










CONSTANS, a HUB for all seasons: How photoperiod pervades plant physiology regulatory circuits

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Abstract

How does a plant detect the changing seasons and make important developmental decisions accordingly? How do they incorporate daylength information into their routine physiological processes? Photoperiodism, or the capacity to measure the daylength, is a crucial aspect of plant development that helps plants determine the best time of the year to make vital decisions, such as flowering. The protein CONSTANS (CO) constitutes the central regulator of this sensing mechanism, not only activating florigen production in the leaves but also participating in many physiological aspects in which seasonality is important. Recent discoveries place CO in the center of a gene network that can determine the length of the day and confer seasonal input to aspects of plant development and physiology as important as senescence, seed size, or circadian rhythms. In this review, we discuss the importance of CO protein structure, function, and evolutionary mechanisms that embryophytes have developed to incorporate annual information into their physiology.

Introduction

The correct timing for developmental and physiological processes such as growth, responses to stresses, and phase transitions is controlled by a complex network of inducing and inhibiting pathways integrating environmental and internal signals. Daylength is a robust environmental signal with major effects on life on Earth, and photoperiod sensing is the basis of many physiological decisions in animals and plants (Bradshaw and Holzapfel 2007; Gendron and Staiger 2023). An adequate seasonal decision for physiological and

developmental processes is crucial for reproductive success and crop yield, and the photoperiod pathway is the main mechanism that senses daylength in plants. The photoperiod pathway is an ancient regulatory system that is evolutionarily conserved from microalgae to vascular plants (Dring 1988; Romero and Valverde 2009; Serrano-Bueno et al. 2017).

The photoperiod signaling mechanism is present in all known plant species and involves several genes as well as a set of input and output genes (Reeves and Coupland 2000;

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Romero-Campero et al. 2013; de los Reyes et al. 2017; Serrano-Bueno et al. 2017) and is best known as the inducer of flowering in the model plant *Arabidopsis* (*Arabidopsis thaliana*). The information received from light, daylength, and the circadian clock is channeled through the photoperiod pathway to modulate the BBX transcription factor (TF) CONSTANS (CO), which is considered the key regulator gene controlling photoperiodic flowering. CO coordinates light and clock inputs in leaves to trigger the expression of the mobile florigen FLOWERING LOCUS T (FT) (Valverde et al. 2004; Abe et al. 2005; Wigge et al. 2005; Yoo et al. 2005).

Although the CO-FT module is widely conserved in plants, the output of the signal may differ. In *Arabidopsis* and several annual species, flowering is induced when the expression of CO concurs with its protein stability in the evening of a long day (LD) by activating FT production in what is known as the “coincidence model” for flowering (Valverde et al. 2004; Austen et al. 2017). However, in rice, a short-day (SD) plant, HEADING DATE 1 (Hd1), a CO ortholog, acts as a repressor in noninductive LD (Suárez-López et al. 2001; Hayama et al. 2003).

CO has been considered the key regulatory gene controlling the timing of flowering through the photoperiod, but in recent years, increasing evidence points to new roles of CO as a hub gene/protein integrating a set of inputs and outputs involved in different developmental and physiological processes, not only in the flowering transition (Romero-Campero et al. 2013). In this way, CO has a predominant role in a regulatory network that can provide daylength information to processes such as carbon metabolism (Ortiz-Marchena et al. 2014), responses to hormones (Wang et al. 2016; Xu et al. 2016), stomatal opening (Ando et al. 2013), stresses, photoprotection (Gabilly et al. 2019; Tokutsu et al. 2019; Park et al. 2023), proline synthesis (Mattioli et al. 2009), lipid metabolism (Deng et al. 2015), floral organ senescence, and abscission (Serrano-Bueno et al. 2021, 2022) and can interact with elements of the circadian clock (de los Reyes et al. 2023).

In addition to photoperiod, flowering can also be controlled by other routes: vernalization, gibberellins (GAs), age, autonomous, and ambient temperature pathways. Recent reviews have been dedicated to different aspects of photoperiodic flowering regulation (Shim et al. 2017, Kinoshita and Richter 2020; Cao et al. 2021; Takagi et al. 2023). In this review, although it is necessary to introduce the role of CO in flowering, we focus on CO functions other than photoperiodic flowering in different plant species, in providing some ideas about CO protein structure and function, and in the evolution of photoperiodic sensing. Finally, we discuss recent advances in agricultural and biotechnological applications of CONSTANS and its structural homologs, the *CONSTANS-like* (*COL*) genes.

Control of flowering by CO

In *Arabidopsis*, CO constitutes a hub in the photoperiodic flowering pathway that, at the end of a LD, triggers flowering by inducing the expression of the florigen *FT* in leaves

(Samach et al. 2000; Andrés and Coupland 2012). FT protein, and possibly its mRNA as well, moves to the shoot apical meristem to generate the “flowering complex” that induces the transition to flowering (Corbesier et al. 2007; Tamaki et al. 2007; Andrés and Coupland 2012; Shibuya and Kanayama 2014; Osnato et al. 2022; Gendron and Staiger 2023).

Regulation of CO activity in LD and SD plants

The flowering mechanism described above varies between LD and SD plants; to understand these changes, *Arabidopsis* will be shown as a model for LD plants and rice for SD plants (Fig. 1).

In *Arabidopsis*, a complex set of effectors cooperate to finely regulate CO transcript and protein levels throughout the day in a light-dependent manner (Fig. 1A). Thus, superimposed on the circadian regulation that produces the CO mRNA peak at the middle of the dark period (Suárez-López et al. 2001), the regulators CYCLING DOF FACTORS (CDFs) and TOPLESS (TPLs) repress CO expression and therefore keep mRNA levels reduced at the end of the night and during the morning and afternoon of a LD (Goralogia et al. 2017). In the evening, however, the CO mRNA level increases as the repression is released due to the blue light-dependent, GIGANTEA (GI)/FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1)-mediated degradation of the repressive elements (Sawa et al. 2007), while activators such as TEOSINTE BRANCHED1/CYCLOIDEA/PCF (TCPs) (Kubota et al. 2017), FLOWERING BHLHs (FBHs) (Ito et al. 2012), and JUMANJI28 (JM28) (Hung et al. 2021) intervene to trigger transcription (Fig. 1A, LD). CO protein content is also finely regulated throughout the day. Early in the morning, a peak of CO protein is present, probably due to the stabilizing effect of its interaction with FK506 BINDING PROTEIN 12 (FKBP12) (Serrano-Bueno et al. 2020). During midday and afternoon, protein levels are kept low as red-light dependent, PHYTOCHROME B (PHYB)-mediated phosphorylation of CO favors its degradation by the ubiquitin-ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES1 (HOS1) (Lázaro et al. 2012, 2015), while elements such as ZEITLUPE (ZTL) counteract the stabilizing effect of PSEUDO RESPONSE REGULATORS (PRRs) proteins (Hayama et al. 2017). In addition, the circadian clock-component EARLY-FLOWERING 3 (ELF3) directly interacts with CO and promotes its degradation (Song et al. 2018). When evening arrives, the CO content increases as the mRNA level rises, coinciding with a stabilization of the synthesized protein due to PHYTOCHROME-DEPENDENT LATE-FLOWERING (PHL) impairment of PHYB action (Endo et al. 2013), far-red light photoreceptor (PHYA) stabilization (Valverde et al. 2004), and blue light photoreceptors FKF1 and CRYPTOCHROMES 1 and 2 (CRY1 and CRY2) inactivating the degradation of SUPPRESSOR OF *phyA*-105/CONSTITUTIVE PHOTOMORPHOGENIC 1 (SPA1/COP1) complex (Holtkotte et al. 2017). This inactivation does not take place in the absence of light; therefore, during the night, the SPA/COP1 ubiquitin ligase complex is active in the nucleus, and phosphorylated CO is degraded (Jang et al. 2008; Sarid-Krebs et al. 2015). Under SD, the genes that release the inhibition of CO transcription are not expressed in the late

