Plant Communications
Review article

CellPress
Partner Journal

Photoperiodic flowering in *Arabidopsis*: Multilayered regulatory mechanisms of *CONSTANS* and the florigen *FLOWERING LOCUS T*

Hiroshi Takagi[1,2], Andrew K. Hempton[1] and Takato Imaizumi[1,2,*]

[1]Department of Biology, University of Washington, Seattle, WA 98195-1800, USA

[2]Center for Gene Research, Nagoya University, Nagoya 464-8602, Japan

***Correspondence: Takato Imaizumi (takato@uw.edu)**

https://doi.org/10.1016/j.xplc.2023.100552

ABSTRACT

The timing of flowering affects the success of sexual reproduction. This developmental event also determines crop yield, biomass, and longevity. Therefore, this mechanism has been targeted for improvement along with crop domestication. The underlying mechanisms of flowering are highly conserved in angiosperms. Central to these mechanisms is how environmental and endogenous conditions control transcriptional regulation of the *FLOWERING LOCUS T* (*FT*) gene, which initiates floral development under long-day conditions in *Arabidopsis*. Since the identification of FT as florigen, efforts have been made to understand the regulatory mechanisms of *FT* expression. Although many transcriptional regulators have been shown to directly influence *FT*, the question of how they coordinately control the spatiotemporal expression patterns of *FT* still requires further investigation. Among *FT* regulators, CONSTANS (CO) is the primary one whose protein stability is tightly controlled by phosphorylation and ubiquitination/proteasome-mediated mechanisms. In addition, various CO interaction partners, some of them previously identified as *FT* transcriptional regulators, positively or negatively modulate CO protein activity. The *FT* promoter possesses several transcriptional regulatory "blocks," highly conserved regions among *Brassicaceae* plants. Different transcription factors bind to specific blocks and affect *FT* expression, often causing topological changes in *FT* chromatin structure, such as the formation of DNA loops. We discuss the current understanding of the regulation of *FT* expression mainly in *Arabidopsis* and propose future directions related to this topic.

Key words: photoperiodic flowering, *Arabidopsis thaliana*, circadian clock, photoreceptor, *CONSTANS*, *FLOWERING LOCUS T*

Takagi H., Hempton A.K., and Imaizumi T. (2023). Photoperiodic flowering in *Arabidopsis*: Multilayered regulatory mechanisms of *CONSTANS* and the florigen *FLOWERING LOCUS T*. Plant Comm. **4**, 100552.

INTRODUCTION

Plants possess a suite of regulatory mechanisms that optimize flowering events by coordinating the timing of flowering with seasonal and environmental changes. Precise control of flowering time is crucial not only in nature but also in modern agricultural management, in which the induction and timing of flowering have significant effects on yield. Although efforts have been made to understand the regulation of flowering for decades, advances in molecular genetics have dramatically accelerated our mechanistic understanding of plant flowering. Most significantly, the FLOWERING LOCUS T (FT) protein was identified in *Arabidopsis* as florigen, a systemic signal responsible for the induction of flowering at the shoot apical meristem (SAM) whose existence had been predicted since the 1930s (for the history of florigen, see Zhu et al. [2021]). FT protein is transported to the SAM through the phloem, interacting with the SAM-specific bZIP transcription factor FD (Abe et al., 2005). Formation of the FT–FD protein complex is crucial for induction of downstream flowering components such as *APETALA 1* (*AP1*) and *LEAFY* (FT downstream events are summarized in recent reviews: Yamaguchi, 2021; Zhu et al., 2021).

Published by the Plant Communications Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and CEMPS, CAS.

The expression profile of *FT* is controlled through complicated regulatory networks. The *FT* gene shows a unique spatiotemporal expression pattern in *Arabidopsis* subjected to laboratory long-day (LD) conditions (16-h light/8-h dark); it is highly expressed in the phloem companion cells in the distal part of leaves and peaks at dusk (Yanovsky and Kay, 2002; Takada and Goto, 2003; Song et al., 2018). The amount of *FT* induced under longer day-length conditions (longer than 12-h day length) correlates with the timing of flowering (Kinmonth-Schultz et al., 2016). As the flower-inducing ability of *FT* showed a dosage-dependent increase, *FT* levels have been thought to determine the timing of flowering under spring LD conditions in *Arabidopsis*. Because *Arabidopsis* is a facultative LD plant, it eventually flowers in short-day (SD) conditions (8-h light/16-h dark, 22°C) in the laboratory without the induction of *FT* (Song et al., 2015; Cao et al., 2021). The phytohormone gibberellin (GA) pathway is responsible for the floral transition in SD conditions (Wilson et al., 1992). Although the GA pathway also contributes to the floral transition in LD conditions (in part through *FT* transcription in leaves as well as the SAM) (Porri et al., 2012), the day-length- and temperature-dependent robust induction of *FT* is a crucial regulatory step for the control of flowering time in spring. Thus, one of the important research objectives is to understand how environmental conditions control *FT* expression in LD conditions.

Among the numerous components that modulate *FT* expression in photoperiodic flowering, the B-box (BBX) transcription factor CONSTANS (CO) is the most important in *Arabidopsis*. CO is expressed in leaf phloem tissues, where it binds directly to the *FT* promoter, upregulating *FT* expression. CO protein is stabilized in LD conditions but degraded in SD conditions (Valverde et al., 2004). This difference in CO stability has been thought to largely account for the difference in *FT* level between LD and SD conditions in *Arabidopsis*. Although CO is necessary for *FT* induction, many other regulatory components (including proteins that modulate CO DNA-binding activity and stability) also affect *FT* transcriptional levels, mainly in LD conditions.

Recent reviews have summarized broader aspects of flowering-related mechanisms, including circadian clock regulation, FT protein transport, and FT downstream regulation at the SAM (Bouché et al., 2016; Shim et al., 2017; Cao et al., 2021; Kinoshita and Richter, 2021). Here, we highlight recent advances in our understanding of the regulatory mechanisms that influence *FT* transcription by focusing on various control mechanisms surrounding the LD-specific master regulator CO and also explicitly describing other modulators of *FT* transcription, mainly in *Arabidopsis*.

REGULATION OF CO ACTIVITY AT THREE DIFFERENT LEVELS: TRANSCRIPTION, PROTEIN STABILITY, AND PROTEIN-BINDING PARTNERS

Regulation of *CO* transcription

CYCLING DOF FACTOR 1 (CDF1) to CDF5 were the first *CO* regulators identified, strongly repressing *CO* transcription by recruiting TOPLESS(TPL)/TPL-RELATED (TPR) co-repressors to the *CO* promoter (Figure 1; Supplemental Table 1) (Imaizumi et al., 2005; Fornara et al., 2009; Goralogia et al., 2017). Recent research has revealed that CDF6, a close homolog of CDF5, also downregulates *CO* transcription (Krahmer et al., 2019). Transcription and protein stability of CDFs are regulated by the circadian clock and light conditions throughout the day (Imaizumi et al., 2005; Fornara et al., 2009). Timing of CDF1 protein degradation is controlled by a light-dependent ubiquitin ligase complex comprised of FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) and GIGANTEA (GI) (Sawa et al., 2007), resulting in release of *CO* repression in the LD afternoon.

As transcriptional activators of *CO*, FLOWERING BHLHs (FBHs), basic helix–loop–helix (bHLH)-type transcription factors, directly upregulate *CO* transcription (Ito et al., 2012). Several environmental stress conditions influence *CO* and *FT* induction and flowering time, in part through modulation of FBH function. For instance, nitrogen (N) limitation accelerates flowering, and this is caused in part by modulation of FBH activity through phosphorylation (Sanagi et al., 2021). Specifically under ample N conditions, FBH4 is highly phosphorylated by SNF1-RELATED KINASE 1 (SnRK1) and accumulates preferentially in the cytosol (Sanagi et al., 2021). In response to low N conditions, de-phosphorylated FBH4 changes cellular localization from the cytosol to the nucleus and activates *CO* transcription, leading to earlier flowering.

The *FBH* family members are phosphorylated by other cellular signaling components, abscisic acid (ABA) and mitogen-activated protein kinases, which are tied to drought and pathogen stress-induced responses, respectively (Popescu et al., 2009; Takahashi et al., 2013). FBH3 (also known as ABA-responsive kinase substrate 1) is phosphorylated by SnRK2.6 in response to an increase in ABA level. SnRK2.6 phosphorylates different residues in FBH proteins than SnRK1, and this phosphorylation event reduces FBH3 DNA binding ability (Takahashi et al., 2016; Sanagi et al., 2021). These findings suggest that *CO* transcript levels can be finely tuned by multi-phosphorylation regulation of FBH proteins in response to various environmental stress conditions.

Recent studies have also shed light on the mechanism of FBH-mediated *CO* transcriptional activation. FBHs enhance *CO* transcription by recruiting chromatin-remodeling factor JUMANJI 28 (JMJ28), an H3K9 demethylase, to the *CO* promoter (Hung et al., 2021). As H3K9 demethylation typically results in enhancement of gene expression, JMJ28 positively affects *CO* gene expression.

In addition to FBHs, class II TEOSINTE BRANCHED 1/CYCLOIDEA/PROLIFERATING CELL NUCLEAR ANTIGEN FACTORs (TCPs) activate *CO* transcription (Kubota et al., 2017; Liu et al., 2017). In addition to the previously known role of GI in CDF degradation, Kubota et al. (2017) found that TCPs required functional GI for *CO* upregulation and that loss of *GI* significantly decreased TCP4 binding to the *CO* promoter. TCP4 is also associated with FBH1 via the mediator PFT1/MED25 (Liu et al., 2017), suggesting that TCPs and FBHs synergistically upregulate *CO* transcription. Curiously, GI forms complexes not only with TCP4 but also with FBH1 (Kubota et al., 2017), suggesting that GI may contribute to formation of the large protein complex consisting of TCP4 and FBH1. Given that GI has previously been shown to exhibit a molecular chaperone-like function (Cha et al.,

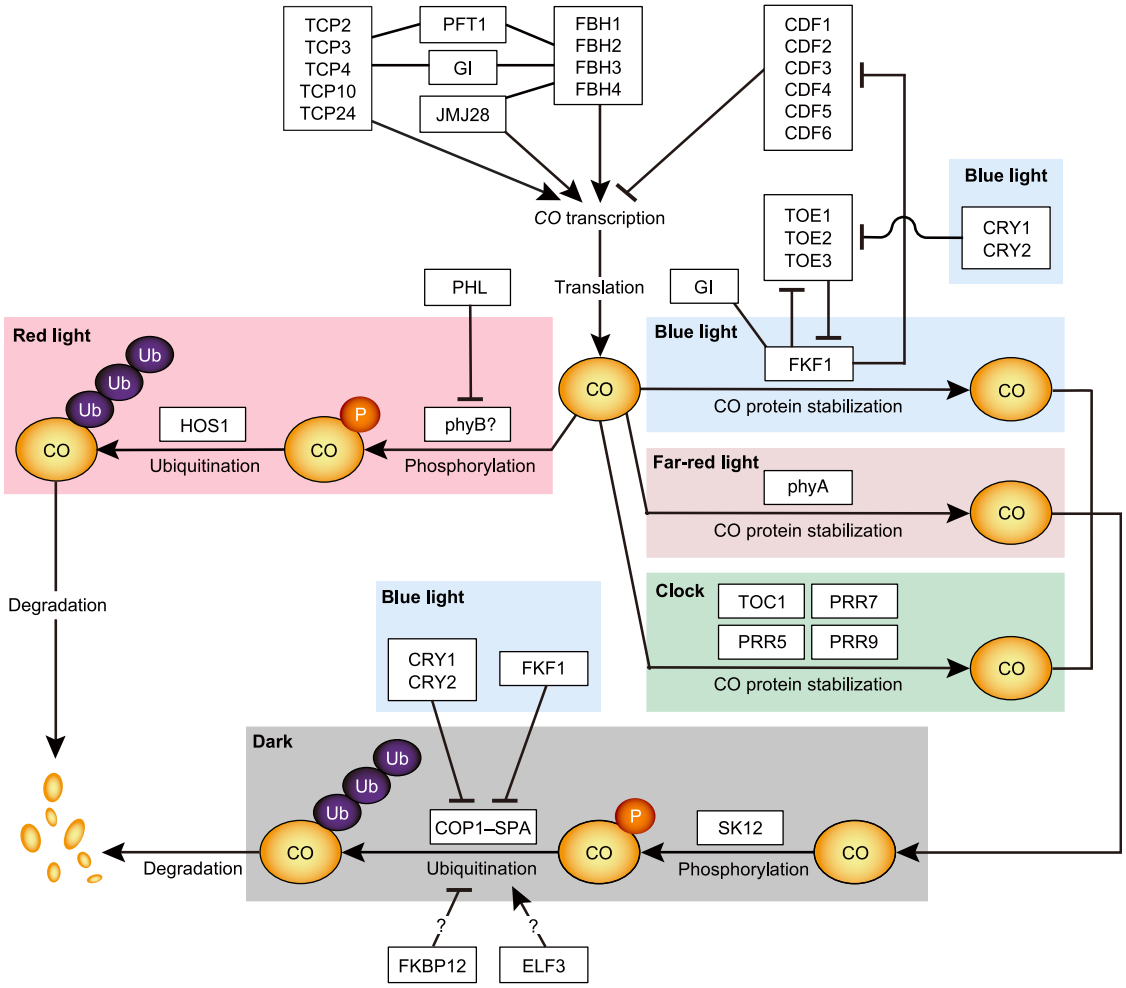

Figure 1. Regulation of CO activity through *CO* transcription, CO protein modification, and CO stability.
CO transcription is upregulated by the positive regulators FBHs and class II TCPs. PFT1 is a mediator that bridges FBH and TCP proteins. JMJ28 relaxes the structure of the *CO* promoter in an FBH-dependent manner. CDF proteins are direct and robust repressors of *CO*. The FKF1–GI complex is formed when FKF1 absorbs blue light, and this complex destabilizes CDF proteins in the LD afternoon. FKF1 also directly interacts with CO protein to stabilize it under blue light. TOE proteins compete with FKF1 for CO, and the interaction with TOE protein destabilizes CO due to the lack of access to FKF1. Similar to FKF1, CRY1/CRY2 also attenuate the activity of TOEs and the COP1–SPA E3 ubiquitin ligase complex under blue light. Far-red light stabilizes CO proteins in a phyA-dependent manner. Clock components PRRs (TOC1, PRR5, PRR7, and PRR9) also directly bind to CO to stabilize it. In the dark, SK12 phosphorylates CO proteins, and the COP1–SPA ubiquitin E3 ligase complex then ubiquitinates the phosphorylated CO proteins. FKBP12 prevents the degradation of phosphorylated CO proteins through direct interaction with CO, whereas ELF3 promotes CO degradation, potentially through interaction with the COP1–SPA complex. Under red light, CO is phosphorylated, potentially through interaction with phyB. PHL represses the function of phyB by an uncharacterized mechanism. The E3 ubiquitin ligase HOS1 ubiquitinates CO proteins under red light and is primarily responsible for degradation of CO proteins in the morning.

2017), GI may facilitate the function of interacting transcription factors by properly folding these proteins. It should be noted that not all TCP and FBH binding sites are located within close proximity on the *CO promoter*, suggesting that the protein–protein interaction between TCP4 and FBH1 may play a role only in particular regions on the *CO* promoter. Each gene family of *CO* transcriptional regulators comprises multiple members with some overlapping functions. It is possible that multiprotein complexes with various combinations of FBHs and TCPs and their interaction partners may exist on the *CO* promoter. Because each component may be influenced by different environmental conditions (e.g., low N conditions alter FBH4 intracellular localization pattern and transcriptional activity), CO regulation may be a point of signal crosstalk between environmental information and flowering. Because multiple stress factors exist simultaneously in the real world, our next challenge may be to understand the spatio-temporal dynamics of interactions among these factors under these complex conditions.

Regulation of CO protein stability

In addition to *CO* transcriptional regulation, CO protein stability mechanisms also play important roles in *FT* induction and, in turn, flowering. *CO* transcripts are expressed in wider areas of leaf vascular tissues than *FT* transcripts (Takada and Goto, 2003; An et al., 2004), and cellular differences in posttranscriptional regulation of CO protein may account for this spatial difference between *CO* and *FT* expression.

