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# Photoperiodic Flowering: Time Measurement Mechanisms in Leaves

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## Keywords

photoperiodism, seasonal flowering, external coincidence model, CONSTANS, FLOWERING LOCUS T, florigen

## Abstract

Many plants use information about changing day length (photoperiod) to align their flowering time with seasonal changes to increase reproductive success. A mechanism for photoperiodic time measurement is present in leaves, and the day-length-specific induction of the *FLOWERING LOCUS T* (*FT*) gene, which encodes florigen, is a major final output of the pathway. Here, we summarize the current understanding of the molecular mechanisms by which photoperiodic information is perceived in order to trigger *FT* expression in *Arabidopsis* as well as in the primary cereals wheat, barley, and rice. In these plants, the differences in photoperiod are measured by interactions between circadian-clock-regulated components, such as CONSTANS (CO), and light signaling. The interactions happen under certain day-length conditions, as previously predicted by the external coincidence model. In these plants, the coincidence mechanisms are governed by multilayered regulation with numerous conserved as well as unique regulatory components, highlighting the breadth of photoperiodic regulation across plant species.

## Contents



**Photoperiodic responses:** responses regulated by changes in day length (photoperiod); the most well characterized of these responses in plants is photoperiodic flowering

**CONSTANS (CO):** a transcription activator that is expressed in leaf phloem companion cells and possesses two B-box and CCT domains

***FLOWERING LOCUS T* (*FT*):** a gene encoding a small phospholipid-binding protein that directly binds to transcription factors such as FD and BRANCHED 1 and regulates their activities

## 1. INTRODUCTION

The ways in which plants respond to changes in day length (photoperiod) were first described through experiments performed in 1920 (29). Numerous studies subsequently aimed at understanding the underlying mechanisms (122), but it took decades for the concept now best supported by molecular research to be proposed. This model, termed the external coincidence model because it describes the coincidence of a fluctuating internal signal with a periodic external signal, is a simple concept (**Figure 1*b***).

Research into photoperiodic responses has been carried out for many years using *Arabidopsis thaliana*, the plant in which the timing of flowering as controlled by fluctuations of the *CONSTANS* (*CO*) gene and protein products has been best characterized. This research has demonstrated that appropriately timing the fluctuations of the internal oscillator requires many layers of control. These layers are repressive, inductive, transcriptional, and posttranslational and are mediated by photoreceptors responsive to specific wavelengths of light.

Here, we begin by describing the theories leading up to the proposal of the external coincidence model as well as the model itself. We then review the complex regulatory mechanisms in *Arabidopsis* that restrict CO protein activity to a narrow window in the late afternoon and ensure that the downstream *FLOWERING LOCUS T* (*FT*) gene is expressed only during the long days of summer. We also review recent discoveries regarding the photoperiodic mechanisms present in the leaves of the agricultural crops wheat, barley, and rice.

Because the physiological responses of these crops have been well studied, they allow us to highlight how photoperiodic control changes throughout the lifetime of a plant, as in the case of wheat and barley. Although these species utilize mechanisms similar to those in *Arabidopsis* (3, 110), they have also developed distinct pathways such as the *Grain number, plant height, and heading date 7* (*Ghd7*)–*Early heading date 1* (*Ehd1*)–*Heading date 3a* (*Hd3a*)/*RICE FLOWERING LOCUS T1* (*RFT1*) pathway in rice (44, 46, 108). The agricultural community is now exploiting the molecular underpinnings of the photoperiodic response to predict the timing of phenological shifts (6, 136),

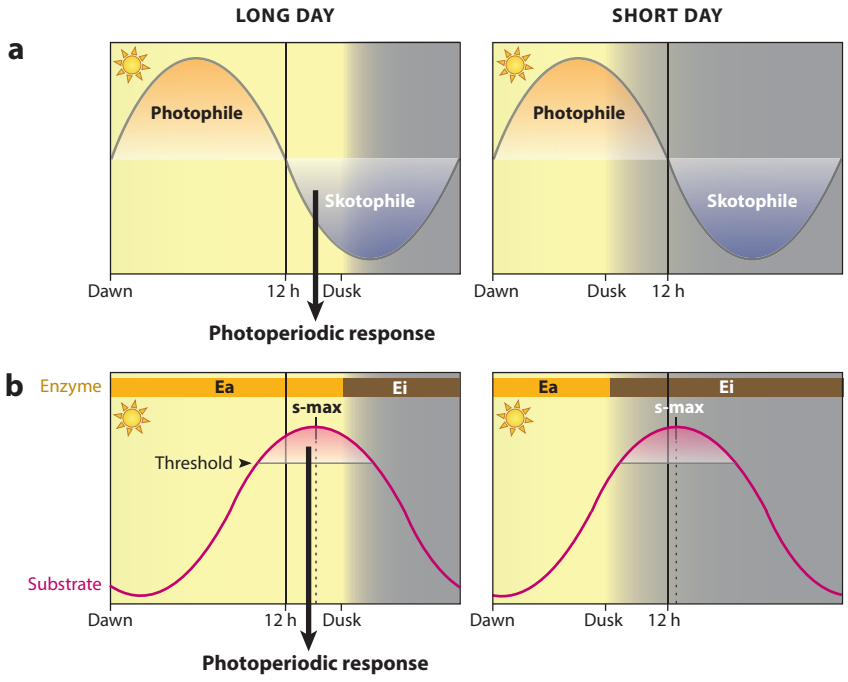



**Figure 1**

Models of induction of the photoperiodic response. (*a*) Bünning's hypothesis. In this model, organisms possess 12-h-long photophile and skotophile phases delimited by an internal oscillator. When daylight lengthens into the skotophile phase, the photoperiodic response is induced in long-day plants and repressed in short-day plants. (*b*) The external coincidence model. This model proposes that a photoperiodic response is induced by the activity of a hypothetical enzyme and the presence of its hypothetical substrate. The enzyme is present throughout the day, and light triggers the enzyme to change from the inactive form (Ei) to the active form (Ea). The expression patterns of the substrate are regulated by the circadian clock. Light and temperature change throughout the day and reset the clock each day by adjusting the phases of the clock components. The time when resetting occurs changes throughout the year, causing the phase of the substrate to also change slightly. Therefore, the phases of the maximal amount of the substrate (s-max) are slightly different in long- and short-day conditions. The photoperiodic response is induced only when the amount of substrate is higher than a required threshold and Ea is present at the same time.

adjust flowering time while maintaining yield (128), and increase or maintain yields in light of a warming climate (60).

Early in the twentieth century, scientists proposed that night length was the factor determining photoperiodic response. When phytochrome, a red/far-red-light photoreceptor, absorbs red light during the day, it changes into the physiologically active Pfr (far-red-light-absorbing) form. Upon absorption of far-red light, Pfr is converted back to the inactive Pr (red-light-absorbing) form. Pfr molecules also gradually revert to the inactive Pr at night in a process called dark reversion. Phytochrome was proposed as a photoperiodic timer, a concept that is easily illustrated in plants that flower during short days. In these plants, when the day is long and the night is short, fewer Pfr molecules change into Pr during the night, leading to Pfr-dependent repression of flowering; by contrast, when the day is short and the night is long, more Pfr molecules change into Pr during the night, diminishing this repression. This type of hypothesis, often known as an hourglass hypothesis, was the major explanation of photoperiodism in plants until the early 1960s. Although

**Wheat and barley:** ancestrally winter annuals; cultivated strains are classified as either winter (vernalization requiring) or spring and as either photoperiod sensitive or insensitive

fascinating, this proposal was eventually rejected because a circadian rhythm of sensitivity to a red-light pulse during an extended dark period was discovered (17, 50, 91, 122).

In 1936, German scientist Erwin Bünning proposed that an internal timekeeping mechanism separated each day into two 12-h periods. The first 12-h period, beginning at dawn, was called the photophile ("light-requiring") phase, and the second 12-h period was called the skotophile ("dark-requiring") phase. When the light period is longer than 12 h, such that light is still present in the beginning of the skotophile phase, flowering is induced in long-day plants and repressed in short-day plants (**Figure 1*a***). This idea is known as Bünning's hypothesis (8). It was not viewed favorably until the 1950s, when other scientists began to recognize the validity of an internal timekeeper, referred to as the circadian clock.

In 1964, chronobiologists Colin Pittendrigh and Dorothea Minis proposed a model (later referred to as the external coincidence model) that was based on Bünning's hypothesis but modified in two key ways (92) (**Figure 1*b***). First, instead of the 12-h skotophile phase, they proposed the presence of two factors: (*a*) a substrate whose levels oscillate throughout the day that induces a photoperiodic response when it is processed, and (*b*) an enzyme that is active only under light. The photoperiodic response is triggered only when the peak of the substrate coincides with the presence of the active enzyme. Second, because the circadian clock regulates the timing (phase) of the substrate peak, the phase of this peak changes depending on day length owing to variations in the timing of dawn and dusk throughout the year, which entrain (reset) the circadian clock each day. The effects of light entrainment, which can be classified as no change, phase advance, or phase delay, differ depending on when the light signals occur.

