

Photoreceptors and Regulation of Flowering Time¹

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One of the most important environmental factors affecting flowering time is the daily duration of light, the photoperiod, which was first discovered by Garner and Allard in the 1920s (Thomas and Vince-Prue, 1997, and refs. therein). Plants in which flowering occurs or is accelerated in short days (SD) or long days (LD) are known as SD plants or LD plants, respectively. LD plants often flower in later spring or early summer (when the daylength becomes longer) to set seeds in a favorable season. SD plants generally flower in fall (when photoperiods are getting shorter) to finish reproduction before the cold winter arrives. Synchronization of flowering time with a reliable environmental cue such as the photoperiod also increases the chance of out-breeding and genetic recombination. The photoperiodic control of flowering is brought about by the interactions of genes involved in the developmental control of floral initiation, the regulation of the circadian clock, and the signal transduction of photoreceptors (Thomas and Vince-Prue, 1997). Recent molecular genetic studies in a facultative LD plant, *Arabidopsis*, have made notable progress in identifying genetic pathways and molecular components associated with the control of flowering time and the function of the circadian clock, which have been discussed in two recent *Updates* (Pineiro and Coupland, 1998; Somers, 1999). This *Update* focuses on the recent advances in our understanding of plant photoreceptors phytochromes and cryptochromes, and their roles in the regulation of flowering time.

CONTROL OF PLANT FLOWERING TIME

Genetic Pathways Control Flowering Time

Flower formation is initiated by the transition of the apical meristem from a vegetative fate to a floral fate. Mechanisms that control the timing of floral initiation have been extensively studied in *Arabidopsis* by the identification of mutations that flower earlier or later than the wild type but otherwise remain

healthy (Koornneef et al., 1998). These mutations are known as flowering-time mutations and the corresponding genes are known as flowering-time genes. In addition, many genes that were initially studied for their roles in other aspects of plant development, such as light perception, hormone metabolism, signal transduction, and floral meristem specification, also play roles in the regulation of flowering time and are sometimes also referred to as flowering-time genes. Based on phenotypic and genetic epistasis analysis of these mutations, flowering-time genes have been grouped into several signal transduction pathways that either suppress or promote floral initiation. These signaling pathways transmit either the developmental or environmental signals to regulate the expression of the floral-meristem-identity genes that control the formation of the floral meristem. Readers are referred to two recent reviews for detailed discussions of genes associated with these pathways in *Arabidopsis* (Koornneef et al., 1998; Levy and Dean, 1998).

Genes of the Photoperiodic Pathway

One of the major signal transduction pathways regulating flowering time is known as either the LD promotion pathway (Koornneef et al., 1998) or the photoperiodic pathway (Levy and Dean, 1998), which relays light and photoperiodic timing signals to the floral initiation process. Mutations of genes in this pathway reduce a plant's responsiveness to photoperiods. As a facultative LD plant, *Arabidopsis* grown in LD conditions flowers earlier than when grown within SD. Misexpression of genes associated with the LD pathway may also delay the flowering of *Arabidopsis* plants grown in LD, but does not alter the flowering time of plants grown in SD, resulting in reduced sensitivity (hyposensitive) to photoperiod. Mutations in genes such as *CO* (*CONSTANS*; Putterill et al., 1995), *PHYA* (phytochrome *A*; Johnson et al., 1994; Reed et al., 1994), *CRY2* (cryptochrome 2; Guo et al., 1998), and *GI* (*GIGANTEA*; Fowler et al., 1999; Park et al., 1999), are of this type. The elevated expression of the *CCA1* (circadian clock associated; Wang and Tobin, 1998) and *LHY* (late elongated hypocotyl; Schaffer et al., 1998) genes also results in photoperiod-hyposensitive late-flowering.

On the other hand, a mutant that flowers earlier than the wild type in both LD and SD may also have reduced sensitivity to photoperiod. Early-flowering

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mutations in genes such as *PHYB* (phytochrome B; Goto et al., 1991), *PHYD* (phytochrome D; Aukerman et al., 1997; Devlin et al., 1999b), *PHYE* (phytochrome E; Devlin et al., 1999a), *ELF3* (early flowering; Hicks et al., 1996; Zagotta et al., 1996), and *PEF* (phytochrome early flowering; Ahmad and Cashmore, 1996) belong to this group. We often assume that a late-flowering mutation corresponds to a gene product that normally promotes floral initiation, whereas an early-flowering mutant implies that the corresponding gene product is a suppressor of floral initiation. Not surprisingly, many genes isolated to date that are associated with the photoperiodic pathway encode either photoreceptors or proteins associated with the circadian clock.

PLANT PHOTORECEPTORS

The primary photosensory receptors of higher plants are the red/far-red light receptors called phytochromes and the blue/UV-A light receptors called cryptochromes (Kendrick and Kronenberg, 1994). Blue light (approximately 400–500 nm) and red light (approximately 600–700 nm) are the two spectra of solar radiation that are most effectively absorbed and utilized by the photosynthetic system of plants. Therefore, the regulation of plant development by phytochromes and cryptochromes allows plants to optimize their developmental processes in coordination with the availability of energy and metabolite resources.

Phytochromes

Phytochromes are photochromic proteins that exist as two photo-interconvertible isomeric forms: the red-light-absorbing form (Pr) and the far-red-light-absorbing form (Pfr; Kendrick and Kronenberg, 1994; Hughes, 1999). *Arabidopsis* has five phytochrome genes, *PHYA* to *PHYE*, which encode the apoproteins of *PHYA* to *PHYE*, respectively (Quail et al., 1995). Mutations in four of the *Arabidopsis* phytochrome genes have been isolated and studied (see below). Different phytochromes regulate either distinct light responses or similar responses under different light conditions (light quantity, quality, and timing). Taking the well-characterized light-inhibition of hypocotyl elongation as an example (Quail et al., 1995), the *phyA* mutant is impaired in hypocotyl inhibition in far-red light, but not in red light. Conversely, the *phyB* mutant loses the ability to inhibit hypocotyl elongation in red light, but not in far-red light, suggesting that although *phyA* and *phyB* both mediate light inhibition of hypocotyl elongation, *phyA* functions primarily in far-red light, whereas *phyB* acts mainly in red light.