Phosphorylated CO proteins are preferentially ubiquitinated for degradation by the E3 ubiquitin ligase complex of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and SUPPRESSOR OF PHYA-105s (SPAs) in the dark (Figure 1; Supplemental Table 1) (Jang et al., 2008; Sarid-Krebs et al., 2015). Although the phosphorylation of CO proteins has been established as the first step in directing CO proteins to the ubiquitin/proteasome pathway, the molecular components involved in CO phosphorylation have only recently been identified. SHAGGY-like kinase 12 (SK12) phosphorylates CO proteins in the nucleus (Chen et al., 2020). SK12-dependent phosphorylation of threonine 119 (T119) of the CO protein, located just after the second B-box domain, promotes CO protein degradation. COP1 binds a separate region of the CO protein, the C-terminal CCT (CO, CO-LIKE, and TOC1) domain, for degradation (Jang et al., 2008). It may be worth investigating the mechanism by which T119 phosphorylation promotes COP1-dependent CO degradation. *SK12* expression levels are higher in LD than in SD conditions, but whether environmental signals such as photoperiod and light quality affect the enzymatic activity of SK12 remains unknown. *SK12* is widely expressed across various tissues, including the leaf vasculature (Chen et al., 2020), and it is involved in plant growth and stress-related metabolic regulation (Li et al., 2021). Because reproductive organs are large sink tissues, plants may coordinate the timing of flowering with processes related to growth by controlling CO protein levels through SK12-dependent phosphorylation.

Conversely, a separate study revealed that the small protein FK506-binding protein (FKBP12) prevents the degradation of phosphorylated CO proteins (Serrano-Bueno et al., 2020). FKBP12 in animals is involved in multiple signaling pathways and is the primary target of the immunosuppressive drugs FK506 and rapamycin (Kang et al., 2008). Overexpression and knockout mutations of *FKBP12* increase and decrease the proportion of phosphorylated CO proteins, respectively. Although FKBP proteins possess prolyl isomerase activity that potentially affects protein structures (Serrano-Bueno et al., 2020), the molecular mechanisms by which FKBP12 stabilizes phosphorylated CO protein in *Arabidopsis* are not yet fully understood. But given that FKBP12 interacts with the CO CCT domain, the FKBP12–CO interaction may interfere with the interaction of CO with COP1. These findings shed light on regulatory mechanisms of CO protein phosphorylation that fine-tune its stability and activity.

Although the COP1–SPA complex is a major player in the degradation of CO in the dark, the *cop1* mutation did not affect CO degradation under either red light (R) or during the morning to midday (Jang et al., 2008), suggesting the influence of other proteins that independently ubiquitinate CO under these conditions. HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1), for example, is an E3 ubiquitin ligase that ubiquitinates CO specifically under R and is responsible for CO degradation from the morning to the afternoon in LD conditions (Lazaro et al., 2012, 2015). Another study showed that HOS1 also promotes CO degradation under cold environments, indicating that HOS1 regulates temperature-dependent flowering in part by controlling CO stability (Jung et al., 2012). HOS1 strongly localizes at the nuclear envelope and forms a protein complex with Nucleoporin 96, a component of the nuclear pore complex, for their mutual stabilization (Lazaro et al., 2012; Cheng et al., 2020), suggesting that molecular events at the nuclear pores are important for HOS1 function. It is unclear whether HOS1 also recognizes the phosphorylation status of CO as a signal for ubiquitination; however, this is likely the case, as CO is strongly phosphorylated under R (Sarid-Krebs et al., 2015). Further evidence supporting this mechanism is the apparent requirement of the red/far-red light (R/FR) photoreceptor phytochrome B (phyB) for HOS1-mediated CO degradation (Lazaro et al., 2015). phyB-mediated phosphorylation and degradation of PHYTOCHROME-INTERACTING FACTORs (PIFs) are well described (summarized in Pham et al., 2018). However, despite the confirmation of direct interactions between phyB and CO proteins (Lazaro et al., 2015), it remains unclear whether phyB is directly responsible for R-dependent phosphorylation of CO. In fact, a recent study describing the 3D structure of phyB claimed that phyB has most likely lost its function as a histidine kinase because the predicted ATP-binding pocket shows a closed structure (Li et al., 2022). Consistent with the crystal structure data, the authors biochemically confirmed that phyB lacked kinase activity, placing into question previous results demonstrating the kinase activity of plant phytochromes. Therefore, it is possible that phyB may function as a signaling component that promotes CO ubiquitination rather than phosphorylating CO directly.

PHYTOCHROME-DEPENDENT LATE-FLOWERING (PHL) is a repressor of phyB in photoperiodic flowering (Endo et al., 2013). Although its molecular function remains elusive, PHL attenuates phyB-dependent CO degradation by directly binding to CO and phyB. The studies described above highlight the negative effects of phyB on CO stability; however, phyB also recruits the *FT* positive regulator TANDEM ZINC-FINGER-PLUS (TZP) into the nucleus (Kaiserli et al., 2015). Moreover, in the context of photomorphogenesis, phyB Pfr interferes with formation of COP1–SPA1 protein complexes under R by binding SPA1, resulting in accumulation of photomorphogenesis-promoting proteins such as ELONGATED HYPOCOTYL 5 (Lu et al., 2015; Sheerin et al., 2015). It is possible that the reduction in COP1–SPA1 complex formation by phyB may contribute to CO stabilization around dawn and/or in the LD afternoon when COP1 is involved in degradation of CO protein (Jang et al., 2008).

In contrast to the degradation effects of R, FR enhances the stability of CO proteins in a phyA-dependent manner (Valverde et al., 2004). Although *FT* expression peaks at dusk under typical laboratory conditions (R/FR ratio > 2.0), a recent study revealed that supplementation with FR, mimicking the R/FR ratio of natural sunlight (R/FR = approximately 1.0), induces an additional *FT* peak in the morning that accelerates flowering time (Figure 2) (Song et al., 2018). This morning *FT* expression is relevant to flowering time determination, as the *phyA* mutant that largely lacks the *FT* morning peak shows late flowering, whereas the *fkf1* and *CRYPTOCHROME 2* (*cry2*) mutants that possess only the morning *FT* peak flower at almost the same time as wild-type plants under LD conditions with supplemental FR (Song et al., 2018). This morning *FT* peak is mediated through enhanced CO stabilization and phyA function, although the detailed mechanisms of phyA-mediated enhancement of CO stability remain unknown. The circadian clock component

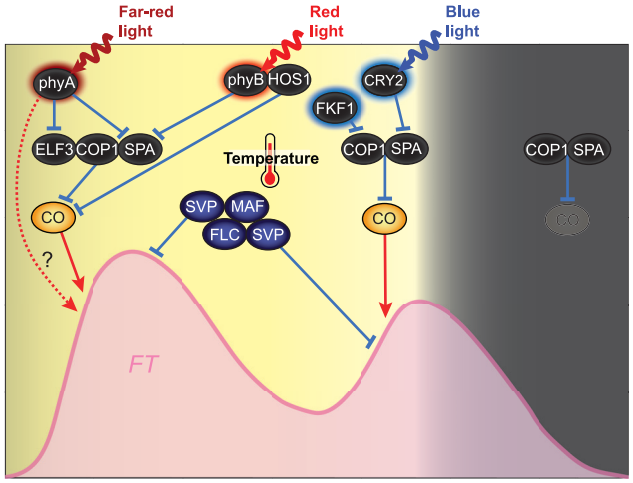

Figure 2. A model for *FT* transcriptional regulation under natural LD conditions.

This model is based on the *FT* expression profiles of mutants grown in simulated natural LD conditions (LD + supplemental far-red light + daily temperature oscillation). *FT* expression peaks in the morning and evening in natural LD conditions. The far-red light photoreceptor phyA is necessary for *FT* induction in the morning. In part, phyA directly attenuates ELF3-dependent CO degradation, whereas the phyB-interacting ubiquitin ligase HOS1 degrades CO in a red light-dependent manner. It is likely that phyA may have different mechanisms (currently unknown) to affect *FT* levels in the morning. The blue-light photoreceptors CRYs and FKF1 contribute to the evening induction of *FT*, but their contributions are relatively weak in the morning. Triple mutations of the temperature-sensitive MADS-box *FT* repressors *SVP*, *FLM* (*MAF1*), and *FLC* result in augmented *FT* morning and evening peaks, although the single mutation of *SVP* only enhances *FT* levels in the morning. These MAD-box transcription factors can form heterodimers (possibly heterotetramers like other MAD-box transcription factors). CO is degraded by the COP1–SPA complex in the dark.

EARLY FLOWERING 3 (ELF3) directly interacts with CO and promotes CO degradation under laboratory conditions with relatively low FR irradiance (R/FR > 2.0) (Song et al., 2018). A previous study showed that ELF3 forms a complex with COP1, and the ELF3–COP1 complex suppresses *FT* expression through the degradation of GI (Yu et al., 2008). Therefore, ELF3 may enhance degradation of CO by interacting with E3 ubiquitin ligases such as COP1. Another recent analysis revealed that ELF3 forms a protein complex with COP1, SPAs, and phyA *in vivo* (Huang et al., 2016), suggesting that phyA may affect the ubiquitin ligase activity of COP1–SPA through an interaction with the ELF3 complex.

Like FR, blue light (B) has been shown to enhance the stability of CO proteins (Valverde et al., 2004), but in contrast to the largely-unexplored mechanisms of FR-mediated CO stability, the mechanisms of B-mediated CO stability are more thoroughly understood. FKF1 was initially identified as a CDF-destabilizing B photoreceptor but was later found to interact directly with CO proteins, enhancing their stability (Figure 1) (Song et al., 2012b). Moreover, FKF1 prevents the formation of COP1 homodimers through direct interaction with COP1, resulting in attenuation of COP1-dependent CO degradation (Lee et al., 2017). The AP2-like proteins TARGET OF EAT1 (TOE1), TOE2, and TOE3—well-known *FT* repressors—compete with FKF1 for CO, resulting in CO destabilization due to a lack of access to FKF1 proteins (Zhang et al., 2015). As reviewed in Song et al. (2015), the dual functions of B-exposed FKF1—in CDF degradation to de-repress *CO* and CO protein stabilization—play a critical role in photoperiodic sensitivity. Similar to FKF1, the B photoreceptor proteins CRY1 and CRY2 physically impair TOE1 and TOE2 (Du et al., 2020) and the COP1–SPA complex in a B-dependent manner (Liu et al., 2011; Zuo et al., 2011; Holtkotte et al., 2017). CRY2 plays a major role in flowering regulation, whereas CRY1 mediates B-dependent de-etiolation responses (Ahmad and Cashmore, 1993; Guo et al., 1998). Although B signals generally stabilize CO proteins, the direct binding of ZEITLUPE (ZTL), another B photoreceptor closely related to FKF1, results in CO degradation (Song et al., 2014). Although the mechanism by which ZTL negatively controls CO protein abundance remains unknown, the time-dependent tripartite interactions among ZTL, FKF1, and GI, which occur differently in the cytosol and nucleus (Hwang et al., 2019), appear to be important mechanisms for regulation of plant development.

The circadian clock components PSEUDO RESPONSE REGULATOR (PRR) proteins, including TIMING OF CAB2 EXPRESSION 1 (TOC1), PRR5, PRR7, and PRR9, also directly stabilize CO proteins (Figure 1) (Hayama et al., 2017). Because the expression of these clock components exhibits rhythmic patterns, the authors concluded that PRR9 contributes to CO accumulation in the morning, whereas PRR5, PRR7, and TOC1 function in the evening. Interestingly, expression of both FKF1 and PRR5 proteins peaks at the end of the day in LD conditions, when CO is most stabilized, and these proteins physically interact (Kiba et al., 2007; Baudry et al., 2010), suggesting that PRRs and FKF1 may form a CO-stabilizing protein complex. Recent studies have shown that *CDF*s are highly expressed when the expression of *PRR*s is low, also suggesting that PRRs may repress the transcription of *CDF*s (Toda et al., 2019).

Positive regulation of CO activity by interaction partners

CO function can be modulated by interacting proteins. The CO protein comprises two major domains: the N-terminal tandem B-box and C-terminal CCT domains. Although CO itself forms oligomers through B-box domains to enhance its transcriptional activation (Lv et al., 2021; Zeng et al., 2022), it also binds to a number of transcription factors and chromatin remodelers. These interactions either enhance or repress CO DNA-binding activity on the *FT* promoter (Figure 3; Supplemental Table 1). CO protein by itself is capable of binding to specific sequences called CO-responsive elements (COREs) (TGTG(N2–3)ATG) located proximal to the transcription start site (TSS) of *FT* (Tiwari et al., 2010). However, formation of a complex with the pioneer transcription factors Nuclear Factor-YBs (NF-YBs) and NF-YCs through the C-terminal CCT domain enhances CO's binding affinity to CORE sequences (Wenkel et al., 2006; Kumimoto et al., 2010; Gnesutta et al., 2017; Lv et al., 2021). The flowering phenotypes of both *nf-yb* double and *nf-yc* triple mutants resembled those of *co* mutants, and CO requires NF-Ys to induce flowering, suggesting that *CO* and *NF-YB/NY-YC* function in the same flowering pathway (Kumimoto et al., 2008, 2010). Because B-box and CCT domains are required for CO oligomerization and interaction with NF-Ys, respectively, NF-Ys are most likely interacting with oligomerized CO (Lv et al., 2021). The CO/NF-YB/NF-YC trimer

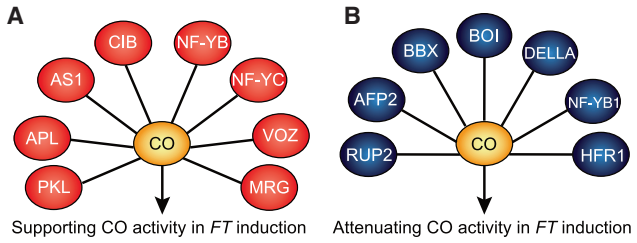

Figure 3. Interactomes of CO proteins with positive and negative transcriptional regulators.

CO protein directly forms protein complexes with groups of proteins that act together with CO to enhance *FT* transcription **(A)** and attenuate CO activity on the *FT* promoter **(B)**. Positive regulators are shown in red circles **(A)**, and negative regulators are in blue circles **(B)**. These interactomes do not contain the factors that modify CO protein or control CO protein stability shown in Figure 1. Among these interactors, NF-YB–NF-YC interaction is essential to form a basic functional unit of CO, the CO/NF-YB/NF-YC tripartite complex. The rest of the interactors can work together or independently with CO. CO is stabilized in the LD afternoon, and most of these interactions therefore happen in the LD afternoon when *FT* is expressed. It is proposed that the interaction of CO with morning-expressed negative BBX regulators attenuates CO transcriptional activity in the morning.

complex also binds to CCACA sequences in the regulatory *FT* promoter regions called P1 and P2 near the TSS (Adrian et al., 2010; Gnesutta et al., 2017; Chaves-Sanjuan et al., 2021; Lv et al., 2021). However, it should be noted that NF-YBs do not always act as positive regulators of *FT*. Wenkel et al. (2006) showed that NF-YB1 interacted with CO, and its overexpression resulted in a late-flowering phenotype.

The MYB domain protein ASYMMETRIC LEAVES 1 (AS1) was the first transcription factor identified as a CO interaction partner other than the NF-Ys (Song et al., 2012a). Although the role of AS1 in leaf patterning has been reported previously (Byrne et al., 2000), it was also shown to bind to the B-box domains of CO. AS1 is a CO-interactor but itself binds to the *FT* promoter as a positive regulator (Song et al., 2012a).

VASCULAR PLANT ONE-ZINC FINGER 1/2 (VOZ1/2), which physically interact with phyB, are known as positive regulators of flowering in *Arabidopsis* (Yasui et al., 2012). Celesnik et al. (2013) reported that *VOZ1/2* suppress *FLOWERING LOCUS C* (*FLC*), a strong repressor of *FT*, as the *voz1 voz2* double mutants show late-flowering phenotypes with higher expression levels of *FLC* and lower levels of *FT*. Recent studies have further implicated VOZ proteins in the acceleration of flowering, primarily through formation of CO–VOZ complexes but not by changes in *FLC* level (Kumar et al., 2018). Kumar et al. (2018) examined the impact of the *flc* mutation with *voz1 voz2* and found that the *FT* level was still low in the *flc voz1 voz2* triple mutant even though *FLC* no longer functioned to repress *FT* in the mutant, indicating that the increased FLC level in *voz1 voz2* was not the cause of the low *FT* level. However, they genetically showed that the *voz1 voz2* flowering phenotype and *FT* levels depend on functional *CO*, implying that the CO–VOZ interaction plays the primary role in VOZ-dependent *FT* regulation and flowering.

