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Photoperiodic control of flowering: not only by coincidence

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The timing of floral transition has a direct impact on reproductive success. One of the most important environmental factors that affect the transition is the change in day length (photoperiod). Classical experiments imply that plants monitor photoperiods in the leaf, and transmit that information coded within an elusive signal dubbed florigen to the apex to reprogram development. Recent advances in *Arabidopsis* research indicate that the core of the day-length measurement mechanism lies in the circadian regulation of CONSTANS (CO) expression and the subsequent photoperiodic induction of the expression of *FLOWERING LOCUS T* (*FT*) gene, which might encode a major component of florigen. In this review, we introduce current perspectives on how, when and where the floral signal is generated.

Introduction of photoperiodism and florigen paradigms

In temperate zones, the duration of daylight changes with the seasons. Other environmental factors can vary from year to year, but day-length changes follow a predictable pattern. Therefore, many organisms have evolved mechanisms to prepare for upcoming seasonal changes by integrating day-length information into developmental programs. In this way, they improve survival and reproductive success. These responses were first described in plants in the early 20th century. In 1920, Wightman Garner and Henry Allard discovered that shortening day length induced flowering in Maryland Mammoth tobacco plants. They explored day-length-dependent flowering responses in other plant species and ascertained that the length of the day is the major determinant of flowering in many plants. They introduced the terms 'photoperiod' (a daily recurring pattern of light and dark periods) and 'photoperiodism' (response or capacity to respond to photoperiod). They classified plants according to photoperiodic responses into long-day (LD) plants, in which flowering occurs when the day becomes longer than some crucial length, short-day (SD) plants, in which flowering occurs when the day becomes shorter, and day-neutral plants in which flowering is not regulated by photoperiod [1]. Not long after these fundamental observations, the day-length-sensing domain was determined to be a mature leaf. For instance, treatment of a single leaf with inductive SD conditions was sufficient to induce flowering in *Xanthium strumarium* [2]. The photoperiod is measured in the leaf but the distal bud develops into a flower; therefore, there must be signal(s) transmitted from the leaf to the shoot apex. In the 1930s, based on grafting experiments, Mikhail Chailakhyan postulated the presence of such a flowering signal and named it 'florigen' [3,4]. Various grafting experiments have predicted the characteristics of florigen but subsequent biochemical attempts to identify florigen have not been that successful. Florigen is a graft-transmissible compound(s) that is thought to move through phloem with the assimilate stream. Florigen generated in the leaf of one species or one photoperiodic-response type (LD, SD, or day-neutral plants) can induce flowering in grafts of different species or response type [5,6]. Tremendous effort has been devoted to characterizing photoperiodic responses (e.g. flowering, dormancy and tuberization) in numerous plant species since photoperiodism was discovered. Nevertheless, fundamental questions still remain largely unanswered at the molecular level: how do plants sense photoperiod in the leaf? What is the nature of florigen?Within the past few years, crucial findings have brought new insight into the molecular mechanisms of

Glossary

B-box-type zinc finger: a type of zinc-binding motif. One zinc ion is captured by two cysteine residues and two histidine residues in this motif. B-box domains mediate protein–protein interactions.
Dof transcription factor: a plant-specific transcription factor that contains a subtype of the zinc-finger DNA binding domains referred to as the Dof (DNA-binding with one finger) domain. All Dof transcription factors characterized bind to the AAAG core sequences.
F-box protein: a protein containing the F-box domain. F-box domains are protein–protein interaction domains. Most of the F-box proteins are components of the SCF (Skp1, Cullin and F-box)-type E3 ubiquitin ligases that are involved in proteasome-dependent protein degradation. Within the SCF complex, F-box proteins are the ones that interact with specific proteins targeted for degradation.
Floral integrator: a gene that is regulated by more than one flowering pathway. The major genetic flowering pathways can currently be classified into photoperiodic, vernalization, autonomous and gibberellin pathways in *Arabidopsis*.
MADS-box transcription factor: a transcription factor containing the MADS (MCM1, AGAMOUS, DEFICENS and SRF)-box DNA binding domain. MADS-box transcription factors are found in various organisms including plants, animals and insects. Many plant MADS-box transcription factors are known to be involved in floral development.
MYB transcription factor: a transcription factor containing the MYB DNA binding domain. The MYB domain was first identified in the retroviral oncogene product v-Myb, and MYB transcription factors exist in various species. MYB domains comprise up to three repeats of helix-turn-helix structures. Plant MYB transcription factors are classified into three subfamilies based on the numbers of repeats in the MYB domains. All the circadian-associated MYB proteins identified contain a single repeat in the MYB domains.
RING-finger domain: a protein–protein interaction domain structurally related to a zinc-finger domain. A protein containing the RING (Really Interesting New Gene)-finger domain often acts as an E3 ubiquitin ligase.
Scaffold protein: a protein that plays a role in recruiting other interacting proteins to maintain a functional complex.

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photoperiodic flowering, and there have been several recent breakthroughs in the study of photoperiodism and the attributes of florigen using the LD plant *Arabidopsis thaliana*.

A model of day-length measurement in plants

Several models have been proposed to explain the mechanisms by which photoperiod information is integrated into the regulation of development [7]. Among them, 'the external coincidence model' is currently the most consistent with the genetic evidence in plants [7–9]. Erwin Bünning proposed the original hypothesis in 1936 based on studies of the circadian and photoperiodic response of soybeans [10]; this was later modified by others based on knowledge obtained from circadian analysis in insects [10]. In this model, light plays two crucial roles. One is resetting the circadian clock, which is important for generating the daily oscillation of a key regulatory component with peak expression in the late afternoon. The other is regulating the activity of this component. Photoperiodic responses will only be triggered when regulator levels above the threshold coincide with daylight, the external signal (Figure 1). In LD plants, the function of the key regulator is to promote flowering. Given that the circadian clock always sets peak expression of the regulator in the late afternoon, coincidence with light occurs more under LD but less under SD. Thus, the regulator is most active under LD, resulting in the acceleration of flowering. In SD plants, the clock-regulated factor functions as a suppressor of flowering.

The photoperiodic flowering pathway can be separated into two functional domains: a circadian clock and a circadian-regulated day-length measurement mechanism. Plants defective in this pathway do not demonstrate day-length-specific acceleration of flowering. These mutants are said to be photoperiod insensitive and flower at the same time in LD and SD.

The *Arabidopsis* circadian clock

There have been recent, excellent reviews of the plant circadian clock [11–13], so we will summarize the molecular features of the *Arabidopsis* clock briefly. In *Arabidopsis*, the circadian clock is reset by light signals that are perceived by phytochrome (phyA to phyE) red and far-red light photoreceptors and cryptochrome (cry1 and cry2) blue-light photoreceptors. Sensitivity to light changes throughout the day and is regulated by the circadian clock in a phenomenon referred to as the gating response. One such gating factor is the clock-regulated EARLY FLOWERING 3 (ELF3) protein, which functions as a cyclic repressor of light signaling [14,15].