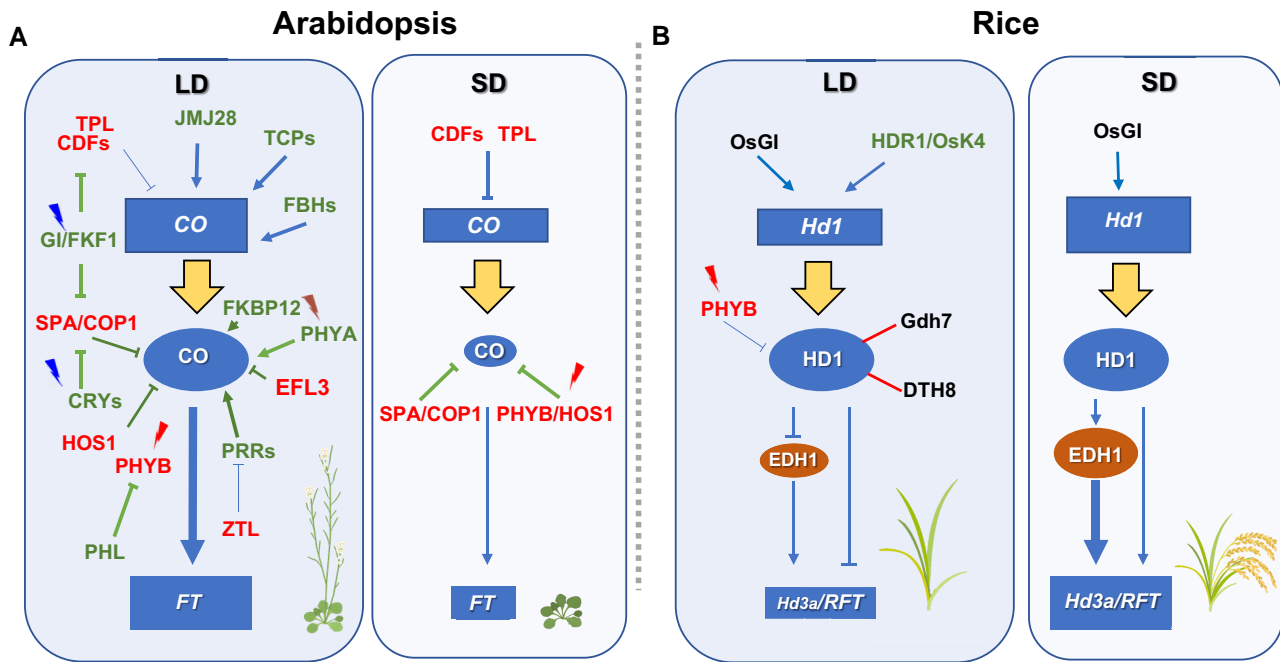


Figure 1. Regulation of flowering in LD and SD plants. Arabidopsis **A**) and rice **B**) were chosen as models. Arrows indicate positive effects, and blunt ends indicate negative effects. Red rods indicate functional interactions. Rectangles denote gene expression and ovals protein content. Green indicates positive effectors, and red indicates negative effectors. Green lines indicate protein effects, and blue lines indicate transcriptional effects. Blue, red, and brown-orange sparks represent blue, red, and far-red light, respectively.

afternoon/evening; therefore, the repressive elements keep CO mRNA levels low at that point (Fig. 1A, SD). CO protein is degraded in the morning by the PHYB/HOS1 pathway and in the dark by the SPA/COP1 ubiquitin ligase complex, as described above. Therefore, the absence of CO protein is one of the main causes of the late-flowering phenotype in SD. However, CO is also able to inhibit flowering under SD through activation of the repressor *TERMINAL FLOWER 1* (*TFL1*). Therefore, CO mediates flowering via *FT* in LD and delays it via *TFL1* in SD (Luccioni et al. 2019).

In rice, a model for SD plants, *Hd1* directly affects the expression of the florigen gene *HEADING DATE 3a* (*Hd3a*) (*FT* ortholog) (Kojima et al. 2002) and its regulator *EARLY HEADING DATE 1* (*Edh1*, not found in Arabidopsis) (Doi et al. 2004) in both light regimes (Fig. 1B). *Hd1* expression is under circadian regulation by *OsGI*, and the mRNA peaks in the middle of the dark period (Tsuji et al. 2011). In LDs, *Hd1* expression is activated by the *HEADING DATE REPRESSOR 1* (*HDR1*)/*OsK4* complex (Sun et al. 2016) and red light-modified *Hd1* protein (McGarry and Ayre 2012), which interacts with *GRAIN NUMBER, PLANT HEIGHT AND HEADING DATE 7* (*Gdh7*) and *DAYS TO HEADING 8* (*DTH8*) to negatively regulate *Hd3a* expression (both directly and through the repression of *Edh1*); this then leads to flowering inhibition (Fig. 1B, LD) (Du et al. 2017). Conversely, in SD, when *Gdh7* and *DTH8* are not present, *Hd1* induces flowering by activating *Hd3a* and *Edh1* expression (Fig. 1B, SD) (Zong et al. 2021).

Photoperiod and GA pathway

It has been shown that photoperiod and GAs cooperate to modulate flowering under LDs. GAs function as essential growth regulators that mediate diverse aspects of plant development. DELLA proteins are key regulators of GA signaling and control flowering by modulating the expression of *FT* in a CO-dependent manner. Under LD, the DELLA-CO physical interaction is disrupted by GA-dependent degradation of DELLA, which allows CO to induce flowering (Wang et al. 2016). Additionally, it was reported that *REPRESSOR OF GA1-3* (*RGA*) represses the interaction of CO-NFYB in a dose-dependent manner, which is required for CO-mediated activation of *FT* in vivo, so that tight regulation of the DELLA dose may also be critical for flowering (Xu et al. 2016; Conti 2017). DELLAs also recruit the repressor of flowering time *BOTRYTIS SUSCEPTIBLE1 INTERACTORS* (*BOIs*) in chromatin positions normally occupied by CO. *BOIs* can interact with CO via the CCT domain, which probably interferes with its DNA binding activity (Conti 2017).

Stress response and flowering

Photoperiod has been associated with several stress conditions, and in many of these, CO or a *COL* gene is involved.

Flowering is induced by drought stress as a drought escape response (DE). The phytohormone abscisic acid (ABA) plays a pivotal role in mediating certain drought adaptive mechanisms. In Arabidopsis, *FT* and *TWEEN SISTER OF FT* (*TSF*) mRNA