ALTERED PHLOEM DEVELOPMENT (APL) was originally identified as a transcription factor necessary for phloem development (Bonke et al., 2003). Later studies found that the *Arabidopsis fe* mutant known for its late-flowering phenotype possesses a single amino acid substitution in the APL protein (Koornneef et al., 1991; Abe et al., 2015). APL interacts with CO and NF-YB, acting as a co-activator of *FT* expression (Shibuta and Abe, 2017). APL also upregulates the FT protein-transport gene *FT-INTERACTING PROTEIN 1* (*FTIP1*), suggesting that APL simultaneously promotes *FT* expression and FT transport to accelerate flowering (Shibuta and Abe, 2017).

In many cases, the DNA-binding ability of transcription factors is affected by the chromatin structures of their target DNA sequences. Although acetylation of histone lysine (K) residues generally relaxes chromatin structure, leading to transcriptional activation, methylation can either promote or repress transcription depending on the K position—methylation of H3K9 or H3K27 typically leading to gene silencingwhereas methylation of H3K4 or H3K36 causes transcriptional activation (Peng et al., 2018). MORF-RELATED GENE 1 (MRG1) and MRG2 are methylated H3K4 and H3K36 readers but also act as CO interaction partners to enhance *FT* levels (Figure 3A; Supplemental Table 1) (Bu et al., 2014; Xu et al., 2014). The *mrg1 mrg2* double mutant exhibits a reduction in CO binding to the *FT* promoter, indicating that MRG1 and MRG2 may play supporting roles in physically influencing CO DNA-binding activity (Bu et al., 2014). MRGs promote H4K5 acetylation on the *FT* promoter through interaction with HISTONE ACETYLTRANSFERASE OF THE MYST FAMILY 1 (HAM1) and HAM2 (Xu et al., 2014), suggesting that MRGs relax and facilitate the shaping of DNA structure, allowing more CO proteins to bind to the *FT* promoter.

Recently, two independent studies found new factors that inhibit the action of MRGs on *FT* regulation (An et al., 2020; Guo et al., 2020). The NAP1-related proteins NRP1 and NRP2 are histone chaperone proteins that preferentially bind to damaged DNA to relax the DNA structure, most likely contributing to DNA repair (Zhu et al., 2006; Gao et al., 2012; González-Arzola et al., 2017). The *nrp1 nrp2* double mutant shows altered root morphology (Zhu et al., 2006, 2017) and accelerated flowering (An et al., 2020; Wang et al., 2020). Both studies showed that absence of NRP1/2 decreased the expression of *FLC*. However, in parallel, An et al. (2020) found that NRP1 inhibits CO–MRG protein binding, although NRP1 does not directly bind to CO. Consistent with this finding, in the *nrp1 nrp2* mutant, MRG2 was more enriched on the *FT* promoter and enhanced H4K5 acetylation of the *FT* promoter (An et al., 2020).

Similarly, HISTONE DEACETYLASE 2C (HD2C) affects flowering by modifying chromatin status at the *FT* locus and CO–MRG interactions (Guo et al., 2020). HD2C binds to *FT* chromatin in an MRG-dependent manner. It causes histone deacetylation of the *FT* promoter, especially during the night, consistent with the peak expression of *HD2C* mRNA around dusk. In addition to histone deacetylase activity, HD2C inhibits the interactions between CO and MRGs, and mutation of *HD2C* causes the retention of CO proteins on the *FT* promoter for an extended period, suggesting that HD2C functions in the removal of CO from the *FT* promoter (Guo et al., 2020). Chromatin status affects the accessibility of DNA to transcription factors. Physical chromatin structures often influence transcription, which is the case in CO-dependent *FT* transcriptional regulation.

Reminiscent of MRGs, CO requires another chromatin remodeler, PICKLE (PKL), for its transcriptional activity on the *FT* promoter, likely because PKL binding on the *FT* promoter promotes H3 acetylation (Jing et al., 2019a). PKL also forms a protein complex with ARABIDOPSIS TRITHORAX 1 (ATX1), which is required to overcome *FT* repression by polycomb group proteins (Jing et al., 2019b). These results indicate that CO multimers can act as a scaffold for recruiting transcription factors and chromatin remodelers near the *FT* TSS to modify the local chromatin conditions suitable for induction of *FT*.

Negative regulation of CO activity by interaction partners

All the examples of CO interaction partners described above concern positive regulation of CO activity. However, CO also interacts with proteins that downregulate its activity in *FT* induction (Figure 3B; Supplemental Table 1). CO is one of over 30 BBX proteins and binds to the *FT* repressor type of BBXs. BBX30 and BBX31 (also known as microprotein 1a [miP1a] and miP1b) interact with CO through the B-box domains (Graeff et al., 2016). Both *BBX30* and *BBX31* peak around dawn in LD conditions (and at the end of the night in SD conditions), and their overexpression causes late-flowering phenotypes by repressing *FT* expression. BBX30 and BBX31 lack the CCT domain but bind to the TPL general transcriptional co-repressor (Graeff et al., 2016). By directly binding to CO, BBX30/31 can thus recruit the TPL co-repressor into the CO complex to repress *FT*, likely during the LD morning. Similarly, the other BBX proteins COL12, BBX17, BBX19, BBX28, and BBX29 interfere with CO activity through direct interaction (Wang et al., 2014, 2021; Ordoñez-Herrera et al., 2018; Liu et al., 2020; Xu et al., 2022). COL5 is a BBX protein that promotes flowering, similar to CO (Hassidim et al., 2009), but it is unknown whether COL5 forms a complex with CO to induce flowering.

Although early flowering caused by a shade avoidance response is believed to reduce future competition with neighboring plants (Casal, 2012), plants also possess a mechanism to suppress shade-dependent flowering induction through the modulation of CO transcriptional activity. HFR1 (LONG HYPOCOTYL IN FAR-RED) is a non-DNA-binding HLH protein that acts as a repressor of the shade avoidance response, forming non-DNA-binding heterodimers with shade-avoidance-associated DNA-binding bHLH factors, including PIFs (Sessa et al., 2005; Hornitschek et al., 2009). Direct interaction with HFR1 attenuates the transcriptional activity of PIF on shade marker genes. In addition, a recent study showed that HFR1 represses *FT* expression through direct interaction not only with PIFs but also with CO (Zhang et al., 2019). The transcription of *HFR1* is induced under shade conditions (Sessa et al., 2005), suggesting that HFR1 may prevent precocious flowering by suppressing CO transcriptional activity under shade conditions.

Similar to that of HFR1 under shade conditions, CO transcriptional activity is affected by the modulator of ultraviolet light (UV) responses REPRESSOR OF UV-B PHOTOMORPHOGENESIS 2 (RUP2, also known as EFO2) (Wang et al., 2011; Arongaus et al., 2018). UV-B perception causes monomerization of the UV-B photoreceptor UVR8 (UV RESISTANCE LOCUS 8) (Rizzini et al., 2011). Monomerized UVR8 directly interacts with COP1, leading to activation of downstream UV-B responses. RUP2 was originally identified as a key player in the negative feedback regulation of UV-B responses; *RUP2* is a UV-B-inducible gene, and its protein directly prevents monomerization of UVR8 (Gruber et al., 2010; Heijde and Ulm et al., 2013). However, a later study showed that RUP2 also directly interacts with CO and suppresses its transcriptional activity, especially under SD + UV-B conditions, without affecting *CO* transcription levels (Arongaus et al., 2018).

CO also integrates with plant hormone signaling components to modulate its activity. GAs promote flowering in both LD and SD conditions (Wilson et al., 1992; Porri et al., 2012). DELLA proteins—major repressors of GA signaling that are degraded after GA perception—interact with CO (Wang et al., 2016; Xu et al., 2016). The release of CO protein from DELLA plays a role in GA-dependent flowering induction, and the *co* mutation rescued early-flowering phenotypes of multiple *della* mutants (*gai-t6 rga-t2 rgl1-1 rgl2-1* mutant). BOTRYTIS SUSCEPTIBLE 1 INTERACTOR (BOI) acts as a co-repressor with DELLA (Park et al., 2013). BOI represses *FT* expression simultaneously through interaction with DELLA and attenuation of CO DNA-binding on the *FT* promoter (Nguyen et al., 2015). BOI exhibits E3 ubiquitin ligase activity and may thus affect CO stability. FKF1 is another E3 ubiquitin ligase that directly binds to DELLA proteins and regulates their stability through the proteasome pathway to regulate flowering in LD conditions (Yan et al., 2020). FKF1 destabilizes DELLA proteins in the LD afternoon, and this regulation influences the levels of *FT* but not *CO*. The GA pathway also feeds back to regulate *FKF1* transcription. These findings suggest that crosstalk between the photoperiod and GA flowering pathways plays an important role in *FT* expression. DELLA proteins also affect *FT* expression through direct interaction with other transcription factors in addition to CO, as described in the section below.

In addition to GA signaling components, ABI5/ABF-BINDING PROTEINs (AFPs)—suppressors of ABA responses—also directly modulate the transcriptional activity of CO (Chang et al., 2019). AFPs are small proteins homologous to NOVEL INTERACTOR OF JAZ (NINJA). NINJA is well known for its negative regulatory role in jasmonic acid (JA) signaling through direct interaction with JA repressive components, the JASMONATE-ZIM-DOMAIN PROTEINs (JAZs). The EAR motif of NINJA recruits the transcriptional co-repressors TPL and TPRs to the MYC–JAZ protein complex, which results in repression of the MYC-dependent JA signaling pathway (Pauwels et al., 2010). Although AFP2 is a homolog of NINJA, AFP2 does not interact with any JAZ proteins (Pauwels et al., 2010). Instead, it binds directly to the bZIP transcription factor ABA-INSENSITIVE 5 (ABI5) and attenuates ABI5-dependent ABA responses such as seed dormancy (Lopez-Molina et al., 2003; Garcia et al., 2008). Chang et al. (2019) found that AFP2 physically associates with CO and negatively affects CO's transcriptional activity. AFP2 recruits TPR2 through its EAR motif and forms a CO/AFP2/TPR2 protein complex (Chang et al., 2019). Recruitment of TPR2 not only weakens CO-dependent *FT* induction but also promotes deacetylation of histone H3, most likely because TPR2 brings histone deacetylase complexes to the *FT* promoter (Chang et al., 2019). Although AFP2 interacts with TPL in yeast (Pauwels et al., 2010), a *tpr2* single mutation is sufficient to cancel the late-flowering effect of AFP

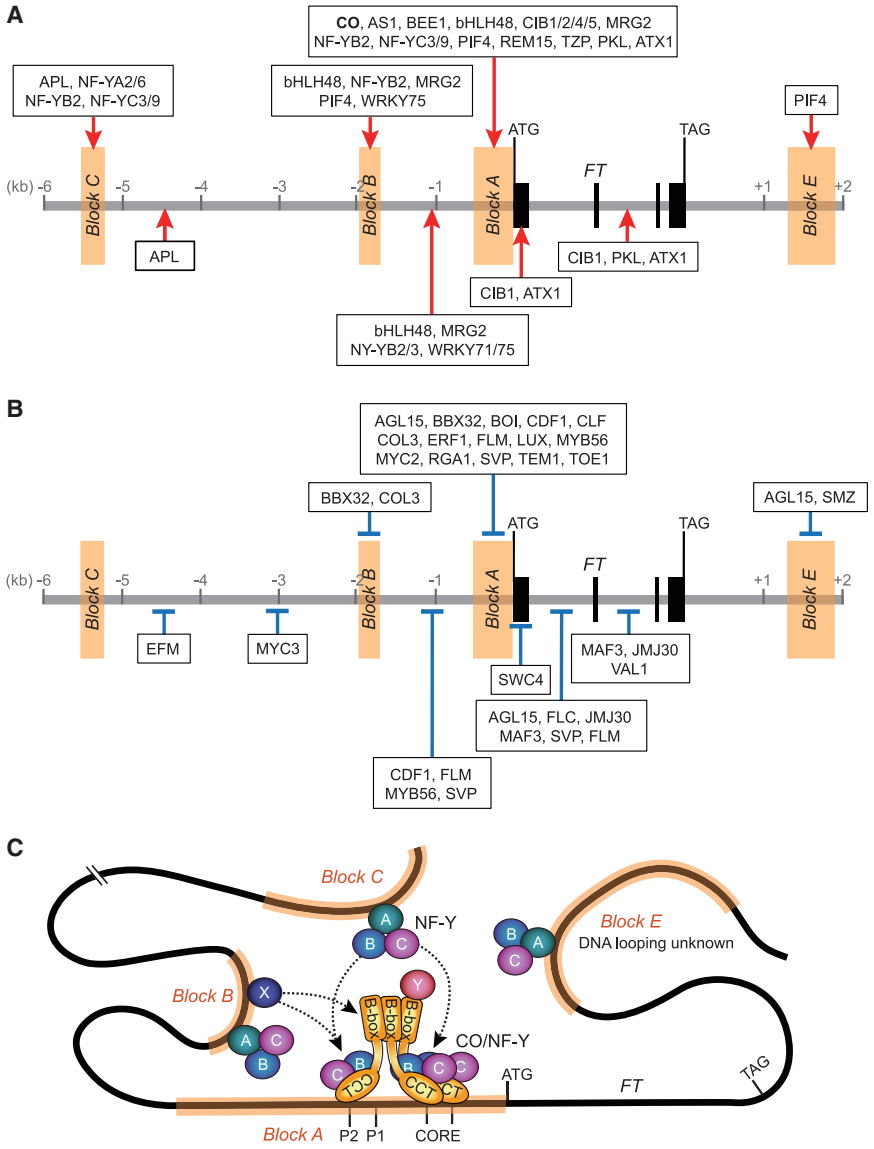

Figure 4. DNA binding sites of *FT*-regulating factors and structural changes in the *FT* regulatory region.

(A and B) The locations of DNA binding sites of *FT* positive **(A)** and negative **(B)** regulators on the *FT* genomic region. Blocks A, B, C, and E are important regulatory regions that are highly conserved in the *Brassicaceae* family. Black boxes depict the positions of exons. To simplify the figure, arrows point to the approximate but not exact locations of transcription factor binding sites. Chromatin remodelers that bind to broader chromatin areas in the *FT* locus (such as LHP1, PRC2 complexes, etc.) are not included.

(C) A model of the effect of NF-Y proteins on CO transcriptional activity and looping structures of the *FT* locus. NF-YB and NF-YC physically bind to the CCT domain of CO, which allows CO to bind to the P1P2 and CORE regulatory elements. NF-YA, B, and C form a protein complex and bind to several regions of the *FT* promoter. NF-Y protein complexes change the DNA structure of Block C and bring this region close to Block A, where CO–NF-Y protein complexes bind. However, because CO and NF-YA bind to the same position of NF-YB–C protein complexes, it is still unclear how Block A and C can be physically associated. Block B also forms a loop structure, but unlike Block C, it is independent of the existence of NF-Ys, suggesting the presence of an unknown factor (X) responsible for the loop structure of Block B. Factor Y represents another CO-interacting protein.

overexpression, suggesting that TPR2 is the primary partner of AFP2 in repression of CO activity. In addition to *AFP2*, *AFP3* knockout mutations and overexpression cause early and late flowering, respectively, likely through the same mechanism as *AFP2* (Chang et al., 2019). These results demonstrate that CO could also bring repressive chromatin remodelers directly to the *FT* locus in LD conditions, emphasizing the need for finer spatiotemporal resolution in our understanding of CO–FT-dependent flowering molecular mechanisms.

CO IS THE MASTER REGULATOR OF *FT* TRANSCRIPTION, BUT OTHER FACTORS ALSO CONTRIBUTE TO THIS REGULATION

DNA looping mediated by NF-Ys

Although shorter promoter sequences are sufficient to induce the spatial expression patterns of many genes, more than 5 kb of upstream sequences are required to recreate the unique leaf-phloem-specific spatial expression pattern of *FT* and rescue the late-flowering phenotype of the *ft* mutant, indicating that such a long promoter is needed to fully induce *FT* expression in *Arabidopsis* (Adrian et al., 2010). Three DNA regions in the long *FT promoter sequence* are conserved within *Brassicaceae*, referred to as Blocks A, B, and C (Figure 4A and 4B) (Adrian et al., 2010). In addition to these three blocks, a recent study identified an additional enhancer region conserved in *Brassicaceae* named Block E (Zicola et al., 2019). Although Block E has only recently been identified, the *FT* positive regulator PIF4 and the negative regulators AGAMOUS-LIKE 15 (AGL15), SCHLAFMÜTZE (SMZ), and LIKE HETEROCHROMATIN PROTEIN1 (LHP1) have already been reported to bind to a location overlapping with Block E (Mathieu et al., 2009; Adrian et al., 2010; Fernandez et al., 2014; Galvão et al., 2019).