Cryptochromes

Cryptochromes are flavoproteins that share amino acid sequence similarity with DNA photolyases that catalyze blue/UV-A light-dependent DNA repairing (Sancar, 1994; Cashmore et al., 1999). Cryptochromes have no DNA photolyase activity; they usually have a C-terminal domain with little sequence homology to photolyase and they show characteristics of blue/UV-A light receptors in plants. *Arabidopsis* has at least two cryptochrome genes, *CRY1* and *CRY2*. Similar to phytochromes, genetic studies of *Arabidopsis* cryptochrome mutations affecting light-dependent hypocotyl inhibition have played a critical role in our understanding of cryptochromes. The isolation of an *Arabidopsis* mutant, *hy4*, which has an elongated hypocotyl in blue light, allowed the cloning of the first cryptochrome gene (Koornneef et al., 1980; Ahmad and Cashmore, 1993).

The *HY4* gene, later referred to as *CRY1*, encodes a protein associated with a flavin chromophore (FAD) that absorbs blue/UV-A light, as was previously suspected for a plant blue/UV-A light receptor (Lin et al., 1995b). A plant's sensitivity to blue/UV-A light can be altered by changing the expression levels of *cry1* (Lin et al., 1995a). The second *Arabidopsis* cryptochrome gene, *CRY2*, was cloned using *CRY1* cDNA as the hybridization probe (Lin et al., 1998). The amino acid sequence of *CRY2* and *CRY1* are about 50% identical, but most of the sequence similarity is concentrated in the N-terminal photolyase-like domain, whereas the C-terminal domains are quite diverged (Lin et al., 1998). Interestingly, *cry2* protein is rapidly degraded in etiolated seedlings exposed to blue light (Lin et al., 1998; Guo et al., 1999), which is reminiscent of the red-light-induced degradation of *phyA* (Clough et al., 1999, and refs. therein). It is not clear what functional role the light-induced proteolysis of *phyA* and *cry2* may play, but no diurnal change in the protein expression levels has been reported for *cry2*.

Based on the observation that transgenic plants overexpressing *CRY2* were hypersensitive to blue light, a genetic screen was designed to look for additional *Arabidopsis* mutants exhibiting a long hypocotyl in blue light (Guo et al., 1998; Lin et al., 1998). Surprisingly, the resulting *cry2* mutants derived from this screen showed a more apparent abnormality in flowering time than in hypocotyl inhibition and turned out to be allelic to *fha*, a photoperiod-hyposensitive late-flowering mutation previously characterized by Koornneef (1991; Guo et al., 1998).

Since the isolation of the *Arabidopsis* *CRYs*, cryptochrome genes have been isolated from not only other plant species and algae, but also animals including fruit fly, mouse, and human (Cashmore et al., 1999, and refs. therein). Studies of mouse and fruit fly cryptochromes have indicated that these proteins play important roles in the function and regulation of

animal circadian clocks (Thresher et al., 1998; Ceriani et al., 1999).

HOW PHOTORECEPTORS WORK

How do photoreceptors convey light signals to affect cellular processes? What are the early steps of photoreceptor signal transduction? A photoreceptor may relay light signals to other molecules by a light-dependent enzymatic activity, or it may do so by changing its conformation and thus its interaction with signaling partners. It appears that at least for phytochromes, the early steps of the signal transduction involve both types of reactions: phytochromes are protein kinases and can interact with signal transducing proteins in a light-dependent manner.

Phytochrome Kinases and a Phytochrome-Regulated Kinase

Plant phytochromes were first proposed to be protein kinases more than a decade ago (Wong et al., 1986), but this has remained a controversial proposition until recently. In light of concurring evidence, the phytochrome kinase hypothesis has gradually gained general acceptance (Elich and Chory, 1997; Cashmore, 1998; Yeh and Lagarias, 1998; Fankhauser and Chory, 1999; Hughes, 1999).

A pivotal question that has arisen from this decade-long debate is the identification of the bona fide substrates of the phytochrome kinases concerning photo-signal transduction in flowering plants. Two recent reports have directly addressed this question (Ahmad et al., 1998; Fankhauser et al., 1999). One contender for the substrate of phytochrome kinases turned out to be the newly discovered cryptochrome. It was reported that cryptochromes can interact with phyA *in vitro*, and in the yeast two-hybrid assay, the recombinant CRY1 could be phosphorylated *in vitro* by recombinant oat phyA protein. Also, the *in vitro* phosphorylation of cry1 by phyA was more efficient in red light or blue light than in the dark (Ahmad et al., 1998). The phosphorylation of cry1 was also found to occur *in vivo* in a red-light-dependent and far-red-light-reversible manner, which again suggested the involvement of a phytochrome. However, the physiological relevance of phytochrome-dependent phosphorylation of cry1 remains unclear.

Another possible substrate for phytochrome kinase is PKS1 (phytochrome kinase substrate 1; Fankhauser et al., 1999). The gene encoding PKS1 was isolated from a yeast two-hybrid screen using the C-terminal domain of Arabidopsis PHYA as the "bait." PKS1 can be phosphorylated *in vitro* by the recombinant oat phyA. Although PKS1 binds to both the Pr and Pfr forms of phyA, Pfr is a more active kinase than Pr in the phosphorylation of PKS1. In keeping with PKS1 being a substrate of phytochromes, PKS1 was phosphorylated *in vivo* in a red-light-dependent manner.