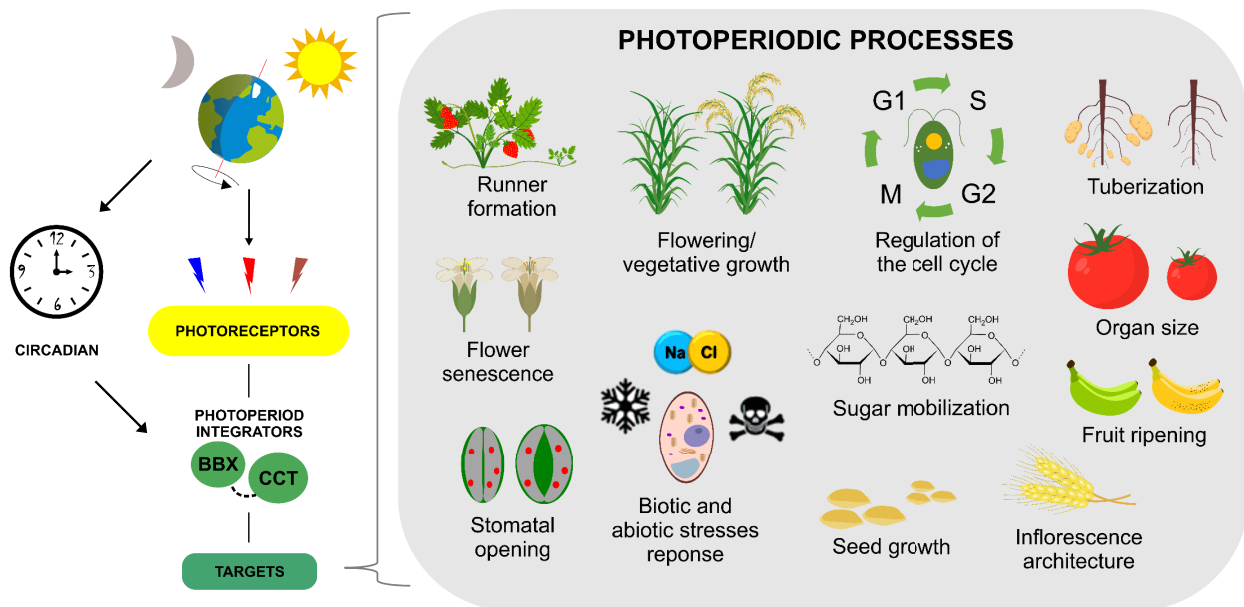


Figure 2. Model of photoperiod reception and response in plants. Seasonal variations in daylength are first sensed by photoreceptors (e.g. cryptochromes, phytochromes) and the circadian clock. This information is transmitted to photoperiod integrators containing B-box and/or CCT elements, such as CO and PRR proteins. These integrators then activate specific targets, which can act as activators or repressors in several photoperiodic processes.

accumulation in response to water deficit only occur under LD conditions, reflecting the photoperiodic nature of DE (Riboni et al. 2013). In addition, the positive regulator of ABA signaling EID1-like protein 3 (EDL3) is also an activator of CO expression. The ABA signaling cascade ends with the phosphorylation of ABA-responsive TFs (ABFs), which allow ABA/stress-response gene transcription by direct binding of ABA-responsive elements (AREB) on their promoters. Triple *abf2/3/4* and quadruple *abf1/2/3/4* mutants show late-flowering phenotypes, which reduces CO expression and its transcriptional activator *FBH3*. This fact reveals that ABFs activate CO expression to induce flowering under drought conditions. ABA also promotes GI-dependent florigen activation. An interplay between GI and CO is necessary for *FT* activation under drought stress because drought or ABA alone cannot activate *FT* expression in *co* mutants. In contrast, *TSF* can be activated in *co* mutants under water deficit conditions in a GI-dependent manner. This fact indicates that in some cases, the interplay between GI and ABA is sufficient to promote florigen expression (Riboni et al. 2014; Martignago et al. 2020). However, the contribution of ABA signaling in the floral transition is still controversial, as the essential transcription factor of ABA signaling ABSCISIC ACID-INSENSITIVE 3 (*ABI3*) interacts with the CO CCT domain, which hinders CO binding to the *FT* promoter. This could explain why *abi3* mutants are early flowering under SD and LD but *ABI3* overexpression results in an increased vegetative phase under LD (Riboni et al. 2014; Hong et al. 2019). As ABA negatively regulates *ABI3* by mediating its ubiquitination and proteasome-dependent degradation, it could also allow *FT* upregulation by CO through *ABI3* degradation (Zhang et al. 2005; Conti 2017).

High and low ambient temperatures also affect flowering. Under warm ambient temperatures, the DELLA-regulated TF PHYTOCHROME INTERACTING FACTOR 4 (*PIF4*) binds to the *FT* promoter and contributes to its activation in cooperation with CO. When *PIF4* interacts with DELLA proteins, it loses its DNA-binding ability. Therefore, GAs may broadly impact how plants sense variations in temperature, which translates into changes in flowering time, through modulating the interaction between DELLA and *PIF4* or other *PIF*-like TFs (Conti 2017). On the other hand, the small BBX proteins *BBX28* and *BBX29* interact with CO to improve transcriptional activation of *FT*, especially at low ambient temperatures (Wang et al. 2021).

Nonflowering functions of CO

As photoperiodic hubs, CO homologs can play several different functions (Fig. 2). In fact, CO orthologs existed as early as in microalgae, and different roles have been assigned to them.

Role in growth and metabolism

The chlorophyte alga *Chlamydomonas reinhardtii* expressing high levels of the CO ortholog *CrCO* shows alterations in several circadian output processes, such as the onset of the expression of genes that regulate the cell cycle (Serrano et al. 2009). In addition, *CrCO* RNAi strains showed accumulation of lipids and triacyl glycerides, whereas *CrCO* overexpression resulted in the opposite effect, suggesting a possible role in the regulation of lipid accumulation (Deng et al. 2015). Interestingly, *CrCO* and the nuclear TF *CrNF-Ys* also form a

complex in *Chlamydomonas* that regulates light-dependent photoprotective responses (Gabilly et al. 2019; Tokutsu et al. 2019). CrCO controls the capacity for rapidly reversible non-photochemical quenching via cis-regulatory CrCO-binding sites at key photoprotection genes, while SPA1 and CUL4, components of a conserved E3 ubiquitin ligase complex, regulate the repression of the same genes in low light. In this unicellular alga, it has also been described that CrCO can alter *GRANULE BOUND STARCH SYNTHASE* (*CrGBSS*) transcript levels (Serrano et al. 2009). Similarly, CO regulates the level and timing of expression of the *GBSS* gene in *Arabidopsis* so that the photoperiod modification of starch homeostasis is crucial to promote the sugar mobilization demanded by the floral transition (Ortiz-Marchena et al. 2014). Furthermore, the effect of CO on *GBSS* could also contribute to the florigenic signal by coordinating the induction of flowering by photoperiod and carbon mobilization (Ortiz-Marchena et al. 2015).

Role in stomatal function

CO has a positive effect on stomatal opening in plants. A constitutive open-stomata phenotype was observed in CO-overexpressing plants with associated changes in the transcription of *FT* and *TSF*, while mutations in *GI*, *CO*, *FT*, and *TSF* suppressed stomatal opening induced by light (Ando et al. 2013). There is a strong parallelism between the mechanisms regulating photoperiodic flowering and stomatal aperture, both of which are based on the temporal expression pattern of *FT* in response to CO activity. In guard cells, low levels of *FT* in SD correlated well with a change in stomatal aperture, and the pattern of stomatal closure at night and rapid opening just before dawn observed in LD conditions is lost (Hassidim et al. 2017).

Biotic and abiotic stress

Photoperiodic control of biotic and abiotic stress responses involving CO homologs has been widely documented. The accumulation of the banana (*Musa acuminata*) *MaCOL1* transcript was enhanced by abiotic and biotic stresses, such as chilling and pathogen *Colletotrichum musae* infection (Chen et al. 2012). Overexpression of mango *MiCOL16A* and *MiCOL16B* in *Arabidopsis* improved salt and drought tolerance, conferring longer roots and higher survival rates under drought and salt stress, but did not significantly affect the floral transition (Liu et al. 2022). Similar results were recently found in soybean, where *GmCOL1a*-overexpressing plants exhibited enhanced salt and drought tolerance, with higher relative water levels, greater proline concentrations, and lower malondialdehyde levels. Reduced reactive oxygen species (ROS) production compared with wild-type plants was also observed, showing opposite phenotypes in the *GmCOL1a* knockout mutant (Xu et al. 2023).

Role in flowers and fruits

CO functions are not limited to vegetative growth; roles in reproductive tissues have also been described. Recently, a novel function of CO in promoting flower senescence and abscission

by augmenting jasmonate (JA) signaling and response in *Arabidopsis* petals has been described (Serrano-Bueno et al. 2022). In this case, mutations in CO amino acids (NIKY motif) that conferred the interaction with JAZ3 prevented JA signaling but had no effect on flowering time. Additionally, a key role of CO in the photoperiod control of plant seed size has been described (Yu et al. 2023) in *Arabidopsis* and soybean. In these species, CO directly repressed the seed development negative regulatory gene *APETALA2* (*AP2*) in the photoperiod favorable for reproductive growth by regulating seed coat epidermal cell proliferation in a maternal-dependent manner. Likewise, the accumulation of *MaCOL1* transcripts in banana pulp increased during natural or ethylene-induced fruit ripening, suggesting that *MaCOL1* might be associated not only with biotic and abiotic stresses but also with pulp ripening of the fruit (Chen et al. 2012).