Block A contains the CO-binding sites CORE and P1P2 (Adrian et al., 2010; Tiwari et al., 2010; Lv et al., 2021). The most upstream block, Block C, contains an NF-Y binding site (CCAAT), indicating that the NF-Y pioneer transcription factors might be involved in *FT* transcription from that site (Figure 4A) (Cao et al., 2014; Siriwardana et al., 2016). NF-Ys not only prevent the deposition of H3K27 trimethylation on the *FT* promoter (Liu

et al., 2018) but also are important for formation of the looping structure of the *FT* promoter (Figure 4C) (Cao et al., 2014). The timing of DNA loop formation matches the timing of *FT* induction, suggesting that *FT* transcription is accompanied by structural changes in chromatin. It was initially proposed that CO interacted with the NF-YA/NF-YB/NF-YC tripartite complex to form the DNA looping structure together (Cao et al., 2014); however, a recent crystallographic study revealed that NF-YA and CO interact with the same domain of the NF-YB/NF-YC complex through similar interfaces (Lv et al., 2021). Thus, it is unlikely that both NF-YA and CO simultaneously bind to the same NF-YB/NF-YC complex, and the detailed mechanism by which NF-Ys bring Block C close to Block A remains to be clarified.

Block B also contains an NF-Y binding site, and this area forms a DNA loop structure (Cao et al., 2014). However, unlike Block C, the looping of Block B was not affected by the *nf-yb2/3* double mutations. On the other hand, the *co* mutation significantly reduced the loop structure of Block B, suggesting that some unknown components directly bind to CO to form the loop structure. Although it is still unknown whether Block E also forms a similar loop structure, this region contains an NF-Y binding site. It is tempting to postulate that Block E also forms a similar DNA loop in an NF-Y-dependent manner to participate in *FT* transcriptional regulation (Figure 4C).

Evolutionary conservation of CO and NF-Y interaction

NF-YB and NF-YC form a protein complex with CO not only in *Arabidopsis* but also in other plant species. The formation of the tripartite complex of CO homologs with NF-YB and NF-YC is important for controlling *FT* homologs for flowering in the SD crop rice (*Oryza sativa*) and the LD crop wheat (*Triticum aestivum*).

In rice, in contrast to *Arabidopsis*, Heading date 1 (Hd1), a rice homolog of CO, behaves as a flowering repressor under LD conditions but is converted into an activator under SD conditions (Yano et al., 2000; Izawa et al., 2002; Hayama et al., 2003). Because Hd1 acts as a major repressor of the rice *FT homolog Hd3a* in a day-length-dependent manner, humans have selected rice *Hd1* mutants for rice cultivation in northern latitudes, as day length is longer in the north than in the south during summer. Among such early-heading cultivars, some have a mutation in the CCT domain of Hd1 that impairs its physical association with DTH8 (days to heading on chromosome 8; also named OsNF-YB11/Ghd8/Hd5) and OsNF-YCs (Goretti et al., 2017). This mutation causes a severe loss of Hd1 DNA-binding activity on the *Hd3a* promoter, which results in an early-heading phenotype under LD conditions. A recent crystallographic study demonstrated that the interaction with DTH8 and OsNF-YC2 strengthens the DNA-binding activity of $Hd1^{CCT}$ on the *Hd3a* locus (Shen et al., 2020). These results indicate that, as in *Arabidopsis*, interactions with NF-YB and NF-YC at the CCT domain are crucial for DNA-binding activity of Hd1 in rice, despite its mode of action being opposite to that in *Arabidopsis*. It appears that the functional switching of Hd1 between LD and SD conditions is partially explained by its interaction with DTH8. Hd1 enhances the levels of H3K27me3 at the *Hd3a* locus to repress gene expression in a DTH8-dependent manner under LD conditions, whereas Hd1 acts as a positive regulator of *Hd3a* under SD conditions, regardless of the presence of functional DTH8 (Du et al., 2017). Based on these observations, the authors speculated that Hd1 alone acted as a positive regulator of Hd3a, but physical association with DTH8 switched its function, and that abundance of the Hd1–DTH8 protein complex might be altered between LD and SD conditions.

Consistent with this idea, a recent study using multiple knockout mutant lines showed that Hd1 behaves like a positive regulator of flowering in the absence of DTH8 and another well-known flowering CCT-domain repressor protein, Ghd7. The triple mutation of *Hd1*, *DTH8*, and *Ghd7* delayed heading compared with the double mutation of *DTH8* and *Ghd7* (Zong et al., 2021). Because Ghd7 interacts with Hd1 and DTH8 (Nemoto et al., 2016; Shen et al., 2020; Zong et al., 2021), it appears that DTH8, together with Ghd7, converts Hd1's function to that of a flowering repressor. Taken together, these results suggest that physical association with NF-YB and NF-YC may be critical for the rice CO homolog Hd1 to control SD-induced expression of the rice florigen *Hd3a*.

In wheat, the CO/NF-Y complex may be involved in both vernalization and photoperiodic flowering responses (Li et al., 2011). Winter wheat requires exposure to low temperatures to facilitate a transition to the reproductive stage, a process tightly regulated by *VERNALIZATION* (*VRN*) genes. The *VRN2* locus consists of two tandemly duplicated genes, *ZCCT1* and *ZCCT2*. ZCCTs are CCT proteins that strongly repress the expression of *VRN3*, an *FT* homolog in wheat (Yan et al., 2004, 2006). Once the expression of *VRN2* genes (especially *ZCCT1*) is depleted after vernalization, *VRN3* (wheat *FT*) is derepressed (Yan et al., 2004, 2006). One of the wheat CO homologs, CO2, functions as a positive regulator of flowering and physically interacts with various NF-Ys at the CCT domain (Nemoto et al., 2003; Li et al., 2011). Li et al. (2011) found that the CCT domain of ZCCT1 competes with CO2 for NF-Ys. NF-Ys potentially work as negative transcriptional co-regulators of *VRN3* before vernalization, together with ZCCTs, but they work as positive co-regulators of CO2 after vernalization, although it is still unclear whether interaction with NF-Ys is necessary for DNA-binding activity of the ZCCTs.

It appears that the protein complex comprised of CO/NF-YB/NF-YC exists in terrestrial plants and modern chlorophytes. In the green algae *Chlamydomonas reinhardtii*, the CO homolog CrCO forms protein complexes with NF-YB and NF-YC to facilitate its transcriptional activity (Tokutsu et al., 2019). CrCO targets the promoters of *LHCSR* genes encoding light-harvesting complex proteins in *C. reinhardtii* (Gabilly et al., 2019; Tokutsu et al., 2019). Because the interaction with NF-YB is essential for transcriptional activity of CrCO, loss of either CrCO or NF-YB decreases *LHCSR* transcription; as a result, LHCSR-dependent photoprotection is severely attenuated (Tokutsu et al., 2019). CrCO was first reported for its positive activity on *Arabidopsis FT* gene expression, and the authors claimed that the basic function of CO originated in ancient chlorophyte species (Serrano et al., 2009). However, this argument was questioned based on a detailed phylogenetic analysis of *CO* homologs (Ballerini and Kramer, 2011). The phylogenetic analysis showed that *CrCO* was more closely related to different *CO*-like proteins that control light signaling than to *CO* and *Hd1*

(Ballerini and Kramer, 2011). In addition, Simon et al. (2015) suggested that *CO* homologs in photoperiodic flowering in different flowering plants arose by convergent evolution rather than evolution from a common ancestor. Although the *CO* homologs may not have originated from a single ancestral gene from chlorophytes to angiosperms, the CO (or COL)/NF-YB/NF-YC transcriptional unit has been utilized to precisely respond to surrounding light environments.

DNA-binding patterns of *FT* regulators

Figure 4A and 4B illustrate the approximate binding sites of *FT*-regulating transcription factors (for a list of transcription factors and specific binding regions, see Supplemental Table 2). These figures depict most CO binding partners (AS1, CIBs, NF-Ys, MRG2, BOI, RGA1, and PKL, but not APL) that bind to Block A, regardless of whether they act as positive or negative regulators (Li et al., 2008; Song et al., 2012a; Liu et al., 2013; Bu et al., 2014; Nguyen et al., 2015). If these CO-interacting factors are expressed in the same cells, they might compete or cooperate with each other to fine-tune *FT* expression. Moreover, the CO-binding chromatin remodeling factor PKL and its partner ATX1 overlap in their binding sites on the *FT* promoter, consistent with their physical association (Jing et al., 2019b).

Two related G2-like transcription factors exhibit opposite roles in *FT* transcriptional regulation—APL and EARLY FLOWERING MYB PROTEIN (EFM) (also known as HHO4). APL binds 5.3 kb (Block C) and 4.5 kb upstream of *FT* (Shibuta and Abe, 2017). It is plausible that APL binds to Block C through interaction with NF-YB. In contrast to APL, EFM is a negative regulator of *FT*. Both APL and EFM bind the *FT* promoter at 4.5 kb upstream (Yan et al., 2014), and it is therefore possible that they compete for this locus. EFM physically binds to the H3K36me2 demethylase JUMONJI30 (JMJ30), indicating that EFM helps to change *FT* DNA-folding structures. A chromatin immunoprecipitation assay confirmed that JMJ30 also binds to the *FT* promoter; however, its binding sites are different from that of EFM (Figure 4B) (Yan et al., 2014).

SHORT VEGETATIVE PHASE (SVP), FLM/MAF1, and FLC are all MADS-box transcription factors that bind to specific CArG box sequences to repress *FT*. They work as heterodimers (SVP–FLC, SVP–FLM, FLC–FLM, etc.) or possibly hetromultimers (Figure 2) (Lee et al., 2007; Li et al., 2008; Gu et al., 2013; Lee et al., 2013; Posé et al., 2013) and bind to the first intron of *FT* (Figure 4B) (Searle et al., 2006; Lee et al., 2007, 2013). The SVP–FLM protein complex function is titrated to regulate *FT* levels under different ambient temperatures through temperature-dependent alternative splicing of *FLM*, and the significance of different *FLM* splicing isoforms is a topic of active discussion. Ambient temperature changes alter the splicing patterns of *FLM* transcripts, resulting in the generation of several different *FLM* splicing variants. Among them, *FLMβ* encodes a full-length protein, whereas *FLMδ* encodes a truncated protein that lacks a part of the MADS-box DNA-binding domain. *FLMδ* is preferentially produced in higher-temperature environments. It was proposed that a high proportion of inactive (non-DNA-binding) SVP–FLMδ formation sequesters SVP from the active SVP–FLMβ complex, thus causing early flowering in high-temperature environments (Posé et al., 2013). The other mechanism is that the SVP protein is destabilized under higher temperatures, contributing to a reduction in the active SVP–FLMβ complex (Lee et al., 2013).

However, as recently reviewed by John et al. (2021), follow-up studies questioned the importance of FLMδ in flowering regulation. Studies using *Arabidopsis* natural accession lines revealed that expression levels of *FLMβ* but not *FLMδ* significantly affect flowering timing (Lutz et al., 2015, 2017). Moreover, a genome-edited mutant line expressing only *FLMδ* but not *FLMβ* showed similar flowering timing compared with the *flm-3* null mutant, suggesting that *FLMδ* does not play an active role in repressing SVP's repressive influence on flowering (Capovilla et al., 2017). In fact, there are many different *FLM* splice variants in addition to *FLMβ and FLMδ* (Sureshkumar et al., 2016; Capovilla et al., 2017). Sureshkumar et al. (2016) showed that many of them contain early stop codons, and they are induced by high ambient temperature. Truncated splice variants are preferentially degraded through nonsense-mediated mRNA decay, resulting in depletion of total *FLMβ* mRNA levels, and this is potentially the primary mechanism of temperature-dependent flowering regulation. In addition to regulation surrounding *FLM* splicing, Gu et al. (2013) highlighted the importance of interactions between FLC and MAF proteins (FLM/MAF1 and MAF2–4). According to this study, FLC and MAF proteins form a protein–protein network that influences temperature-dependent flowering, supporting the notion that interactions among MADS-box proteins fine-tune flowering regulation under different temperature conditions. Under simulated natural LD conditions (LD + supplemental FR + temperature oscillation), the *svp* single mutation enhances *FT* expression in the morning, whereas the *svp/flm/flc* triple mutation results in augmented *FT* expression in both the morning and evening (Figure 2) (Song et al., 2018). These results suggest that these MADS-box transcription factors participate in generating the daily *FT* expression profile not only under colder temperatures but also under regular growth temperatures.

In addition to colder temperatures, warmer temperatures (e.g., 27°C) also influence the timing of photoperiodic flowering. In SD conditions (22°C), *Arabidopsis* plants usually flower later because *FT* is not induced. However, when the ambient temperature is high, for example 27°C, even SD-grown plants show early flowering with induction of *FT* (Kumar et al., 2012). This is in part controlled by eviction of the histone variant H2A.Z from the +1 nucleosome located just downstream of the *FT* TSS (Kumar et al., 2012), which negatively regulates transcription initiation. The SWR1 complex (SWR1-C) exchanges histone H2A in the +1 nucleosome with the histone variant H2A.Z to titrate the expression of target genes. Although it was known that the *Arabidopsis* SWR1-C component mutant *swc6* shows early flowering in LD conditions (March-Diaz and Reyes 2009), the mechanism by which SWR1-C was recruited to the *FT* locus was not known. A recent work revealed the molecular mechanism of SWR1-C targeting to the *FT* TSS. SWR6 (SWR1-C component)-associated DNA-binding protein SWC4 binds to AT-rich sequences at the *FT* TSS region, and binding of SWC4 to the *FT* TSS facilitates recruitment of SWR1-C to the *FT* TSS for H2A.Z deposition (Gomez-Zambrano et al., 2018). It would be interesting to investigate how higher temperature regulates H2A.Z eviction and whether this H2A.Z-related regulation is modulated by day-length conditions.

Environmental responses of protein–protein interactions of *FT* regulators

We highlighted the protein–protein interactions between CO and other proteins in Figures 1 and 3. Here, to enhance our understanding of the role of protein–protein interactions related to *FT* transcriptional regulation, we constructed an interaction network model of flowering regulators without the network interacting with CO (Figure 5; Supplemental Table 3). At first glance, the entire known interactome is quite complicated (Figure 5A). However, based mainly on the daily expression patterns of mRNA or protein and the presence or absence of light signals, proposed models of time-resolved interaction networks seem to be less complicated (Figure 5B, 5C, and 5D). In the LD morning, B and R/FR signals repress COP1–SPA degradation mechanisms, but genes (e.g., *CDF*s, *BBX*s, and *PRR9*) that peak in the morning are mainly repressors that bind to TPL/TPRs (Figure 5B). These repressors may keep *FT* levels low during the morning, even though CO protein is temporarily stabilized at the beginning of the morning (Valverde et al., 2004). Most of the interactions take place in the afternoon when *FT* is induced (Figure 5C). Although they all exist at the time of *FT* induction, none of these analyzed genes show spatial expression patterns similar to that of *FT*, indicating that investigating unknown posttranscriptional and translational regulation and protein activity of these factors where they are expressed may be important to further understand their mechanistic contributions to *FT* regulation. At night, most of the transcription factors in this diagram become less stable and are known to interact with the COP1–SPA complex for degradation (Figure 5D), possibly resetting the entire network for responding to seasonal information on the next day.