This hypothetical enzymatic reaction was used to explain the concept of the model (**Figure 1*b***), but the mechanism can actually be any cellular event—gene and protein expression, protein modification, controlled degradation, etc. Although Pittendrigh (90) later proposed another model, known as the internal coincidence model, that was based on the study of *Drosophila pseudoobscura* pupation, the external coincidence model is currently the model most strongly supported by known molecular mechanisms of photoperiodism in *Arabidopsis*, wheat, barley, and rice. In this review, we summarize the molecular basis of time measurements in these plants.

***Grain number, plant height, and heading date 7 (Ghd7):*** a gene isolated from QTLs and associated with increased grain number, plant height, and late flowering

***Early heading date 1 (Ehd1):*** a gene isolated from QTLs and associated with early flowering, independent of *Hd1*, in both long and short days

**Phytochrome:** a photoreceptor that absorbs red and far-red light spectra to change its structure between active Pfr and inactive Pr forms

**Long-day plants:** plants that show a photoperiodic response when the day length is longer than a certain threshold (critical day length)

**Florigen:** a hypothetical substrate explaining floral induction at the shoot apical meristem in response to exposure of leaves to inductive conditions

**FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1):** a blue-light photoreceptor that forms an SCF-type E3 ubiquitin ligase complex

## 2. PHOTOPERIODIC FLOWERING MECHANISMS IN *ARABIDOPSIS*

In many plant species, the timing of flowering depends largely on seasonal changes in the expression of the *FT* gene, which encodes a systemic signaling molecule that is a key component of the long-sought florigen, because it is synthesized in the leaves but moves to the shoot apex to induce flowering. The photoperiodic flowering mechanism induced by *FT* expression is most well characterized in the long-day plant *Arabidopsis thaliana*. In *Arabidopsis*, long-day conditions induce high levels of *FT* expression that consequently accelerate flowering, whereas short-day conditions lead to very low levels of *FT* expression (53). The day-length-dependent induction of *FT* is governed mainly by the transcriptional activator CO (101, 112, 121). Consistent with the external coincidence model, accumulation of *CO* transcripts occurs from the afternoon to night by the circadian clock, thereby coinciding with light primarily in the summer, when the CO protein is stabilized and *FT* expression occurs (**Figure 2**).

### 2.1. Generation of Rhythmic Expression Patterns of the *CO* Gene

To restrict CO protein activity to the long-day afternoon for proper *FT* induction, both circadian-clock regulation of *CO* transcription and photoreceptor regulation of CO protein abundance are necessary (112, 113, 121). Day-length-dependent differences in *CO* transcript abundance under light are tightly correlated with the amount of the FLAVIN-BINDING, KELCH REPEAT,

F-BOX 1 (FKF1)–GIGANTEA (GI) complex. This complex is formed in a blue-light-dependent manner and mediates the degradation of *CO* transcriptional repressors known as CYCLING DOF FACTORs (CDFs) (26, 39, 103).

The CDF family members (CDF1–CDF5), of which CDF1 is the most well characterized, function as repressors of flowering through direct repression of *CO* transcription in the morning (39, 96, 103). This repression in the morning is an important feature to allow *Arabidopsis* plants to differentiate between long days and short days (**Figure 2**). The *cdf1 cdf2 cdf3 cdf5* quadruple mutant, in which *CO* expression levels are highly elevated in the morning regardless of photoperiod, no longer distinguishes changes in day length (26). In addition, among wild-type accessions, natural variations in the number of repeats of CDF-binding sites (two to four repeats) located in tandem near the transcription start site of the *CO* locus are tightly correlated with differences in *CO* transcript abundance (96). A higher number of repeats leads to later flowering, indicating that these *cis*-regulatory variations contribute to adaptation to local environments by adjusting flowering time.

Because precise timing of daily *CDF* expression is crucial for proper timing of flowering, multiple core clock components regulate *CDF* expression (30, 37, 42, 80, 82). Two related morning Myb transcription factors, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), induce *CDF1* expression in the morning (82, 104, 123) (**Figure 2**). In the afternoon, *CDF* transcription is repressed by the PSEUDO-RESPONSE REGULATOR (PRR) family of transcriptional repressor proteins (30, 37, 80). The PRR5, PRR7, and PRR9 proteins each directly bind to at least three of the *CDF* (*CDF2*, *CDF3*, and *CDF5*) promoters (80, 81). Concomitantly, expression of the *CCA1* and *LHY* genes is also repressed by the PRR proteins (80, 81). These mechanisms stop *CDF* transcription in the afternoon (**Figure 2**).

In addition to transcriptional regulation, posttranscriptional regulation plays an important role. In long days, the degradation of CDF1, CDF2, and most likely other CDF proteins is controlled by the FKF1-GI ubiquitin ligase complex (103). This regulation fits nicely with the external coincidence model. Expression of the *FKF1* and *GI* genes is regulated by the circadian clock, and the FKF1 and GI proteins show similar diurnal expression patterns, peaking at the end of the day in long days (18, 27, 38, 40, 51, 103) (**Figure 2**). When the protein expression profiles of FKF1 and GI synchronize in the afternoon in long days, the FKF1 LOV (light, oxygen, or voltage) domain absorbs blue light, activating the protein. Blue-light absorption enables FKF1 to form a protein complex with GI through the LOV domain and, simultaneously, to recognize the CDF protein family members through the binding of the KELCH repeat domain. FKF1 then mediates ubiquitin-dependent degradation of the CDF proteins to alleviate repression of the *CO* promoter (103). In short days, FKF1 does not contribute much to the control of CDF stability. FKF1 expression occurs mainly at night, and out-of-phase expression of FKF1 and GI proteins results in significantly reduced formation of the FKF1-GI complex in short days. The amount of CO protein therefore remains low under light and subsequently causes little *FT* expression throughout the day (103).

In addition to FKF1, ZEITLUPE (ZTL) and LOV KELCH PROTEIN 2 (LKP2) interact with GI and contribute to the removal of *CO* repression, partly through the degradation of CDF2 (26, 51). CDF stability is also controlled by a small ubiquitin-related modifier (SUMO)–targeted ubiquitin ligase (STUbL). The *Arabidopsis* STUbL4 (AT-STUbL4) protein is a RING-finger-type E3 ubiquitin ligase that localizes in the nucleus and targets SUMO-attached (i.e., SUMOylated) proteins for ubiquitination and degradation (24). CDF2 is SUMOylated and targeted by AT-STUbL4 for degradation (7, 24). Thus, in long days, degradation of CDF proteins by these E3 ubiquitin ligases toward the late afternoon restricts the transcriptional repression of the *CO* gene to the morning.

**GIGANTEA (GI):** a plant-specific large nuclear protein that does not have any known functional domains

**Dof factors:** plant-specific transcription factors that possess a single zinc-finger domain called the Dof (DNA-binding-with-one-finger) domain

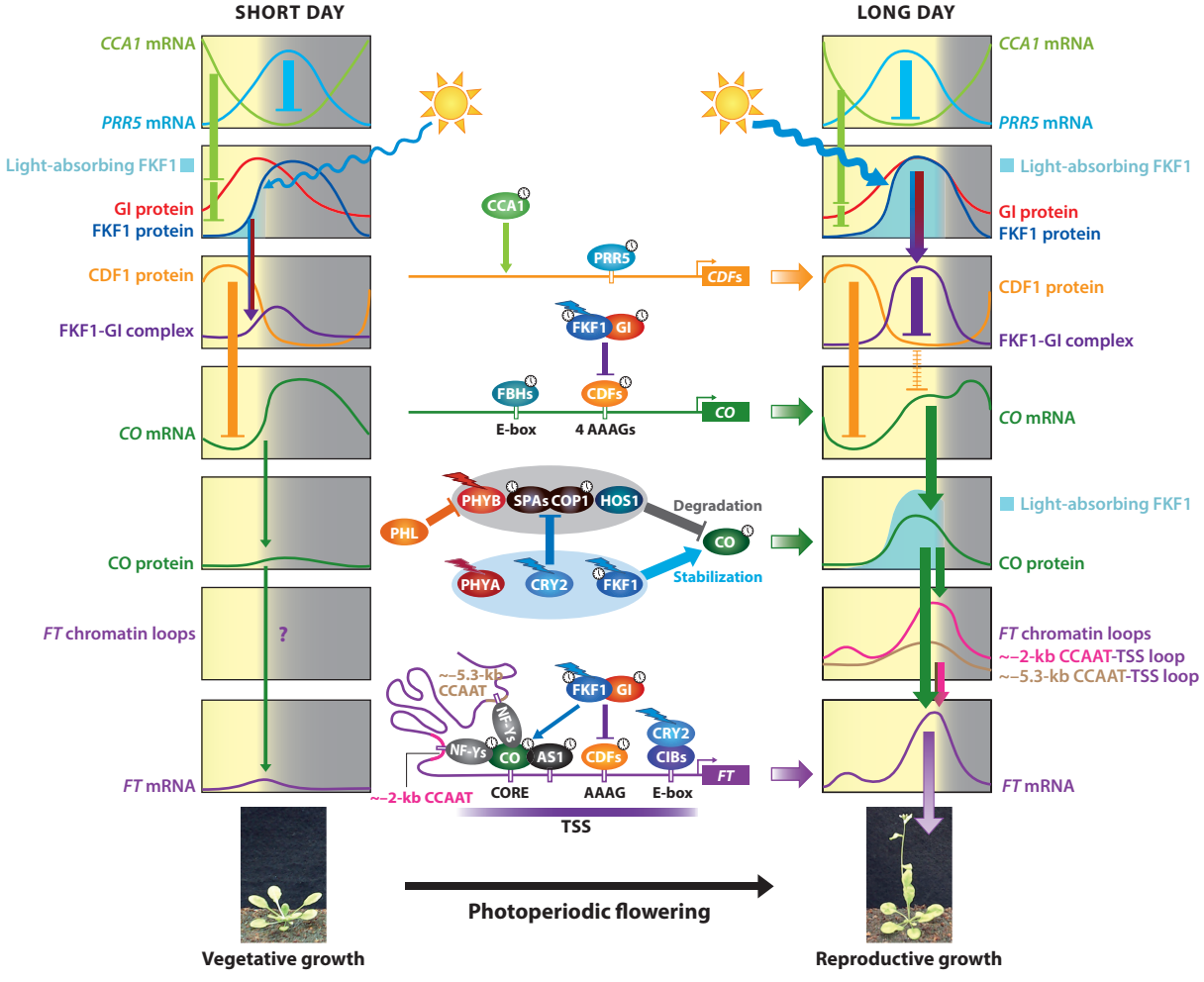
**LOV (light, oxygen, or voltage) domain:** a flavin-containing domain that is important for blue-light sensing and functions as a protein-protein interaction domain