Role in tuber and runner formation

CO activity has been reported in reproductive structures other than the flower. The potato (*Solanum tuberosum*) CO-like gene *StCO* affects, in a photoperiod-dependent manner, the regulation of tuber induction. Potato plants overexpressing *StCO* tuberized later than wild-type plants under a weakly inductive photoperiod, while *StCO* silencing promoted tuberization under both repressive and weakly inductive photoperiods but did not have any effect under strongly inductive SDs (González-Schain et al. 2012). Overexpression of *Arabidopsis AtCO* in potato impaired tuberization in SD inductive conditions; however, *AtCO*-overexpressing lines required prolonged exposure to SD to form tubers (Martínez-García et al. 2002).

The interaction between CO and members of the *CETS* (*CENTRORADIALIS*; *TFL1*; and *SELF-PRUNING*) gene family is observed in the tuberization process in potato. The mobile signal responsible for inducing tuberization (so-called tuberigen) is a protein encoded by the *SELF PRUNING 6A* (*StSP6A*) gene, an FT homolog (Navarro et al. 2011). Under noninductive LD conditions, *StSP6A* expression is downregulated by another FT homolog, known as *StSP5G*, which in turn is directly activated by the *StCOL1* protein (homolog of CO), repressing tuberization (Abelenda et al. 2016).

The ortholog of CO in the perennial rosaceous model species woodland strawberry, *FvCO*, has an opposite role in the control of flowering and vegetative reproduction through runners (Kurokura et al. 2017). *FvCO*-overexpressing plants produced a higher number of new inflorescences than wild-type plants, whereas runner production was significantly suppressed. In contrast, RNAi lines continuously produced new runners, and inflorescence production was reduced. Taken together, these opposite roles indicate that *FvCO* affects the balance between vegetative and generative development in strawberries.

Photoperiodic regulation by CO and COLs in different species

Several works have identified and studied COL proteins with a protein structure close to that of CO (B-boxes, intermediate

Table 1. CO homologs from several families and their main functions

Family	Species	CO/COL homologue	Function	Non-CO/COL-homologues with functional equivalence to AtCO	Cultivar variability	Reference
<i>Poaceae</i>	Rice	Hd1	Flowering repressor in LD Flowering activator in SD	Module GHD7-Ehd1	Local adaptation	McGarry and Ayre 2012 Kojima et al. 2002
	Maize	CONZ1		ZmPRR37 Module GHD7-Ehd1	Domestication from teosinte	Miller et al. 2008
	Sorghum	SbCO	Flowering activator in SD	SbPRR37	Energy cultivars	Yang et al. 2014
	Barley	HvCO1/2	Flowering activator in LD	Module GHD7-Ehd1 Ppd-H1	Sensitivity for photoperiod	Campoli et al. 2012
	Wheat	TtCO1/2	Flowering repressor in SD	HvPRR37 PPD1 TtPRR37 BvBBX19 + BTC1	Sensitivity for photoperiod	Shaw et al. 2020
<i>Amaranthaceae</i>	Sugar beet	BvCOL1				Dally et al. 2014
<i>Vitaceae</i>	Grapevine	VvCO	Increase fruitfulness in LD			Almada et al. 2009
<i>Theaceae</i>	Tea-oil tree	CoCO	Flowering			Guo et al. 2022
<i>Magnoliaceae</i>	Chinese tulip tree	LcCO/ LcCOL3	Flowering			Cui et al. 2023
<i>Salicaceae</i>	Poplar	CO1/2	Tree size			Hsu et al. 2011
<i>Rosaceae</i>	Peach	PpCO	Flowering			Zhang et al. 2015
	Strawberry	FvCO	Flowering repressor in LD	TFL1	Perpetual flowering	Muñoz-Avila et al. 2022
	Rose	RcCO/COL4	Activator LD/ Activator SD	TFL1	Perpetual flowering	Lu et al. 2020
<i>Solanaceae</i>	Potato	StCO	Tuberization repressor in LD			Abelenda et al. 2016
	Tomato	SICO1	Flowering repressor in LD			Cui et al. 2022
<i>Fabaceae</i>	Soybean	COL1a/b	Flowering repressor in LD	E1	Loss of photoperiodic sensitivity	Cao et al. 2015
	Common bean	PvCOL2	Flowering repressor in LD	E1	Loss of photoperiodic sensitivity	González et al. 2021

Summary of CO or COL homologs (underlined) identified in other species from several families and their primary function. Alternative genes performing photoperiodic-flowering regulation on each species are listed, as well as the contribution of those genes to the cultivar variability observed to date.

domain, and CCT domain) in many species (Table 1). They include diverse taxonomic groups, from microalgae such as *C. reinhardtii* (Serrano et al. 2009) to trees such as *Prunus persica* L. and *Liriodendron chinense* (Zhang et al. 2015; Cui et al. 2023). Furthermore, ornamentals such as *Petunia hybrida* and *Pharbitis nil* (Liu et al. 2001; Hayama et al. 2007; Khatun et al. 2021) and economically important crops such as the cereals rice, barley, wheat, maize, and sorghum (Kojima et al. 2002; Miller et al. 2008; Campoli et al. 2012; Yang et al. 2014; Shaw et al. 2020); cotton (Cai et al. 2017); hemp (Pan et al. 2021); tomato (Yang et al. 2020); grapevine (Almada et al. 2009); and strawberry (Kurokura et al. 2017) also show the involvement of COLs in development.

Crops

Although highly conserved, the mechanisms by which COL genes operate vary among species. We have already discussed the different flowering behaviors between an LD plant such as *Arabidopsis* and a SD plant such as rice (Fig. 1). In rice, Hd1

also controls rice flowering through a monocot-specific pathway (Nemoto et al. 2016), which is regulated by the GHD7-Ehd1-Hd3a/RFT1 module. Under noninductive LD conditions, Hd1 and Ghd7, CCT domain proteins unique to monocots, interact and form a complex that binds to a cis-regulatory region of the *Ehd1* gene and represses its expression (Nemoto et al. 2016). *Ehd1* always acts as an inducer of florigen genes (*Hd3a* in SD or *RFT* in LD), and its mRNA level is regulated by multiple elements under different photoperiod conditions, such as its expression activation by blue light in the morning (Doi et al. 2004; Tsuji et al. 2011). *Ehd1* is functionally similar to *Arabidopsis* CO, although at the structural level, it is different: while CO is a BBX protein similar to Hd1, *Ehd1* is a type-B Response Regulator (RR) without a clear *Arabidopsis* ortholog (Doi et al. 2004; Lee and An 2015).

Ehd1 and *Ghd7* are also involved in the photoperiodic response of other monocot crops, such as maize and sorghum, together with the *PRR37* gene (Yang et al. 2014; Stephenson et al. 2019; Zhao et al. 2023). *PRR* genes encode proteins that contain

2 conserved regions, a response regulator receiver (REC) and a CCT domain (Mizuno and Nakamichi 2005). In energy sorghum (*Sorghum bicolor*), light and the circadian clock regulate the expression of *SbPRR37*, which is a floral repressor and inhibits *SbCO*, a floral activator, resulting in delayed flowering under LD conditions. In SD, *SbCO* induces *SbEhd1* and its targets, *SbCN8* and *SbCN12* (*FT* homologs), promoting flowering (Yang et al. 2014). As in sorghum, in maize (*Zea mays* ssp. *mays* L.), a day-neutral plant domesticated from the SD plant teosinte (*Z. mays* ssp. *parviglumis*), there is also a *ZmPRR37* that controls flowering through *ZCN8* (*FT* homolog) (Zhao et al. 2023). *CONZ1* is the *CO* homolog in maize (Miller et al. 2008), but even when it has a different expression pattern in LD and SD, its function seems more associated with the circadian clock (Minow et al. 2018). *ZmCCT10* has also been identified as a major QTL controlling photoperiod sensitivity in maize (Stephenson et al. 2019).