The observation that PKS1 was hyperphosphorylated in transgenic plants overexpressing phyB suggested an involvement of phyB in the phosphorylation of PKS1 *in vivo*. PKS1 played a negative role in phyB signaling, because transgenic plants overexpressing PKS1 showed the phenotype similar to that of a *phyB* mutant: transgenic plants overexpressing PKS1 had elongated hypocotyls in red light but not in blue or far-red light (Fankhauser et al., 1999).

Phytochrome may also regulate the activity of other protein kinases. For example, a recently identified phyA-interacting protein, nucleotide diphosphate kinase 2 (NDPK2), appears to be such an enzyme (Choi et al., 1999). In an *in vitro* binding assay, the Pfr form of phyA could bind to NDPK2 about three to four times better than the Pr form. The binding of Pfr (but not Pr) to NDPK2 increased the substrate affinity of this kinase in an *in vitro* NDPK2 enzymatic assay. NDPK2, localized in both the cytosol and nucleus, may play a positive role in phytochrome signal transduction. An Arabidopsis mutant with the *NDPK2* gene interrupted by a T-DNA insertion showed decreased sensitivity to both red light and far-red light in cotyledon opening and greening (Choi et al., 1999).

Plant Photoreceptors Can Enter the Nucleus and Interact with Nuclear Proteins

Where do photoreceptors work in the cell? Phytochromes and cryptochromes are both soluble proteins, and it seems clear now that both types of photoreceptors can enter the nucleus, either constitutively or in a light-dependent manner. The intracellular localization of phytochromes and cryptochromes has been studied using fusion protein assays. In these studies, a transgene encoding a fusion protein of a photoreceptor and a marker enzyme such as β -glucuronidase or green fluorescence protein is expressed in plants, and the intracellular localization of the photoreceptor is identified by monitoring the location of the visible marker enzyme. These studies demonstrated that phyA and phyB stay mostly in the cytosol in the dark, but are translocated to the nucleus in the light (Sakamoto and Nagatani, 1996; Kircher et al., 1999; Kleiner et al., 1999). Arabidopsis cry1 and cry2 are also nuclear proteins, although no light regulation of the nuclear transportation of cryptochromes has been reported (Cashmore et al., 1999; Guo et al., 1999; Kleiner et al., 1999).

Differential nuclear compartmentation is commonly found for receptor molecules in eukaryotes (Adam, 1999). In the absence of a ligand, a receptor can be bound to a cytosolic protein and thus be retained in the cytosol. Interaction with the ligand may induce the translocation of the receptor to the nucleus. A similar sport may also be played by plant photoreceptors. For example, it has been suggested that PKS1 may act as a cytosolic-retention protein for

phytochromes in non-inductive conditions such as the dark (Fankhauser et al., 1999; Smith, 1999). Light induces the nuclear compartmentation of the phytochrome. Once in the nucleus, a photoreceptor may interact with other nuclear proteins to affect light-regulated gene expression (Smith, 1999).

Recently, two phytochrome-signaling nuclear proteins, SPA1 and PIF3, have been identified. The SPA1 gene was identified by positional cloning of the *spa1* (suppressor of *phyA-105*) mutation that was isolated as an allele-specific suppressor of a *phyA* mutation (Hoecker et al., 1998, 1999). SPA1 is a nuclear protein that has WD repeats, a coil-coil domain, and a protein kinase domain (Hoecker et al., 1999). *spa1* mutant plants exhibited an exaggerated hypocotyl inhibition in response to light, suggesting that SPA1 is a negative regulator of *phyA*. The expression of SPA1 was up-regulated in light through the action of phytochromes, indicating a possible feedback regulation on the phytochrome signal transduction.

The gene encoding another phytochrome-signaling nuclear protein, PIF3 (phytochrome interacting factor), was isolated on the basis of its interaction with the C-terminal domain of PHYB in a yeast two-hybrid assay (Ni et al., 1998). PIF3 contains a PAS protein-protein interaction domain and a basic helix-loop-helix (bHLH) domain that may have a role in the interaction with promoters of light-regulated genes. The *in vitro* interaction between PIF3 and phytochromes was dependent on red light: PIF3 interacted strongly with the Pfr form, but only weakly with the Pr form of Arabidopsis *phyB* (Ni et al., 1999). In contrast to SPA1, the expression of PIF3 was down-regulated in light (Halliday et al., 1999). PIF3 is a positive regulator of phytochrome function, because PIF3-antisense transgenic plants showed a reduced hypocotyl inhibition in response to light. Consistent with the hypothesis that nuclear compartmentation and binding of phytochrome to a nuclear protein such as PIF3 may be part of the signal transduction leading to the regulation of gene expression, PIF3-antisense transgenic plants exhibited reduced light responsiveness for expression of various light-regulated genes (Ni et al., 1998).

It is interesting that although every Arabidopsis photoreceptor studied to date has been shown to play a role in both light-regulated hypocotyl inhibition and floral initiation, most mutations or transgenic plants misexpressing the phytochrome-signaling genes described above, except *PIF3*, have no reported alteration in flowering time. Since some of these phytochrome-signaling factors bind to phytochrome, it may be argued that there are two separate phytochrome signal transduction pathways leading to the two different developmental responses. However, some of the flowering-time genes associated with the photoperiodic pathway (e.g. *ELF3*, *CCA1*, *LHY*, *COPI*, and *DET1*) have functions in both hypocotyl inhibition and flowering time (Levy and Dean, 1998, and refs. therein). These obser-

vations may not be satisfactorily explained by models involving linear signal transduction pathways of photoreceptors, even though we frequently use the term "pathway" in this *Update*.