The *Arabidopsis* core oscillator consists of a negative-feedback loop with morning factors, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) proteins, and evening factors, TIMING OF CAB EXPRESSION 1 (TOC1), EARLY FLOWERING 4 (ELF4) and LUX ARRHYTHMO (LUX) proteins [16–22]. CCA1 and LHY proteins, which are two related MYB transcription factors (see Glossary), suppress the expression of *TOC1* and *LUX* during the morning by direct binding to specific *cis*-elements called the evening elements [23] in the *TOC1* and *LUX* promoters [19,21]. TOC1, ELF4 and LUX proteins are involved in activating *CCA1* and *LHY* transcription [19–22], probably through indirect mechanisms.

The list of clock-associated proteins is expanding rapidly. Four PSEUDO-RESPONSE REGULATOR (PRR3, PRR5, PRR7 and PRR9) proteins, which are homologs of TOC1, are associated with clock function because they comprise the interlocking loops in the CCA1–LHY circuit [24–26]. The post-transcriptional regulation of clock proteins such as phosphorylation of CCA1 protein [27] and controlled degradation of TOC1 protein by a clock-associated F-box protein ZEITLUPE (ZTL) [28] are also important mechanisms. Because the circadian clock takes part in the day-length measurement mechanism described next, the alteration of clock function often influences photoperiodic responses.

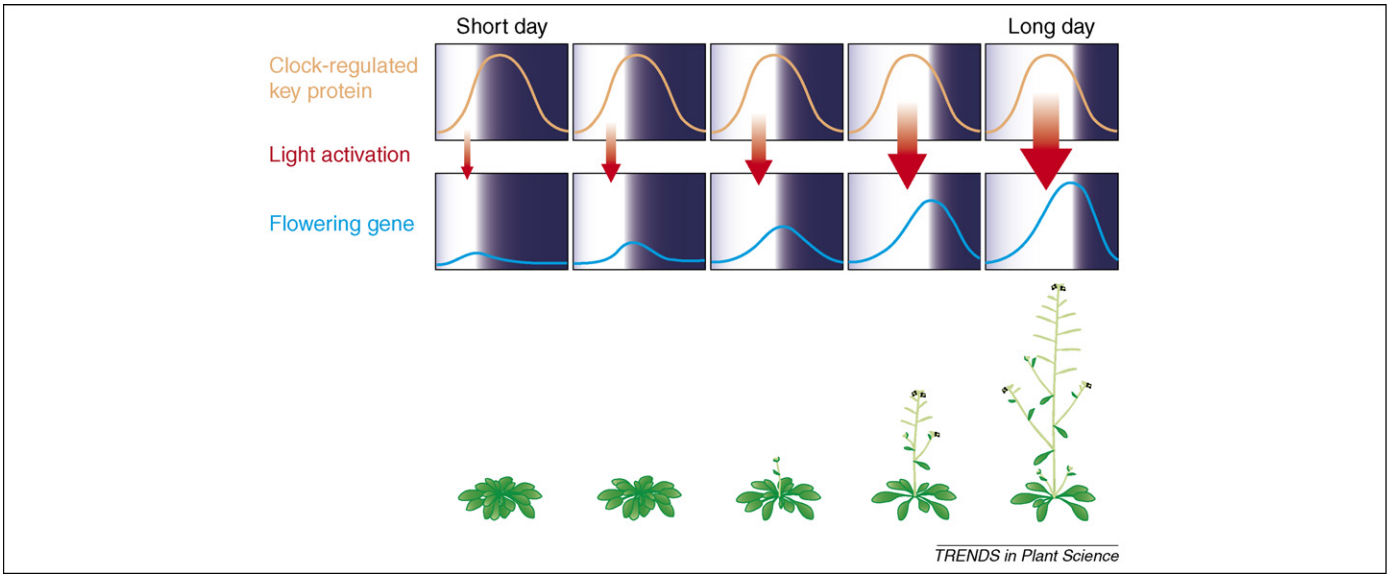

Figure 1. The external coincidence model: an example of the photoperiodic flowering response in long-day (LD) plants. The function of the clock-regulated key regulator, which induces the expression of the flowering gene, is regulated by light, therefore, flowering will be accelerated when the late-afternoon expression of the key regulator and the presence of daylight coincide.

The day-length measurement mechanism in *Arabidopsis*

The crucial aspects of the day-length measurement mechanism are the circadian regulation of *CONSTANS* (*CO*) gene expression and the light-regulation of CO protein stability and activity [8]. The *CO* gene encodes a B-box-type zinc-finger transcriptional activator that induces the expression of the floral integrator *FLOWERING LOCUS T* (*FT*) gene in a light-dependent manner [29]. Given that the circadian clock sets *CO* expression in the afternoon in LD and light activates CO to induce *FT*, this process might be explained by the external coincidence model [7,8] (Figures 1 and 2). Thus, elucidating the molecular mechanisms underlying this process is the most