**Basic helix-loop-helix (bHLH):** a transcription factor that possesses a bHLH DNA-binding domain and works in a dimer

Degradation of CDF proteins on the *CO* promoter also facilitates access by transcriptional activators to the *CO* promoter. Four basic helix-loop-helix (bHLH) transcription factors—FLOWERING BHLH 1 (FBH1), FBH2, FBH3, and FBH4—induce *CO* transcription by binding to the E-box *cis*-elements in the *CO* locus (43). Although FBH1 protein is expressed at a similar level throughout the day, more FBH1 binds to the *CO* promoter in the afternoon than in the morning. Thus, the temporal interplay among repressors and activators restricts the *CO* gene expression during the late afternoon when daylight remains.

## 2.2. Light-Dependent Control of CO Protein Stabilization

Posttranslational regulation of CO protein is another key element of the photoperiodic induction of *FT* transcription. The abundance of CO protein changes depending on day length and dynamically fluctuates between day and night (78, 121) (**Figure 2**). Various light signal components control CO stability throughout the day, as far-red- and blue-light signals stabilize CO but red-light signals destabilize it (78, 121). Light signaling modulates the ubiquitin-dependent degradation mechanisms of CO at different times of day (47, 62, 70). Phytochrome B (PHYB)



and two RING-finger E3 ubiquitin ligases, CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1), are involved in CO degradation (47, 62, 70, 121) (**Figure 2**). In the morning, HOS1 directly binds to CO and degrades the protein in a proteasome-dependent manner (62). In addition, PHYB destabilizes CO at the same time (121).

The molecular mechanisms mediating PHYB-dependent destabilization of CO have not been well elucidated. Recent evidence that the function of PHYTOCHROME DEPENDENT LATE FLOWERING (PHL) counteracts the ability of PHYB to regulate flowering suggests that the stability change in CO mediated by PHYB is intricate. PHL interacts with both PHYB and CO under red light (25). Because HOS1 and PHYB play a similar role in CO protein stability, they might function in the same pathway. During the night in both long days and short days, CO is actively degraded by a complex between COP1 and SUPPRESSOR OF *PHYA-105* 1 (SPA1). The SPA family members SPA1, SPA3, and SPA4 interact with CO and redundantly destabilize the protein (47, 61, 99). This dark-dependent degradation of CO is particularly important for preventing flowering in short days.

In contrast to red light, blue and far-red light accelerate flowering through an increase in CO protein abundance in long days (121). The antagonistic function of blue and far-red light

**Figure 2**

Photoperiodic regulation of *FT* induction in *Arabidopsis*. The abundance of *CCA1* transcript oscillates throughout the day; it is high in the early morning in both long and short days. CCA1 and its homolog LHY bind to promoters of *PRR5*, *FKF1*, and *GI* to repress their expression in the morning. Daily oscillation patterns of *PRR5* mRNA expression are antiphasic to those of *CCA1*. PRR5 protein binds to the *CCA1* promoter to form a feedback loop between morning and evening clock components. PRR5 also negatively controls the expression of *CDF* genes. CDF proteins (CDF1, CDF2, CDF3, and CDF5) act as transcriptional repressors that likely bind to the Dof-binding site (AAAG) in the *CO* promoter in both long and short days. Daily expression profiles of CDF1 are regulated by the FKF1-GI complex. During long days, the peak expression of FKF1 and GI proteins, which are regulated by the circadian clock, occurs in the afternoon. When FKF1 absorbs blue light, it interacts with GI. The photo-induced FKF1-GI complex accumulates to high levels in long-day afternoons and degrades CDF proteins on the *CO* promoter. Once the repression of *CO* transcription by CDFs is relieved, FBH proteins activate *CO* gene expression by directly binding to the E-box elements in the *CO* locus. In contrast to their response to long-day conditions, the expression of FKF1 and GI proteins is out of phase during short days, and FKF1 is expressed mainly in the dark. This causes a low level of FKF1-GI complex formation in the afternoon, and consequently the abundance of *CO* mRNA remains very low under light. CO protein is the primary activator of *FT* transcription and shows daily oscillation patterns. The protein accumulates to high levels only in the late afternoon in long days, and its stability is regulated by several factors. PHYB, the COP1-SPA complex, and HOS1 are involved in the degradation of CO. Among these, COP1, SPAs, and HOS1 directly bind to and degrade the protein. PHYB is a red-light photoreceptor, and the function of PHYB is inhibited by PHL through the formation of a protein complex under red light. PHL also interacts with CO. By contrast, the far-red-light photoreceptor PHYA and the blue-light photoreceptors CRY2 and FKF1 stabilize CO. Blue-light-stimulated CRY2 interacts with both COP1 and the SPAs, and the interactions lead to sequestration of CO protein away from the COP1-SPA complex. Through another FKF1-dependent mechanism that aligns with the external coincidence model, FKF1 directly binds to CO in a blue-light-enhanced manner and promotes the stability of the protein in the late afternoon in long days. Many factors regulate *FT* expression throughout the day during long days. In the morning, CDFs repress *FT* transcription through direct association with this gene's promoter. The degradation of CDFs is controlled by the FKF1-GI complex, which also exists on the *FT* promoter. CO, which is stabilized by FKF1, strongly induces *FT* expression around dusk in long days by directly binding to the CORE region in the *FT* promoter as well as by interacting with other *FT* regulators, namely NF-Y complexes and AS1. NF-Ys bind to the CCAAT boxes located approximately 2 kb and 5.3 kb upstream from the transcription start site (TSS) of the *FT* gene. These CCAAT-box regions form loops with the CORE region within the TSS, and the timing of loop formation shows diurnal oscillation in long days. This type of *FT* chromatin dynamic has not been studied in short-day conditions, but it demonstrates how *cis*-acting sequences away from the TSS influence the activation of *FT* transcription. In addition to CO, CIB proteins are involved in the activation of *FT* transcription. CIB proteins, which interact with CRY2 under blue light, directly bind to the E-box located near the TSS on the *FT* promoter. Hence, the functions of CO and other factors enable *FT* to be strongly expressed at the end of the day only in long days, which accelerates the time to flowering. Clock marks on each protein symbol indicate that the circadian clock regulates its expression.

in relation to red light enables CO to accumulate only in the late afternoon in long days, which causes the highest *FT* expression to occur at dusk. Three kinds of photoreceptors—FKF1, PHYA, and cryptochromes (CRY), especially CRY2—are involved in CO protein stabilization (**Figure 2**). PHYA is a red/far-red-light photoreceptor that stabilizes CO under far-red light (121). CRY2 acts as a blue-light photoreceptor that is phosphorylated and negatively regulated by the blue-light-induced function of casein kinase 1 (CK1) proteins (118). Photoactivated CRY2 forms a protein complex with SPA1, which enhances the binding of CRY2 to COP1 in response to blue light. The complexes of CRY2 with COP1 and SPA1 suppress COP1/SPA1 activity, which in turn causes CO to accumulate and *FT* transcription to be activated during the daytime (137). However, the function of PHYA and CRY2 cannot fully explain how the CO protein is stabilized only in the late afternoon in long days, because both photoreceptors are constitutively expressed throughout the day.

In addition to alleviating the repression of *CO* gene transcription, FKF1 plays a critical role in stabilizing CO in long-day afternoons (**Figure 2**). The diurnal rhythm of FKF1 protein abundance in long days is similar to that of CO (40, 112, 121). FKF1 directly binds to CO through its LOV domain. This binding is enhanced by blue light and leads to an increase in CO protein stability in the late afternoon in long days (112). Again, this mechanism fits with the external coincidence model. Therefore, FKF1 acts as a photoperiodic sensor in *Arabidopsis*.