HOW INDIVIDUAL PHOTORECEPTORS REGULATE FLOWERING TIME

In searching for photoreceptors regulating photoperiodic responses, action spectra have been extensively analyzed in different plants to investigate how light qualities affect flowering time. These early studies, along with observations of the effect of light on germination and stem elongation, led to the discovery of phytochrome (Thomas and Vince-Prue, 1997, and refs. therein). The more recent studies of photoreceptor mutations have allowed us to assign specific functions of individual photoreceptors in the regulation of flowering time.

Effect of Light Quality on Arabidopsis Flowering Time

Two of the most frequently used experimental approaches to analyze the action spectra of light regulation of flowering time are the day extension and the night break methods (Thomas and Vince-Prue, 1997). In a day extension experiment, the SD photoperiod is extended by applying a low-fluence-rate light at the end of the main photoperiod. For a night break experiment, the additional light exposure is often inserted in the middle of a long night. Both conditions mimic the LD photoperiod that promotes flowering in LD plants, and are referred to as "quasi LD" in the following discussion. In Arabidopsis, far-red light, blue light, and red light were all effective at promoting flowering in night break experiments, although red light was the least effective (Goto et al., 1991; Carre, 1998). Day extensions with far-red light or light rich in far-red spectra (e.g. incandescent light) are also very effective in promoting flowering (Goto et al., 1991; Bagnall et al., 1995).

Continuous illumination with light of different wavelengths is another method used to investigate how different photoreceptors regulate floral initiation. Although the night-break and day-extension methods can more effectively minimize the interference of photosynthesis than the continuous-light methods, the latter condition can simplify the situation by eliminating light/dark cycles (and the influence of the circadian clock) to allow an assessment of the direct effect of photoreceptors on floral initiation. Arabidopsis plants grown under continuous light with a high red- to far-red-light ratio (poor in far-red light) flower later than plants grown in light of a low red- to far-red-light ratio (i.e. rich in far-red light; Halliday et al., 1994). Moreover, plants grown in continuous red light flower significantly later than those grown in continuous blue light (Guo et al., 1998; Fig. 1). Therefore, the rule of thumb seems to be

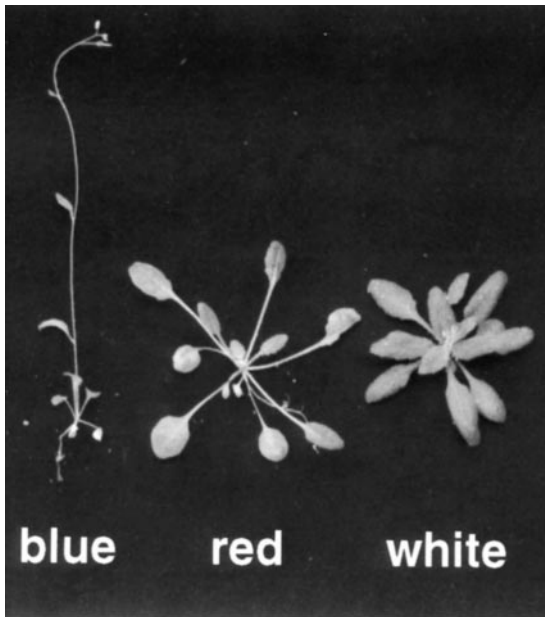


Figure 1. Effect of red and blue light on Arabidopsis flowering time. The 21-d-old Arabidopsis (ecotype Columbia) plants shown were imbibed at 4°C for 4 d in the dark and then grown under continuous blue, red, or white light (all approximately 100 $\mu\text{mol s}^{-1} \text{m}^{-2}$). The plant grown in blue light had flowered for 6 d, that in white light for 1 d, and the plant grown in red light was not flowering (it was about 20 d before flowering).

that, at least for Arabidopsis, far-red light and blue light promote flowering, whereas red light is often inhibitory.

phyA

phyA promotes flowering. The Arabidopsis *phyA* mutant flowers later than wild-type plants in LD (Johnson et al., 1994; Neff and Chory, 1998) or quasi-LD conditions with either night breaks (Reed et al., 1994) or day extensions (Johnson et al., 1994; Neff and Chory, 1998). Consistent with the notion that phyA plays a promotive role in flowering, transgenic Arabidopsis plants overexpressing phyA flowered earlier than the wild type in both SD and quasi-LD conditions (Bagnall et al., 1995). Both *phyA* mutant plants and *phyA*-overexpressing transgenic plants had decreased sensitivity to photoperiod, because they flowered at about the same time in SD as in the quasi-LD conditions. The *phyA* mutant of pea, another LD plant, also showed a phenotype similar to that of the Arabidopsis *phyA* mutant. The pea *phyA* mutant (*fun1*) flowered normally in SD photoperiods but failed to respond to day-extension treatments with incandescent light; therefore, the pea *phyA* mutant flowered at about the same time in SD and quasi-LD conditions (Weller et al., 1997). Interestingly, the pea *phyA* mutant accumulated a graft-transmissible inhibitor that could delay the flowering of the grafting recipient plants, suggesting that phyA

signaling may suppress the biosynthesis of a floral suppressor (Weller et al., 1997).