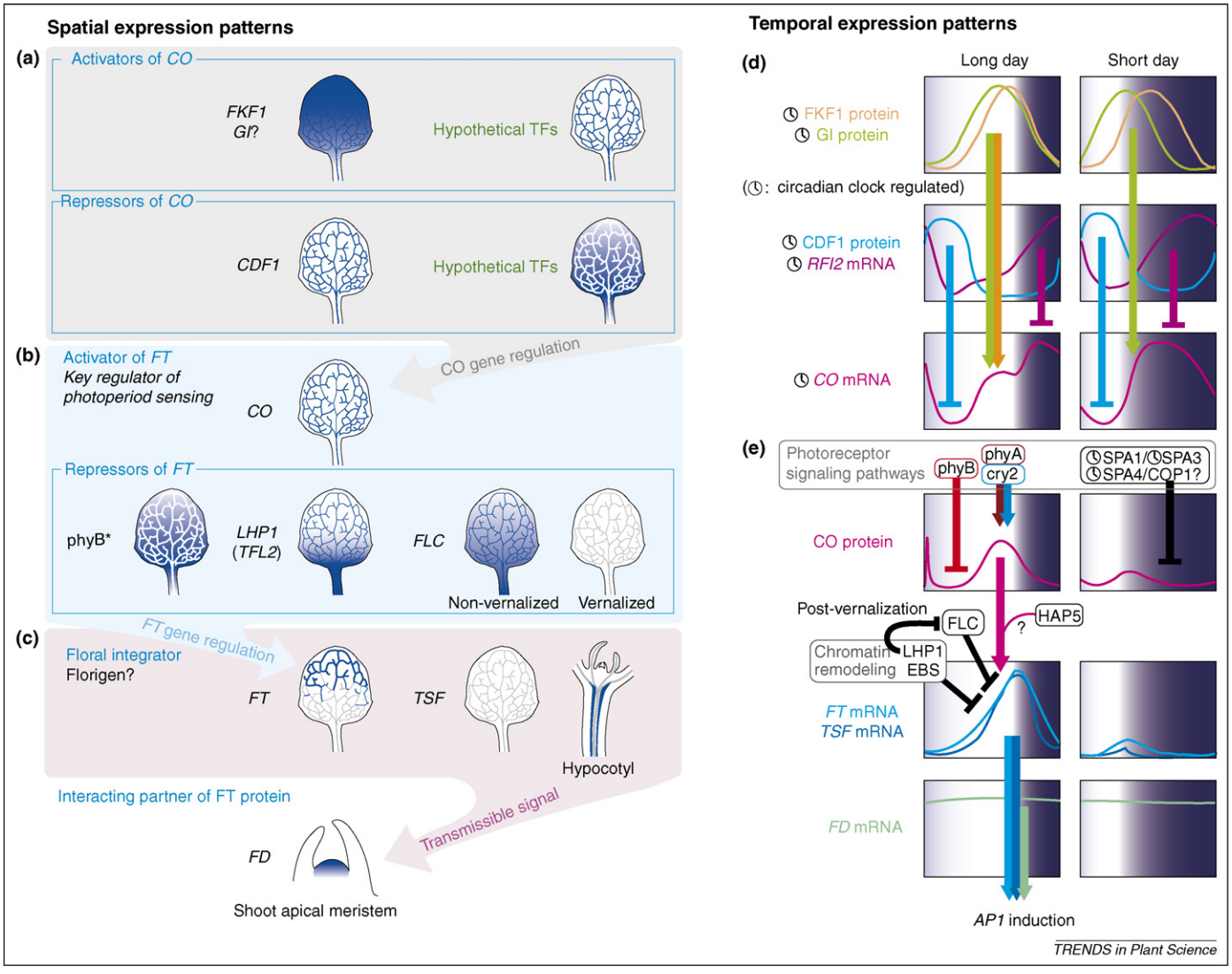

Figure 2. Spatial and temporal regulation of *CO* and *FT* expression under different photoperiods. The current understanding of the spatial and temporal regulation of *CO* and *FT* expression is depicted. **(a–c)** The spatial gene expression patterns in the leaf (except for *TSF* and *FD* expression patterns) of known components (depicted in black font) and of hypothetical transcription factors (TFs) (depicted in green font) that are involved in the regulation are shown in blue. Most of the results of the gene expression patterns were obtained by promoter-fused β-glucuronidase (*GUS*) gene expression analyses. **(a)** Spatial expression patterns of the genes that are involved in the regulation of *CO* transcription. A repressor of *CO* transcription, *CDF1*, is expressed in the vascular tissue, which is similar to the *CO* expression pattern; however, *CO* activators, *FKF1* and possibly *GI*, are expressed broadly in the leaf. This implies that there might be unidentified *CO* activator(s) that are expressed only in the phloem, and/or unidentified *CO* repressor(s) that are expressed somewhere other than the phloem to restrict the spatial pattern of *CO* expression. **(b)** Spatial expression patterns of the genes involved in regulating *FT* transcription. CO is an activator of *FT* transcription that is expressed in the leaf phloem, whereas phyB, LHP1 and FLC act as repressors of *FT* expression. The phyB molecule (phyB*), which is involved in the repression of *FT* expression, is expressed in parts of the leaf other than vascular tissues, although phyB is expressed in the whole leaf. This indicates that phyB signaling molecule(s) that are involved in the repression of *FT* move from mesophyll cells to phloem cells. *LHP1* (also known as *TFL2*) has higher expression levels in the proximal part of the leaf than in the distal part of the leaf. This polarized expression of *LHP1* in the leaf could be the reason that *FT* expression is restricted in the distal part of the leaf. The *FLC* expression pattern in the leaf changes after vernalization treatment. Without vernalization, FLC is involved in the repression of *FT* expression. **(c)** Spatial expression patterns of *FT*, *FT* homolog, *TSF*, and FT-interacting partner *FD*. *FT* is expressed in the phloem of the distal part of the leaf whereas *TSF* is expressed in the stem phloem. *FD* expression is restricted in the shoot apical meristem (SAM). As discussed in the text, it is likely that *FT* mRNA and FT protein are transmitted from the leaves to the SAM. **(d,e)** The daily temporal expression patterns of known components in the photoperiodic flowering pathway are shown under long-day (LD) and short-day (SD) conditions. The circadian clock-regulated genes are marked with a clock symbol. Protein function (activation or repression) is also indicated. **(d)** Temporal expression patterns of the gene and the proteins that play roles in *CO* transcription. The expression of all identified *CO* transcriptional regulators is clock-regulated. Both activators and repressors of *CO* are expressed at different times of the day to regulate *CO* expression patterns precisely. **(e)** Temporal expression patterns of the components that are involved in the induction of LD-specific *FT* and *TSF* expression. The components in the photoreceptor signaling pathways play crucial roles in the regulation of CO protein stability and activity. The CO protein expressed in the late afternoon under LD is protected from proteasome-dependent degradation and induces the expression of *FT* and *TSF* transcripts. The vernalization pathway attenuates photoperiod sensitivity partly by FLC suppressing the expression of *FT* and *TSF*. Chromatin remodeling factors also affect the expression of both genes, although LHP1 might not be involved in regulating *TSF* chromatin. FT and TSF, together with FD, activate *AP1* expression at the SAM; consequently, the floral primordium develops.

crucial step towards understanding the day-length measurement mechanisms. *CO* and *FT* exhibit different daily expression patterns depending on the photoperiod (Figure 2d,e). In addition, both genes show distinct tissue-specific expression patterns in which *CO* is expressed in both leaf and stem phloem, and *FT* is expressed only in the leaf phloem [30,31] (Figure 2b,c). Classical analyses have indicated the importance of temporal and spatial aspects in regulating photoperiodic flowering so we will review recent findings regarding transcriptional and post-transcriptional regulation of CO by considering these aspects of the components in this pathway.

How does the circadian clock regulate CO transcription?

Currently, an increasing number of factors, such as FLAVIN-BINDING, KELCH REPEAT, AND F-BOX 1 (FKF1), GIGANTEA (GI), ELF3, CYCLING DOF FACTOR 1 (CDF1), and RED AND FAR-RED INSENSITIVE 2 (RFI2) proteins are known to be involved in regulating *CO* transcription [32–36] (Figure 2a,d). FKF1 and GI are activators of *CO* transcription, whereas ELF3, CDF1 and RFI2 are repressors. The circadian clock regulates the expression of all five genes [15,32,35–37]. The promoter regions of *FKF1* and *GI* genes contain several evening elements, suggesting that the core clock components CCA1 and LHY directly regulate transcription of both evening genes [34,38].