This diagram shows that most protein–protein interactions are directly or indirectly influenced by B signaling. Of particular note are CRY1 and CRY2, which appear to influence several processes from the top of the network. CRY1/2 proteins directly activate CIBs and BR ENHANCED EXPRESSION 1 (BEE1) bHLH transcription factors (Liu et al., 2008; Wang et al., 2019) and deactivate the AP2-like repressors TOE1 and TOE2 (Du et al., 2020) under B conditions, although BEE1 and TOE1/2 are expressed from the morning, whereas CIBs peak in the LD afternoon. CRY1/2 proteins also bind to the COP1–SPA complex when CRY1/2 absorb B, impairing the E3 ubiquitin ligase activity (Liu et al., 2011; Zuo et al., 2011; Holtkotte et al., 2017). Because GI is a target of the ELF3–COP1 complex (Yu et al., 2008), activation of CRYs appears to stabilize GI indirectly. GI forms a complex with another B photoreceptor, FKF1, regulating CDF and PRR protein stabilities (Sawa et al., 2007; Baudry et al., 2010). FKF1 also enhances CIB1–CIB3 heterodimerization under B, which enhances CIB1–CIB3 activity to increase *FT* in the LD afternoon (Zhou et al., 2021). Moreover, *GI* is required for type II TCPs to activate *CO* transcription (Kubota et al., 2017). The FKF1 homologs LKP2 and ZTL also contribute to CDF/PRR degradation through their interaction with GI (Fornara et al., 2009; Krahmer et al., 2019).

Another large interaction hub is related to GA responses, indicating that GA perception simultaneously affects several different pathways, primarily through DELLA degradation (Figure 5). DELLAs directly inhibit several *FT* positive regulators such as bHLH48, bHLH60, PIF4, PKL, and WRKY75 (De Lucas et al., 2008; Zhang et al., 2014, 2018; Li et al., 2017). A recent study revealed another *FT*-regulating pathway mediated by DELLAs (Fukazawa et al., 2021). GAI-ASSOCIATED FACTOR 1 (GAF1) is a DELLA interactor. After GA perception, GAF1 is released from DELLAs and subsequently associates with TPR4. GAF1–TPR4 functions as a transcriptional repressor complex that suppresses expression of the flowering repressors *ELF3*, *SVP*, *TEM1*, and *TEM2*. Thus, DELLAs regulate *FT* transcription levels in part by controlling levels of the GAF1–TPR4 protein–protein interaction (Fukazawa et al., 2014, 2021). DELLAs also contribute to several repressors' actions. MYC3 not only binds to the *FT* promoter (Figure 4B) but also prevents the DNA-binding activity of CO through interaction with DELLA proteins (Bao et al., 2019). Moreover, the CO-interacting protein BOI binds to the *FT* promoter with the help of DELLA (Park et al., 2013). Recent studies demonstrated that CRY1 stabilizes DELLA proteins through direct interaction, whereas FKF1 degrades DELLA proteins to control diurnal changes in DELLA protein stability through ubiquitination (Yan et al., 2020, 2021; Xu et al., 2021). DELLA protein levels are low during the night because of GA-signaling-dependent degradation (Arana et al., 2011). These results suggest that B perception controls the photoperiodic flowering pathway in part by modulating the GA-dependent flowering pathway during the day.

In contrast to well-characterized B- and GA-dependent flowering activation, Figure 5 shows few examples of FR-dependent protein–protein interactions, despite the strong effect of FR on *FT* expression. This relative lack of understanding may be a consequence of typical artificial laboratory light conditions, which often subject plants to R/FR ratios greater than 2. Under such conditions, the importance of the FR signal is easily overlooked simply because of insufficient FR, although FR is a necessary component for the morning peak of *FT* under natural LD conditions (Figure 2) (Song et al., 2018). Only two direct mechanisms that affect *FT* transcription under FR have been characterized to date. First, CO is stabilized to a greater extent under conditions with supplemental FR in a phyA-dependent manner (Valverde et al., 2004; Song et al., 2018), although the detailed mechanism is still unknown. Second, PIF proteins are stabilized because phyB is converted from the active Pfr to the inactive Pr form under FR. phyB physically interacts with and destabilizes the flowering activators PIF4, 5, and 7 (Huq and Quail, 2002; Lorrain et al., 2008; Kidokoro et al., 2009), and FR supplementation therefore results in PIF protein accumulation. Among the PIFs, PIF7 plays the primary role in shade-induced flowering and is subject to negative feedback by HFR1 (Zhang et al., 2019).

POST-TRANSCRIPTIONAL AND POST-TRANSLATIONAL REGULATION AFFECT FT PROTEIN LEVELS

Although most studies have focused on the mechanism controlling *FT* transcription levels, Seo et al. (2011) reported that the WD40 protein WEREWOLF (WER) is required for *FT* transcript stabilization. WER is known for its role in root hair patterning. It forms protein complexes with TRANSPARENT TESTA GLABRA1 (TTG1), GLABRA3 (GL3), and ENHANCER OF GLABRA3 (EGL3) to suppress the generation of root hairs. However, null mutation and overexpression of *WER* cause late and

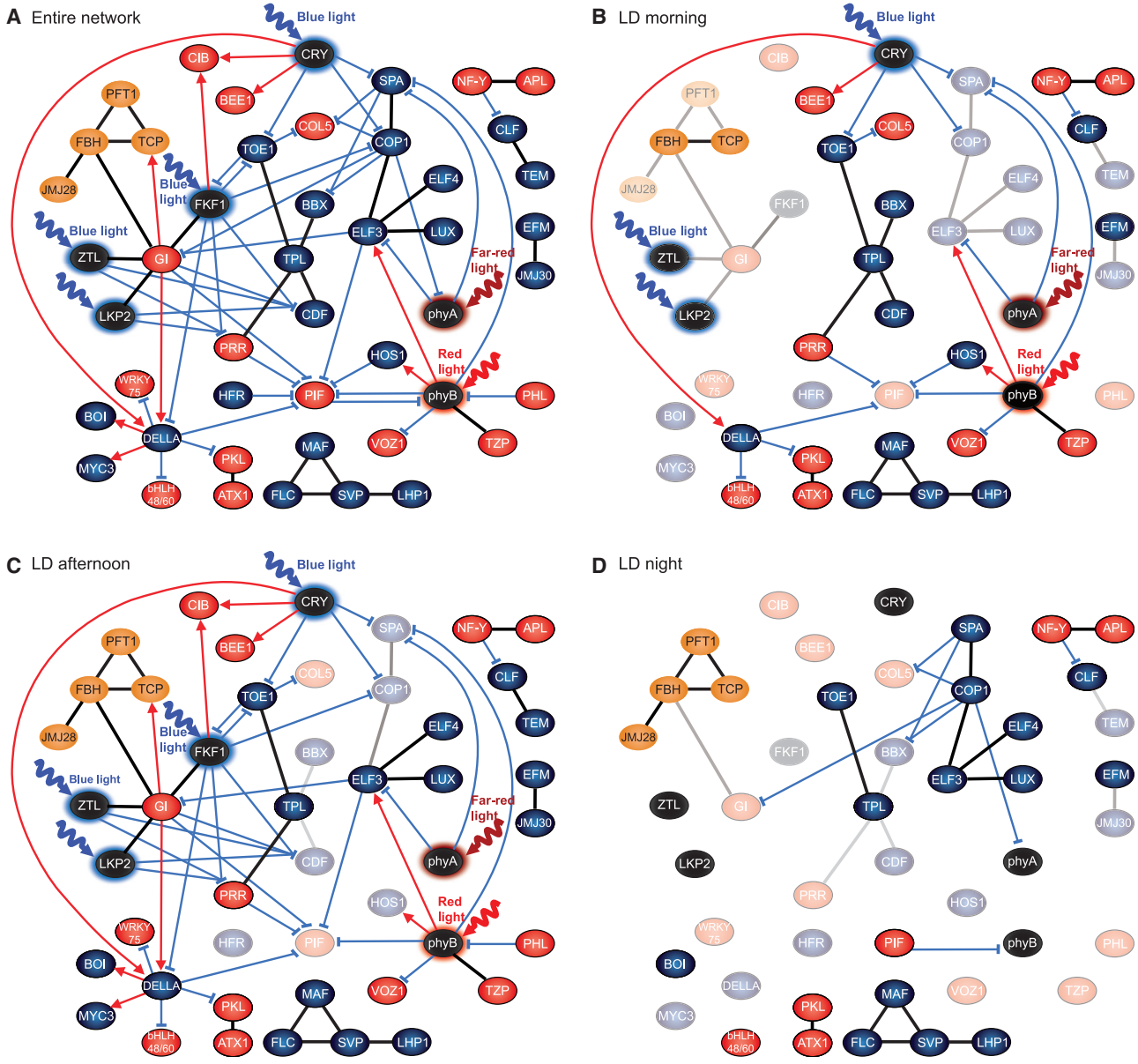

Figure 5. Working models for time-dependent protein–protein interactions of *FT*-regulating factors.

Protein interactomes of *CO*- and *FT*-regulating factors without CO and direct CO-interacting factors (Figures 1 and 3) are shown. Protein–protein interactions without functional characterization are not included in this network. Red arrows and blue lines indicate functional activation and repression, respectively. Black lines indicate the formation of functional units (often having mutual positive interactions). Black, red, blue, and orange circles indicate photoreceptors, positive regulators of *FT*, negative regulators of *FT*, and positive regulators of *CO*, respectively. Although PRRs are transcriptional repressors that work with TPL/TPRs, because *prr* multiple mutants showed late-flowering phenotypes with low *FT* expression (likely due to destabilization of CO), PRRs are designated as *FT* positive regulators in this diagram. Faded circles and lines in (**B–D**) indicate lower activities due to either lower expression levels or clear light-dependent control (e.g., light-induced repression of COP1–SPA activity). The activity classification (high or low) proposed here is a very simplified way to describe the complicated interactome based on the assumption that higher expression levels of mRNA or protein equal higher activity. For some factors, diurnal expression pattern results or light-dependent regulation results were not available (e.g., these types of results are lacking for many chromatin remodelers). In that case, their activities were deduced from *FT* expression changes in their mutants (e.g., *FT* levels are high in the *clf* mutant throughout the entire day and night in LD conditions, and we thus assume that CLF is active in both day and night). We propose these interactomes as working models (not solid facts) to guide further investigation of their interactions in the future.

(A) Shows all interactions described in **(B–D)**, clearly depicting the presence of complicated connections among factors that regulate *FT* transcription.

(B) In the LD morning, photoreceptor (phyA, phyB, and CRY1/2) signaling represses activity of the COP1–SPA complex. Some *FT* negative regulators (e.g., CDFs, BBXs) peak in the morning to repress *FT*.

(C) Most of the interactions depicted in **(A)** take place in the LD afternoon. These regulators are expressed and often stabilized in the LD afternoon when *FT* is induced.

(D) In the LD night, the amounts of many of these regulators become low, and some are known to be degraded by the COP1–SPA complex.

early flowering, respectively, under LD conditions. Curiously, mutations of the WER binding partners TTG1, GL3, and EGL3 also caused late flowering. The *wer-1* mutation does not change *FT* transcription activity as measured by GUS assay; however, it clearly decreases *FT* transcript levels. The authors also found that the *wer-1* mutation specifically makes *FT* transcripts unstable, but not other transcripts. These results suggest that WER1 somehow selectively stabilizes *FT* mRNA, despite the fact that *WER* appears to show little expression in leaf vascular tissues.

Arabidopsis controls FT protein levels with another layer of regulatory mechanisms—post-translational regulation. Like those of many other protein-coding genes, FT protein levels do not necessarily coincide with *FT* transcript levels (Kim et al., 2016). FT protein was stable and present throughout the day in LD conditions and became less stable once the floral transition had taken place. This observation suggested that the overall amount of *FT* transcript determined the amount of FT protein. The results demonstrated that the proteasome system targeted FT for degradation through its C-terminal end, which is important for its flowering-promoting function, and overexpression of a C-terminally truncated version of FT caused a dominant late-flowering phenotype. It would be interesting to know whether FT protein is destabilized before or after it functions and whether there are tissue-specific differences in FT stability.

FUTURE PERSPECTIVES

The large number of *FT* regulators reflects the significance of *FT* regulation for plant survival and reproduction. The use of multiple regulators may enable plants to respond to subtle variations in their growing environments and to flower under the most appropriate conditions. Despite the breadth and depth of current knowledge regarding *FT* transcriptional regulation, ongoing investigations continue to uncover novel modes of *FT* regulation. Given the central conserved role of *FT* in plant seasonal development, further exploration of *FT*-regulating processes in various plants will yield valuable insights into mechanisms of seasonal adaptation.

Notably, the influence of FR on the induction of *FT* in the mornings remains largely undescribed—a consequence of the use of fluorescent and LED light sources that fail to accurately simulate the R/FR ratio found in natural sunlight, masking its significance in *FT* induction. As implied in Figures 3 and 5, which show large B- and GA-dependent protein–protein networks, detailed mechanisms of FR-dependent CO stabilization and *FT* activation remain elusive. Although phyA is implicated in FR-dependent CO stabilization (Valverde et al., 2004), recent studies have conversely revealed that ELF3 negatively affects CO stabilization and the FR-dependent morning peak of *FT* (Figure 2) (Song et al., 2018), suggesting a yet-unrevealed mechanism of phyA-dependent modulation of ubiquitin ligase activity through its interactions with COP1–SPA, possibly via ELF3. Investigating the COP1–SPA and ELF3 protein complex may prove a productive approach to gaining a more complete understanding of CO stabilization and *FT* induction. It may shed light on the different contributions of phyA to induction of the morning and evening *FT* peaks (Song et al., 2018).

Another mechanism that remains to be fully clarified involves changes in the physical DNA structure of the *FT* locus. Many *FT*-regulating factors appear to affect the topology of genomic DNA in the *FT* region critical for gene expression. Notably, the formation of a loop structure by NF-Ys is crucial for *FT* induction (Cao et al., 2014). NF-YA, B, and C protein complexes contribute to the loop formed from Block C to A (Figure 4C), and CO requires interaction with NF-YB and NF-YC to bind to the region of Block A (Gnesutta et al., 2017). However, the mechanisms by which these complexes interact to control the DNA loop structure remain unknown. Recent advances have suggested the involvement of additional factors bridging the NF-Y complex and CO (Chaves-Sanjuan et al., 2021; Lv et al., 2021), but the exact nature of these crucial regulatory elements and contributing transcription factors for DNA looping remain elusive. Furthermore, Block E has only recently been identified as a regulatory region approximately 1 kb downstream of *FT* (Zicola et al., 2019). Thus far, a few *FT* regulators, PIF4, AGL15, SMZ, and LHP1, have been found to bind to this region (Mathieu et al., 2009; Adrian et al., 2010; Fernandez et al., 2014; Galvão et al., 2019). Previous studies of DNA–protein interaction (e.g., chromatin immunoprecipitation assays) have been limited primarily to investigating the upstream *FT* promoter to CDS regions; given the apparent importance of the Block E region, further exploration of its regulatory influence may yield promising advances in understanding aspects of the topological regulation of *FT* and genomic DNA.

Relatively little attention has been given to *FT* post-transcriptional and FT post-translational mechanisms compared with *FT* transcriptional regulation, despite a total lack of understanding of detailed mechanisms. For example, Kim et al. (2016) showed that an unknown protease regulates FT protein degradation, and the specific mechanism responsible remains to be clarified. Whether plants change *FT* transcript and FT protein stability in an environmentally responsive manner also remains to be discovered. Because accumulation of FT protein is the driving force for flowering, such information will be significant for understanding plant flowering regulation.

A recurring challenge has been clarifying the role of these regulators in mediating the unique spatial expression patterns of FT. Generally speaking, the roles of *FT* regulators have been investigated by analyzing *FT* levels during their period of peak expression (at dusk in LD conditions) or over LD time courses using whole tissue samples. *FT* is expressed in specific leaf phloem companion cells (Chen et al., 2018). Characterization of *FT* regulators often includes the use of endogenous promoter-driven GUS (β-glucuronidase) expression analysis to reveal their tissue-specific expression patterns. However, whether these regulators are expressed in the exact same cells where *FT* is expressed remains unclear. Therefore, tissue-specific gene expression analysis may be a powerful approach for obtaining more mechanistic and spatially appropriate insights into *FT* gene expression. Several different tissue-specific transcriptome approaches have recently been developed. For example, nuclei isolation methods from companion cells such as isolation of nuclei tagged in specific cell types and fluorescence-activated cell sorting have been reported (You et al., 2019; Shi et al., 2021; Tian et al., 2021). Laser microdissection may also be an effective avenue for exploring differences in tissue-specific gene expression (Berkowitz et al., 2021). Because each method possesses pros and cons (e.g., nuclear sorting typically

provides very specific but low RNA yields), the use of different approaches will be necessary to gain a comprehensive understanding of the often-overlooked mechanisms of tissue-specific regulation of gene expression.