phyB

phyB plays an inhibitory role in floral initiation. The Arabidopsis *phyB* mutant flowered earlier than the wild type in both LD and SD conditions, but the early-flowering phenotype of the *phyB* mutant is more pronounced in SD than in LD conditions (Goto et al., 1991; Mockler et al., 1999). *phyB* mutations of pea (*Iv-1*; Weller and Reid, 1993), and sorghum (*Ma₃^R*; Pao and Morgan, 1986; Childs et al., 1997) showed early-flowering and decreased photoperiodic sensitivities. More interestingly, in contrast to the *phyB* mutant of the LD plant pea that flowered early in SD but not in LD, the *phyB* mutant of the SD plant sorghum flowered early in LD but not in SD (Pao and Morgan, 1986). Therefore, phyB inhibits floral initiation in both LD plants and SD plants, but the phyB inhibition of flowering appears more apparent in the photoperiod that normally suppresses flowering in the respective plant. However, the function of phyB in floral initiation may be more complex than simply as a floral inhibitor (such as that shown in the model in Fig. 2). For example, transgenic Arabidopsis plants overexpressing phyB also flowered earlier than the wild type, which could not be easily explained (Bagnall et al., 1995).

phyD

An Arabidopsis *phyD* mutation was identified as a naturally occurring allele of the wild-type Wasilewskija ecotype, which encoded no functional phyD protein (Aukerman et al., 1997). This *phyD* mutant allele was introgressed into various genetic backgrounds and used to study phyD function. The monogenic *phyD* mutant plants had no obvious phenotypic abnormality, whereas plants impaired in both the *PHYB* and the *PHYD* genes flowered earlier than the *phyB* monogenic mutation in both LD and

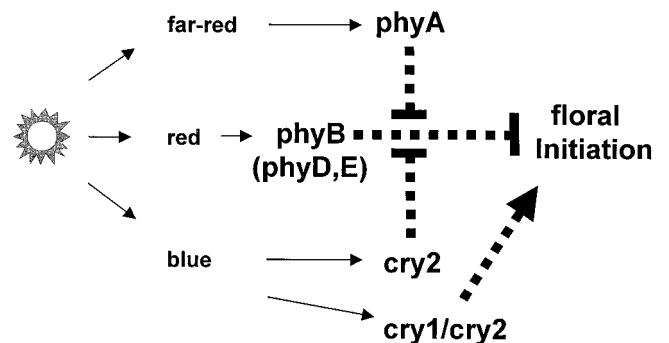


Figure 2. Antagonistic or redundant functions of photoreceptors in regulating floral initiation of Arabidopsis. The broken lines represent signal transduction pathways of photoreceptors; arrows denote the positive effects; and lines terminated with a bar denote inhibitory effects.

SD conditions (Aukerman et al., 1997; Devlin et al., 1999b). This indicated that, like *phyB*, *phyD* inhibits flowering. The triple mutant *phyAphyBphyD* still retained the ability to respond to the end-of-day far-red light treatment by developing elongated rosette internodes and accelerated flowering—the responses collectively known as the “shade avoidance syndrome” (Smith and Whitelam, 1997; Devlin et al., 1999a; Morelli, 1999). Accelerated flowering under shade, in which there is more far-red light, may allow plants to complete their life cycle before the canopy of other plants becomes too dense. It has been hypothesized that *Arabidopsis* has at least one other phytochrome associated with shade avoidance responses (Devlin et al., 1999b).

phyE

Based on the hypothesis that *Arabidopsis* has another phytochrome associated with shade avoidance responses (Devlin et al., 1999b), a genetic screen was carried out to look for mutations that exhibited elongated rosette internodes, resulting in the isolation of the *phyE* mutation (Devlin et al., 1999a). The *phyE* mutant showed no phenotypic alteration unless it was in the *phyB* mutant background. This indicated the function of *phyE* is also similar to that of *phyB*. Among other phenotypes, the *phyBphyE* double mutant flowered much earlier than the *phyB* monogenic mutant in SD conditions (Devlin et al., 1999a). In SD conditions, plants containing mutations in both the *PHYB* and *PHYE* genes flowered so early that the end-of-day far-red-light treatment no longer caused further acceleration of flowering (Devlin et al., 1999a). It appears that *phyB* and *phyE* normally inhibit flowering in a redundant manner, but their effects can be suppressed by an end-of-day far-red-light treatment. Plants containing mutations in both *PHYB* and *PHYE* genes have reduced suppression of floral initiation, so that they flower early with or without the end-of-day far-red-light treatment.

The two *Arabidopsis* mutants impaired in phytochrome chromophore biosynthesis, *hy1* and *hy2*, flowered earlier than the wild type in both LD and SD conditions (Goto et al., 1991). Because the deficiency of phytochrome-chromophore synthesis is likely to indiscriminately affect all phytochromes, it may be expected that the collective outcome of the actions of individual phytochromes would be largely inhibitory with respect to the floral initiation of *Arabidopsis*. The fact that the majority of *Arabidopsis* phytochromes play inhibitory roles in flowering appears to be consistent with this view.

cry1

The function of *cry1* in flowering seems complicated, although it may have a promotive effect. The *hy4* mutant (in the Landsberg *erecta* ecotype back-

ground) was shown to flower late under SD conditions (Mozley and Thomas, 1995). It was also reported that *hy4* mutant alleles in the Columbia ecotype background flowered late in both SD and quasi-LD conditions with either day extensions or night breaks, and that night breaks with blue light had a stronger effect than night breaks with white light or red light (Bagnall et al., 1996). However, in contrast to other photoreceptors, there is a great deal of inconsistency in the flowering time of the *cry1* mutant (Goto et al., 1991; Mozley and Thomas, 1995; Bagnall et al., 1996; Zagotta et al., 1996; Mockler et al., 1999). Some of these inconsistencies may be explained by allele-specific effects. For example, contrary to other *cry1* mutant alleles, *hy4-3* (in the wild-type Wassilewskija ecotype background) and *hy4-6* (in the Columbia ecotype background) flowered earlier than the wild type in SD conditions, which was interpreted as being the result of the direct interaction between *phyB* and the mutant CRY1 protein (Ahmad et al., 1998). However, the inconsistency in flowering time can also be found in reports concerning the identical *cry1* mutant allele (Bagnall et al., 1996; Zagotta et al., 1996; Mockler et al., 1999). The mode of action of *cry1* in floral initiation remains unclear.

cry2

cry2 promotes flowering. *cry2* mutants are allelic to the photoperiod-hyposensitive late-flowering *fla* mutant, although the *cry2* alleles (in the Columbia ecotype background) had stronger phenotype than the *fla* alleles (in the Landsberg *erecta* background; Guo et al., 1998; Koornneef et al., 1998). *cry2* mutant plants flowered late in LD but not in SD conditions, transgenic plants overexpressing *cry2* flowered early in SD but not in LD conditions. Therefore, either a mutation or an overexpression of the CRY2 gene resulted in the reduced sensitivity to photoperiods.

