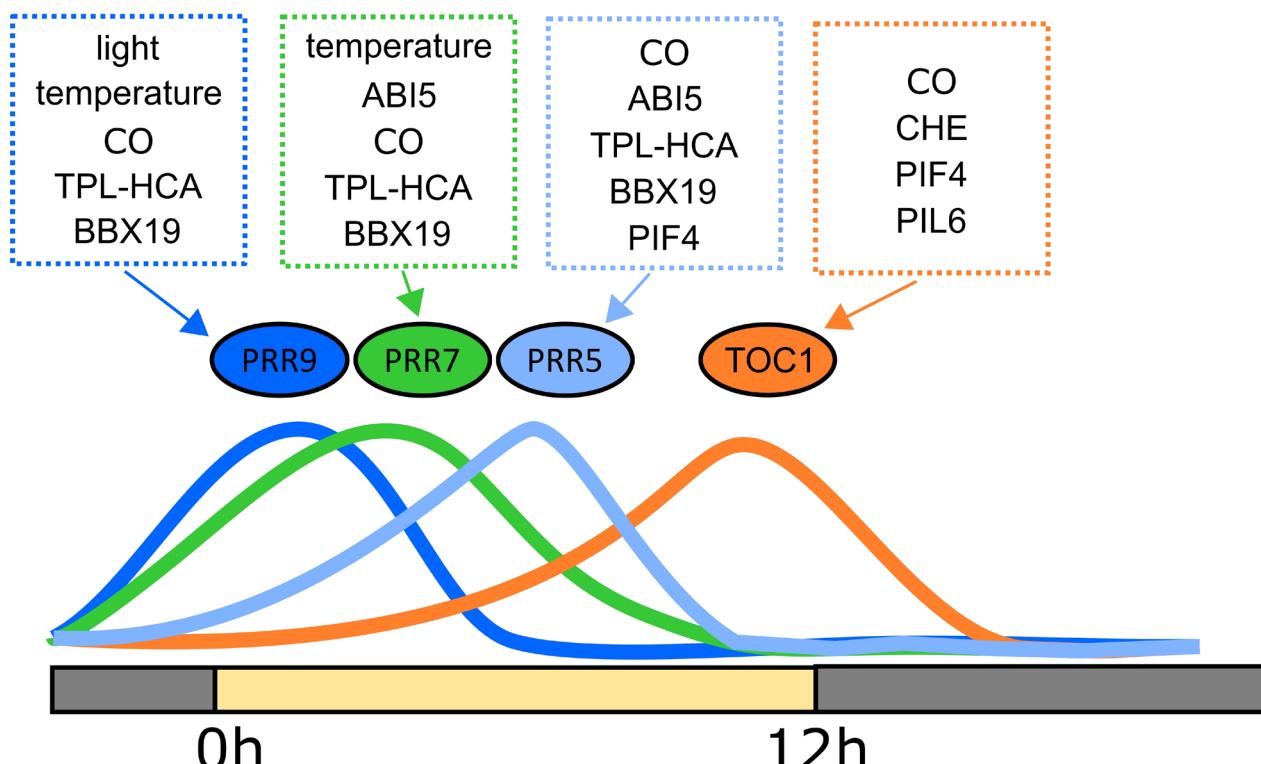


Figure 5 – Regulators of the PRR proteins in *Arabidopsis thaliana*. AtPRR9 (dark blue), AtPRR7 (green), AtPRR5 (light blue) and AtTOC1 (orange) are expressed during the daytime, forming waves of expression. The PRR proteins make protein-protein complexes that regulate their DNA binding activity.

The protein complexes formed by PRRs can also act as input pathways to the core oscillator, integrating information about light, temperature, and energy status. AtPRR9 is light-responsive but not the other PRRs, and thus it is one point of entry of light signalling into the core oscillator (Farré *et al.*, 2005; Ito *et al.*, 2005; Zeilinger *et al.*, 2006). Double mutants of *AtPRR7* and *AtPRR9* cannot entrain to temperature changes, nor can they compensate for temperature, suggesting that these genes are part of the temperature input pathways into the circadian oscillator (Salomé and McClung, 2005; Salomé *et al.*, 2010). Finally, energy status regulates the circadian oscillator by inhibiting AtPRR7 through the transcription factor AtbZIP63 downstream of the SnRK1/KIN10 signalling pathway (Haydon *et al.*, 2013; Frank *et al.*, 2018; Viana *et al.*, 2021).