FKF1 is an F-box protein that is involved in inducing the LD-specific daytime peak of *CO*. In fkf1 mutants, the daytime CO peak is absent but night-time CO expression is unaltered in LD and SD [34]. High levels of *CO* mRNA can only be found in the daytime when light coincides with high levels of FKF1 protein, suggesting that FKF1 acts in a light-dependent manner to regulate the transcription of *CO* (Figure 2a,d). This mechanism is strikingly consistent with the external coincidence model [34]. One of the molecular mechanisms by which FKF1 regulates daytime *CO* transcription is the degradation of CDF1 *CO* suppressor in the late afternoon [35]. CDF1 is a Dof transcription factor, and is the only protein known to bind directly to the *CO* promoter [35].

Although the expression pattern of the GI protein is similar to that of FKF1 in LD [39], *CO* gene expression in the *gi* mutant is depressed all day under both LD and SD [33,38]. These results indicate that GI is necessary for the expression of *CO* transcription regardless of photoperiod. Given that GI is a large nuclear protein with unknown molecular function [32], it might act as a scaffold protein and recruit transcription factors that are expressed throughout the day to regulate *CO* transcription.

In *elf3* mutants, the expression level of the *CO* gene is higher throughout the day than in wild-type plants [33], and the expression levels of both *FKF1* and *GI* genes are also elevated [40]. This indicates that ELF3 can suppress *FKF1* and *GI* expression, consequently suppressing *CO* expression. The *rfi2* mutant was originally isolated as a long hypocotyl mutant under both red and far-red light conditions, and genetic analysis placed the RFI2 protein within both the phyA and phyB signaling pathways [41]. The *rfi2* mutants also exhibited an early flowering phenotype, particularly under LD conditions [36]. The RFI2 protein contains a RING-finger domain, suggesting that it is involved in protein degradation. *RFI2* transcripts peak at the end of the night under both LD and SD, which is when higher expression of *CO* transcripts is observed in *rfi2* mutants than in wild-type plants [36] (Figure 2d). ELF3 and RFI2 affect phyB signals that partly affect CO stability and activity, hence both proteins might have additional roles in the post-transcriptional regulation of *CO* protein.

Another possible activator of *CO* transcription is PRR7. Evidence for this comes from the observation that the barley PRR7 homolog Ppd-H1 (Photoperiod-H1) is involved in activating the transcription of *CO* homologs [42]. In addition, when the *prr7* mutation was combined with either *prr5*, *prr9*, or *prr5 prr9* mutations, these double and triple mutations caused strong late-flowering phenotypes in LD [43]. Because the circadian phenotype of these double and triple mutants varies [43], it is difficult to explain how the similar late-flowering phenotypes of these mutants could be caused by different circadian rhythm defects. These results suggest that PRR7 plays an important role in flowering regulation under LD in addition to the role in the circadian clock, and that PRR5 and PRR9 share overlapping functions with PRR7.

Generating the LD-specific daytime expression of *CO* is the first important process for producing day-length-dependent *FT* expression in the photoperiodic flowering pathway. The complex picture in which both activators and suppressors are expressed at different times of the day to generate the *CO* waveform precisely is emerging (Figure 2d). Our next challenge will be to investigate how these factors coordinate functions. Within these components, only one encodes a transcription factor, suggesting that we are still missing key components of *CO* transcription.

How is CO protein stability regulated?

CO protein stability is highest in the late afternoon in LD and appears to be regulated by light signals perceived by phyA, phyB, cry1 and cry2 photoreceptors [44] (Figure 2e). To regulate CO protein stability, phyA and cry signals function in an antagonistic manner to phyB signals: phyA and cry signals protect CO protein from degradation whereas phyB signals promote degradation.

Under SD conditions, CO protein is unstable throughout the day [44]. Recent findings suggest that the negative regulator of phyA signaling, SUPRESSOR OF PHYA-105 (SPA1), and its homologs, SPA3 and SPA4, are involved in regulating CO stability [45,46]. The *spa1* mutants flower early under SD but not LD conditions and the *spa3 spa4* double mutations enhance the early flowering phenotype of the *spa1* mutants [46]. The expression of *FT* transcript was increased in *spa1* mutants and *spa1 spa3 spa4* triple mutants under SD, although the CO transcriptional level was not altered. The daily expression patterns of *FT* in SD-grown *spa1* mutants were somewhat different in two different studies: however, in both of them, *FT* is expressed in the dark in the *spa1* mutants under SD [45,46]. This is an interesting observation because it is known that CO function is activated by light [29]. It implies that SPA1 might play a crucial role in light-dependent activation (or

dark-dependent suppression) of CO activity. Furthermore, the SPA1 protein physically interacts with the CO protein *in vitro* and *in planta* [46]. Moreover, CO proteins are more stable in the *spa1 spa3 spa4* triple mutants [46]. These results suggest that SPA1 directly influences CO protein stability. SPA1 also physically interacts with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) RING finger E3 ubiquitin ligase to regulate the stability of several transcription factors [47–51]. One protein targeted for degradation by COP1 is CONSTANS-LIKE 3 (COL3), which is a homolog of CO [51]. This implies that COP1, together with SPA1, could be involved in regulating CO protein stability, at least under SD conditions (Figure 2e).

How is CO protein activity regulated?

Recently, it has been shown that the phyB molecules within mesophyll cells, but not those in the vascular bundle in the cotyledons, affect flowering and suppress *FT* expression [52]. There is no direct evidence that the phyB expressed in mesophyll cells affects CO stability in phloem because this can also be explained by attenuating CO activity without changing CO stability. This suggests that there is intercellular signaling downstream from the phyB pathway that regulates *FT* expression (Figures 2b and 3). In the SD plant rice, phyB signals mediate suppression of the expression of the rice *FT* ortholog *Hd3a* (*Heading date 3a*) in response to short light pulses given during the night [53]. This suggests the presence of conserved phyB-dependent light regulation in *FT* expression in both LD and SD plants. Although it is uncertain whether the mechanism of CO protein stability regulation by itself explains light-dependent regulation of CO activity, phyA and cry2 signals are also important for activating CO function [29].