## 2.3. Induction of *FT* Gene Expression in Long Days

Not only photoperiod but also other environmental and endogenous factors, including temperature and hormones, converge on the regulation of *FT* transcription to create flexible yet precise seasonal responses (3, 73, 111). Even in the photoperiodic flowering pathway, various *FT* transcriptional repressors, which counteract the activity of CO, have been identified (3, 73, 111). GI plays several roles in regulating *FT* repressor expression and activity (49, 102, 112). For instance, through a microRNA pathway, GI negatively regulates the expression of *SCHLAFMÜTZE* (*SMZ*) and related genes that encode *APETALA 2* (*AP2*)–related transcription factors (49). Stimulated by GI, the expression of microRNA172 targets the *SMZ* and related mRNAs and reduces their abundance (49, 72). SMZ protein directly associates with the 3′ untranslated region of the *FT* locus and represses *FT* transcription in long days (72). In addition, two GI-interacting proteins, TEMPRANILLO 1 (TEM1) and TEM2, also repress *FT* transcription throughout the day in long days (11, 102). TEM1 directly binds to the 5′ untranslated region of *FT* and represses the transcription redundantly with TEM2 (11).

Other repressors are involved in the production of the daily expression profiles of the *FT* gene. Similar to the transcriptional regulation of *CO*, the repression mechanism mediated by CDF proteins exists in the expression of the *FT* gene. CDF1 associates with *FT* promoter regions in the morning and represses the gene expression together with other CDFs (112) (**Figure 2**). Because the FKF1-GI complex degrades CDF proteins, direct interaction of FKF1 and GI proteins with the *FT* locus implies that CDF proteins are degraded on the *FT* promoter by the blue-light-activated FKF1 complex in the afternoon (112).

Once the repression of the *FT* gene is relieved, two classes of transcription factors, CO and the CRYPTOCHROME-INTERACTING BASIC HELIX-LOOP-HELIX (CIB) transcription factor proteins, activate *FT* gene expression in long days (66, 67, 93, 95, 112, 121) (**Figure 2**). Therefore, blue-light signaling plays a key role in the accumulation of both the CO and CIB1 proteins in *Arabidopsis* (66, 67, 112, 121). At dusk, blue-light-stabilized CO protein associates with the *FT* locus and strongly induces *FT* transcription through two modes of activation. First, the protein directly binds to the CONSTANS-responsive element (CORE) in the *FT* promoter

through the C-terminal CCT (CONSTANS, CONSTANS-like, and TOC1) domain. Second, the protein is recruited to the *FT* promoter by physical interaction with the ASYMMETRIC LEAVES 1 (AS1) protein and the CCAAT-box-binding nuclear factor Y (NF-Y) proteins (10, 58, 59, 112, 125). A recent study revealed that the interaction between CO and NF-Y dynamically changes the structure of the *FT* promoter region throughout the day in long days (10, 58, 59, 112, 125) (**Figure 2**).

Another transcription factor family, comprising CIB1, CIB2, CIB4, and CIB5, also positively regulates *FT* expression in the afternoon. CIB proteins interact with CRY2 under blue light and redundantly activate *FT* transcription via direct binding to the *FT* promoter (67, 71). CIB1 stability is enhanced by ZTL and LKP2 in a blue-light-dependent manner (66). Heterodimer complexes that form between CIB1 and the other CIBs facilitate the binding of CIB1 to the noncanonical E-box elements in the *FT* promoter (71). In addition to these transcription factors, multiple chromatin-remodeling factors play important roles in regulating *FT* transcription (35).

In long days, after the complex regulation of *FT* transcription, *FT* mRNA is synthesized in the distal part of the leaf phloem companion cells (2, 115). FT protein, as a major component of florigen, moves from the leaf to the shoot apical meristem and triggers the phase transition from vegetative to reproductive growth (for details, see 31, 69, 89) (**Figure 2**).

Since the first evidence for the molecular mechanism of photoperiodic flowering that fits with the external coincidence model was reported in 2001 (113), we have learned in great detail about the photoperiodic flowering mechanisms in *Arabidopsis*. The knowledge obtained from *Arabidopsis* research has greatly facilitated our understanding of the mechanisms of photoperiodic flowering in other plant species. In the remainder of this review, we discuss the current understanding of photoperiodic flowering in wheat, barley, and rice.

## 3. PHOTOPERIODIC FLOWERING IN AGRICULTURAL CROPS

Knowledge of photoperiodic flowering has broad applications in agriculture. Through targeted breeding, our knowledge of photoperiodic flowering can be used to lengthen or shorten the duration of growth to adapt crops to regional conditions. In the United States, analysis of historical management and climate data has revealed that planting dates for maize and soybean have trended earlier over the last 30 years, and the use of longer-season cultivars has increased (98). Photoperiodic sensitivity in wheat (a long-day plant) may allow growers in the northern United States to take advantage of these earlier planting dates and longer growing seasons, as photoperiod-sensitive strains may accumulate more biomass before flowering, contributing to higher yields (60). Conversely, the transition to cultivars with reduced photoperiodic sensitivity in rice (a short-day plant) has increased the heat requirement needed for development and stabilized yields over the last 30 years, helping to offset the negative impacts of warming (68).

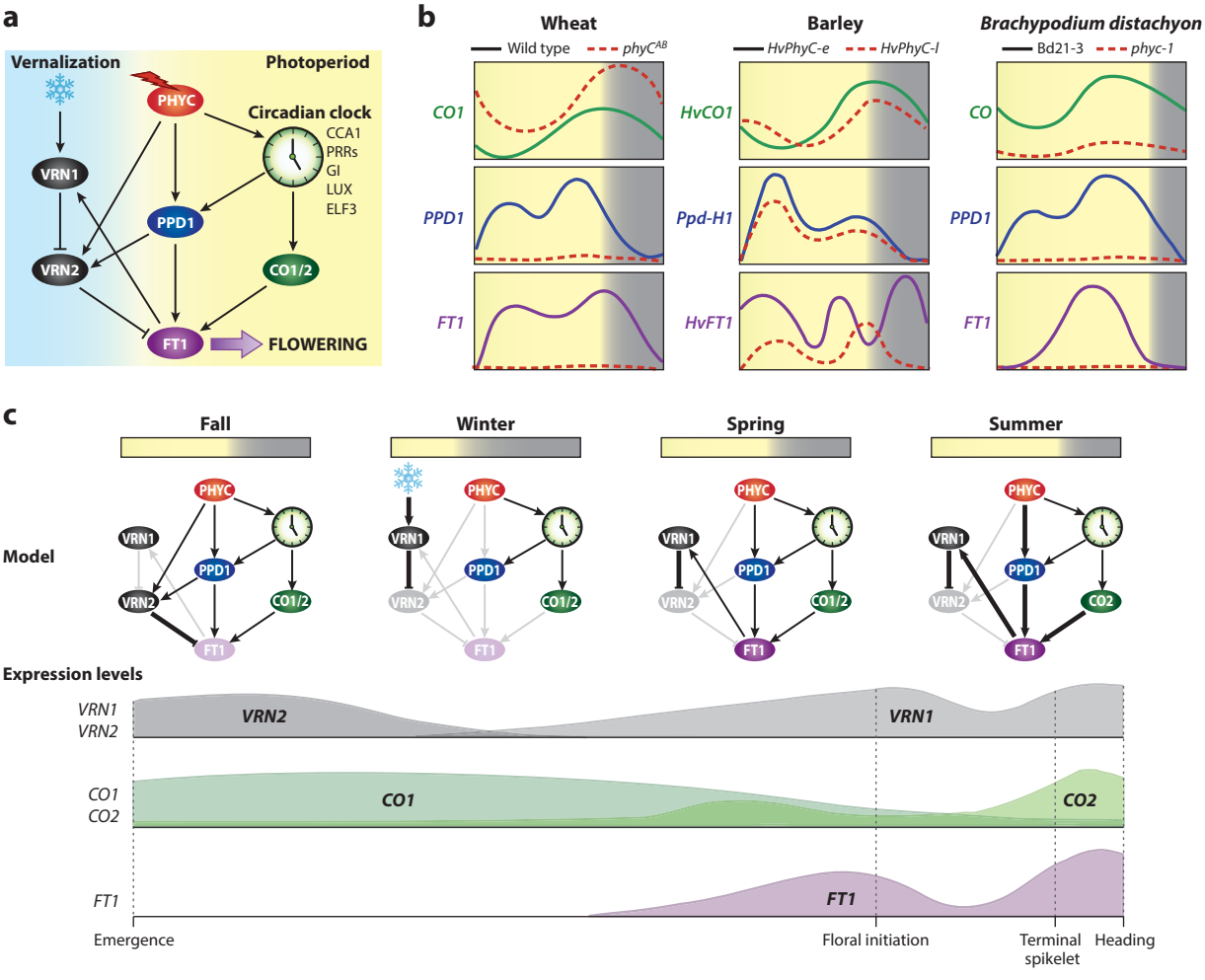
### 3.1. Photoperiodic Sensing in Wheat and Barley

Variation in photoperiodic sensitivity within the long-day cereals is conferred primarily through the *PHOTOPERIOD 1* (*PPD1*) genes (4, 119) (**Figure 3**). Photoperiod insensitivity in wheat varieties, characterized by early flowering in short days, occurs through several naturally occurring mutations, including a 1,085-base-pair (bp) deletion, a 2,089-bp deletion, and a 308-bp insertion located above the transcription start sites of the *PPD-A1*, *PPD-D1*, and *PPD-B1* alleles, respectively (4, 84, 126). [Note that wheat can be diploid, tetraploid, or hexaploid, and wheat genomes are classified as A, B, or D (as in *PPD-A1*); the barley genome is classified as H. Photoperiod-insensitive variants are indicated with an *a*, as in *PPD-A1a*.] These three modifications either span or interrupt

a 95-bp region that is conserved across wheat, barley, rice, and *Brachypodium distachyon*; this region likely contains a key *cis*-regulatory element involved in light perception and has been proposed to be the binding site of an unknown transcriptional repressor (84). Increased copy numbers of *PPD-B1* are associated with photoperiod insensitivity as well (19). Interestingly, the relative influence of each *PPD1* locus located on the A, B, or D genomes in hexaploid wheat differs, providing a means to fine-tune the photoperiodic response (106).