Conclusions

The PRR gene family is an integral part of the circadian oscillator, with a role in the core oscillator and the input and output pathways. The PRRs can make protein-protein and protein-DNA interactions, interacting with many proteins and promoters. The three clades of PRRs have a different evolutionary history, with only one copy of *TOC1/PRR1* in Angiosperms and multiple copies of *PRR7/3* and *PRR9/5*. When the numerous genome-wide duplications are considered, many copies of these genes were lost, probably to maintain the correct gene dosage. However, evidence of subfunctionalisation of the *PRR7/3* clade in monocots suggests that the roles of these genes may vary among the different plant species. Consequently, sequence similarities and mutant complementation using heterologous genes may not be enough to establish functional homology among other species. The function of these genes may not lie in their structure but in their protein and DNA binding partners. Until most of the protein complexes formed by PRRs are described, it will be difficult to fully understand the whole function of PRR proteins in the plant circadian clock.

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Conflict of Interest

The author has no competing interests to declare.

Author Contributions

CTH Writing – Original Draft; Writing – Review & Editing.

References

Alabadí D, Oyama T, Yanovsky MJ, Harmon FG, Más P and Kay SA (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. *Science* 293:880-883.

Beales J, Turner A, Griffiths S, Snape JW and Laurie DA (2007) A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor Appl Genet* 115:721-733.

Bendix C, Marshall CM and Harmon FG (2015) Circadian clock genes universally control key agricultural traits. *Mol Plant* 8:1135-1152.

Campoli C, Shtaya M, Davis SJ and von Korff M (2012) Expression conservation within the circadian clock of a monocot: Natural variation at barley *Ppd-H1* affects circadian expression of flowering time genes, but not clock orthologs. *BMC Plant Biol* 12:97.

Chanderbali AS, Jin L, Xu Q, Zhang Y, Zhang J, Jian S, Carroll E, Sankoff D, Albert VA, Howarth DG *et al.* (2022) *Buxus* and *Tetracentron* genomes help resolve eudicot genome history. *Nat Commun* 13:643.

Chow BY, Helfer A, Nusinow DA and Kay SA (2012) ELF3 recruitment to the PRR9 promoter requires other Evening Complex members in the *Arabidopsis* circadian clock. *Plant Signal Behav* 7:170-173.

Corellou F, Schwartz C, Motta J-P, Djouani-Tahri EB, Sanchez F and Bouget F-Y (2009) Clocks in the green lineage: Comparative functional analysis of the circadian architecture of the picoeukaryote *ostreococcus*. *Plant Cell* 21:3436-3449.

Dodd AN, Salathia N, Hall A, Kévei E, Tóth R, Nagy F, Hibberd JM, Millar AJ and Webb AAR (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* 309:630-633.

Eriksson ME, Hanano S, Southern MM, Hall A and Millar AJ (2003) Response regulator homologues have complementary, light-dependent functions in the *arabidopsis* circadian clock. *Planta* 218:159-162.

Farré EM and Liu T (2013) The PRR family of transcriptional regulators reflects the complexity and evolution of plant circadian clocks. *Curr Opin Plant Biol* 16:621-629.

Farré EM, Harmer SL, Harmon FG, Yanovsky MJ and Kay SA (2005) Overlapping and distinct roles of PRR7 and PRR9 in the *Arabidopsis* circadian clock. *Curr Biol* 15:47-54.

Frank A, Matioli CC, Viana AJC, Hearn TJ, Kusakina J, Belbin FE, Wells Newman D, Yochikawa A, Cano-Ramirez DL, Chembath A *et al.* (2018) Circadian entrainment in *Arabidopsis* by the sugar-responsive transcription factor bZIP63. *Curr Biol* 28:2597-2606.e6.

Fujimori T, Yamashino T, Kato T and Mizuno T (2004) Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in *Arabidopsis thaliana*. *Plant Cell Physiol* 45:1078-1086.

Gendron JM, Pruneda-Paz JL, Doherty CJ, Gross AM, Kang SE and Kay SA (2012) *Arabidopsis* circadian clock protein, TOC1, is a DNA-binding transcription factor. *Proc Natl Acad Sci U S A* 109:3167-3172.