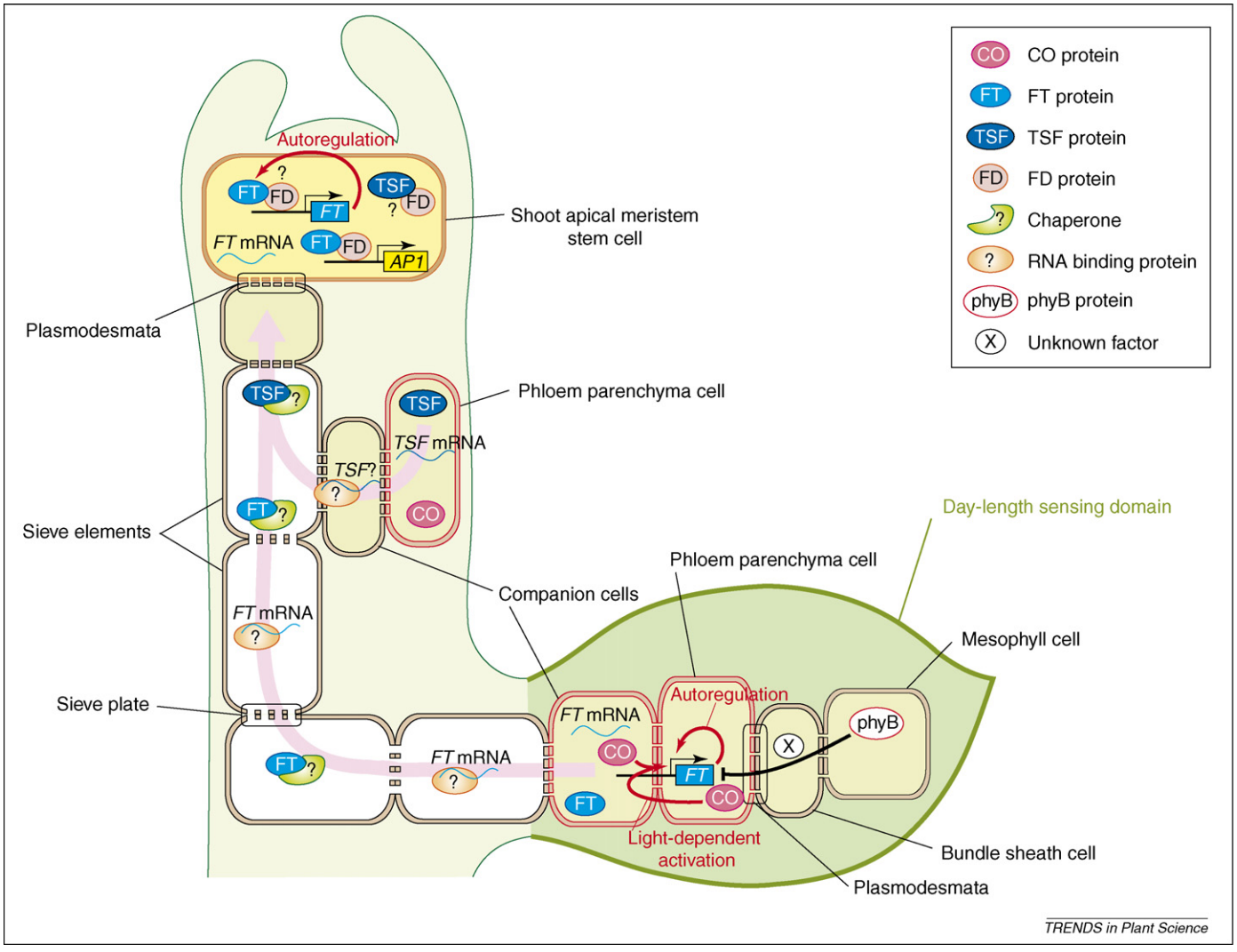

Figure 3. Schematic diagram of the *FT* and *TSF* mRNA and protein long-distance transport. The photoperiod is measured in the leaf, and *FT* and *TSF* expression induced by CO in phloem (parenchyma cells and companion cells) is thought to be an output of the photoperiodic pathway (see details in Figure 2). The plasmodesmata (cytoplasmic connections between cells) allow materials in the cytoplasm to pass directly from one cell to the next. This intercellular system called the symplast plays an important role in florigen signaling. An unknown phyB signal might be transmitted by the symplastic pathway from the mesophyll cells to the companion cells to repress *FT* expression. *FT* (and *TSF*) mRNA and FT (TSF) protein expressed in the companion cells might move through the plasmodesmata into the sieve elements. The sieve elements are differentiated cells without nucleus and ribosomes and specialized for material transport. *FT* (*TSF*) mRNA and FT (TSF) protein might be translocated through the sieve elements to the shoot apex in combination with putative RNA binding proteins and protein chaperones, respectively. After *FT* (*TSF*) mRNA and protein reach the cells in the SAM, FT protein (possibly TSF protein as well) interacts with the FD bZIP transcription factor and induces *AP1* expression. FT protein is also involved in the amplification of its own transcription (autoregulation) in the leaf and at the apex.

Although CO induces *FT* expression, CO protein does not contain a typical DNA binding domain and, hence, it was postulated that the CO protein interacts with transcription factors that directly bind to the *FT* promoter. Recently, one such possible mechanism was reported. CO physically interacts with HAP5 which, together with HAP2 and HAP3, constitute the heterotrimeric CCAAT-binding factor complex [54]. Under warm LD conditions (28/22°C day/night temperature in LD), ectopic expression of the tomato *HAP5* homolog caused a slightly earlier flowering phenotype in *Arabidopsis*, indicating that HAP5 might be involved in regulating flowering. There are several putative CCAAT-binding sites in the *FT* promoter; therefore, HAP5 might recruit CO protein to the *FT* promoter under some circumstances.

Although *FT* is an important target, it might not be the only gene directly regulated by CO in the photoperiodic flowering pathway. CO also activates the transcription of the closest homolog of *FT*, *TWIN SISTER OF FT* (*TSF*), in a similar manner to *FT* regulation [55,56]. The daily expression pattern of *TSF* transcript resembles that of *FT* (Figure 2e). Overexpression of *TSF* causes precocious flowering, whereas the *tsf* mutation enhances the late-flowering phenotype of *ft* mutants [55,56]. Although the tissue-specific expressions of *FT* and *TSF* are different (*FT* is expressed in the leaf phloem whereas *TSF* is expressed in the stem phloem) [56] (Figure 2c), these results suggest that FT and TSF play a similar role in the promotion of flowering.