In addition to isolation of specific cell types, single-cell RNA sequencing (scRNA-seq) technology has become a powerful approach for analyzing gene expression in different plant cell types. The availability of plant tissue-specific gene expression data is steadily increasing. Although the body of scRNA-seq data from aerial plant tissues continues to increase, accessible datasets describing gene expression specific to leaf companion cells remain scarce—in large part due to the difficulty in isolating strongly embedded companion cells. Improved methods of protoplast isolation from vascular tissues have recently been optimized (Kim et al., 2021). Although these protocols have significantly improved the yield of parenchyma and bundle sheath protoplasts, the yield from companion cells remains limited. Moreover, the long digestion times and high solution osmolarity required for protoplasting may be less valuable in studying *FT* regulation owing to the time-of-day and environmentally dependent *FT* expression profiles.

Many regulatory factors are involved in *FT* regulation of floral initiation and are required to facilitate the optimal timing of flowering in seasonal environments. Mathematical modeling and bioinformatic approaches may therefore be helpful for enhancing our understanding of the complicated, coordinated action of *FT regulators*. Through the combination of tissue-specific gene expression and mathematical modeling, we anticipate a promising yield of novel insights into the still-elusive processes that underlie the unique spatial and temporal expression patterns of *FT*. Although many intriguing questions remain to be answered, our mechanistic understanding of seasonal flowering has continuously progressed, particularly in *Arabidopsis*. We hope that the knowledge obtained from *Arabidopsis* continues to facilitate our understanding of flowering mechanisms in other plant species.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Plant Communications Online*.

FUNDING

This work is supported by grants from the National Institutes of Health (R01GM079712) and MEXT KAKENHI grants (20H05910 and 22H04978).

AUTHOR CONTRIBUTIONS

H.T., A.K.H., and T.I. conceived the contents and wrote the manuscript. H.T. and T.I. prepared the figures and tables.

ACKNOWLEDGMENTS

We apologize for not being able to cite all relevant works due to space limitations. No conflict of interest is declared.

Received: July 22, 2022
Revised: December 20, 2022
Accepted: January 18, 2023
Published: January 20, 2023

REFERENCES

Abe, M., Kaya, H., Watanabe-Taneda, A., Shibuta, M., Yamaguchi, A., Sakamoto, T., Kurata, T., Ausín, I., Araki, T., and Alonso-Blanco, C. (2015). FE, a phloem-specific Myb-related protein, promotes flowering through transcriptional activation of *FLOWERING LOCUS T* and *FLOWERING LOCUS T INTERACTING PROTEIN 1*. Plant J. **83**:1059–1068.

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K., and Araki, T. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science **309**:1052–1056.

Adrian, J., Farrona, S., Reimer, J.J., Albani, M.C., Coupland, G., and Turck, F. (2010). *cis*-regulatory elements and chromatin state coordinately control temporal and spatial expression of *FLOWERING LOCUS T* in *Arabidopsis*. Plant Cell **22**:1425–1440.

Ahmad, M., and Cashmore, A.R. (1993). *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature **366**:162–166.

An, H., Roussot, C., Suárez-López, P., Corbesier, L., Vincent, C., Piñeiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C., et al. (2004). CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. Development **131**:3615–3626.

An, Z., Yin, L., Liu, Y., Peng, M., Shen, W.H., and Dong, A. (2020). The histone methylation readers MRG1/MRG2 and the histone chaperones NRP1/NRP2 associate in fine-tuning Arabidopsis flowering time. Plant J. **103**:1010–1024.

Arana, M.V., Marín-de la Rosa, N., Maloof, J.N., Blázquez, M.A., and Alabadí, D. (2011). Circadian oscillation of gibberellin signaling in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **108**:9292–9297.

Arongaus, A.B., Chen, S., Pireyre, M., Glöckner, N., Galvão, V.C., Albert, A., Winkler, J.B., Fankhauser, C., Harter, K., and Ulm, R. (2018). *Arabidopsis* RUP2 represses UVR8-mediated flowering in noninductive photoperiods. Genes Dev. **32**:1332–1343.

Ballerini, E.S., and Kramer, E.M. (2011). In the light of evolution: a reevaluation of conservation in the *CO–FT* regulon and its role in photoperiodic regulation of flowering time. Front. Plant Sci. **2**:81.

Bao, S., Hua, C., Huang, G., Cheng, P., Gong, X., Shen, L., and Yu, H. (2019). Molecular basis of natural variation in photoperiodic flowering responses. Dev. Cell **50**:90–101.e3.

Baudry, A., Ito, S., Song, Y.H., Strait, A.A., Kiba, T., Lu, S., Henriques, R., Pruneda-Paz, J.L., Chua, N.H., Tobin, E.M., et al. (2010). F-Box proteins FKF1 and LKP2 act in concert with ZEITLUPE to control Arabidopsis clock progression. Plant Cell **22**:606–622.

Berkowitz, O., Xu, Y., Liew, L.C., Wang, Y., Zhu, Y., Hurgobin, B., Lewsey, M.G., and Whelan, J. (2021). RNA-seq analysis of laser microdissected *Arabidopsis thaliana* leaf epidermis, mesophyll and vasculature defines tissue-specific transcriptional responses to multiple stress treatments. Plant J. **107**:938–955.

Bonke, M., Thitamadee, S., Mähönen, A.P., Hauser, M.T., and Helariutta, Y. (2003). APL regulates vascular tissue identity in Arabidopsis. Nature **426**:181–186.

Bouché, F., Lobet, G., Tocquin, P., and Périlleux, C. (2016). FLOR-ID: an interactive database of flowering-time gene networks in Arabidopsis thaliana. Nucleic Acids Res. **44**:D1167–D1171.

Bu, Z., Yu, Y., Li, Z., Liu, Y., Jiang, W., Huang, Y., and Dong, A.W. (2014). Regulation of *Arabidopsis* flowering by the histone mark readers MRG1/2 via interaction with CONSTANS to modulate *FT* expression. PLoS Genet. **10**:e1004617.

Byrne, M.E., Barley, R., Curtis, M., Arroyo, J.M., Dunham, M., Hudson, A., and Martienssen, R.A. (2000). *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. Nature **408**:967–971.

Cao, S., Kumimoto, R.W., Gnesutta, N., Calogero, A.M., Mantovani, R., and Holt, B.F. (2014). A distal *CCAAT*/NUCLEAR FACTOR Y complex promotes chromatin looping at the *FLOWERING LOCUS T* promoter and regulates the timing of flowering in *Arabidopsis*. Plant Cell **26**:1009–1017.

Cao, S., Luo, X., Xu, D., Tian, X., Song, J., Xia, X., Chu, C., and He, Z. (2021). Genetic architecture underlying light and temperature mediated flowering in *Arabidopsis*, rice, and temperate cereals. New Phytol. **230**:1731–1745.

Capovilla, G., Symeonidi, E., Wu, R., and Schmid, M. (2017). Contribution of major FLM isoforms to temperature-dependent flowering in *Arabidopsis thaliana*. J. Exp. Bot. **68**:5117–5127.

Casal, J.J. (2012). Shade avoidance. Arabidopsis Book **10**:e0157.

Celesnik, H., Ali, G.S., Robison, F.M., and Reddy, A.S.N. (2013). Arabidopsis thaliana VOZ (vascular plant one-zinc finger) transcription factors are required for proper regulation of flowering time. Biol. Open **2**:424–431.

Cha, J.Y., Kim, J., Kim, T.S., Zeng, Q., Wang, L., Lee, S.Y., Kim, W.Y., and Somers, D.E. (2017). GIGANTEA is a co-chaperone which facilitates maturation of ZEITLUPE in the *Arabidopsis* circadian clock. Nat. Commun. **8**:3.

Chang, G., Yang, W., Zhang, Q., Huang, J., Yang, Y., and Hu, X. (2019). ABI5-BINDING PROTEIN2 coordinates CONSTANS to delay flowering by recruiting the transcriptional corepressor TPR2. Plant Physiol. **179**:477–490.

Chaves-Sanjuan, A., Gnesutta, N., Gobbini, A., Martignago, D., Bernardini, A., Fornara, F., Mantovani, R., and Nardini, M. (2021). Structural determinants for NF-Y subunit organization and NF-Y/DNA association in plants. Plant J. **105**:49–61.

Chen, Q., Payyavula, R.S., Chen, L., Zhang, J., Zhang, C., and Turgeon, R. (2018). *FLOWERING LOCUS T* mRNA is synthesized in specialized companion cells in *Arabidopsis* and Maryland Mammoth tobacco leaf veins. Proc. Natl. Acad. Sci. USA **115**:2830–2835.

Song, S., Gan, Y., Chen, Y., Jiang, L., Yu, H., Shen, L., Jiang, L., Yu, H., and Shen, L. (2020). SHAGGY-like kinase 12 regulates flowering through mediating CONSTANS stability in *Arabidopsis*. Sci. Adv. **6**:eaaw0413.

Cheng, Z., Zhang, X., Huang, P., Huang, G., Zhu, J., Chen, F., Miao, Y., Liu, L., Fu, Y.-F., and Wang, X. (2020). Nup96 and HOS1 are mutually stabilized and gate CONSTANS protein level, conferring long-day photoperiodic flowering regulation in Arabidopsis. Plant Cell **32**:374–391.

De Lucas, M., Davière, J.M., Rodríguez-Falcón, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blázquez, M.A., Titarenko, E., and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature **451**:480–484.

Du, A., Tian, W., Wei, M., Yan, W., He, H., Zhou, D., Huang, X., Li, S., and Ouyang, X. (2017). The DTH8-Hd1 module mediates day-length-dependent regulation of rice flowering. Mol. Plant **10**:948–961.

Du, S.S., Li, L., Li, L., Wei, X., Xu, F., Xu, P., Wang, W., Xu, P., Cao, X., Miao, L., et al. (2020). Photoexcited cryptochrome2 interacts directly with toe1 and toe2 in flowering regulation. Plant Physiol. **184**:487–505.

Endo, M., Tanigawa, Y., Murakami, T., Araki, T., and Nagatani, A. (2013). Phytochrome-dependent late-flowering accelerates flowering through physical interactions with phytochrome B and CONSTANS. Proc. Natl. Acad. Sci. USA **110**:18017–18022.

Fernandez, D.E., Wang, C.T., Zheng, Y., Adamczyk, B.J., Singhal, R., Hall, P.K., and Perry, S.E. (2014). The MADS-domain factors AGAMOUS-LIKE15 and AGAMOUS-LIKE18, along with SHORT VEGETATIVE PHASE and AGAMOUS-LIKE24, are necessary to block floral gene expression during the vegetative phase. Plant Physiol. **165**:1591–1603.

Fornara, F., Panigrahi, K.C.S., Gissot, L., Sauerbrunn, N., Rühl, M., Jarillo, J.A., and Coupland, G. (2009). Arabidopsis DOF transcription factors act redundantly to reduce *CONSTANS* expression and are essential for a photoperiodic flowering response. Dev. Cell **17**:75–86.

Fukazawa, J., Ohashi, Y., Takahashi, R., Nakai, K., and Takahashi, Y. (2021). DELLA degradation by gibberellin promotes flowering via GAF1-TPR-dependent repression of floral repressors in Arabidopsis. Plant Cell **33**:2258–2272.

Fukazawa, J., Teramura, H., Murakoshi, S., Nasuno, K., Nishida, N., Ito, T., Yoshida, M., Kamiya, Y., Yamaguchi, S., and Takahashi, Y. (2014). DELLAs function as coactivators of GAI-ASSOCIATED FACTOR1 in regulation of gibberellin homeostasis and signaling in Arabidopsis. Plant Cell **26**:2920–2938.

Gabilly, S.T., Baker, C.R., Wakao, S., Crisanto, T., Guan, K., Bi, K., Guiet, E., Guadagno, C.R., and Niyogi, K.K. (2019). Regulation of photoprotection gene expression in *Chlamydomonas* by a putative E3 ubiquitin ligase complex and a homolog of CONSTANS. Proc. Natl. Acad. Sci. USA **116**:17556–17562.

Galvão, V.C., Fiorucci, A.-S., Trevisan, M., Franco-Zorilla, J.M., Goyal, A., Schmid-Siegert, E., Solano, R., and Fankhauser, C. (2019). PIF transcription factors link a neighbor threat cue to accelerated reproduction in *Arabidopsis*. Nat. Commun. **10**:4005.

Gao, J., Zhu, Y., Zhou, W., Molinier, J., Dong, A., and Shen, W.H. (2012). NAP1 family histone chaperones are required for somatic homologous recombination in *Arabidopsis*. Plant Cell **24**:1437–1447.

Garcia, M.E., Lynch, T., Peeters, J., Snowden, C., and Finkelstein, R. (2008). A small plant-specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating *Arabidopsis* seeds and seedlings. Plant Mol. Biol. **67**:643–658.

Gnesutta, N., Kumimoto, R.W., Swain, S., Chiara, M., Siriwardana, C., Horner, D.S., Holt, B.F., and Mantovani, R. (2017). CONSTANS imparts DNA sequence specificity to the histone fold NF-YB/NF-YC Dimer. Plant Cell **29**:1516–1532.

Gómez-Zambrano, Á., Crevillén, P., Franco-Zorrilla, J.M., López, J.A., Moreno-Romero, J., Roszak, P., Santos-González, J., Jurado, S., Vázquez, J., Köhler, C., et al. (2018). Arabidopsis SWC4 binds DNA and recruits the SWR1 complex to modulate histone H2A.Z deposition at key regulatory genes. Mol. Plant **11**:815–832.

González-Arzola, K., Díaz-Quintana, A., Rivero-Rodríguez, F., Velázquez-Campoy, A., De La Rosa, M.A., and Díaz-Moreno, I. (2017). Histone chaperone activity of *Arabidopsis thaliana* NRP1 is blocked by cytochrome *c*. Nucleic Acids Res. **45**:2150–2165.

Goralogia, G.S., Liu, T.K., Zhao, L., Panipinto, P.M., Groover, E.D., Bains, Y.S., and Imaizumi, T. (2017). CYCLING DOF FACTOR 1 represses transcription through the TOPLESS co-repressor to control photoperiodic flowering in Arabidopsis. Plant J. **92**:244–262.

Goretti, D., Martignago, D., Landini, M., Brambilla, V., Gómez-Ariza, J., Gnesutta, N., Galbiati, F., Collani, S., Takagi, H., Terauchi, R., et al. (2017). Transcriptional and post-transcriptional mechanisms limit Heading date 1 (Hd1) function to adapt rice to high latitudes. PLoS Genet. **13**:e1006530.

Graeff, M., Straub, D., Eguen, T., Dolde, U., Rodrigues, V., Brandt, R., and Wenkel, S. (2016). MicroProtein-Mediated recruitment of CONSTANS into a TOPLESS trimeric complex represses flowering in Arabidopsis. PLoS Genet. **12**:e1005959.

Gruber, H., Heijde, M., Heller, W., Albert, A., Seidlitz, H.K., and Ulm, R. (2010). Negative feedback regulation of UV-B-induced photomorphogenesis and stress acclimation in *Arabidopsis*. Proc Natl Acad Sci U S A **107**:20132–20137.

Gu, X., Le, C., Wang, Y., Li, Z., Jiang, D., Wang, Y., and He, Y. (2013). *Arabidopsis* FLC clade members form flowering-repressor complexes coordinating responses to endogenous and environmental cues. Nat. Commun. **4**:1947.

Guo, H., Yang, H., Mockler, T.C., and Lin, C. (1998). Regulation of flowering time by *Arabidopsis* photoreceptors. Science **279**:1360–1363.

Guo, Z., Li, Z., Liu, Y., An, Z., Peng, M., Shen, W.H., Dong, A., and Yu, Y. (2020). MRG1/2 histone methylation readers and HD2C histone deacetylase associate in repression of the florigen gene *FT* to set a

proper flowering time in response to day-length changes. New Phytol. **227**:1453–1466.

Hassidim, M., Harir, Y., Yakir, E., Kron, I., and Green, R.M. (2009). Over-expression of *CONSTANS-LIKE 5* can induce flowering in short-day grown *Arabidopsis*. Planta **230**:481–491.

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

Hayama, R., Yokoi, S., Tamaki, S., Yano, M., and Shimamoto, K. (2003). Adaptation of photoperiodic control pathways produces short-day flowering in rice. Nature **422**:719–722.