Hayama R, Sarid-Krebs L, Richter R, Fernández V, Jang S and Coupland G (2017) PSEUDO RESPONSE REGULATORs stabilise CONSTANS protein to promote flowering in response to day length. *EMBO J* 36:904-918.

Haydon MJ, Mielczarek O, Robertson FC, Hubbard KE and Webb AAR (2013) Photosynthetic entrainment of the *Arabidopsis thaliana* circadian clock. *Nature* 502:689-692.

Higgins JA, Bailey PC and Laurie DA (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. *PLoS One* 5:e10065.

Holm K, Källman T, Gyllenstrand N, Hedman H and Lagercrantz U (2010) Does the core circadian clock in the moss *Physcomitrella patens* (Bryophyta) comprise a single loop? *BMC Plant Biol* 10:109.

Hotta CT (2021) From crops to shops: How agriculture can use circadian clocks. *J Exp Bot* 72:7668-7679.

Hotta CT, Gardner MJ, Hubbard KE, Baek SJ, Dalchau N, Suhita D, Dodd AN and Webb AAR (2007) Modulation of environmental responses of plants by circadian clocks. *Plant Cell Environ* 30:333-349.

Huang W, Pérez-García P, Pokhilko A, Millar AJ, Antoshechkin I, Riechmann JL and Mas P (2012) Mapping the core of the

Arabidopsis circadian clock defines the network structure of the oscillator. *Science* 336:75-79.

Ito S, Nakamichi N, Matsushika A, Fujimori T, Yamashino T and Mizuno T (2005) Molecular dissection of the promoter of the light-induced and circadian-controlled APRR9 gene encoding a clock-associated component of *Arabidopsis thaliana*. *Biosci Biotechnol Biochem* 69:382-390.

Koo B-H, Yoo S-C, Park J-W, Kwon C-T, Lee B-D, An G, Zhang Z, Li J, Li Z and Paek N-C (2013) Natural variation in OsPRR37 regulates heading date and contributes to rice cultivation at a wide range of latitudes. *Mol Plant* 6:1877-1888.

Kumar S, Stecher G, Suleski M and Hedges SB (2017) Timetree: A resource for timelines, timetrees, and divergence times. *Mol Biol Evol* 34:1812-1819.

Lagercrantz U, Billhardt A, Rousku SN, Leso M, Reza SH and Eklund DM (2021) DE-ETIOLATED1 has a role in the circadian clock of the liverwort *Marchantia polymorpha*. *New Phytol* 232:595-609.

Lee HG, Mas P and Seo PJ (2016) MYB96 shapes the circadian gating of ABA signaling in Arabidopsis. *Sci Rep* 6:17754.

Legnaioli T, Cuevas J and Mas P (2009) TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought. *EMBO J* 28:3745-3757.

Li M-W and Lam H-M (2020) The modification of circadian clock components in soybean during domestication and improvement. *Front Genet* 11:571188.

Li M-W, Liu W, Lam H-M and Gendron JM (2019) Characterisation of two growth period QTLs reveals modification of PRR3 genes during soybean domestication. *Plant Cell Physiol* 60:407-420.

Linde A-M, Eklund DM, Kubota A, Pederson ERA, Holm K, Gyllenstrand N, Nishihama R, Cronberg N, Muranaka T, Oyama T *et al.* (2017) Early evolution of the land plant circadian clock. *New Phytol* 216:576-590.

Martín G, Rovira A, Veciana N, Soy J, Toledo-Ortiz G, Gommers CMM, Boix M, Henriques R, Minguez EG, Alabadi D *et al.* (2018) Circadian waves of transcriptional repression shape PIF-Regulated photoperiod-responsive growth in Arabidopsis. *Curr Biol* 28:311-318.e5.

Más P, Kim W-Y, Somers DE and Kay SA (2003) Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. *Nature* 426:567-570.

Matsushika A, Makino S, Kojima M and Mizuno T (2000) Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana*: Insight into the plant circadian clock. *Plant Cell Physiol* 41:1002-1012.

McClung CR (2021) Circadian clock components offer targets for crop domestication and improvement. *Genes (Basel)* 12:374.