Expression of the wild-type variant of *PPD1* (designated *PPD1b*) is upregulated throughout the light period in both long and short days and in constant light (13, 106), declining at night and remaining at basal levels in constant darkness. Although *PPD1a* variants also require light to be expressed (13), they lose their cyclic expression profile and are expressed throughout the day and night (19, 106, 126). The majority of the photoperiod-insensitive strains of hexaploid wheat that were instrumental during the green revolution carry the *PPD-D1a* allele. However, even strains designated as photoperiod insensitive display accelerated flowering in response to longer photoperiods, likely as a result of photoperiod-responsive variants of *PPD1* in other genomes (32).

Wheat and barley *PPD1* are homologous to *Arabidopsis PRR7*, a gene integral to the circadian clock in *Arabidopsis* (119). However, the timing of the peak expression of the core clock genes



*TOC1* and *GI1* and the clock output genes *CDF1* and *CO1* are not altered in wheat carrying the constitutively active *PPD1a* alleles in short days (106). The same is true of *Ppd-H1* in barley (9). Rather, both the timing of peak *PPD1b* expression and the amount of expression are altered in two clock mutants—the Igri [*early maturity 8* (*eam8*)] barley variety and wheat carrying a deletion of *PHYTOCLOCK 1*, a homolog of *Arabidopsis LUX ARYTHMO/PHYTOCLOCK 1*, which indicates that *PPD1* is an output of the circadian clock (77, 120).

Red light acts through *PHYC* and *PPD1* to regulate *FT1* and flowering (**Figure 3*a***). Upregulation of *PPD1* is accompanied by upregulation of *FT1* (also called *VRN3*) in long days in vernalized plants and in strains not requiring vernalization (13, 52, 106). Neither gene is upregulated when both variants of PHYC in tetraploid wheat are nonfunctional, and flowering is delayed in both long and short days (13). PHYC can form dimers with wheat PHYB and with itself in rice cultured cells. PHYC preferentially dimerizes in the light, whereas both dimers and monomers are present in the dark when it is expressed in the *Arabidopsis phyA phyB phyC phyD phyE* quintuple mutant. As *PHYC* is also expressed at higher levels than *PHYB* in wheat, taken together, these data indicate that red light causes PHYC to dimerize with itself and PHYB and to move into the nucleus, where it elicits transcription of *PPD1* and *FT1* (13). This process differs from that in *Arabidopsis*, where PHYC requires the presence of PHYA and PHYB to function (16). The important function of PHYC in flowering-time regulation is conserved in barley and *Brachypodium* (83, 127), and it is tantalizing to ask whether PHYC acts to degrade a repressor situated on the conserved 95-bp region in the *PPD1* promoter (**Figure 3*b***). It is possible that light signals perceived by PHYC and the presence of PPD1 represent the point at which external coincidence occurs (13). However, the molecular mechanism of the interaction remains elusive.

## 3.2. Involvement of *CO* in Photoperiodic Sensing in Long-Day Cereals

*CO1*, which in barley has close homology to *Arabidopsis CO* (107), displays an oscillating diurnal expression profile in wheat that peaks approximately 16 h after dawn in both long and short days

**Figure 3**

Photoperiodic control in the leaves of the long-day cereals wheat, barley, and *Brachypodium distachyon*. (*a*) Regulation of *FT1* via the vernalization and photoperiodic pathways. The latter pathway may be governed by the coincidence of circadian-clock control of *PPD1* and *CO* as well as by red-light signals mediated through PHYC, which influences the expression of circadian-clock genes in wheat, barley, and *Brachypodium*. VRN2, a negative regulator of *FT1* gene expression, is downregulated by vernalization through *VRN1*. *VRN2* is induced in long days in a PHYC-dependent manner, potentially through PPD1. Whether CO acts in parallel or cooperatively with PPD1 is not known. (*b*) Diurnal patterns in the gene expression of the key floral-regulator genes *CO1* (or *CO* in *Brachypodium*), *PPD1*, and *FT1* in strains carrying wild-type or hyperfunctional alleles (*solid lines*) and strains with reduced or null PHYC activity (*dashed red lines*). PHYC is nonfunctional in the $phyC^{AB}$ and *phyc-1* lines in wheat and *Brachypodium*, respectively, whereas *HvPhyC-e* from an early-flowering barley variety is likely hyperfunctional relative to *HvPhyC-l* in a late-flowering variety. In the strains carrying nonfunctional *phyC* alleles (wheat and *Brachypodium*), the expression of all three floral regulators is altered, whereas the expression of *Ppd-H1* is only slightly decreased and that of *HvCO1* is not significantly altered in the barley *HvPhyC-l* line. *FT1* expression is significantly decreased across the three species in all three of those strains. Wheat *CO1* is upregulated, perhaps owing to the release of a negative feedback from *FT1* in $phyC^{AB}$ lines. (*c*) The changing influence of day length throughout the year as mediated by PHYC. During fall, in winter varieties (i.e., those requiring vernalization), afternoon light causes upregulation of *VRN2* gene expression. *VRN2* may be downstream of *PPD1* and also acts antagonistically to *PPD1* to repress *FT1* and delay flowering. Cold winter temperatures repress *VRN2* expression via *VRN1*. *CO1* and *PPD1* genes continue to be transcribed. In spring, day length acts through *PHYC*, *PPD1*, and *CO1* to activate *FT1* expression, which feeds back to further upregulate *VRN1* and maintain repression of *VRN2*. In summer, activation by light further facilitates this process. In wheat, around the time of floral initiation, *CO1* begins to decline, perhaps owing to negative feedback from *FT1*. *CO2* begins to be upregulated, perhaps maintaining *FT1* expression through the terminal spikelet stage and heading.

and continues to oscillate in constant light, indicating the involvement of the circadian clock (13, 106). Consistent with CO's light-dependent activation of *FT* in *Arabidopsis*, barley *HvCO1* overexpression results in activation of *HvFT1* only in long days (9). However, *CO1* expression declines over time as wheat transitions from vegetative to reproductive stages in both long and short days, whereas *FT1* expression remains high or at least peaks again in later life stages (52, 105) (**Figure 3*c***). This has led some to propose that FT feeds back to repress *CO1* expression (105, 106). Consistent with this, in wheat, nighttime *CO1* expression is inversely proportional to *FT1* expression (105). However, this hypothesis requires further testing.

**Floral initiation:** the stage at which the rate of bud primordium formation (leaves or fruit) at the shoot apex accelerates (5)

**Terminal spikelet:** the last primordium formed at the shoot apex; the terminal spikelet stage follows floral initiation and precedes heading (5)

One candidate for maintenance of *FT1* expression after *CO1* declines is *CO2* (also called *TaHd1*) (52). Expression of *CO2* increases as *CO1* declines in both long and short days (52) (**Figure 3*c***). In tetraploid wheat carrying constitutively active *Ppd1a* alleles, *FT1* transcription is induced in short days, concurrent with increased expression of *CO2* (52). This implies that, in contrast to CO protein activity in *Arabidopsis*, CO2 is stabilized in short days in the presence of active PPD1. Alternatively, PPD1 could lead to *FT1* expression directly (13). Interestingly, the wild-type tetraploid wheat *PPD1b* strain flowers only slightly later than the constitutively active *PPD1a* strain in short days, and overexpression of barley *HvCO1* leads to early flowering in short days, although *FT1* is not expressed in either case (9, 52). Therefore, downstream factors other than *FT1* can induce flowering.