Since blue light is known to promote flowering of *Arabidopsis*, one may expect that the *cry2* mutant, which is impaired in a blue light receptor, would show a delayed flowering in blue light. Surprisingly, this was not the case. The *cry2* mutant flowered at the same time as the wild type in continuous blue light or red light, but the late-flowering phenotype of *cry2* in white light could be phenocopied in blue-plus-red light (Guo et al., 1998; Mockler et al., 1999). Therefore, the flowering promotion function of *cry2* is dependent on both blue and red light.

Teamwork of Photoreceptors

Why is the late-flowering phenotype of the *cry2* mutation only revealed in the presence of both blue light and red light? In other words, why does the function of a blue light receptor, *cry2*, require red light? It was proposed that a cryptochrome may need

to be phosphorylated by a phytochrome in red light to become fully active (Ahmad et al., 1998). However, this model may not explain how *cry2* regulates flowering time, because genetic studies have demonstrated that phytochromes and *cry2* often had opposite effects on floral initiation. Furthermore, the function of *cry2* in flowering appeared to require the simultaneous presence of red and blue light, implying that either the red-light-activated *cry2* is extremely short-lived, or that red light is not directly required for the biochemical activity of *cry2* (Mockler et al., 1999). The latter scenario, as depicted in the double-negative model in Figure 2, predicts that *cry2* promotes flowering through its suppression of the phyB-mediated red-light inhibition of floral initiation (Guo et al., 1998; Mockler et al., 1999). Indeed, *phyB* mutant plants showed a much more pronounced early-flowering phenotype in continuous red light than in continuous white light or continuous red-plus-blue light.

It appears that, similar to its function in hypocotyl elongation, the inhibitory action of phyB in floral initiation is also dependent on red light (Guo et al., 1998). Consistent with the hypothesis that phyB inhibits flowering whereas *cry2* inhibits phyB action, a *phyBcry2* double mutant flowered as early as the *phyB* mutant in red-plus-blue light. The *phyB* mutation did not completely suppress the *cry2* mutant phenotype in white light (Mockler et al., 1999), which may be explained by the redundant function of phyD and phyE. However, whether phyD- or phyE-mediated inhibition of floral initiation is dependent on red light remains to be investigated.

An antagonistic interaction may also exist between phyB/D/E and phyA—another photoreceptor known to promote flowering. *phyAphyB* double mutant plants flowered earlier than the *phyA* mutant, and in certain conditions, the double mutant flowered almost as early as the *phyB* monogenic mutant (Reed et al., 1994; Devlin et al., 1996; Neff and Chory, 1998). These observations indicate that phyA may also inhibit the function of phyB, and possibly phyD and phyE as well. Because the *phyA* mutant flowered late in response to a day extension with incandescent light rich in far-red spectra (Johnson et al., 1994), it is tempting to speculate that, analogous to the antagonism between *cry2* and phyB and to the far-red-light-dependent phyA function in hypocotyl inhibition, phyA may mediate a far-red-light-dependent inhibition of the phyB function (Fig. 2). One test of this hypothesis would be to compare the flowering time of the Arabidopsis wild type with *phyA* or *phyAphyB* mutants grown in red-plus-far-red light or in far-red light under conditions allowing plants to flower in the absence of photosynthesis (Araki and Komeda, 1993).

In addition to antagonistic actions, photoreceptors can work in redundant ways to regulate floral initiation. As described previously, phyB, phyD, and phyE inhibit flowering in a redundant manner. Sim-

ilar redundancy has also been found for *cry1* and *cry2*. The *cry1/cry2* double mutant flowered late in continuous blue light, although neither the monogenic *cry1* or *cry2* mutant exhibited delayed flowering in such conditions (Mockler et al., 1999). This observation was interpreted to mean that *cry2*, in addition to its antagonism to phyB, also mediates a blue-light-dependent promotion of floral initiation, but the latter action of *cry2* is redundantly carried out by *cry1* (Mockler et al., 1999; Fig. 2).

Photoreceptors Regulate the Expression of Flowering-Time Genes

How does the action of a photoreceptor affect floral initiation? Given that both phytochrome and cryptochrome can enter the nucleus, there is the possibility that photoreceptors regulate expression of flowering-time genes without invoking second messages for signal transduction. It has been shown that some flowering-time genes are differentially expressed in different photoperiods. For example, the activity of the *LEAFY* promoter was more quickly up-regulated in LD than in SD conditions (Blazquez et al., 1997). This is significant because floral initiation is determined to a large degree by the level of *LEAFY* expression (Weigel and Nilsson, 1995). Consistent with the important role of *LEAFY* on floral initiation and the opposite effect of phyB and *cry2* on flowering time, the mutation of the *CRY2* or *PHYB* genes has been shown to repress or activate *LEAFY* promoter activity, respectively (Nilsson et al., 1998; Blazquez and Weigel, 1999). Expression of another flowering-time gene, *CO*, is also dependent on photoperiod, and is expressed at higher levels in LD than in SD conditions (Putterill et al., 1995).

