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Arabidopsis photoperiodic regulator CONSTANS feeds back to control the circadian clock

Human beings live in a world defined by daily cycles of light and darkness caused by the Earth's rotation around its axis. Plants, like most living organisms, have evolved internal circadian clocks that time biological processes in anticipation of these daily environmental changes (Young and Kay, 2001). The plant clock relies on core genes encoding transcription factors (TFs), which regulate each other's expression through intricate networks of interlocking transcriptional-translational feedback loops (Nohales and Kay, 2016). These loops ultimately control the expression of thousands of genes, allowing plants to adapt to daily fluctuations in light, temperature, and humidity (Covington et al., 2008). In addition to daily rhythms, the Earth experiences yearly seasonal cycles marked by longer, warmer days in spring and summer, followed by shorter, cooler days in autumn and winter. Seasonal changes in day length and temperature intensify with distance from the equator. Consequently, the ability of plants to adjust their growth and development in anticipation of seasonal changes determines their latitudinal distribution (McMillan, 1960).

Plants anticipate seasonal changes by monitoring day length (photoperiod). The molecular mechanisms underlying photoperiodic regulation of flowering time have been best characterized in *Arabidopsis*. In this species, the gene *CONSTANS* (*CO*) is a key integrator of circadian clock activity and photoreceptor signals to control photoperiodic flowering (Takagi et al., 2023). The circadian clock regulates *CO* mRNA levels, causing its expression to peak at night (Suárez-López et al., 2001). Conversely, photoreceptors influence CO protein stability (Valverde et al., 2004). As a result, during the short days of winter, *CO* expression is limited to the dark period, and due to its instability in darkness, CO protein does not accumulate. As days lengthen in spring, periods of light coincide with times when *CO* mRNA levels are relatively high. This overlap stabilizes the recently synthesized CO protein, allowing it to accumulate and promote expression of the *FLOWERING LOCUS T* (*FT*) gene, which triggers the floral transition (Valverde et al., 2004).

Light influences photoperiodic flowering in two ways: first by regulating CO protein stability and second by entraining the circadian clock that controls the waveform of *CO* mRNA expression. Light entrainment of the circadian clock is mediated by the distinct effects of light at dawn and dusk on the phase of circadian rhythms (Wang et al., 2022). However, full photoperiods also play a crucial role in establishing appropriate phase relationships between different biological rhythms throughout the year (Flis et al., 2016). While the effects of light pulses on clock components have been studied extensively, how photoperiod affects clock function in plants is less understood. In a recent article published in *Molecular Plant*, de los Reyes et al. (2024) propose the existence of a feedback loop in which CO communicates photoperiodic information back to the circadian clock system, thereby modulating its function. The authors began by characterizing the intricate relationships between circadian and photoperiodic signaling pathways by constructing a transcriptional network called CircadianFloralNet. This network integrates published chromatin immunoprecipitation sequencing data from 33 TFs involved in light signaling, circadian clock function, and flowering time regulation. Then, they integrated photoperiodic signaling into this network by developing a chromatin immunoprecipitation sequencing dataset of CO-binding sites in the *Arabidopsis* genome. They discovered that CO binds to 3214 regions across 2418 putative target genes, including light signaling genes such as *PHYTOCHROME INTERACTING FACTOR1* (*PIF1*), *PIF3*, and *PIF4*; flowering time genes like *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) and *SHORT VEGETATIVE PHASE* (*SVP*); as well as several clock genes, including *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), *TIMING OF CAB EXPRESSION1* (*TOC1*)/*PSEUDO RESPONSE REGULATOR1* (*PRR1*), *PRR5*, *PRR7*, and *PRR9*. Notably, the topological characterization of the resulting network revealed a significant and common motif representing interactions between the circadian clock and photoperiod: a "feedback loop with an output," indicating a robust interconnection between these processes. This finding aligns with their observations of mutual regulation between PRR5 and CO, as these two proteins bind to each other's gene promoters, and suggests that CO may also regulate clock function. Analyses of the transcriptomes of *35S:CO* and *SUC2:CO* plants as well as available data from the *co-10* mutant identified 70 genes regulated by CO that were common across all three datasets. Enrichment analysis of Gene Ontology terms indicated that rhythmic processes and the circadian clock were among the most prominently enriched biological processes associated with these genes. Furthermore, the authors demonstrated that the circadian period of leaf movement rhythms is lengthened in *co* mutants and shortened in *CO*-overexpressing plants, confirming that *CO* functions not only as an output of the clock but also as a regulator of its function. Interestingly, the effect of *CO* on the circadian clock, combined with the photoperiodic regulation of CO protein levels, results in the photoperiodic modulation of clock function. Indeed, the expression waveform of several clock genes was influenced by CO, particularly in the afternoon of a long day (de los Reyes et al., 2024).