Although wheat *CO1* and *CO2* likely contribute to *FT1* expression, whether and (if so) how *PHYC*, *PPD1*, and the *CO* homologs interact remain open questions. Nishida et al. (83) proposed that barley PHYC acts to upregulate *FT1* independently of *CO1*, on the basis that *FT1* expression is altered in functional *phyC* mutants, whereas expression of *CO1* is not changed (**Figure 3*b***). However, null *phyC* mutations in wheat and *Brachypodium* showed altered expression of *CO1* and *CO2* (13, 127) (**Figure 3*b***), indicating that PHYC does regulate the expression of *CO* homologs. In the case of *PPD1*, tetraploid wheat carrying either the constitutively active *PPD1a* allele or the wild-type *PPD1b* allele differ in their expression levels of *CO1* and *CO2* across the lifetime of the plant (52). Nighttime *CO1* expression inversely correlates with the number of null *ppd1* alleles contained within hexaploid wheat strains (105). Together, these results indicate that PHYC and PPD1 do influence expression of *CO1* and *CO2*. However, because the expression profiles of several circadian-clock genes are also altered in wheat *phyC* null mutants (13) and *FT1* expression is altered in strains carrying active forms of *PPD1a*, it is still unclear whether *PHYC* and *PPD1* act directly on the *CO* homologs, whether *PHYC*'s influence occurs through the circadian clock, or whether their influence on *CO1* expression is through feedback from *FT1* in wheat.

### 3.3. Interaction Between Photoperiod and Vernalization Response

Like *Arabidopsis*, photoperiod and vernalization interact to regulate flowering in wheat (**Figure 3*a***), but the interaction changes depending on the developmental stage of the plant (5, 6, 136). By analyzing spring and winter strains of Batten wheat grown in several combinations of vernalization temperatures and photoperiods, Brown et al. (6) demonstrated that final leaf number at heading correlates strongly with the leaf number at floral initiation when plants are exposed to different vernalization temperatures in long days. By contrast, exposure to short days reduces the leaf number at the floral initiation stage but extends the leaf number at the stage when a terminal spikelet forms. Together, these data indicate that vernalization influences the timing of floral initiation but has little influence on later stages. Long photoperiods delay flowering if experienced prior to floral initiation, but they accelerate flowering later. Long photoperiods also seem to decrease the time between terminal spikelet formation and heading (109).

The mechanisms underlying the interaction between photoperiod and vernalization have been difficult to pinpoint because of the existence of feedback among the three key flowering loci in wheat; however, much progress has been made (12, 13, 20, 21, 107). *VRN1* (also called *FUL1* or *WAP1*) has high homology to *AP1/FRUITFUL* in *Arabidopsis* (131) and acts in both the leaves and the shoot apex to promote flowering. In the apex, it appears to act downstream of *FT1* similarly to what occurs in *Arabidopsis*. An increase of *FT1* in the leaves correlates with an increase of *VRN1* in the apex (64). Further, FT1 forms a complex with TaFDL2 (a homolog of *Arabidopsis* FD) and binds the promoter of *VRN1* in vitro. However, in the leaves, *VRN1* seems to maintain suppression of *VRN2* after vernalization, as *VRN2* declines during vernalization in both *vrn1* and wild-type TILLING wheat strains but increases after vernalization in the *vrn1* mutants (12) (**Figure 3*c***). The *VRN2* locus, which contains the similar *ZCCT1* and *ZCCT2* genes (130), acts to suppress *FT1* and delay flowering, so an increase of *VRN1* expression as a result of vernalization results in upregulation of *FT1* and earlier flowering. Interestingly, if vernalization has not occurred, *VRN2* is upregulated in long days and as a result of exposure to light (20, 23) but downregulated in *phyC* mutants (13, 127), indicating that *VRN2* is regulated by photoperiod through *PHYC* in a mode similar to photoperiodic regulation of *PPD1* (**Figure 3*a***).

It is possible that PHYC acts through PPD1 to regulate *VRN2* expression in response to changes in photoperiod (120, 127). In wheat carrying the active *PPD-D1a* allele, even after a prolonged exposure to short days known as short-day vernalization, *VRN2* expression was still upregulated, and consequently flowering was delayed. The same was true of a vernalization-requiring barley variety that had been crossed to gain the early-flowering Igri (*eam8*) mutation (120). This mutation affects a key clock gene that is orthologous to *Arabidopsis EARLY FLOWERING 3* (*ELF3*). In both strains, *PPD1* expression is elevated. Consistently, in *Brachypodium phyC* mutants, *PPD1* and *VRN2* are expressed only at basal levels; however, cold-temperature regulation of *VRN2* appears to be independent of *PHYC* function (127).

Coupling the physiological and mechanistic data, it appears that long photoperiods induce the expression of *VRN2* in developmental stages prior to floral initiation and delay flowering. Once the vernalization requirement has been met through suppression of *VRN2* by *VRN1*, long photoperiods induce the expression of *FT* and shorten the timing of onset for floral initiation, terminal spikelet formation, and perhaps heading (6, 109).

**Short-day vernalization:** exposure to a longer period of short days during early development, mimicking the effect of vernalization treatment (which represses *VRN2*) and accelerating flowering

**Quantitative trait loci (QTLs):** regions of chromosomes that contain or are near genes that underlie a quantitative phenotype

## 4. PHOTOPERIODIC FLOWERING IN RICE

Rice is an important food resource in most parts of the world. As in wheat and barley, control of flowering time (or heading date) in rice is closely related to grain production. Early or late flowering in rice causes reduced grain production through insufficient growth of vegetative organs or poor fertility (128). Although rice is considered a short-day plant, cultivars have been developed through continued domestication and breeding that initiate the reproductive transition under many different photoperiods, making it possible to cultivate rice in a broad range of latitudes and to contribute to yield increases (45).

Through analysis of the quantitative trait loci (QTLs) associated with differences in flowering among cultivars, several regulators involved in photoperiodic flowering have been characterized (3, 108, 110). *Hd3a* and *RFT1*, orthologs of *Arabidopsis FT*, encode rice florigens (54, 55, 117). As in *Arabidopsis*, in rice these genes are expressed in leaf vasculatures and move to the shoot apical meristem to induce flowering (54, 117). Photoperiodic flowering of rice is regulated by two distinct pathways: the *Hd1-Hd3a* module, similar to the *Arabidopsis CO-FT* module, and the unique