Other factors known to affect *FT* expression

Chromatin remodeling factors are likely to be involved in regulating the chromatin status of the *FT* locus because mutations in several of these factors affect *FT* expression (Figure 2e). The *early bolting in short days* (*ebs*) mutant and the *like heterochromatin protein 1* (*lhp1*) [also known as *terminal flower 2* (*tfl2*)] mutant showed early flowering phenotypes under both LD and SD conditions and *FT* expression was elevated in both mutants [57,58]. *EBS* is a putative chromatin-remodeling factor [57]. LHP1 is an *Arabidopsis* homolog of HETEROCHROMATIN PROTEIN 1, which is a key component of heterochromatin in fission yeast, *Drosophila* and mammals [59]. In *Arabidopsis*, LHP1 is involved in silencing several other genes in euchromatic regions [60,61]. LHP1 also maintains the epigenetically repressed heterochromatin-like state of *FLOWERING LOCUS C* (*FLC*) locus after vernalization [62]. Although FLC also acts as a suppressor of *FT* expression, the elevated *FLC* expression level did not repress *FT* expression in the *lhp1* mutants under LD, but did repress it under SD, suggesting that there might be a mechanism by which photoperiod affects the chromatin status of the *FT* locus.

FLC belongs to the MADS-box transcription factor family and is the key regulator of both the vernalization and autonomous flowering pathways [63]. Winter annual accessions that possess dominant alleles of *FRIGIDA* (*FRI*) and *FLC* genes require vernalization to be able to gain sensitivity to the perceived photoperiod [64]. In winter annuals, *FLC* is expressed in different types of tissues, including the shoot apical meristem and vascular tissues, and vernalization strongly represses *FLC* expression through a series of histone modifications essential for chromatin status at the *FLC* locus [65,66] (Figure 2b). Without vernalization, FLC represses *FT* transcription in phloem and reduces the expression of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FD* in the meristem [67]. SOC1 is another MADS-box transcription factor that acts as a flowering activator [68] and its expression is partially regulated by FT [55,69]. FLC is likely to bind directly to the CArG-box DNA motifs present in the first intron of the *FT* gene and in the promoters of *FD* and *SOC1* [67,70]. This constitutes another important mechanism for plants to limit photoperiod sensitivity during specific times of the year to prevent misjudging their optimal season to commit to flowering.

FT functions as a possible systemic signaling molecule

How do FT transcripts expressed in the leaf induce floral development at the shoot apex?

The differences in *FT* and *FT*-homolog expression levels under different day-length conditions are likely to be the defining outputs of the photoperiodic time measurement mechanism in plants. It is clear that *FT* levels define flowering behavior because *FT* can induce flowering in a dosage-dependent manner [71,72]. *FT* is expressed in leaf phloem, which raises an important question: how does *FT* expressed in the leaf induce floral transition at the shoot apical meristem (SAM)? *FT* encodes a 20 kDa protein with some homology to the Raf kinase inhibitor protein [71,72]; however, the biochemical function remained elusive until its interacting protein FD was identified. FD, which is a bZIP transcription factor, interacts with FT *in vivo* [73]. FD is required for the function of FT that initiates the gene expression cascade of floral induction by inducing floral meristem identity genes such as *APETALA1* (*AP1*), *FRUITFULL* (*FUL*) and *CAULIFLOWER* (*CAL*) genes at the SAM [73,74]. *FD* is expressed at the SAM whereas *FT* is expressed in phloem. In addition, the ectopic expression of *FT* in the SAM could rescue the *ft* mutant phenotype, indicating that *FT* functions at the shoot apex. These observations suggest the possibility that *FT* mRNA, FT protein or both encode transmissible signals that are translocated through the phloem to the meristem during the transition to flowering (Figure 3).

Indeed, two recent observations strongly indicate that both *FT* mRNA and FT protein can be transported from the leaf to the apex [75,76]. One report shows that the heat-shock inducible promoter-driven *FT* transcript, which was transiently induced in one leaf, could initiate flowering under SD conditions. Furthermore, after 6 h, and later after the induction of *FT* in the leaf, *FT* transcript was detected in the shoot apex. Moreover, the movement velocity of *FT* transcripts was within the same range as that of the unknown floral transmissible signal. During the course of these experiments, the presence of the positive auto-regulatory mechanism of *FT* transcription, by which it is presumed to amplify its own signal, was found in both the leaf and the apex [75]. Plants that have this self-amplification mechanism of *FT* transcription could explain observations from classical grafting experiments (e.g. a *Perilla*

crispa leaf that had been treated once with floral inductive photoperiod could continuously induce flowering in multiple grafts; in *Xanthium*, non-induced leaves from a stock induced to flower by grafting floral-induced leaves could initiate flowering in another non-induced stock [2]). Another report shows that the phloem sap exuded from *Brassica napus* plants grown under LD conditions contained *Brassica* homologs of FT and TSF proteins, as well as several RNA-binding proteins and chaperones [76]. These RNA-binding proteins and chaperones might be involved in the movement of *FT* mRNA and FT protein in phloem, respectively (Figure 3). Together with the findings regarding the molecular mechanism of FT–FD-dependent floral induction in the SAM, these results imply that *FT* mRNA and FT protein could be the long-sought florigen signals.

Could FT be a florigen?

If FT is a florigen, FT should fulfill the other criteria of florigen suggested by classical experiments. Results of interspecific grafting experiments indicated that florigen should function and be transmissible between species, often in between different photoperiodic groups. *FT* and *FT* homologs can accelerate flowering within different species. Ectopic expression of *FT* homologs, such as *Hd3a* from the SD plant rice, *SINGLE FLOWER TRUSS* (*SFT*) from the day-neutral plant tomato, *PtFT1* from the LD plant *Populus trichocarpa* and *FT* from *Citrus unshiu* induced early flowering in the LD plant *Arabidopsis* [77–81]. Both *SFT* and *FT* also induced early flowering in SD and day-neutral tobacco plants [79], indicating that the functions of FT orthologs are highly conserved among diverse plant species. In addition, experiments performed using the tomato *FT* ortholog *SFT* have provided evidence that the FT signal might be transmissible through graft unions. Grafted tomato scion with overexpressed *SFT* rescued flowering phenotypes of *sft* mutant stock and also induced early flowering in both SD and day-neutral tobacco stocks despite photoperiod. These results further support the possibility that FT (mRNA and/or protein) is a florigen, or that FT plays a crucial role in directly generating a florigen signal.

Future perspective

We now have a better understanding of the molecular mechanisms by which plants sense photoperiodic changes within the leaf and integrate the information to alter their developmental fate in the SAM. New components involved in the regulation of CO expression and activity need to be identified, and the functional interactions between known factors, as well as newly identified ones, must be examined. Given that the regulation of the precise spatial and temporal expression patterns of *CO* and *FT* is crucial to this pathway, it is necessary to describe the spatial and temporal expression patterns of all components to contemplate the molecular mechanisms underlying their regulation. There remains a need for additional discussion and further study regarding the molecular nature of florigen (for example, the plant hormone gibberellins also fulfill many of the criteria of florigen [82,83]). Assuming that FT is one of the components of florigen, our next challenge will be to obtain insight into the long-distance targeted delivery networks through which *FT* mRNA and FT protein are selectively transported to the SAM. This exploration will include an attempt to find transport machineries that comprise *FT* mRNA interacting RNA binding proteins and chaperones for FT protein present in phloem sap. Another challenge will be to substantiate how much the CO–FT module is functionally conserved among various plant species. It has been shown that even tree species possess a circadian system similar to *Arabidopsis* [84], and a CO–FT module [80] to regulate floral transition and other growth aspects in response to seasonal changes. Because of their sessile nature, plants have evolved complex sensing systems to monitor predictable environmental changes in existing organs, mainly leaves, and long-distance signaling systems to transmit the information obtained from the sensing organs to the newly developing organs (the stem cells in the meristem) to maximize their adaptation and survivability. Thus, understanding the substantial network underlying photoperiodism remains challenging but also highly intriguing for many researchers.