Temperate cereals, such as barley (*Hordeum vulgare*) and wheat (*Triticum* spp.), exhibit a strong interaction between photoperiod and vernalization pathways (Fernández-Calleja et al. 2021). The photoperiodic flowering pathway is mainly regulated by a *PRR* gene, known as *Photoperiod-H1* (*Ppd-H1*) in barley and *PHOTOPERIOD1* (*PPD1*) in wheat, which activate *VRN-H3/VRN3* expression and consequently promote flowering (Turner et al. 2005; Beales et al. 2007). In addition, these *PRR* genes also control agronomic traits such as leaf size and inflorescence architecture in barley and wheat, respectively (Boden et al. 2015; Digel et al. 2016; Errum et al. 2023).

As in cereals, sugar beet also recruits a *PRR* gene to regulate photoperiodic flowering, known as *BOLTING TIME CONTROL 1* (*BTC1*), but its regulation depends on another gene, *BvBBX19* (Dally et al. 2014; 2018). *BTC1* encodes a *PRR3/7* with homology to the barley *Ppd-H1* (Turner et al. 2005; Pin et al. 2012), while *BvBBX19* encodes a DOUBLE B-BOX TYPE ZINC FINGER protein with 2 B-box domains that act in epistasis to *BTC1* (Dally et al. 2014). These genes regulate the activity of 2 *FT* homologs, *BvFT1* (a floral repressor) and *BvFT2* (a floral inducer) (Pin et al. 2010; Dally et al. 2014, 2018). Thus, a model was proposed in which *BvBBX19* (BBX domain) and *BTC1* (CCT domain) form a heterodimer in vivo and acquire a *CO* function to regulate their targets, repressing *BvFT1* and activating *BvFT2* expression (Dally et al. 2014, 2018).

Trees and herbaceous species

Although *CO* has been extensively studied in grasses and annual Eudicot plants, recent efforts have been made to understand the role of *CO* and *COL* genes in woody or semiwoody species. These species have a longer life cycle and relatively complicated reproductive processes (Sun et al. 2022), and *CO* proteins seem to act as an important factor that integrates photoperiodic signals and circadian rhythms. In grape (*Vitis vinifera*), bud fruitfulness is increased in LD, where the amplitude of *VvCO* (homolog of Arabidopsis *CO*) expression is greater. Importantly, the temporal and spatial coexpression of *VvCO*, *VFY* (ortholog of *AtLFY*), and *VvMADS8* (ortholog of *SOC1*) in latent buds suggests a role in the seasonal periodicity of

flowering in grapevines (Almada et al. 2009). In peach (*Prunus persica* L.), *PpCO* and *PpFT* were identified, and their sequences have high homology with those of Arabidopsis (Zhang et al. 2015). They are expressed when vegetative growth is interrupted by flowering signals (Penso et al. 2020).

In the tea-oil tree *Camellia oleifera*, *CoFT1* showed diurnal rhythm- and photoperiodic-dependent expression (Lei et al. 2017). Analysis of photoperiodic sensitivity showed that an increase in light exposure promoted earlier flowering, and the circadian clock may also have a role (Yan et al. 2022). Importantly, transcriptome analysis of floral initiation from old leaves revealed that the circadian rhythm interacted with the photoperiod pathway to induce floral initiation, and the Arabidopsis *CO* homolog (*CoCO*) was identified as one of the key genes involved (Guo et al. 2022). In *Liriodendron chinense*, a rare relict plant that has tulip-like flowers and is now popularly used as an ornamental tree species, *LcCO* and *LcCOL3* displayed seasonal expression patterns (low expression levels in the middle of the year) (Cui et al. 2023).

A previous report showed that the *PtCO2/PtFT1* hub controlled the onset of reproduction and seasonal growth cessation in poplar, a model perennial woody plant (Böhlenius et al. 2006). However, the overexpression of *PtCO1* and *PtCO2* individually or together affects poplar tree size without impacting reproductive onset, spring bud break, or fall dormancy (Hsu et al. 2011). All these examples illustrate the complexity of the control of photoperiod-dependent flowering in trees because photoperiod affects not only the transition from vegetative to reproductive phase, as in herbaceous plants, but also bud dormancy (Hussain et al. 2022). These 2 complex developmental processes directly influence the year-round cyclic regulation of flowering, which varies between trees adapted to different climates (Sun et al. 2022).

The photoperiod-dependent flowering of commercial strawberry (*Fragaria × ananassa* Duch.) and the diploid model woodland strawberry (*Fragaria vesca* L.) is regulated by the systemic antiflorigen *FveTFL1*, an *AtTFL1* homolog that represses flowering in LD (Gaston et al. 2021). Mutation of the *FveTFL1* gene leads to a perpetual flowering phenotype; that is, the plants bloomed independently of daylength (Koskela et al. 2012). *FvCO* is an important regulator of photoperiodic flowering and runner formation in strawberries (Kurokura et al. 2017), acting as an activator of the *FT1-SOC1-TFL1* module (Muñoz-Avila et al. 2022). *FvCO* induced *FvFT1* expression, which in turn activated *TFL1* expression, repressing flowering. However, in the *tff1* mutant background, *FvFT1* functions as a promoter of flowering (Koskela et al. 2012; Nakano et al. 2015; Kurokura et al. 2017). Moreover, other strawberry *COLs*, *FvCO3* and *FvCO5*, could bind to the promoter of *FvFT1* and activate its expression (Zhao et al. 2022). Thus, in strawberry, *CO* and *COLs* are versatile photoperiod-dependent flowering regulators that can act as inhibitors or activators of flowering depending on the *TFL1* allele present in the plants.

Interestingly, plants of the genus *Rosa* harboring the functional allele of *TFL1* also required SD to bloom (Bendahmane et al. 2013). In the popularly known China rose (*Rosa*

chinensis) *tfl1* mutant, flowering time is regulated by a balance between 2 CO genes, *RcCO* and *RcCOL4*, whose expression levels are affected by daylength. Under LD conditions, *RcCO* expression increased and promoted *RcFT* activation by binding directly to its promoter. In contrast, *RcCO* mRNA levels were reduced in SD, but *RcCOL4* was upregulated and physically interacted with *RcCO*, which increased the *RcCO* transcriptional activity of *RcFT* and consequently promoted flowering. This balance in expression levels and protein interactions contributes to day-neutral flowering in modern roses (Lu et al. 2020). However, as the *TFL1* gene is also a limiting factor for the photoperiodic response in roses, it is possible that the CO/FT/TFL1 hub acts similarly in rose and strawberry.

Other crops

SISP5G is an important component in the photoperiod pathway of tomato (*S. lycopersicum*), along with another FT homolog, *SIFTL1* (Song et al. 2020). Wild tomato relatives exposed to LD exhibited increased *SISP5G* expression, which was accompanied by repression of the florigen *SINGLE FLOWER TRUSS* (*SFT*), *FT* ortholog, and consequently late flowering (Soyk et al. 2017). Conversely, in SD, *SFT* expression is induced by *FLL1*, resulting in early flowering (Song et al. 2020). Mutations in both loci (*SP5G* and *FLL1*) during tomato domestication reduced the tomato response to photoperiod (Song et al. 2020) and allowed its cultivation at different latitudes.