It was reported that *CO* was expressed at lower levels in *cry2* mutant plants than the wild type in LD conditions, whereas *CO* expression was elevated in transgenic plants overexpressing *CRY2* in both LD and SD conditions (Guo et al., 1998). On the other hand, the expression of *CO* did not seem to be altered in the *phyB* mutant (Blazquez and Weigel, 1999). Given the redundant function of phyB/D/E, it will be interesting to see how the expression of *CO* may be affected in the *phyBphyD* or *phyBphyE* double mutant plants. A systematic survey of the expression of more flowering-time genes in various photoreceptor mutations and under different photoperiodic conditions may provide a clearer picture of the role of different photoreceptors in the regulation of expression of the flowering-time genes.

HOW PHOTORECEPTORS REGULATE FLOWERING TIME IN RESPONSE TO PHOTOPERIODS

We have so far conveniently overlooked the question of how photoreceptors regulate flowering time in response to different photoperiods. Apparently,

the signal transduction of photoreceptors needs to interact with the circadian clock to regulate flowering time in different daylengths, but the molecular aspects of such interactions remains unclear. It is possible that a photoreceptor regulates the pace and activity of the circadian clock, which in turn regulates floral initiation (Fig. 3). Another compelling hypothesis is the external coincidence model, which was initially proposed in the 1930s and later modified to explain why light applied to plants at different times of a dark treatment had different effects on flowering time (Thomas and Vince-Prue, 1997; Carre, 1998). According to this hypothesis, the functions of photoreceptors are 2-fold: first, photoreceptors regulate operation of the circadian clock, and secondly, photoreceptors mediate signal transductions that directly affect floral initiation (Fig. 3).

The action of the circadian clock governs, at any given time, the effect of a photoreceptor (or a plant's responsiveness to the light signal) on floral initiation, which often exhibits the photoperiodic response rhythm. Under the appropriate experimental conditions (such as transferring plants grown in a specified photoperiod to continuous darkness and treating them with light at different times), the effect of light on floral initiation may be permitted or denied at certain times of day by the actions of the circadian clock (Carre, 1998). A regulation of the signal transduction of photoreceptors by the circadian clock has been referred to as gating (Millar and Kay, 1996).

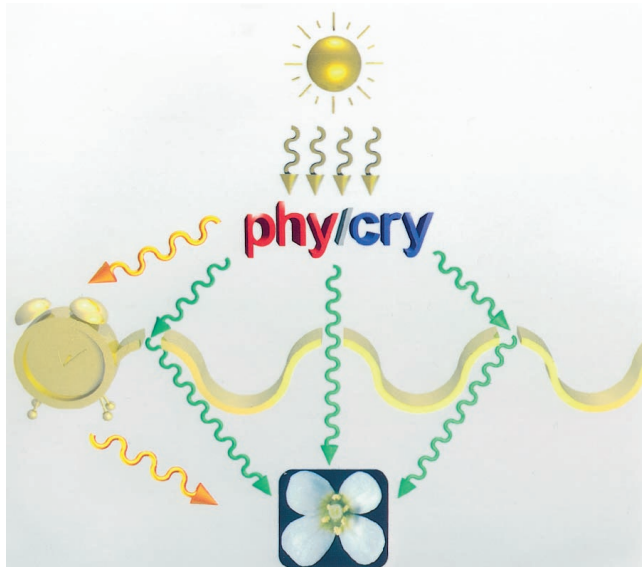


Figure 3. How photoreceptors may regulate flowering time in response to different photoperiods. The daylength signal may be transmitted by photoreceptors, through the circadian clock, to affect floral initiation (orange arrows). Alternatively, the circadian clock may regulate (gate) the signal transduction of photoreceptors affecting floral initiation, as depicted by the photoreceptor signal transduction (green arrows) being permitted at the peak, but denied (no arrow) at the trough of the circadian rhythm of an output pathway of the circadian clock (yellow wavy line).

Circadian Clock and Regulation of Flowering Time

The circadian clock is an internal oscillator, or it may be more broadly defined as the signaling system that is made up of three functional components—an internal oscillator (or central pacemaker) that generates the circadian oscillation, an input pathway that resets (entrains) the pacemaker according to the environmental cues such as light, and an output pathway that renders oscillations of the pacemaker to overt circadian rhythms (Dunlap, 1999; Somers, 1999). Although the molecular basis of the circadian clock in plant remains unclear, studies in other organisms have established a transcriptional negative feedback loop as the essential component of the central pacemaker (Dunlap, 1999). Several *Arabidopsis* flowering-time genes have been recently isolated and shown to be associated with the function of the circadian clock. Mutations or misexpression of these genes resulted in a decreased photoperiodic response of flowering, which provided the direct evidence for the essential role of the circadian clock in the regulation of photoperiodic flowering. The clock-related genes known to affect flowering time include *ELF3*, *TOC1*, *CCA1*, *LHY*, and *GI*.

elf3 was isolated as a photoperiod-hyposensitive early-flowering mutation that flowered early in both LD and SD conditions, and it also exhibited an elongated hypocotyl in red and blue light (Zagotta et al., 1996). The *elf3* mutant lacked circadian rhythms for both *CAB2* promoter activity and leaf movement when assayed under constant light, but the *elf3* mutant retained rhythmicity in constant dark, suggesting a possible function of *ELF3* in the input pathway (Hicks et al., 1996).

The *Arabidopsis toc1* was isolated as a circadian clock mutation that has a short period in every overt rhythm analyzed (Somers et al., 1998b). The effect of daylength on flowering time was diminished in the *toc1* mutant in the C24 ecotype background, and was nearly eliminated when the *toc1* mutation was introgressed into the Landsberg *erecta* background. It is particularly interesting that the *toc1-1* alleles of the C24 ecotype flowered earlier or later than the wild type in SD or LD conditions, respectively (Somers et al., 1998b), whereas almost all other flowering-time mutations affect flowering time in only one direction (either early or late). Isolation of the *TOC1* gene may provide more insight into photoperiodic flowering.