To better understand how CO regulates gene expression, the authors performed an unbiased DNA motif discovery analysis. They found that CO binding peaks were enriched in a G-box motif (CACGTG), in addition to CCACA and CORE-like motifs associated previously with CO binding to the *FT* promoter (de los

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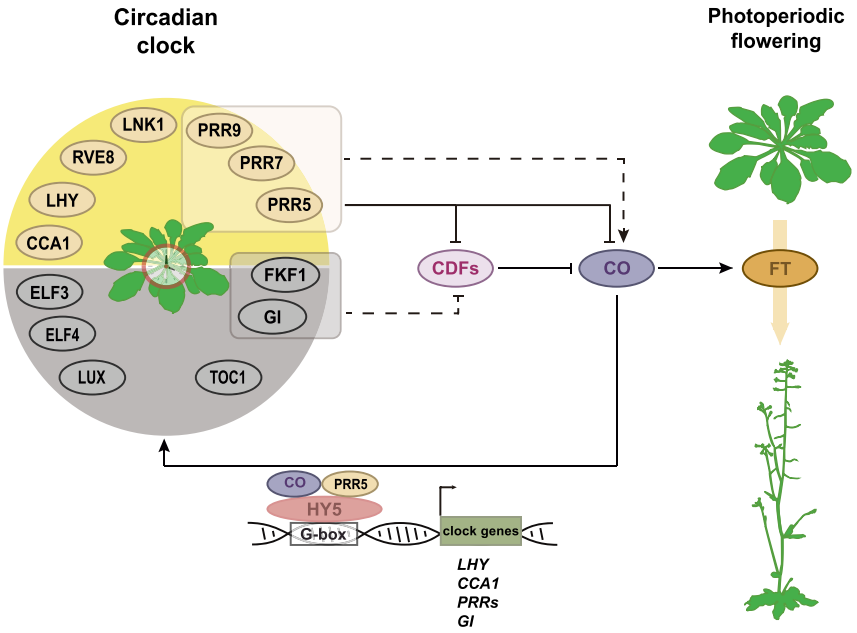

Figure 1. Schematic representation of photoperiodic regulation of clock function and flowering time.

The circadian clock is composed of three transcriptional loops: the core loop (*CCA1*, *LHY*, and *TOC1*), the morning loop (*PRR9*, *PRR7*, *PRR5*, *REVEILLE8* [*RVE8*], and *NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED GENE1* [*LNK1*]), and the evening loop (*EARLY FLOWERING3* [*ELF3*], *ELF4*, *LUX ARRHYTHMO* [*LUX*], *GIGANTIA* [*GI*], and *FLAVIN-BINDING, KELCH REPEAT, F-BOX1* [*FKF1*]). The circadian clock regulates the expression of *CO* through several mechanisms. It facilitates the degradation of CYCLING DOF FACTORS (CDFs), which repress *CO* expression, via the GI-FKF1 protein complex. At the same time, PRRs inhibit CDFs at the transcriptional level. Additionally, PRR5 binds to the *CO* promoter, adding another layer of regulation to *CO* expression. As a result of these mechanisms, *CO* mRNA levels increase during the afternoon under long-day conditions. This accumulation, combined with the stabilization of CO protein by the PRRs and the effect of light through various photoreceptors, leads to elevated levels of CO protein. Ultimately, this increase activates the transcription of the florigen *FT* and induces the floral transition. CO itself regulates the circadian clock, binding to G-box elements present in the promoter of circadian-related genes in complex with PRR5 and HY5, enhancing their expression. Solid lines indicate transcriptional regulation, while dotted lines indicate posttranslational regulation.

Reyes et al., 2024; Romero et al., 2024). The basic region/leucine zipper motif (bZIP) TF LONG HYPOCOTYL5 (HY5) has been shown to bind G-box motifs, and *hy5* mutants have been reported to alter clock function (Hajdu et al., 2018). HY5 is known to interact with some members of the B-box (BBX) protein family (Gangappa and Botto, 2014), to which CO belongs, suggesting that HY5 might act together with CO and PRR5 to regulate clock gene expression. Indeed, the authors found a strong overlap in the binding sites of these three proteins in core clock genes and observed that they interact physically *in planta*. The circadian phenotypes of *hy5*, *prr5*, and *co* mutants and *CO*-overexpressing plants indicate that CO accelerates the clock, while HY5 and PRR5 slow it down (de los Reyes et al., 2024). It appears that the binding of CO to PRR5 and HY5 may trigger a switch in the activity of the protein complex, changing it from a repressor to an activator complex that binds to G-box elements in the promoter of core clock genes at the end of a long day in spring (Figure 1).

Light and circadian regulation of the photoperiodic flowering pathway has been studied for decades, identifying CO as a central integrator of these signals in *Arabidopsis* (Romero et al., 2024). However, the mechanisms by which photoperiod regulates circadian signaling pathways have largely remained unknown until now. The work by de los Reyes et al. firmly establishes that CO is part of a novel feedback loop that connects the photoperiodic pathway with the circadian clock (de los Reyes et al., 2024). These findings provide valuable insights into how plants adjust their internal clocks in response to varying day lengths, ensuring timely physiological and developmental transitions throughout the year.

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