Interestingly, all the species mentioned thus far recruit at least 1 protein with a C-terminal CCT domain to coordinate its photoperiodic response, which suggests that CCT is a key domain for plants to perceive changes in daylength, via either a PRR or a COL protein (Fig. 2). However, in leguminous species such as soybean and common bean, there is a specific gene called *E1* that has a strong impact on photoperiodic regulation and does not contain a CCT domain (Table 1). This gene encodes a protein that contains a putative bipartite nuclear localization signal and a region related to the DNA-binding B3 domain and acts as a transcriptional repressor of several florigen (*FT-like*) genes (Xia et al. 2012). In soybean, *E1* expression is upregulated under LD conditions in a process dependent on the photoreceptor *GmPHYA* and represses the flowering activators *GmFT2a* and *GmFT5a* (Xia et al. 2012). Nonetheless, *E1* expression can also be modulated by soybean *COL* genes through a negative feedback loop (Cao et al. 2015); that is, although the *E1* protein does not have a CCT domain, it is regulated by proteins that have this domain.

Control of flowering in legumes shows differences in the molecular mechanisms controlling photoperiodic flowering. In *Medicago truncatula*, *CO* and *COL* genes seem not to have a central role in photoperiodic flowering or have lost it (Wong et al. 2014). It has been shown that *MtSOC1c* regulates flowering in *M. truncatula*, suggesting that *SOC1* proteins, and not *CO*, have a crucial role in flowering time (Fudge et al. 2018; Yuan et al. 2023). Besides, González et al. (2021) showed that the partial loss of the photoperiodic response in common bean (*Phaseolus vulgaris*) was due to

mutations in *PvCOL2*, which acts downstream of the photoreceptor *PHYA3*, possibly in a parallel mechanism to the legume-specific *E1* gene, repressing multiple *FT* genes in LD. Bean insensitivity to photoperiod is also associated with mutations in the *PHYA3* gene (Weller et al. 2019). Thus, based on the frequencies and distributions of *PvCOL2* and *PHYA3* haplotypes, González et al. (2021) proposed a sequential loss of photoperiod sensitivity during bean domestication, which started with the selection of mutations for *PvCOL2* and later for *PHYA3*.

All these examples show that photoperiod roughly involves a series of elements that are depicted in Fig. 2. Seasonal information is detected by photoreceptors and the clock and sent to photoperiod integrators that contain CCT and B-box elements. These elements then act through targets such as mobile signals (*FT*, *SFT*) or independent pathways (*E1*, *EhD1*) to trigger a plethora of photoperiodic responses. In fact, photoperiodic responses were originally present in species that were adapted to different latitudes through domestication, such as cotton (Song et al. 2017), tomato (Soyk et al. 2017), maize (Minow et al. 2018), barley (Fernández-Calleja et al. 2021), sorghum (Yang et al. 2014), and common bean (González et al. 2021). In these species, the wild ancestors were responsive or more sensitive to the photoperiod control of flowering, while cultivated species and cultivars retained only a residual photoperiod response, likely allowing them to adapt to diverse climates.

Structural characteristics of the CONSTANS protein

Both the central role of *CO* in gene networks and the widespread changes caused by its mutation are features characteristic of hub proteins (Vandereyken et al. 2018), and the structural information available for *CO*, albeit limited, is consistent with this idea. The *CO* monomer (373 aa residues) presents a tandem of B-Box zinc finger domains coordinating 4 Zn^{2+} cations (*BBX*) that correspond to the first 110 residues of the polypeptide chain; another 75 residue-long DNA-binding domain (*CCT*) is located at the C-terminal end (Fig. 3). These 2 distinctive regions are linked by a large central stretch of approximately 200 aa residues (Valverde 2011) predicted to be intrinsically disordered both by AlphaFold 2 (Jumper et al. 2021, <https://alphafold.ebi.ac.uk/>) and ColabFold (Mirdita et al. 2022). While the 2 amino and carboxyl domains are highly structured and have been successfully crystallized, the central, nonorganized domain, or the complete *CO* protein, have not.

The structure of the *BBX* domain was independently established by 2 groups following essentially the same experimental approach: expression of the first 110 residues of *CO* in *E. coli* followed by purification and crystallization of the expressed polypeptide. The experimental evidence shows that in *BBX*, 2 of the Zn^{2+} cations are coordinated by 4 cysteines (C20, C23, C40, C43 and C63, C66, C83, C86), whereas the other 2 are coordinated by *CDH2* motifs (C32, D35, H48,

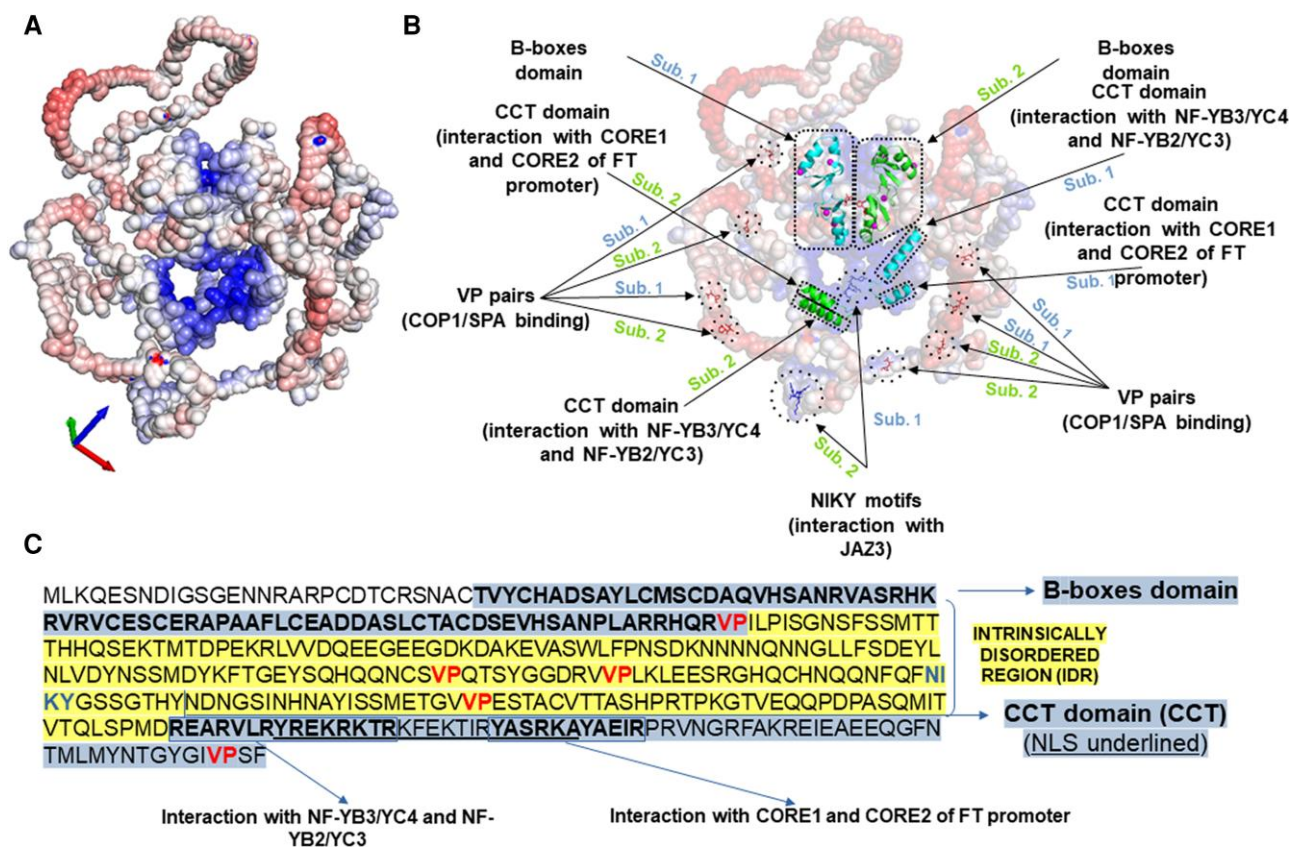


Figure 3. Structure of the CO protein. **A**) Structural model for the dimer of *Arabidopsis thaliana* CO predicted by ColabFold overlapped with the electrostatic potential. **B**) Location of the different domains and motifs identified in CO in the previous dimeric structure (subunit 1 in blue, subunit 2 in green). **C**) CO amino acid sequence domains and motifs shown in the 3D models. Graphical visualizations were obtained with PyMOL.