Michael TP, Salomé PA, Yu HJ, Spencer TR, Sharp EL, McPeek MA, Alonso JM, Ecker JR and McClung CR (2003) Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science* 302:1049-1053.

Millar AJ, Carré IA, Strayer CA, Chua NH and Kay SA (1995) Circadian clock mutants in Arabidopsis identified by luciferase imaging. *Science* 267:1161-1163.

Murakami M, Ashikari M, Miura K, Yamashino T and Mizuno T (2003) The evolutionarily conserved OsPRR quintet: Rice pseudo-response regulators implicated in circadian rhythm. *Plant Cell Physiol* 44:1229-1236.

Murphy RL, Klein RR, Morishige DT, Brady JA, Rooney WL, Miller FR, Dugas DV, Klein PE and Mullet JE (2011) Coincident light and clock regulation of pseudoreponse regulator protein 37 (PRR37) controls photoperiodic flowering in sorghum. *Proc Natl Acad Sci U S A* 108:16469-16474.

Nakamichi N, Kiba T, Henriques R, Mizuno T, Chua N-H and Sakakibara H (2010) PSEUDO-RESPONSE REGULATORS 9, 7, and 5 are transcriptional repressors in the Arabidopsis circadian clock. *Plant Cell* 22:594-605.

Nakamichi N, Kiba T, Kamioka M, Suzuki T, Yamashino T, Higashiyama T, Sakakibara H and Mizuno T (2012) Transcriptional repressor PRR5 directly regulates clock-output pathways. *Proc Natl Acad Sci U S A* 109:17123-17128.

Nakamichi N, Kita M, Ito S, Yamashino T and Mizuno T (2005) PSEUDO-RESPONSE REGULATORS, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of *Arabidopsis thaliana*. *Plant Cell Physiol* 46:686-698.

Para A, Farré EM, Imaizumi T, Pruneda-Paz JL, Harmon FG and Kay SA (2007) PRR3 is a vascular regulator of TOC1 stability in the Arabidopsis circadian clock. *Plant Cell* 19:3462-3473.

Pin PA, Zhang W, Vogt SH, Dally N, Büttner B, Schulze-Buxloh G, Jelly NS, Chia TYP, Mutasa-Göttgens ES, Dohm JC *et al.* (2012) The role of a pseudo-response regulator gene in life cycle adaptation and domestication of beet. *Curr Biol* 22:1095-1101.

Pokhilko A, Fernández AP, Edwards KD, Southern MM, Halliday KJ and Millar AJ (2012) The clock gene circuit in Arabidopsis includes a repressilator with additional feedback loops. *Mol Syst Biol* 8:574.

Pruneda-Paz JL, Breton G, Para A and Kay SA (2009) A functional genomics approach reveals CHE as a novel component of the Arabidopsis circadian clock. *Science* 323:1481-1485.

Salomé PA and McClung CR (2005) PSEUDO-RESPONSE REGULATOR 7 and 9 are partially redundant genes essential for the temperature responsiveness of the Arabidopsis circadian clock. *Plant Cell* 17:791-803.

Salomé PA, Weigel D and McClung CR (2010) The role of the Arabidopsis morning loop components CCA1, LHY, PRR7, and PRR9 in temperature compensation. *Plant Cell* 22:3650-3661.

Salter MG, Franklin KA and Whitelam GC (2003) Gating of the rapid shade-avoidance response by the circadian clock in plants. *Nature* 426:680-683.

Satbhai SB, Yamashino T, Okada R, Nomoto Y, Mizuno T, Tezuka Y, Itoh T, Tomita M, Otsuki S and Aoki S (2011) Pseudo-response regulator (PRR) homologues of the moss *Physcomitrella patens*: insights into the evolution of the PRR family in land plants. *DNA Res* 18:39-52.

Steed G, Ramirez DC, Hannah MA and Webb AAR (2021) Chronoculture, harnessing the circadian clock to improve crop yield and sustainability. *Science* 372:eabc9141.

Strayer C, Oyama T, Schultz TF, Raman R, Somers DE, Más P, Panda S, Kreps JA and Kay SA (2000) Cloning of the Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. *Science* 289:768-771.

Takata N, Saito S, Saito CT and Uemura M (2010) Phylogenetic footprint of the plant clock system in angiosperms: Evolutionary processes of pseudo-response regulators. *BMC Evol Biol* 10:126.