Heijde, M., and Ulm, R. (2013). Reversion of the *Arabidopsis* UV-B photoreceptor UVR8 to the homodimeric ground state. Proc Natl Acad Sci U S A **110**:1113–1118.

Holtkotte, X., Ponnu, J., Ahmad, M., and Hoecker, U. (2017). The blue light-induced interaction of cryptochrome 1 with COP1 requires SPA proteins during Arabidopsis light signaling. PLoS Genet. **13**:e1007044.

Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O., and Fankhauser, C. (2009). Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. EMBO J. **28**:3893–3902.

Huang, H., Alvarez, S., Bindbeutel, R., Shen, Z., Naldrett, M.J., Evans, B.S., Briggs, S.P., Hicks, L.M., Kay, S.A., and Nusinow, D.A. (2016). Identification of evening complex associated proteins in arabidopsis by affinity purification and mass spectrometry. Mol. Cell. Proteomics **15**:201–217.

Hung, F.Y., Lai, Y.C., Wang, J., Feng, Y.R., Shih, Y.H., Chen, J.H., Sun, H.C., Yang, S., Li, C., and Wu, K. (2021). The Arabidopsis histone demethylase JMJ28 regulates *CONSTANS* by interacting with FBH transcription factors. Plant Cell **33**:1196–1211.

Huq, E., and Quail, P.H. (2002). PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in *Arabidopsis*. EMBO J. **21**:2441–2450.

Hwang, D.Y., Park, S., Lee, S., Lee, S.S., Imaizumi, T., and Song, Y.H. (2019). GIGANTEA regulates the timing stabilization of CONSTANS by altering the interaction between FKF1 and ZEITLUPE. Mol. Cell **42**:693–701.

Imaizumi, T., Schultz, T.F., Harmon, F.G., Ho, L.A., and Kay, S.A. (2005). FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. Science **309**:293–297.

Ito, S., Song, Y.H., Josephson-Day, A.R., Miller, R.J., Breton, G., Olmstead, R.G., and Imaizumi, T. (2012). FLOWERING BHLH transcriptional activators control expression of the photoperiodic flowering regulator *CONSTANS* in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **109**:3582–3587.

Izawa, T., Oikawa, T., Sugiyama, N., Tanisaka, T., Yano, M., and Shimamoto, K. (2002). Phytochrome mediates the external light signal to repress *FT* orthologs in photoperiodic flowering of rice. Genes Dev. **16**:2006–2020.

Jang, S., Marchal, V., Panigrahi, K.C.S., Wenkel, S., Soppe, W., Deng, X.W., Valverde, F., and Coupland, G. (2008). Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. EMBO J. **27**:1277–1288.

Jing, Y., Guo, Q., Zha, P., and Lin, R. (2019a). The chromatin-remodelling factor PICKLE interacts with CONSTANS to promote flowering in *Arabidopsis*. Plant Cell Environ. **42**:2495–2507.

Jing, Y., Guo, Q., and Lin, R. (2019b). The chromatin-remodeling factor PICKLE antagonizes polycomb repression of *FT* to promote flowering. Plant Physiol. **181**:656–668.

John, S., Olas, J.J., and Mueller-Roeber, B. (2021). Regulation of alternative splicing in response to temperature variation in plants. J. Exp. Bot. **72**:6150–6163.

Jung, J.H., Seo, P.J., and Park, C.M. (2012). The E3 ubiquitin ligase HOS1 regulates *Arabidopsis* flowering by mediating CONSTANS degradation under cold stress. J. Biol. Chem. **287**:43277–43287.

Kaiserli, E., Páldi, K., O'Donnell, L., Batalov, O., Pedmale, U.V., Nusinow, D.A., Kay, S.A., and Chory, J. (2015). Integration of light and photoperiodic signaling in transcriptional nuclear foci. Dev. Cell **35**:311–321.

Kang, C.B., Hong, Y., Dhe-Paganon, S., and Yoon, H.S. (2008). FKBP family proteins: immunophilins with versatile biological functions. Neurosignals **16**:318–325.

Kiba, T., Henriques, R., Sakakibara, H., and Chua, N.H. (2007). Targeted degradation of PSEUDO-RESPONSE REGULATOR5 by an SCFZTL complex regulates clock function and photomorphogenesis in *Arabidopsis thaliana*. Plant Cell **19**:2516–2530.

Kidokoro, S., Maruyama, K., Nakashima, K., Imura, Y., Narusaka, Y., Shinwari, Z.K., Osakabe, Y., Fujita, Y., Mizoi, J., Shinozaki, K., et al. (2009). The phytochrome-interacting factor PIF7 negatively regulates *DREB1* expression under circadian control in Arabidopsis. Plant Physiol. **151**:2046–2057.

Kim, J.-Y., Symeonidi, E., Pang, T.Y., Denyer, T., Weidauer, D., Bezrutczyk, M., Miras, M., Zöllner, N., Hartwig, T., Wudick, M.M., et al. (2021). Distinct identities of leaf phloem cells revealed by single cell transcriptomics. Plant Cell **33**:511–530.

Kim, S.-J., Hong, S.M., Yoo, S.J., Moon, S., Jung, H.S., and Ahn, J.H. (2016). Post-translational regulation of FLOWERING LOCUS T protein in *Arabidopsis*. Mol. Plant **9**:308–311.

Kinmonth-Schultz, H.A., Tong, X., Lee, J., Song, Y.H., Ito, S., Kim, S.H., and Imaizumi, T. (2016). Cool night-time temperatures induce the expression of *CONSTANS* and *FLOWERING LOCUS T* to regulate flowering in *Arabidopsis*. New Phytol. **211**:208–224.

Kinoshita, A., and Richter, R. (2021). Genetic and molecular basis of floral induction in *Arabidopsis thaliana*. J. Exp. Bot. **71**:2490–2504.

Koornneef, M., Hanhart, C.J., and van der Veen, J.H. (1991). A genetic and physiological analysis of late flowering mutants in Arabidopsis thaliana. Mol. Gen. Genet. **229**:57–66.

Krahmer, J., Goralogia, G.S., Kubota, A., Zardilis, A., Johnson, R.S., Song, Y.H., MacCoss, M.J., Le Bihan, T., Halliday, K.J., Imaizumi, T., et al. (2019). Time-resolved interaction proteomics of the GIGANTEA protein under diurnal cycles in *Arabidopsis*. FEBS Lett. **593**:319–338.

Kubota, A., Ito, S., Shim, J.S., Johnson, R.S., Song, Y.H., Breton, G., Goralogia, G.S., Kwon, M.S., Laboy Cintrón, D., Koyama, T., et al. (2017). TCP4-dependent induction of CONSTANS transcription requires GIGANTEA in photoperiodic flowering in *Arabidopsis*. PLoS Genet. **13**:e1006856.

Kumar, S., Choudhary, P., Gupta, M., and Nath, U. (2018). VASCULAR PLANT ONE-ZINC FINGER1 (VOZ1) and VOZ2 interact with CONSTANS and promote photoperiodic flowering transition. Plant Physiol. **176**:2917–2930.

Kumar, S.V., Lucyshyn, D., Jaeger, K.E., Alós, E., Alvey, E., Harberd, N.P., and Wigge, P.A. (2012). Transcription factor PIF4 controls the thermosensory activation of flowering. Nature **484**:242–245.

Kumimoto, R.W., Adam, L., Hymus, G.J., Repetti, P.P., Reuber, T.L., Marion, C.M., Hempel, F.D., and Ratcliffe, O.J. (2008). The Nuclear Factor Y subunits NF-YB2 and NF-YB3 play additive roles in the promotion of flowering by inductive long-day photoperiods in Arabidopsis. Planta **228**:709–723.

Kumimoto, R.W., Zhang, Y., Siefers, N., and Holt, B.F. (2010). NF-YC3, NF-YC4 and NF-YC9 are required for CONSTANS-mediated,

photoperiod-dependent flowering in *Arabidopsis thaliana*. Plant J. **63**:379–391.

Lazaro, A., Mouriz, A., Piñeiro, M., and Jarillo, J.A. (2015). Red light-mediated degradation of CONSTANS by the E3 ubiquitin ligase HOS1 regulates photoperiodic flowering in Arabidopsis. Plant Cell **27**:2437–2454.

Lazaro, A., Valverde, F., Piñeiro, M., and Jarillo, J.A. (2012). The Arabidopsis E3 ubiquitin ligase HOS1 negatively regulates CONSTANS abundance in the photoperiodic control of flowering. Plant Cell **24**:982–999.

Lee, B.D., Kim, M.R., Kang, M.Y., Cha, J.Y., Han, S.H., Nawkar, G.M., Sakuraba, Y., Lee, S.Y., Imaizumi, T., McClung, C.R., et al. (2017). The F-box protein FKF1 inhibits dimerization of COP1 in the control of photoperiodic flowering. Nat. Commun. **8**:2259.

Lee, J.H., Ryu, H.-S., Chung, K.S., Posé, D., Kim, S., Schmid, M., and Ahn, J.H. (2013). Regulation of temperature-responsive flowering by MADS-Box transcription factor repressors. Science **342**:628–632.

Lee, J.H., Yoo, S.J., Park, S.H., Hwang, I., Lee, J.S., and Ahn, J.H. (2007). Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis*. Genes Dev. **21**:397–402.

Li, C., Distelfeld, A., Comis, A., and Dubcovsky, J. (2011). Wheat flowering repressor VRN2 and promoter CO2 compete for interactions with NUCLEAR FACTOR-Y complexes. Plant J. **67**:763–773.

Li, H., Burgie, E.S., Gannam, Z.T.K., Li, H., and Vierstra, R.D. (2022). Plant phytochrome B is an asymmetric dimer with unique signalling potential. Nature **604**:127–133.

Li, C., Zhang, B., and Yu, H. (2021). GSK3s: nodes of multilayer regulation of plant development and stress responses. Trends Plant Sci. **26**:1286–1300.

Li, D., Liu, C., Shen, L., Wu, Y., Chen, H., Robertson, M., Helliwell, C.A., Ito, T., Meyerowitz, E., and Yu, H. (2008). A repressor complex governs the integration of flowering signals in *Arabidopsis*. Dev. Cell **15**:110–120.

Li, Y., Wang, H., Li, X., Liang, G., and Yu, D. (2017). Two DELLA-interacting proteins bHLH48 and bHLH60 regulate flowering under long-day conditions in *Arabidopsis thaliana*. J. Exp. Bot. **68**:2757–2767.

Liu, B., Zuo, Z., Liu, H., Liu, X., and Lin, C. (2011). *Arabidopsis* cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. Genes Dev. **25**:1029–1034.

Liu, H., Yu, X., Li, K., Klejnot, J., Yang, H., Lisiero, D., and Lin, C. (2008). Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in *Arabidopsis*. Science **322**:1535–1539.

Liu, J., Cheng, X., Liu, P., Li, D., Chen, T., Gu, X., and Sun, J. (2017). MicroRNA319-regulated TCPs interact with FBHs and PFT1 to activate *CO* transcription and control flowering time in Arabidopsis. PLoS Genet. **13**:e1006833.

Liu, X., Yang, Y., Hu, Y., Zhou, L., Li, Y., and Hou, X. (2018). Temporal-specific interaction of NF-YC and CURLY LEAF during the floral transition regulates flowering. Plant Physiol. **177**:105–114.

Liu, Y., Li, X., Li, K., Liu, H., and Lin, C. (2013). Multiple bHLH proteins form heterodimers to mediate CRY2-dependent regulation of flowering-time in *Arabidopsis*. PLoS Genet. **9**:e1003861.

Liu, Y., Lin, G., Yin, C., and Fang, Y. (2020). B-box transcription factor 28 regulates flowering by interacting with CONSTANS. Sci. Rep. **10**:17789.

Lopez-Molina, L., Mongrand, S., Kinoshita, N., and Chua, N.H. (2003). AFP is a novel negative regulator of ABA signaling that promotes ABI5 protein degradation. Genes Dev. **17**:410–418.

Lorrain, S., Allen, T., Duek, P.D., Whitelam, G.C., and Fankhauser, C. (2008). Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant J. **53**:312–323.

Lu, X.-D., Zhou, C.-M., Xu, P.-B., Luo, Q., Lian, H.-L., and Yang, H.-Q. (2015). Red-light-dependent interaction of phyB with SPA1 promotes COP1–SPA1 dissociation and photomorphogenic development in. Mol. Plant **8**:467–478.

Lutz, U., Nussbaumer, T., Spannagl, M., Diener, J., Mayer, K.F., and Schwechheimer, C. (2017). Natural haplotypes of *FLM* non-coding sequences fine-tune flowering time in ambient spring temperatures in Arabidopsis. Elife **6**:e22114.

Lutz, U., Posé, D., Pfeifer, M., Gundlach, H., Hagmann, J., Wang, C., Weigel, D., Mayer, K.F.X., Schmid, M., and Schwechheimer, C. (2015). Modulation of ambient temperature-dependent flowering in *Arabidopsis thaliana* by natural variation of *FLOWERING LOCUS* M. PLoS Genet. **11**:e1005588.

Lv, X., Zeng, X., Hu, H., Chen, L., Zhang, F., Liu, R., Liu, Y., Zhou, X., Wang, C., Wu, Z., et al. (2021). Structural insights into the multivalent binding of the Arabidopsis *FLOWERING LOCUS T* promoter by the CO-NF-Y master transcription factor complex. Plant Cell **33**:1182–1195.

Mathieu, J., Yant, L.J., Mürdter, F., Küttner, F., and Schmid, M. (2009). Repression of flowering by the miR172 target *SMZ*. PLoS Biol. **7**:e1000148.

March-Díaz, R., and Reyes, J.C. (2009). The beauty of being a variant: H2A.Z and the SWR1 complex in plants. Mol. Plant **2**:565–577.

Nemoto, Y., Kisaka, M., Fuse, T., Yano, M., and Ogihara, Y. (2003). Characterization and functional analysis of three wheat genes with homology to the *CONSTANS* flowering time gene in transgenic rice. Plant J. **36**:82–93.

Nemoto, Y., Nonoue, Y., Yano, M., and Izawa, T. (2016). *Hd1*, a *CONSTANS* ortholog in rice, functions as an *Ehd1* repressor through interaction with monocot-specific CCT-domain protein Ghd7. Plant J. **86**:221–233.

Nguyen, K.T., Park, J., Park, E., Lee, I., and Choi, G. (2015). The *Arabidopsis* RING domain protein BOI inhibits flowering via CO-dependent and CO-independent mechanisms. Mol. Plant **8**:1725–1736.

Ordoñez-Herrera, N., Trimborn, L., Menje, M., Henschel, M., Robers, L., Kaufholdt, D., Hänsch, R., Adrian, J., Ponnu, J., and Hoecker, U. (2018). The transcription factor COL12 is a substrate of the COP1/SPA E3 ligase and regulates flowering time and plant architecture. Plant Physiol. **176**:1327–1340.

Park, J., Nguyen, K.T., Park, E., Jeon, J.S., and Choi, G. (2013). DELLA proteins and their interacting RING finger proteins repress gibberellin responses by binding to the promoters of a subset of gibberellin-responsive genes in *Arabidopsis*. Plant Cell **25**:927–943.

Pauwels, L., Barbero, G.F., Geerinck, J., Tilleman, S., Grunewald, W., Pérez, A.C., Chico, J.M., Bossche, R.V., Sewell, J., Gil, E., et al. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. Nature **464**:788–791.

Peng, M., Li, Z., Zhou, N., Ma, M., Jiang, Y., Dong, A., Shen, W.H., and Li, L. (2018). Linking phytochrome-interacting factor to histone modification in plant shade avoidance. Plant Physiol. **176**:1341–1351.

Pham, V.N., Kathare, P.K., and Huq, E. (2018). Phytochromes and phytochrome interacting factors. Plant Physiol. **176**:1025–1038.

Popescu, S.C., Popescu, G.V., Bachan, S., Zhang, Z., Gerstein, M., Snyder, M., and Dinesh-Kumar, S.P. (2009). MAPK target networks in *Arabidopsis thaliana* revealed using functional protein microarrays. Genes Dev. **23**:80–92.

Porri, A., Torti, S., Romera-Branchat, M., and Coupland, G. (2012). Spatially distinct regulatory roles for gibberellins in the promotion of flowering of arabidopsis under long photoperiods. Development **139**:2198–2209.

Posé, D., Verhage, L., Ott, F., Yant, L., Mathieu, J., Angenent, G.C., Immink, R.G.H., and Schmid, M. (2013). Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature **503**:414–417.