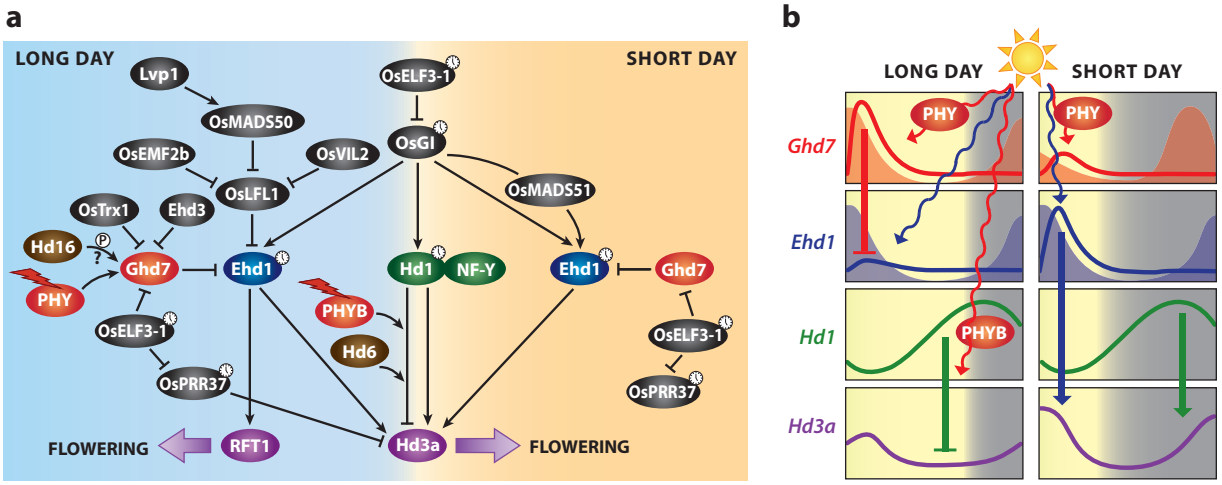



**Figure 4**

Regulation of rice *Hd3a* and *RFT1* expression by photoperiod. (*a*) The regulatory network controlling expression of *Hd3a* and *RFT1*. In rice, the critical day length required for floral induction is determined by two distinct pathways, *Hd1-Hd3a* and *Ghd7-Ehd1-Hd3a/RFT1*, which are regulated by the circadian clock and light signaling. The circadian clock regulates diurnal expression of *Hd1* through *OsGI* function. Hd1, which potentially forms a complex with NF-Y, activates *Hd3a* expression in short days but suppresses it in long days. Red light converts Hd1 activity from activating to repressing *Hd3a* expression via PHYB. This repressive activity is enhanced by *Hd6*, which encodes the α subunit of CK2. Expression of *Ehd1* and *Ghd7* is controlled by the circadian clock and light signaling. *Ehd1* activates expression of *Hd3a* and *RFT1* independently of *Hd1*. *OsGI* regulates *Ehd1* expression by activating *OsMADS51* expression, setting a blue-light-dependent gate around dawn. *Ghd7* acts as a repressor of *Ehd1* expression, and *Hd16* promotes repressive activity of Ghd7, potentially through phosphorylation. The *Ghd7* transcript is induced by light and increased by lengthening photoperiods. Phytochrome is required for light-dependent-induction of *Ghd7*. In short-day conditions, low induction of *Ghd7* allows induction of *Ehd1* to activate *Hd3a* expression. When day length increases above the critical short day length that is required for flowering, *Ghd7* is highly induced and is sufficient to suppress *Ehd1* and *Hd3a* expression. Disruption of the circadian clock by decreasing activity of *OsELF3-1* and *OsPRR37* affects daily expression of floral regulators. *OsELF3-1* negatively regulates expression of *OsGI*, *OsPRR37*, and *Ghd7* in both long- and short-day conditions. *OsPRR37* preferentially affects long-day flowering by suppressing *Hd3a* expression. Long-day-dependent induction of *Ehd1* is promoted when *OsMADS50* suppresses the negative regulators *Ghd7* and *OsLFL1*. *OsLFL1* is also negatively regulated by the OsVIL2-OsEMF2b complex, which is responsible for increasing repressive histone marks (H3K27me3). *Lvp1/SDG724* activates *OsMADS50* expression by increasing H3K36 methylation. Two plant-homeodomain-containing proteins, *OsTrx1/SDG723* and *Ehd3*, downregulate *Ghd7* expression to activate *Ehd1* transcription in long-day conditions. Clock marks on protein symbols indicate that the circadian clock regulates their expression. (*b*) Diurnal expression of floral regulators. *Ghd7* has higher phytochrome-dependent red-light inducibility around dawn in long-day conditions, shifting to midnight in short-day conditions (*orange shaded area*). *Ehd1* has higher blue-light-dependent inducibility around dawn in both long- and short-day conditions (*blue shaded area*). In long days, red light induces *Ghd7* transcription, leading to suppression of *Ehd1* and *Hd3a* expression. Accumulation of *Hd1* transcript in the presence of light suppresses *Hd3a* expression through PHYB function. In short days, weak expression of *Ghd7* allows induction of the *Ehd1* gene, leading to activation of *Hd3a* expression. Under these conditions and through a parallel pathway, *Hd1* expression occurs mainly during nighttime and also acts as an activator of *Hd3a*.

*Ghd7-Ehd1-Hd3a/RFT1* pathway (44, 46, 108, 129). Each pathway is regulated by the coincidence of the internal circadian clock and external photoperiodic information (**Figure 4**).

## 4.1. Transcriptional Regulation of Rice Florigens via the *Hd1-Hd3a* Pathway

Rice *Hd1*, an ortholog of *Arabidopsis CO*, is crucial for short-day photoperiodic induction of *Hd3a* (46) (**Figure 4*a***). *Hd1* expression oscillates, and its peak coincides with light in long-day afternoons (**Figure 4*b***). In the presence of afternoon light, *Hd1* acts as a repressor of *Hd3a* expression to

prevent flowering. When days become shorter, *Hd1* expression coincides with darkness, and Hd1 becomes an activator of *Hd3a* expression to promote flowering. These opposing, photoperiod-dependent effects of Hd1 protein on *Hd3a* gene expression are controlled by the circadian clock and light signaling. Diurnal expression of *Hd1* is regulated by a circadian-clock component, *OsGI*, an ortholog of *Arabidopsis GI* (34). In long-day afternoons, Hd1 is converted from an activator to a repressor of *Hd3a* expression in a functional conversion that is mediated by phytochromes, specifically PHYB (41, 46) (**Figure 4**). In the phytochrome-deficient mutant *photoperiod sensitivity 5* (*se5*), *Hd1* acts as a positive regulator of *Hd3a* in both long- and short-day conditions (41, 46). Functional conversion of *Hd1* by phytochrome light signaling is important for sensing day length, but the precise mechanisms resulting in Hd1-activity conversion are still unknown. The repressive effect of *Hd1* on *Hd3a* expression in long days is enhanced by *Hd6*, which encodes the α subunit of CK2, suggesting that Hd1 activity could be regulated by phosphorylation of unknown regulators (85, 116).

***photoperiod sensitivity 5 (se5):*** a phytochrome-deficient mutant lacking plastid-heme-oxygenase activity and known to function in chromophore biosynthesis

In addition to *OsGI*, other photoperiodic regulators known from *Arabidopsis* are conserved in rice. In *Arabidopsis*, FKF1 forms a complex with GI in a blue-light-dependent manner to induce the expression of *CO* by degrading CDF1. *OsFKF1*, a homolog of *Arabidopsis FKF1*, exists in rice and also shows a diurnal expression pattern (79). In rice, genome analysis has identified 30 different DOF (DNA-binding-with-one-finger)–encoding genes (65). One of them, the transcription factor gene *OsDOF12*, is characterized as a component of photoperiodic regulation of flowering. However, *OsDOF12* affects expression of *Hd3a* but not that of *Hd1*.

Rice also possesses *OsCOP1* and *OsSPA1* genes. Overexpression of *OsCOP1* in the *Arabidopsis cop1* mutant background completely restores the wild-type flowering phenotypes, but transformation of *OsSPA1* under the control of the *Arabidopsis SPA1* promoter into the *spa1 spa3 spa4* triple mutant did not rescue the early-flowering phenotype of this mutant (94). In *Arabidopsis*, the COP1-SPA complex degrades CO protein in the dark to inhibit the activation of *FT* expression during the night (47, 61). In contrast, rice Hd1 protein accumulates during the night in short-day conditions (41), and Hd1 activates expression of *Hd3a* during the night (46). Therefore, the molecular functions of OsCOP1 and OsSPA1 in flowering may differ from those in *Arabidopsis*.

Because of the high similarity of Hd1 to *Arabidopsis* CO, Hd1 is thought to have DNA-binding activity, but direct regulation of *Hd3a* by Hd1 has not yet been reported. The rice *LH8* locus encodes a putative HEME ACTIVATOR PROTEIN 3 (HAP3)/NF-YB subunit that binds to the CCAAT box, a key *cis*-regulatory region in the promoters of several genes (14). In yeast, Hd1 physically interacts with the protein derived from the *LH8* allele associated with late flowering (14). The late-flowering *LH8* alleles have been independently characterized as *Hd5*, *DAYS TO HEADING 8* (*DTH8*), and *Ghd8* (124, 132). When *LH8/Hd5/DTH8/Ghd8* is overexpressed in *Arabidopsis*, the resultant phenotype is similar to that of *Arabidopsis* HAP3/NF-YB (132). *LH8/Hd5/DTH8/Ghd8* activates *Hd3a* expression in short days but suppresses it in long days (132). The *Arabidopsis* HAP/NF-Y proteins facilitate CO binding on the *FT* promoter (10). Therefore, the bifunctionality of *LH8/Hd5/DTH8/Ghd8* resembles that of *Hd1* and suggests that the opposing effects of *LH8/Hd5/DTH8/Ghd8* on *Hd3a* expression in long- and short-day conditions could be caused by a lack of Hd1-NF-Y/HAP complex formation (46, 132). Interestingly, *LH8/Hd5/DTH8/Ghd8* also mediates *Ehd1* expression, which activates *Hd3a* expression independently of *Hd1* (124, 132). This suggests that complex formation with NF-Y/HAP proteins is required for the proper action of two major floral activators, *Hd1* and *Ehd1*. It will be of interest to determine whether Hd1 and LH8/Hd5/DTH8/Ghd8 form a complex in vivo and whether this complex can directly regulate *Hd3a* expression.

## 4.2. Transcriptional Regulation of Rice Florigens via the *Ghd7-Ehd1-Hd3a/RFT1* Pathway

Photoperiodic flowering of rice is also regulated by a rice-specific B-type response regulator, *Ehd1* (22) (**Figure 4*a***). *Ehd1* promotes flowering independently of *Hd1* in short days but also promotes flowering in long days when *Hd1* represses *Hd3a* expression, suggesting that *Ehd1* and *Hd1* determine the degree of florigen expression through distinct pathways under a given photoperiod. The expression pattern of *Ehd1* is regulated by several upstream regulators. *Ehd2* [also known as *Oryza sativa Indeterminate 1* (*OsId1*) and *RICE INDETERMINATE 1* (*RID1*)] and *Ehd4* positively regulate expression of *Ehd1* and its downstream genes under both long- and short-day conditions (28, 76). *OsCO-like 4* (*OsCOL4*) acts as a photoperiod-independent floral repressor by suppressing *Ehd1* expression (63).

*Ghd7* encodes a CCT-domain protein and negatively regulates photoperiodic expression of *Ehd1* (129) (**Figure 4*a***). Lengthening days gradually increase *Ghd7* expression, and this induction requires functional phytochromes (44, 86) (**Figure 4*b***). Modification by phosphorylation may participate in regulation of Ghd7 activity, as *Hd16* encodes CK1 and Ghd7 is a potential target of Hd16 (36). A naturally occurring weak allele of *Hd16* decreases its phosphorylation activity, and plants carrying this weak allele show elevated levels of *Ehd1* and *Hd3a* expression as well as an early-flowering phenotype in long-day conditions, suggesting that kinase activity of Hd16 is required for suppression of *Ehd1* and *Hd3a* in long days (36). Thus, phosphorylation of Ghd7 by Hd16 could increase Ghd7 activity and contribute to preventing flowering in long days. Additional in vivo tests are needed to clarify the function of Hd16 in photoperiodic floral regulation.