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References

1 Salisbury, F.B. (1985) Photoperiodism. *Hortic. Rev. (Am Soc Hortic Sci)* 4, 66–105
2 Zeevaart, J.A. (1976) Physiology of flower formation. *Annu. Rev. Plant Physiol.* 27, 321–348
3 Chailakhyan, M.K. (1968) Internal factors of plant flowering. *Annu. Rev. Plant Physiol.* 19, 1–36
4 Pennazio, S. (2004) Florigen: an intriguing concept of plant biology. *Riv. Biol.* 97, 33–51
5 Lang, A. *et al.* (1977) Promotion and inhibition of flower formation in a dayneutral plant in grafts with a short-day plant and a long-day plant. *Proc. Natl. Acad. Sci. U. S. A.* 74, 2412–2416
6 Zeevaart, J.A. (1982) Transmission of the floral stimulus from a long-short-day plant, *Bryophyllum daigremontianum*, to the short-long-day plant *Echeveria harmsii*. *Annu Bot* 49, 549–552
7 Yanovsky, M.J. and Kay, S.A. (2003) Living by the calendar: how plants know when to flower. *Nat. Rev. Mol. Cell Biol.* 4, 265–275
8 Hayama, R. and Coupland, G. (2004) The molecular basis of diversity in the photoperiodic flowering responses of *Arabidopsis* and rice. *Plant Physiol.* 135, 677–684
9 Putterill, J. *et al.* (2004) It's time to flower: the genetic control of flowering time. *Bioessays* 26, 363–373
10 Saunders, D.S. (2005) Erwin Bünning and Tony Lees, two giants of chronobiology, and the problem of time measurement in insect photoperiodism. *J. Insect Physiol.* 51, 599–608
11 Harmon, F.G. *et al.* (2005) The plant circadian clock: review of a clockwork *Arabidopsis*. *Annu. Plant Rev.* 20, 1–23
12 McClung, C.R. (2006) Plant circadian rhythms. *Plant Cell* 18, 792–803
13 Gardner, M.J. *et al.* (2006) How plants tell the time. *Biochem. J.* 397, 15–24
14 McWatters, H.G. *et al.* (2000) The ELF3 zeitnehmer regulates light signalling to the circadian clock. *Nature* 408, 716–720
15 Covington, M.F. *et al.* (2001) ELF3 modulates resetting of the circadian clock in *Arabidopsis*. *Plant Cell* 13, 1305–1315
16 Wang, Z.Y. and Tobin, E.M. (1998) Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) gene disrupts circadian rhythms and suppresses its own expression. *Cell* 93, 1207–1217