H57 and C75, D78, H91, H100) (Dahal et al. 2022; Zeng et al. 2022). Structural data for this domain can be found at <http://doi.org/10.2210/pdb7wsj/pdb> and <http://doi.org/10.2210/pdb7VSQ/pdb>.

The DNA-binding region at the C terminus of CO contains a domain that is also present in other COL and PRR (including TOC1) proteins, CONSTANS-COL1-TOC1 or CCT. A CCT is also present in the NF-YA subunit of the heterotrimeric TF that binds CCAAT elements in eukaryotic promoters, composed of a histone fold domain (HFD) dimer (subunits NF-YB and NF-YC) and NF-YA, which is responsible for the DNA sequence specificity of the complex (Wenkel et al. 2006). CO CCT can physically interact with the HFD of NF-Y, yielding a complex (NF-CCT) that efficiently binds the core element of the *FT* promoter in *Arabidopsis* (Gnesutta et al. 2017). It was also shown that the cis-elements CORE1, CORE2, the P1, and P2 of the *FT* promoter are direct binding sites of CO that recognize a TGTG motif. These authors determined the crystal structure of the NF-CCT trimer in complex with CORE2 and CORE1 DNA (Lv et al. 2021) and reported that the *FT* promoter could bind multiple NF-CCT complexes, which suggests that the B-boxes mediate a multimeric assembly of NF-CO that might help CO bind to the *FT* promoter with high affinity and specificity.

Much less structural information is available for the large central region located between the BBX and CCT domains, a 193-residue region that shows features of intrinsically disordered proteins (IDPs): (1) low abundance of bulky hydrophobic and aromatic aa (23.8% of Leu, Ile, Val, Trp, Phe, Tyr, Met), (2) abundance of “simpler” uncharged aa (43% of Ser, Pro, Gly, Gln, Asn), (3) charged aa (24% of Asp, Glu, Lys, His, Arg) often located in stretches of opposing charge, and (4) several regions composed mainly of 1 or 2 selected aa such as Gln and/or Asn (Morris et al. 2021). These features probably result in the inability of this region to form a well-defined 3D fold because a hydrophobic core is unlikely to be established; moreover, the abundance of charged residues implies fewer intramolecular hydrogen bonds and more interactions with the surrounding aqueous solvent. The presence of an intrinsically disordered central region probably complicates the task of purifying the full-length CO, as IDP/IDRs usually show unexpected features, such as increased sensitivity to in vitro proteolysis, or peculiar behavior during the purification process (Trivedi and Nagarajaram 2022). This situation, along with the high proportion of small aa, results in a high conformational flexibility, which should allow for promiscuous interactions with many partners (Morris et al. 2021). This would be the case for CO, which seems to interact with many proteins, yielding a variety

of complexes with different functions within the cell (Fig. 3, B and C). The presence of IDR in CO hampers crystallization of the whole protein irrespective of the presence of structured B-boxes and CCT domains that individually have been crystallized. Cryo-electron microscopy and nuclear magnetic resonance are promising techniques, but a combination of different methods will probably be needed to obtain structural information of CO at high resolution.

Recent advances in artificial intelligence, such as AlphaFold 2 (Jumper et al. 2021) and ColabFold (Mirdita et al. 2022), have been a major breakthrough in the prediction of 3D protein structures; however, IDP/IDRs still pose a challenge for this advanced software. The structures predicted by AlphaFold 2 and ColabFold for CO are quite similar: BBX and CCT appear as well-defined ordered structures with α -helices and β -sheets; however, the rest of the polypeptides appear as ribbon-like depictions of very low confidence, as quantified by the predicted local distance difference test (Fig. 3A). Low-confidence regions reflect the conformational heterogeneity essential to allow the interaction of CO with many partners, which results in the formation of complexes with different functions in vivo (Ruff and Pappu 2021) (Fig. 3, B and C).

Some experimental evidence suggests that CO monomers might form oligomers (Dahal et al. 2022), which prompted us to model possible dimers and trimers of CO by using ColabFold. Figure 3A shows the structural model obtained that better fits with the current knowledge of the structural and functional properties of CO. The model suggests that the 2 subunits of the complex would interact mainly through the CO-BBX domains, which is consistent with previous experimental results (Dahal et al. 2022; Zeng et al. 2022). The CCT domains would form a positively charged space (Fig. 3A, in blue) where the DNA would fit. This was corroborated by simulating complexes between the CO dimer and the CORE2 element of the *FT* promoter using the pyDockDNA server (<https://model3dbio.csic.es/pydockdna/>, Rodríguez-Lumbreras et al. 2022).

Evolution of photoperiod sensing

In the course of evolution, cellular organisms have adapted different mechanisms to perceive seasonal cues to time their physiology to the changing environment and inform on the correct timing of crucial developmental decisions (Bradshaw and Holzapfel 2007). This is particularly true of plants, whose main surge of energy is solar light, and thus, photosynthesis must be very efficient to maximize productivity and fitness. The capacity to detect daylight is as ancient as that of photosynthetic bacteria, but the capacity to detect daylength, being a very predictable annual signal, has been present since the origin of eukaryotic microalgae (Serrano et al. 2009).

Photoperiod sensing in unicellular algae

The photoperiod in unicellular algae affects photosynthesis performance, so the longer the light regime is, the better the growth and biomass production parameters are; thus, a

singular regulation is needed (Dring 1988; Serrano et al. 2009). Unicellular algae are already able to perceive daylength and make important developmental and physiological decisions upon reception of photoperiodic signals (Valverde 2011; Ferrari et al. 2019). Photoperiod is closely linked to the capacity to perceive light by photoreceptors and modulate this signal via the clock, but light perception seems to have developed very early in ancient algae, while the clock was simple in early eukaryotic microalgae (de los Reyes et al. 2017; Ferrari et al. 2019). However, unicellular algae already possessed a plethora of different light photoreceptors and a CO ortholog (Serrano et al. 2009) that was able to complement the *co* mutation in *Arabidopsis* (thus hinting at their structure–function conservation). As mentioned above, the unicellular green alga *Chlamydomonas* is able to control the cell cycle, starch biosynthesis, chloroplast function, and lipid accumulation through the photoperiodic response, and homologs of actors involved in the daylength response in angiosperms, such as CRYs, CDFs, COP1, SPA1, HY5, or PIFs, are already present in algae (Serrano-Bueno et al. 2017). The need for more complex responses to light related to multicellularity, land adaptation, and reproduction developed with time a more complex circadian clock (Ferrari et al. 2019) and, in parallel, a more complex photoperiodic response system (Serrano-Bueno et al. 2021).

Photoperiod sensing in early land plants

The evolution of the clock and photoperiod sensing does not always correlate. While early land plants had a simple clock that did not evolve rapidly in early land algae and bryophytes, photoperiod sensing already had all the basic elements in unicellular algae and quickly evolved to several members in Bryophytes (Ferrari et al. 2019). The liverwort *Marchantia* initiates gametangiophore formation in LDs with a far-red light signal (Inoue et al. 2019) and includes 6 *COL* genes in the genome, 2 of which show circadian regulation (Lagercrantz et al. 2020). In fact, one of these genes shows all the features of a CO protein described in the previous section. Additionally, a phytochrome and an MpPIF exist, and the relationship between GI-FKF in sexual reproduction through the clock is present, which opens the possibility of complex photoperiod regulation (Kubota et al. 2014; Inoue et al. 2019). Some mosses respond to photoperiod signals in different ways (Lee et al. 2010). The genome of the moss *Physcomitrella patens* includes several *COLs*, although multiple duplications of its genome may be responsible for this. Some show circadian regulation, but none of the tested *COLs* seemed to produce early-flowering phenotypes when introduced in *Arabidopsis* (Zobell et al. 2005) and may have diverged from the general land plant evolutionary path. However, the presence of cryptochromes, phytochromes, and phosphatidylethanolamine-binding (PEPB) proteins similar to FT may suggest an early CO-FT module in these plants (Hedman et al. 2009). The disruption of *PpCCA1s* compromises the clock and diurnal physiological responses, showing an early link between photoperiod and the clock (Okada et al. 2009); however, it seems to

lack a clear GI ortholog, although it may have proteins with FKF features (Serrano-Bueno et al. 2017, Ferrari et al. 2019).