Rizzini, L., Favory, J.J., Cloix, C., et al. (2011). Perception of UV-B by the *Arabidopsis* UVR8 protein. Science **332**:103–106.

Sanagi, M., Aoyama, S., Kubo, A., Lu, Y., Sato, Y., Ito, S., Abe, M., Mitsuda, N., Ohme-Takagi, M., Kiba, T., et al. (2021). Low nitrogen conditions accelerate flowering by modulating the phosphorylation state of FLOWERING BHLH 4 in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **118**. e2022942118.

Sarid-Krebs, L., Panigrahi, K.C.S., Fornara, F., Takahashi, Y., Hayama, R., Jang, S., Tilmes, V., Valverde, F., and Coupland, G. (2015). Phosphorylation of CONSTANS and its COP1-dependent degradation during photoperiodic flowering of Arabidopsis. Plant J. **84**:451–463.

Sawa, M., Nusinow, D.A., Kay, S.A., and Imaizumi, T. (2007). FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis*. Science **318**:261–265.

Searle, I., He, Y., Turck, F., Vincent, C., Fornara, F., Kröber, S., Amasino, R.A., and Coupland, G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. Genes Dev. **20**:898–912.

Seo, E., Yu, J., Ryu, K.H., Lee, M.M., and Lee, I. (2011). *WEREWOLF*, a regulator of root hair pattern formation, controls flowering time through the regulation of *FT* mRNA stability. Plant Physiol. **156**:1867–1877.

Serrano, G., Herrera-Palau, R., Romero, J.M., Serrano, A., Coupland, G., and Valverde, F. (2009). Chlamydomonas *CONSTANS* and the evolution of plant photoperiodic signaling. Curr. Biol. **19**:359–368.

Serrano-Bueno, G., Said, F.E., de los Reyes, P., Lucas-Reina, E.I., Ortiz-Marchena, M.I., Romero, J.M., and Valverde, F. (2020). CONSTANS–FKBP12 interaction contributes to modulation of photoperiodic flowering in Arabidopsis. Plant J. **101**:1287–1302.

Sessa, G., Carabelli, M., Sassi, M., Ciolfi, A., Possenti, M., Mittempergher, F., Becker, J., Morelli, G., and Ruberti, I. (2005). A dynamic balance between gene activation and repression regulates the shade avoidance response in *Arabidopsis*. Genes Dev. **19**:2811–2815.

Sheerin, D.J., Menon, C., zur Oven-Krockhaus, S., Enderle, B., Zhu, L., Johnen, P., Schleifenbaum, F., Stierhof, Y.D., Huq, E., and Hiltbrunner, A. (2015). Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex. Plant Cell **27**:189–201.

Shen, C., Liu, H., Guan, Z., Yan, J., Zheng, T., Yan, W., Wu, C., Zhang, Q., Yin, P., and Xing, Y. (2020). Structural insight into DNA recognition by CCT/NF-YB/YC complexes in plant photoperiodic flowering. Plant Cell **32**:3469–3484.

Shi, D., Jouannet, V., Agustí, J., Kaul, V., Levitsky, V., Sanchez, P., Mironova, V.V., and Greb, T. (2021). Tissue-specific transcriptome profiling of the Arabidopsis inflorescence stem reveals local cellular signatures. Plant Cell **33**:200–223.

Shibuta, M., and Abe, M. (2017). FE controls the transcription of downstream flowering regulators through two distinct mechanisms in leaf phloem companion cells. Plant Cell Physiol. **58**:2017–2025.

Shim, J.S., Kubota, A., and Imaizumi, T. (2017). Circadian clock and photoperiodic flowering in *Arabidopsis*: CONSTANS is a hub for signal integration. Plant Physiol. **173**:5–15.

Simon, S., Rühl, M., de Montaigu, A., Wötzel, S., and Coupland, G. (2015). Evolution of *CONSTANS* regulation and function after gene duplication produced a photoperiodic flowering switch in the Brassicaceae. Mol. Biol. Evol. **32**:2284–2301.

Siriwardana, C.L., Gnesutta, N., Kumimoto, R.W., Jones, D.S., Myers, Z.A., Mantovani, R., and Holt, B.F. (2016). NUCLEAR FACTOR Y, subunit A (NF-YA) proteins positively regulate flowering and act through FLOWERING LOCUS T. PLoS Genet. **12**:e1006496.

Song, Y.H., Kubota, A., Kwon, M.S., Covington, M.F., Lee, N., Taagen, E.R., Laboy Cintrón, D., Hwang, D.Y., Akiyama, R., Hodge, S.K., et al. (2018). Molecular basis of flowering under natural long-day conditions in *Arabidopsis*. Nat. Plants **4**:824–835.

Song, Y.H., Shim, J.S., Kinmonth-Schultz, H.A., and Imaizumi, T. (2015). Photoperiodic flowering: time measurement mechanisms in leaves. Annu. Rev. Plant Biol. **66**:441–464.

Song, Y.H., Estrada, D.A., Johnson, R.S., Kim, S.K., Lee, S.Y., MacCoss, M.J., and Imaizumi, T. (2014). Distinct roles of FKF1, GIGANTEA, and ZEITLUPE proteins in the regulation of CONSTANS stability in *Arabidopsis* photoperiodic flowering. Proc. Natl. Acad. Sci. USA **111**:17672–17677.

Song, Y.H., Lee, I., Lee, S.Y., Imaizumi, T., and Hong, J.C. (2012a). CONSTANS and ASYMMETRIC LEAVES 1 complex is involved in the induction of *FLOWERING LOCUS T* in photoperiodic flowering in Arabidopsis. Plant J. **69**:332–342.

Song, Y.H., Smith, R.W., To, B.J., Millar, A.J., and Imaizumi, T. (2012b). FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. Science **336**:1045–1049.

Sureshkumar, S., Dent, C., Seleznev, A., Tasset, C., and Balasubramanian, S. (2016). Nonsense-mediated mRNA decay modulates FLM-dependent thermosensory flowering response in *Arabidopsis*. Nat. Plants **2**:16055.

Takada, S., and Goto, K. (2003). TERMINAL FLOWER2, an Arabidopsis homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of *FLOWERING LOCUS T* by CONSTANS in the vascular tissues of leaves to regulate flowering time. Plant Cell **15**:2856–2865.

Takahashi, Y., Ebisu, Y., Kinoshita, T., Doi, M., Okuma, E., Murata, Y., and Shimazaki, K.I. (2013). BHLH transcription factors that facilitate K^+ uptake during stomatal opening are repressed by abscisic acid through phosphorylation. Sci. Signal. **6**:ra48.

Takahashi, Y., Kinoshita, T., Matsumoto, M., and Shimazaki, K.I. (2016). Inhibition of the *Arabidopsis* bHLH transcription factor by monomerization through abscisic acid-induced phosphorylation. Plant J. **87**:559–567.

Tian, H., Li, Y., Wang, C., Xu, X., Zhang, Y., Zeb, Q., Zicola, J., Fu, Y., Turck, F., Li, L., et al. (2021). Photoperiod-responsive changes in chromatin accessibility in phloem companion and epidermis cells of Arabidopsis leaves. Plant Cell **33**:475–491.

Tiwari, S.B., Shen, Y., Chang, H.C., Hou, Y., Harris, A., Ma, S.F., McPartland, M., Hymus, G.J., 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.

Tokutsu, R., Fujimura-Kamada, K., Matsuo, T., Yamasaki, T., and Minagawa, J. (2019). The CONSTANS flowering complex controls the protective response of photosynthesis in the green alga *Chlamydomonas*. Nat. Commun. **10**:4099.

Toda, Y., Kudo, T., Kinoshita, T., and Nakamichi, N. (2019). Evolutionary insight into the clock-associated PRR5 transcriptional network of flowering plants. Sci. Rep. **9**:2983.

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.

Wang, C.Q., Guthrie, C., Sarmast, M.K., and Dehesh, K. (2014). BBX19 interacts with CONSTANS to repress *FLOWERING LOCUS T* transcription, defining a flowering time checkpoint in *Arabidopsis*. Plant Cell **26**:3589–3602.

Wang, F., Gao, Y., Liu, Y., Zhang, X., Gu, X., Ma, D., Zhao, Z., Yuan, Z., Xue, H., and Liu, H. (2019). BES1-regulated BEE1 controls photoperiodic flowering downstream of blue light signaling pathway in *Arabidopsis*. New Phytol. **223**:1407–1419.

Wang, H., Pan, J., Li, Y., Lou, D., Hu, Y., and Yu, D. (2016). The DELLA-CONSTANS transcription factor cascade integrates gibberellic acid and photoperiod signaling to regulate flowering. Plant Physiol. **172**:479–488.

Wang, M.J., Ding, L., Liu, X.H., and Liu, J.X. (2021). Two B-box domain proteins, BBX28 and BBX29, regulate flowering time at low ambient temperature in *Arabidopsis*. Plant Mol. Biol. **106**:21–32.

Wang, Y., Zhong, Z., Zhang, Y., Xu, L., Feng, S., Rayatpisheh, S., Wohlschlegel, J.A., Wang, Z., Jacobsen, S.E., and Ausin, I. (2020). NAP1-RELATED PROTEIN1 and 2 negatively regulate H2A.Z abundance in chromatin in Arabidopsis. Nat. Commun. **11**:2887.

Wang, W., Yang, D., and Feldmann, K.A. (2011). *EFO1* and *EFO2*, encoding putative WD-domain proteins, have overlapping and distinct roles in the regulation of vegetative development and flowering of *Arabidopsis*. J. Exp. Bot. **62**:1077–1088.

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.

Wilson, R.N., Heckman, J.W., and Somerville, C.R. (1992). Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol. **100**:403–408.

Xu, F., Li, T., Xu, P.B., Li, L., Du, S.S., Lian, H.L., and Yang, H.Q. (2016). DELLA proteins physically interact with CONSTANS to regulate flowering under long days in *Arabidopsis*. FEBS Lett. **590**:541–549.

Xu, P., Chen, H., Li, T., Xu, F., Mao, Z., Cao, X., Miao, L., Du, S., Hua, J., Zhao, J., et al. (2021). Blue light-dependent interactions of CRY1 with GID1 and DELLA proteins regulate gibberellin signaling and photomorphogenesis in Arabidopsis. Plant Cell **33**:2375–2394.

Xu, X., Xu, J., Yuan, C., Chen, Q., Liu, Q., Wang, X., and Qin, C. (2022). BBX17 interacts with CO and negatively regulates flowering time in *Arabidopsis thaliana*. Plant Cell Physiol. **63**:401–409.

Xu, Y., Gan, E.S., Zhou, J., Wee, W.Y., Zhang, X., and Ito, T. (2014). Arabidopsis MRG domain proteins bridge two histone modifications to elevate expression of flowering genes. Nucleic Acids Res. **42**:10960–10974.

Yamaguchi, N. (2021). LEAFY, a pioneer transcription factor in plants: a mini-review. Front. Plant Sci. **12**:701406–701409.

Yan, B., Yang, Z., He, G., Jing, Y., Dong, H., Ju, L., Zhang, Y., Zhu, Y., Zhou, Y., and Sun, J. (2021). The blue light receptor CRY1 interacts with GID1 and DELLA proteins to repress gibberellin signaling and plant growth. Plant Commun. **2**:100245.

Yan, J., Li, X., Zeng, B., Zhong, M., Yang, J., Yang, P., Li, X., He, C., Lin, J., Liu, X., et al. (2020). FKF1 F-box protein promotes flowering in part by negatively regulating DELLA protein stability under long-day photoperiod in *Arabidopsis*. J. Integr. Plant Biol. **62**:1717–1740.

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S., and Dubcovsky, J. (2006). The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. Proc. Natl. Acad. Sci. USA **103**:19581–19586.

Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., SanMiguel, P., Bennetzen, J.L., Echenique, V., and Dubcovsky, J. (2004). The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science **303**:1640–1644.

Yan, Y., Shen, L., Chen, Y., Bao, S., Thong, Z., and Yu, H. (2014). A MYB-domain protein EFM mediates flowering responses to environmental cues in *Arabidopsis*. Dev. Cell **30**:437–448.

Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto, K., Umehara, Y., Nagamura, Y., et al. (2000). *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell **12**:2473–2484.

Yanovsky, M.J., and Kay, S.A. (2002). Molecular basis of seasonal time measurement in Arabidopsis. Nature **419**:308–312.

Yasui, Y., Mukougawa, K., Uemoto, M., Yokofuji, A., Suzuri, R., Nishitani, A., and Kohchi, T. (2012). The phytochrome-interacting VASCULAR PLANT ONE-ZINC FINGER1 and VOZ2 redundantly regulate flowering in *Arabidopsis*. Plant Cell **24**:3248–3263.

You, Y., Sawikowska, A., Lee, J.E., Benstein, R.M., Neumann, M., Krajewski, P., and Schmid, M. (2019). Phloem companion cell-specific transcriptomic and epigenomic analyses identify MRF1, a regulator of flowering. Plant Cell **31**:325–345.

Yu, J.W., Rubio, V., Lee, N.Y., Bai, S., Lee, S.Y., Kim, S.S., Liu, L., Zhang, Y., Irigoyen, M.L., Sullivan, J.A., et al. (2008). COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Mol. Cell **32**:617–630.

Zeng, X., Lv, X., Liu, R., He, H., Liang, S., Chen, L., Zhang, F., Chen, L., He, Y., and Du, J. (2022). Molecular basis of CONSTANS oligomerization in *FLOWERING LOCUS T* activation. J. Integr. Plant Biol. **64**:731–740.

Zhang, B., Wang, L., Zeng, L., Zhang, C., and Ma, H. (2015). *Arabidopsis* TOE proteins convey a photoperiodic signal to antagonize CONSTANS and regulate flowering time. Genes Dev. **29**:975–987.

Zhang, D., Jing, Y., Jiang, Z., and Lin, R. (2014). The chromatin-remodeling factor PICKLE integrates brassinosteroid and gibberellin signaling during skotomorphogenic growth in *Arabidopsis*. Plant Cell **26**:2472–2485.

Zhang, L., Chen, L., and Yu, D. (2018). Transcription factor WRKY75 interacts with DELLA proteins to affect flowering. Plant Physiol. **176**:790–803.

Zhang, R., Yang, C., Jiang, Y., and Li, L. (2019). A PIF7-CONSTANS-centered molecular regulatory network underlying shade-accelerated flowering. Mol. Plant **12**:1587–1597.

Zhou, L., Lu, Y., Huang, J., Sha, Z., Mo, W., Xue, J., Ma, S., Shi, W., Yang, Z., Gao, J., et al. (2021). *Arabidopsis* CIB3 regulates photoperiodic flowering in an FKF1-dependent way. Biosci. Biotechnol. Biochem. **85**:765–774.

Zhu, Y., Dong, A., Meyer, D., Pichon, O., Renou, J.P., Cao, K., and Shen, W.H. (2006). Arabidopsis *NRP1* and *NRP2* encode histone chaperones and are required for maintaining postembryonic root growth. Plant Cell **18**:2879–2892.

Zhu, Y., Klasfeld, S., and Wagner, D. (2021). Molecular regulation of plant developmental transitions and plant architecture via PEPB family proteins: an update on mechanism of action. J. Exp. Bot. **72**:2301–2311.

Zhu, Y., Rong, L., Luo, Q., Wang, B., Zhou, N., Yang, Y., Zhang, C., Feng, H., Zheng, L., Shen, W.H., et al. (2017). The histone chaperone NRP1 interacts with WEREWOLF to activate *GLABRA2* in Arabidopsis root hair development. Plant Cell **29**:260–276.

Zicola, J., Liu, L., Tänzler, P., and Turck, F. (2019). Targeted DNA methylation represses two enhancers of *FLOWERING LOCUS T* in *Arabidopsis thaliana*. Nat. Plants **5**:300–307.

Zong, W., Ren, D., Huang, M., Sun, K., Feng, J., Zhao, J., Xiao, D., Xie, W., Liu, S., Zhang, H., et al. (2021). Strong photoperiod sensitivity is controlled by cooperation and competition among Hd1, Ghd7 and DTH8 in rice heading. New Phytol. **229**:1635–1649.

Zuo, Z., Liu, H., Liu, B., Liu, X., and Lin, C. (2011). Blue light-dependent interaction of CRY2 with SPA1 regulates COP1 activity and floral initiation in Arabidopsis. Curr. Biol. **21**:841–847.