17 Schaffer, R. *et al.* (1998) The *late elongated hypocotyl* mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* 93, 1219–1229
18 Strayer, C. *et al.* (2000) Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* 289, 768–771
19 Alabadí, D. *et al.* (2001) Reciprocal regulation between *TOC1* and *LHY/CCA1* within the *Arabidopsis* circadian clock. *Science* 293, 880–883
20 Doyle, M.R. *et al.* (2002) The *ELF4* gene controls circadian rhythms and flowering time in *Arabidopsis thaliana*. *Nature* 419, 74–77
21 Hazen, S.P. *et al.* (2005) *LUX ARRHYTHMO* encodes a Myb domain protein essential for circadian rhythms. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10387–10392
22 Onai, K. and Ishiura, M. (2005) *PHYTOCLOCK 1* encoding a novel GARP protein essential for the *Arabidopsis* circadian clock. *Genes Cells* 10, 963–972
23 Harmer, S.L. *et al.* (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science* 290, 2110–2113
24 Farré, E.M. *et al.* (2005) Overlapping and distinct roles of *PRR7* and *PRR9* in the *Arabidopsis* circadian clock. *Curr. Biol.* 15, 47–54
25 Mizuno, T. (2005) Two-component phosphorelay signal transduction systems in plants: from hormone responses to circadian rhythms. *Biosci. Biotechnol. Biochem.* 69, 2263–2276
26 Mizuno, T. and Nakamichi, N. (2005) Pseudo-Response Regulators (PRRs) or True Oscillator Components (TOCs). *Plant Cell Physiol.* 46, 677–685
27 Daniel, X. *et al.* (2004) CK2 phosphorylation of CCA1 is necessary for its circadian oscillator function in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 3292–3297
28 Más, P. *et al.* (2003) Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. *Nature* 426, 567–570
29 Yanovsky, M.J. and Kay, S.A. (2002) Molecular basis of seasonal time measurement in *Arabidopsis*. *Nature* 419, 308–312
30 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
31 An, H. *et al.* (2004) CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. *Development* 131, 3615–3626
32 Fowler, S. *et al.* (1999) *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J.* 18, 4679–4688
33 Suárez-López, P. *et al.* (2001) *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410, 1116–1120
34 Imaizumi, T. *et al.* (2003) FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. *Nature* 426, 302–306
35 Imaizumi, T. *et al.* (2005) FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*. *Science* 309, 293–297
36 Chen, M. and Ni, M. (2006) RFI2, a RING-domain zinc finger protein, negatively regulates *CONSTANS* expression and photoperiodic flowering. *Plant J.* 46, 823–833
37 Nelson, D.C. *et al.* (2000) *FKF1*, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell* 101, 331–340
38 Mizoguchi, T. *et al.* (2005) Distinct roles of *GIGANTEA* in promoting flowering and regulating circadian rhythms in *Arabidopsis*. *Plant Cell* 17, 2255–2270
39 David, K.M. *et al.* (2006) *Arabidopsis* GIGANTEA protein is post-transcriptionally regulated by light and dark. *FEBS Lett.* 580, 1193–1197
40 Kim, W.Y. *et al.* (2005) Independent roles for *EARLY FLOWERING 3* and *ZEITLUPE* in the control of circadian timing, hypocotyl length, and flowering time. *Plant Physiol.* 139, 1557–1569
41 Chen, M. and Ni, M. (2006) RED AND FAR-RED INSENSITIVE 2, a RING-domain zinc finger protein, mediates phytochrome-controlled seedling deetiolation responses. *Plant Physiol.* 140, 457–465
42 Turner, A. *et al.* (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. *Science* 310, 1031–1034
43 Nakamichi, N. *et al.* (2005) PSEUDO-RESPONSE REGULATORS, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of *Arabidopsis thaliana*. *Plant Cell Physiol.* 46, 686–698
44 Valverde, F. *et al.* (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science* 303, 1003–1006
45 Ishikawa, M. *et al.* (2006) The *Arabidopsis SPA1* gene is required for circadian clock function and photoperiodic flowering. *Plant J.* 46, 736–746
46 Laubinger, S. *et al.* (2006) *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development* 133, 3213–3222
47 Hoecker, U. and Quail, P.H. (2001) The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in *Arabidopsis*. *J. Biol. Chem.* 276, 38173–38178
48 Saijo, Y. *et al.* (2003) The COP1–SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes Dev.* 17, 2642–2647
49 Seo, H.S. *et al.* (2003) LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature* 423, 995–999
50 Yang, J. *et al.* (2005) Repression of light signaling by *Arabidopsis* SPA1 involves post-translational regulation of HFR1 protein accumulation. *Plant J.* 43, 131–141
51 Datta, S. *et al.* (2006) *Arabidopsis* CONSTANS-LIKE3 is a positive regulator of red light signaling and root growth. *Plant Cell* 18, 70–84
52 Endo, M. *et al.* (2005) Phytochrome B in the mesophyll delays flowering by suppressing *FLOWERING LOCUS T* expression in *Arabidopsis* vascular bundles. *Plant Cell* 17, 1941–1952
53 Ishikawa, R. *et al.* (2005) Suppression of the floral activator *Hd3a* is the principal cause of the night break effect in rice. *Plant Cell* 17, 3326–3336
54 Ben-Naim, O. *et al.* (2006) The CCAAT binding factor can mediate interactions between CONSTANS-like proteins and DNA. *Plant J.* 46, 462–476
55 Michaels, S.D. *et al.* (2005) Integration of flowering signals in winter-annual *Arabidopsis*. *Plant Physiol.* 137, 149–156
56 Yamaguchi, A. *et al.* (2005) *TWIN SISTER OF FT* (*TSF*) acts as a floral pathway integrator redundantly with *FT*. *Plant Cell Physiol.* 46, 1175–1189
57 Pineiro, M. *et al.* (2003) EARLY BOLTING IN SHORT DAYS is related to chromatin remodeling factors and regulates flowering in *Arabidopsis* by repressing *FT*. *Plant Cell* 15, 1552–1562
58 Kotake, T. *et al.* (2003) *Arabidopsis* TERMINAL FLOWER 2 gene encodes a heterochromatin protein 1 homolog and represses both FLOWERING LOCUS T to regulate flowering time and several floral homeotic genes. *Plant Cell Physiol.* 44, 555–564
59 Gaudin, V. *et al.* (2001) Mutations in *LIKE HETEROCHROMATIN PROTEIN 1* affect flowering time and plant architecture in *Arabidopsis*. *Development* 128, 4847–4858
60 Libault, M. *et al.* (2005) The *Arabidopsis* LHP1 protein is a component of euchromatin. *Planta* 222, 910–925
61 Nakahigashi, K. *et al.* (2005) The *Arabidopsis* heterochromatin protein1 homolog (TERMINAL FLOWER2) silences genes within the euchromatic region but not genes positioned in heterochromatin. *Plant Cell Physiol.* 46, 1747–1756
62 Sung, S. *et al.* (2006) Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. *Nat. Genet.* 38, 706–710
63 Henderson, I.R. *et al.* (2003) The need for winter in the switch to flowering. *Annu. Rev. Genet.* 37, 371–392
64 Michaels, S.D. and Amasino, R.M. (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* 11, 949–956
65 He, Y. and Amasino, R.M. (2005) Role of chromatin modification in flowering-time control. *Trends Plant Sci.* 10, 30–35
66 Sung, S. and Amasino, R.M. (2005) Remembering winter: toward a molecular understanding of vernalization. *Annu. Rev. Plant Biol.* 56, 491–508
67 Searle, I. *et al.* (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
68 Onouchi, H. *et al.* (2000) Mutagenesis of plants overexpressing CONSTANS demonstrates novel interactions among *Arabidopsis* flowering-time genes. *Plant Cell* 12, 885–900

69 Yoo, S.K. *et al.* (2005) CONSTANS activates SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 through FLOWERING LOCUS T to promote flowering in *Arabidopsis*. *Plant Physiol.* 139, 770–778
70 Helliwell, C.A. *et al.* (2006) The *Arabidopsis* FLC protein interacts directly *in vivo* with *SOC1* and *FT* chromatin and is part of a high-molecular-weight protein complex. *Plant J.* 46, 183–192
71 Kardailsky, I. *et al.* (1999) Activation tagging of the floral inducer *FT*. *Science* 286, 1962–1965
72 Kobayashi, Y. *et al.* (1999) A pair of related genes with antagonistic roles in mediating flowering signals. *Science* 286, 1960–1962
73 Abe, M. *et al.* (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* 309, 1052–1056
74 Wigge, P.A. *et al.* (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* 309, 1056–1059
75 Huang, T. *et al.* (2005) The mRNA of the *Arabidopsis* gene *FT* moves from leaf to shoot apex and induces flowering. *Science* 309, 1694–1696
76 Giavalisco, P. *et al.* (2006) Towards the proteome of *Brassica napus* phloem sap. *Proteomics* 6, 896–909
77 Kojima, S. *et al.* (2002) *Hd3a*, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. *Plant Cell Physiol.* 43, 1096–1105
78 Izawa, T. *et al.* (2002) Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. *Genes Dev.* 16, 2006–2020
79 Lifschitz, E. *et al.* (2006) The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6398–6403
80 Böhlenius, H. *et al.* (2006) *CO/FT* regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* 312, 1040–1043
81 Endo, T. *et al.* (2005) Ectopic expression of an *FT* homolog from *Citrus* confers an early flowering phenotype on trifoliate orange (*Poncirus trifoliata* L. Raf.). *Transgenic Res.* 14, 703–712
82 King, R.W. and Evans, L.T. (2003) Gibberellins and flowering of grasses and cereals: prizing open the lid of the "florigen" black box. *Annu. Rev. Plant Biol.* 54, 307–328
83 King, R.W. *et al.* (2006) Regulation of flowering in the long-day grass *Lolium temulentum* by gibberellins and the *FLOWERING LOCUS T* gene. *Plant Physiol.* 141, 498–507
84 Ramos, A. *et al.* (2005) Winter disruption of the circadian clock in chestnut. *Proc. Natl. Acad. Sci. U. S. A.* 102, 7037–7042

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