Photoperiod sensing in vascular plants

Photoperiod sensing is closely linked to the clock, and the expression of CO and CO-related genes is affected by clock-regulated factors (Suárez-López et al. 2001; Lagercrantz 2009). The GI/FKF-CDF-CO-FT core is conserved in vascular plants, CDFs, and COLs evolving from algal ancestors (Lucas-Reina et al. 2015), following the innovation, amplification, and divergence model of gene evolution by duplication (Romero-Campero et al. 2013). Other complex components of the clock, however, that also regulate photoperiod, such as ZTL and GI, were incorporated as the system became more complex and robust (Serrano-Bueno et al. 2017; Ferrari et al. 2019). However, a loop back from CO to the clock in Arabidopsis was recently described, so that photoperiod signaling can adjust the circadian system, communicating daylength information to the time keeper (de los Reyes et al. 2023). As this system involves the Arabidopsis bZIP TF HY5, which is present and commonly coexpressed with COLs in coexpression gene networks in algae (de los Reyes et al. 2017), it is highly probable that a compensation of the clock by daylength was introduced very early in the evolution of light sensing in plants.

CO protein evolution has also followed an amplification and divergence model from early photosynthetic eukaryotes to spermatophytes. While B-boxes and the CCT domain did not undergo strong changes in amino acids, the intermediate, poorly structured region is the most divergent in CO structure (Robson et al. 2001; Valverde 2011). As this middle domain was recently discovered to have different structure/function-related features, understanding the difference in functions in CO will be closely linked to differences in this region.

Agricultural and biotechnological applications of CO and COL genes

We may be able to avoid the negative effects of global climate change by increasing the robustness of photoperiodic signals as the main plant seasonal information (Ettinger et al. 2021), and CO/COLs may be key targets for modern agricultural strategies to achieve this important goal. In recent decades, several studies have unraveled mutations or natural allelic variations in CO/COL sequences that allowed crop cultivation in different places around the world.

In maize, the autonomous pathway became more important than the photoperiodic pathway via *INDETERMINATE1*, becoming the main controlling gene (Minow et al. 2018). During rice domestication, diversification of flowering time was important to expand areas of cultivation. In contrast to cultivated rice, no functional variations in the *Hd1* gene are found in accessions of wild ancestors. Extensive phylogenetic analyses of *Hd1* alleles in cultivated and wild rice species suggest that the nonfunctional *Hd1* alleles found in cultivated rice originated during domestication (Takahashi and

Shimamoto 2011). Low Hd1 activity in domesticated rice may have helped develop varieties adapted to various environments at multiple locations worldwide. Determining how the regulation of CO and COLs has been modified during domestication could facilitate breeding strategies (e.g. using marker-assisted selection) such that photoperiod insensitivity of temperate inbreds could be maintained while introducing valuable traits from photoperiod-sensitive landraces or even model strategies to establish day-neutral cultivars in important crops.

In soybean, the transcript abundance of *E1* may be regulated by another COL, as a 214-kbp deletion in chromosome 19 reduced the repression of flowering by cool temperatures but did not affect photoperiodic flowering. Interestingly, this deletion harbors the *COL2b* gene, suggesting a possible thermal regulation of flowering by a COL (Zhang et al. 2020). *COL2b* was also positively associated with the regulation of seed development (Yu et al. 2023). These reports demonstrate not only the complexity of CO and CO-like genes but also the applicability of manipulating them in the regulation of plant development, since the loss-of-function of *COL2b* leads to a decrease in the sensitivity of flowering to temperature, with negative consequences on seed size. Thus, the use of genetic editing tools can represent an excellent option in the search for CO and CO-like gene alleles that would favor the tradeoff between flowering and productivity traits.

Activating CO independently of photoperiod can be a strategy to modulate flower and seed development. The overexpression of *HvCO1* combined with a natural genetic variation at the *Ppd-H1* locus (ortholog of *PRR7*) accelerated barley inflorescence development and stem elongation in LD and SD (Campoli et al. 2012). More recently, Zhang et al. (2022) identified a wheat COL gene (*TaCOL-B5*) that had the potential to increase yield by ~12%. The authors showed that constitutive overexpression of the dominant *TaCOL-B5* allele, without the region encoding B-boxes, increased the number of spikelet nodes per spike and produced more tillers without seed size penalties. These examples illustrate the tantalizing possibility that genetically manipulating COL alleles through genome editing may become an attractive option for plant breeders.

In recent decades, it has become clear that phenotypic variation can be achieved even when the genome sequence is unaltered (Tonosaki et al. 2022); thus, modifications in DNA methylation can generate meiotically stable epialleles, which are transmissible through selection and breeding. During the domestication of allotetraploid cottons, DNA methylation changes generated *COL2* epialleles. The *COL2D* epiallele is hypermethylated in wild cottons but highly expressed due to methylation loss in all domesticated cottons evaluated. Inhibiting DNA methylation activates *COL2* expression, promoting early flowering in cotton (Song et al. 2017). Therefore, epialleles of photoperiod-associated genes in combination with classical genetic diversity may be used in plant breeding to fine-tune photoperiodic signaling in crops.

It has been recently shown that Arabidopsis CO is involved in floral senescence by interfering with JA signaling

(Serrano-Bueno et al. 2022). Thus, the cut flower industry could employ this knowledge to lengthen flower longevity by delaying or inhibiting senescence. This phenotype might be achieved by either modifying CO expression or function systemically or by spraying RNA-based molecules to inhibit CO translation specifically in the flowers.

Conclusions

Due to their immobile lifestyle, plants continuously monitor external conditions to optimize growth and development. The length of the day is a sturdy seasonal signal that does not change year after year due to fluctuating earth conditions and therefore has been used by plants since the origin of eukaryotic microalgae to make important physiological decisions. Photoperiod is influenced by the clock and at the same time feeds back information to it, hence keeping the pacemaker continuously tuned to the seasons.

When considering the photoperiod pathway, flowering time often comes to mind first due to the enormous amount of knowledge accumulated in the last 20 years on floral transition. However, increasing evidence suggests that photoperiod pervades many aspects of plant physiology, particularly when these aspects need a daylength input to adapt to the environment. The age of genomics has brought abundant information on how different species respond to photoperiod and the involvement of CO function. In addition, artificial selection has repeatedly favored different alternatives to annulate the CO response when a strict daylength adaptation was a problem for crop domestication.

CO protein structure can help elucidate many of these complex regulatory layers of photoperiod response, the wide variety of tissues where it may be found, and the different physiological processes where it can act. The presence of 2 protein ends with conserved structured domains that respond to photoperiod and an internal flexible domain that can adapt and interact with many partners make it an ideal protein to respond to multiple signals. Since their appearance in microalgae, COLs have changed little structurally but have amplified and diversified enormously in the mechanisms of their regulation and the capacity to control many physiological traits that have become increasingly important in plant physiology since their land life acquisition.

Through the discovery of alleles and mutations in different species, we now have the knowledge and the gene editing tools to manipulate the photoperiod response at will. We could then employ this robust information to modify important physiological responses that will be of utmost relevance for crop yield and contribute to food security and sustainable agriculture in the next decades of uncertain climate change.

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Author contributions

All authors contributed equally to the work.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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