The circadian-clock component *OsELF3-1/Hd17/Early flowering 7* (*Ef7*) participates in regulation of rice photoperiodic flowering through *Ehd1* and *Ghd7* (74, 100, 135) (**Figure 4*a***). Disruption of *OsELF3-1/Hd17/Ef7* function causes elevated expression of *Ghd7* in both long and short days, resulting in reduced *Ehd1* and *Hd3a* expression (100, 135). *OsELF3-1/Hd17/Ef7* also negatively affects *OsGI* expression, which is responsible for *Ehd1* expression, and *OsPRR37*, which suppresses expression of *Hd3a* but not that of *RFT1* under long-day conditions (56, 135) (**Figure 4*a***). The effects on flowering are probably indirectly caused by disruption of the circadian clock, but these data suggest that photoperiodic flowering in rice is closely tied to the circadian clock.

Photoperiodic regulation of *Ehd1* and *Ghd7* expression helps to temper the photoperiodic response in rice, and expression of these genes can be explained by the coincidence mechanism of circadian-clock-controlled genes and light signaling through two separate gating mechanisms (44) (**Figure 4*b***). *Ehd1* is highly induced by blue-light pulses around subjective dawn after entrainment under both long- and short-day conditions, and the peak disappears in *osgi* mutants (44). Conversely, *Ghd7* is induced by red-light pulses around dawn in long-day conditions, but this induction shifts to midnight in short-day conditions. The induction takes place at least partially through *OsGI*, as the early peak of *Ghd7* expression is reduced in *osgi* mutants (44). In long-day conditions, the gating of light-sensitive expression of *Ehd1* and *Ghd7* is timed similarly. Thus, red-light-induced *Ghd7* suppresses the blue-light-dependent *Ehd1* induction, leading to the stable suppression of *Hd3a* expression in long days. However, as day length decreases below a critical threshold, the red-light-dependent peak of *Ghd7* inducibility shifts from dawn to night, resulting in reduced *Ghd7* expression in the morning. This allows blue-light-dependent induction of *Ehd1*, which in turn activates *Hd3a* expression in the morning (**Figure 4*b***).

Red-light-dependent induction of *Ghd7* requires functional phytochrome (44), but the blue-light receptor responsible for *Ehd1* induction is still unknown. Moreover, even though *OsGI* sets the blue-light-inducible gate around dawn for *Ehd1*, *OsGI* expression reaches trough level at dawn, suggesting that *OsGI* controls other components responsible for blue-light induction of *Ehd1*.

Characterization of the molecular components that control the light-dependent inducibility of *Ehd1* and *Ghd7* is necessary to improve our understanding of photoperiodic regulation of flowering in rice.

### 4.3. Photoperiodic Flowering Mechanism in Rice in Long Days

Although rice is considered a short-day species, it can be induced to flower in long days through the induction of *RFT1* by *Ehd1* (22, 54, 55). *OsMADS50*, a homolog of *Arabidopsis SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), positively regulates expression of *Ehd1*, *Hd3a*, and *RFT1* by suppressing their upstream negative regulator *OsLEC2 and FUSCA 3–like 1* (*OsLFL1*), which induces flowering in long-day conditions (97) (**Figure 4*a***). OsLFL1, a putative B3 DNA-binding-domain-containing transcription factor, physically associates with RY motifs in the promoter of *Ehd1* to repress its transcription (87, 88).

Floral induction of rice in long-day conditions is also under the control of histone modification at the level of *Ehd1* and *RFT1* as well as their upstream regulators. These modifications act both to induce and to repress flowering. *Long vegetative phase 1* (*Lvp1*)/*SET DOMAIN GROUP PROTEIN 724* (*SDG724*) enhances H3K36 methylation (H3K36me) of *Ehd1* and *RFT1* to promote flowering in long days (114). *Oryza sativa* VERNALIZATION INSENSITIVE 3–LIKE 2 (OsVIL2) forms a complex with OsEMF2b, a component of Polycomb repressive complex 2, to associate physically with the *OsLFL1* promoter, increasing repressive histone marks (H3K27me3) and suppressing its expression (133). Two plant-homeodomain-containing proteins, *Oryza sativa Trithorax* (*OsTrx1*)/*SDG723* and *Ehd3*, activate *Ehd1* expression by suppressing *Ghd7* in long days (15, 75). OsTrx1 binds to histone H3 and has methyltransferase activity. OsTrx1 forms a heterodimer with Ehd3 through its plant homeodomain to regulate *Ghd7* expression negatively (15), inducing *Ehd1* expression (**Figure 4*a***).

The *Se14* locus encodes a Jumonji C (JmjC)-domain-containing protein that functions as a histone demethylase and participates in long-day-dependent flowering (134). Mutation of *Se14* causes activation of *RFT1* and early flowering in long-day conditions. *Se14* shows high similarity to *Arabidopsis ELF6*, which represses *FT* expression by reducing the level of H3K4me on *FT* (48). Consistent with ELF6 function in *Arabidopsis*, mutation of *Se14* increases the level of H3K4me on the *RFT1* chromatin. Thus, *Se14* regulates photoperiod-dependent flowering by reducing the level of H3K4me on *RFT1* chromatin to repress its expression in long-day conditions. Taken together, the above data indicate that rice possesses numerous genetic pathways to affect the long-day flowering response both positively and negatively.

## 5. CONCLUDING REMARKS

Our knowledge about photoperiodic flowering mechanisms in *Arabidopsis* has greatly facilitated our understanding of these mechanisms in major crops (wheat, barley, and rice). Thus, *Arabidopsis* research plays an instrumental role in the photoperiodic flowering field. In addition, it is critical to study mechanisms in plants that are highly valued in agriculture and horticulture. In these species, mechanisms both similar to and different from those in *Arabidopsis* have been discovered, shedding light on the numerous modes that plants have adopted to ensure developmental progress.

In the photoperiodic flowering pathway in *Arabidopsis*, multiple coherent type-1 feedforward loops (C1-FFLs) (1) exist in blue-light signaling mediated by FKF1 and CRY2 to control *FT* expression. FKF1 directly stabilizes CO protein and simultaneously removes CDFs that repress *CO* and *FT* transcription (26, 39, 103, 112). CRY2 indirectly stabilizes CO by directly repressing the activity of the COP1-SPA1 complex, and activates CIB *FT* activators as well (66, 67, 93, 95,

112, 121, 137). The C1-FFL structural module functions as a persistence detector, which means only a persistent signal (e.g., duration of light), not spontaneous or sporadic signals, can induce the response (1). Because plants must ensure that the day is long enough to initiate flowering, it is logical that plants have regulatory modules that respond only to steady elongation of the light period to induce flowering. In addition, current results indicate that FKF1 acts as the photoperiod sensor. Having a photoreceptor that is expressed only in the afternoon enables plants to monitor light conditions at the end of the day. Thus, FKF1 nicely fits with the characteristics of both the enzyme and substrate in the external coincidence model (**Figure 1*b***). Recent analysis revealed that the liverwort *Marchantia polymorpha* uses an FKF1 ortholog to regulate photoperiodic gametophore formation, leading to reproduction (57). This analysis suggests that the role of FKF1 as a photoperiodic sensor was acquired in an early lineage of land plants.

As discussed in Sections 3 and 4, phytochromes are the major photoreceptors that regulate photoperiodic flowering in many plants. In *Arabidopsis*, both PHYA and PHYB are involved in regulating CO stability and *FT* expression. However, the mechanism by which phytochromes regulate flowering is still underexplored. As with blue-light signaling, we assume that feedforward or feedback loops are involved in phytochrome signaling for flowering. As the contributions of phytochrome to photoperiodic flowering have long been known, gaining an understanding of the molecular mode of phytochrome action is of great interest.

In the study of crop photoperiodism, many interesting questions linger. Gating through control by the circadian clock and light-signal perception has been described in detail in rice, consistent with the external coincidence model (44), but much less is known about parallel mechanisms in wheat and barley. The *PPD1* gene does fluctuate under constant light, indicating the involvement of the circadian clock (13); however, its expression is abolished in the dark, and it is expressed throughout the light period regardless of day length (13, 106). It seems, then, that activation of PPD1 via PHYC could be sensitive to light throughout the day. *CO1* expression does peak at a consistent time in both long and short days (13, 106), aligning with the concept of external coincidence, and it is possible that CO1 facilitates day-length perception in photoperiod-sensitive varieties of wheat and barley. However, how PPD1 and CO1 converge to regulate *FT* expression is still unknown.

Although rice is classified as a short-day plant, it possesses the *Ghd7-Ehd1-Hd3a/RFT1* pathway, which enables flowering responses under various day-length conditions. Investigation of whether this pathway is conserved in other plants, or whether it is unique in rice, is of great interest. In another short-day plant, morning glory, the phases of circadian oscillation of two *FT* orthologs are reset by the light-to-dark transition that occurs at dusk (33). Is this characteristic of the circadian clock conserved in rice or other short-day plants? It is not surprising that each species has developed unique strategies in order to adapt to specific niches. Studying photoperiodic flowering mechanisms in a range of plants enables us to determine which mechanisms are conserved and which are individual among plant species.

Finally, as a cautionary note, sequence variations that impact photoperiodic sensitivity and flowering time may have independent effects on yield components such as grain filling and tiller production (128). It is necessary to assess the broader physiological impacts of alleles that display desirable photoperiodic responses.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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