Tang H, Bowers JE, Wang X, Ming R, Alam M and Paterson AH (2008) Synteny and collinearity in plant genomes. *Science* 320:486-488.

Thommen Q, Pfeuty B, Morant P-E, Corellou F, Bouget F-Y and Lefranc M (2010) Robustness of circadian clocks to daylight fluctuations: Hints from the picoeucaryote *Ostreococcus tauri*. *PLoS Comput Biol* 6:e1000990.

Tiwari SB, Shen Y, Chang H-C, Hou Y, Harris A, Ma SF, McPartland M, Hymus GJ, Adam L, Marion C *et al.* (2010) The flowering time regulator CONSTANS is recruited to the FLOWERING LOCUS T promoter via a unique cis-element. *New Phytol* 187:57-66.

Turner A, Beales J, Faure S, Dunford RP and Laurie DA (2005) The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. *Science* 310:1031-1034.

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A and Coupland G (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science* 303:1003-1006.

Viana AJC, Matioli CC, Newman DW, Vieira JGP, Duarte GT, Martins MCM, Gilbault E, Hotta CT, Caldana C and Vincentz M (2021) The sugar-responsive circadian clock regulator bZIP63 modulates plant growth. *New Phytol* 231:1875-1889.

Wang L, Kim J and Somers DE (2013) Transcriptional corepressor TOPLESS complexes with pseudoreponse regulator proteins and histone deacetylases to regulate circadian transcription. *Proc Natl Acad Sci U S A* 110:761-766.

Webb AAR, Seki M, Satake A and Caldana C (2019) Continuous dynamic adjustment of the plant circadian oscillator. *Nat Commun* 10:550.

Wenkel S, Turck F, Singer K, Gissot L, Le Gourrierec J, Samach A and Coupland G (2006) CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. *Plant Cell* 18:2971-2984.

Yamamoto Y, Sato E, Shimizu T, Nakamichi N, Sato S, Kato T, Tabata S, Nagatani A, Yamashino T and Mizuno T (2003) Comparative genetic studies on the APRR5 and APRR7 genes belonging to the APRR1/TOC1 quintet implicated in circadian rhythm, control of flowering time, and early photomorphogenesis. *Plant Cell Physiol* 44:1119-1130.

Yang M, Han X, Yang J, Jiang Y and Hu Y (2021) The *Arabidopsis* circadian clock protein PRR5 interacts with and stimulates ABI5 to modulate abscisic acid signaling during seed germination. *Plant Cell* 33:3022-3041.

Yang S, Weers BD, Morishige DT and Mullet JE (2014) CONSTANS is a photoperiod regulated activator of flowering in sorghum. *BMC Plant Biol* 14:148.

Yuan L, Yu Y, Liu M, Song Y, Li H, Sun J, Wang Q, Xie Q, Wang L and Xu X (2021) BBX19 fine-tunes the circadian rhythm by interacting with PSEUDO-RESPONSE REGULATOR proteins to facilitate their repressive effect on morning-phased clock genes. *Plant Cell* 33:2602-2617.

Zeilinger MN, Farré EM, Taylor SR, Kay SA and Doyle FJ (2006) A novel computational model of the circadian clock in *Arabidopsis* that incorporates PRR7 and PRR9. *Mol Syst Biol* 2:58.

Zhang L, Chen F, Zhang X, Li Z, Zhao Y, Lohaus R, Chang X, Dong W, Ho SYW, Liu X *et al.* (2020) The water lily genome and the early evolution of flowering plants. *Nature* 577:79-84.

Zhang Y, Pfeiffer A, Tepperman JM, Dalton-Roesler J, Leivar P, Grandio EG and Quail PH (2020) Central clock components modulate plant shade avoidance by directly repressing transcriptional activation activity of PIF proteins. *Proc Natl Acad Sci U S A* 117:3261-3269.

Zhu J-Y, Oh E, Wang T and Wang Z-Y (2016) TOC1-PIF4 interaction mediates the circadian gating of thermoresponsive growth in *Arabidopsis*. *Nat Commun* 7:13692.

Internet Resources

Timetree 4, <http://www.timetree.org/> (accessed 6 April 2022).

Supplementary material

The following online material is available for this article:
Table S1 – List of PRR orthologs used for sequence analysis.

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