CCA1 and *LHY* both encode MYB-related transcription factors, which when overexpressed cause photoperiod-hyposensitive late flowering, elongated hypocotyls in white light, and disrupted overt circadian rhythms in *Arabidopsis* (Schaffer et al., 1998; Wang and Tobin, 1998). The expression of *CCA1* and *LHY* both showed circadian rhythms that could be abolished by the overexpression of the respective gene. The *Arabidopsis cca1* loss-of-function mutant showed a shortened period length of circadian expression of several genes (Green and

Tobin, 1999). *CCA1* and *LHY* may function, in a partially redundant manner, in the regulation of the circadian clock. *CCA1* and *LHY* are regulated by the protein kinase CK2, because CK2 has been shown to interact and phosphorylate both *CCA1* and *LHY* (Sugano et al., 1999). Overexpression of the *CKB3* gene, which encodes a regulatory subunit of CK2, resulted in increased CK2 activity, shortened periods of many clock-related genes, and photoperiod-hyposensitive early flowering (Sugano et al., 1999).

Another photoperiod-hyposensitive late-flowering mutation in *Arabidopsis* is *gi* (Koornneef et al., 1998). The *GI* gene was cloned recently and shown to encode a putative membrane protein (Fowler et al., 1999; Park et al., 1999). It is very interesting how a membrane protein like *GI* may affect the time of floral initiation, which had previously been thought to be determined largely by the regulation of the expression of flowering-time genes. The expressions of *GI*, *CCA1*, *LHY*, and *ELF3* were found to be dependent on each other (Fowler et al., 1999; Park et al., 1999). The expression of *GI* exhibited a circadian rhythm with different cycling phases in LD and SD conditions (Fowler et al., 1999). It is conceivable that the circadian expression of other flowering-time genes, including *CCA1*, *LHY*, *ELF3*, and *CO*, may also have distinct cycling phases in different photoperiods.

The function of these clock-related genes may directly affect the floral initiation process. Alternatively, these genes may act as the gating factors to regulate the signal transduction of a photoreceptor, as predicted by the external coincidence model. Elucidation of how these proteins affect flowering time will likely significantly enhance our understanding of the photoperiodic flowering.

Photoreceptors and the Entrainment of the Circadian Clock

The circadian clock is entrained by the action of photoreceptors to oscillate with a period of about 24 h. In light, the action of photoreceptors generally accelerates the pace of the clock, resulting in shortened period length comparing to that in dark (Millar et al., 1995). It has been shown that mutations of photoreceptor genes *PHYA*, *PHYB*, and *CRY1* causes the circadian rhythm of *CAB2* promoter activity to oscillate at a pace slower (with a longer period length) than that of the wild type under various light conditions (Somers et al., 1998a). This study revealed that in the regulation of the *Arabidopsis* circadian clock, *phyA* acts in low intensities of red light and blue light, *phyB* functions in high-intensity red light, and *cry1* acts in both low and high intensities of blue light. The function of *phyA* in the entrainment of the circadian clock in response to blue light was further demonstrated by showing that the *phyA* mutant was slower in adapting to a new light/dark condition in low- but not in high-fluence blue light compared

with the wild type. Interestingly, the *cry2* mutation, despite its reduced sensitivity to photoperiod, did not significantly affect the circadian clock, at least when it was measured for the *CAB2* promoter activity (Somers et al., 1998a). This result is consistent with a view that *cry2* may not have a major role in mediating light regulation of the circadian clock.

Although *phyA*, *phyB*, and *cry1* are clearly involved in the regulation of the circadian clock, it is difficult to distinguish whether the abnormality in flowering time observed in the *phyA*, *phyB*, and *cry1* mutants is the consequence of the malfunction of regulation of the circadian clock, a manifestation of the direct action of the respective photoreceptor on the floral initiation process, or both. It is interesting that mutations in the *PHYA*, *PHYB*, and *CRY1* genes affected the circadian clock in the similar manner (they all caused longer period length for the circadian expression of the *CAB2* promoter), yet their effects on flowering time were dissimilar and sometimes opposite (e.g. the *phyA* mutant flowered late but the *phyB* mutant flowered early). This phenomenon seems to suggest that the observed alterations in flowering time of the *phyA*, *phyB*, and *cry1* mutants are unlikely to be the direct consequence of a malfunction of the circadian clock. Instead, these photoreceptors may directly affect the floral initiation process, but the signal transduction of photoreceptors may be gated (rather than executed) by the circadian clock, as predicted by the external coincidence model.

The Gated Signal Transduction Paths of Photoreceptors

The function of the circadian clock in regulating flowering time can be demonstrated by the photoperiodic response rhythm or the circadian periodicity of floral induction (or inhibition) in response to light treatment applied at different times of the day (Thomas and Vince-Prue, 1997). For example, *Arabidopsis* plants grown in SD conditions could be promoted to flower by a 3-h far-red-light treatment applied at various times during a 3-d dark period, and the promotion of flowering by such treatments exhibited circadian rhythms (Carre, 1998). This observation can be explained by an external coincidence model, that the action of a photoreceptor on floral initiation is gated by the circadian clock (Fig. 3). It will be interesting to determine whether *phyA* mediates this far-red-light response. It will also be useful to systematically investigate the photoperiodic response rhythms for other spectra of light in various photoreceptor mutations.

The *phyB*-regulated floral initiation may represent another example for gated photoreceptor signal transduction. *phyB* mutations of the SD plant sorghum and the LD plant *Arabidopsis* (or pea) both caused an early-flowering phenotype. This was somewhat surprising given that the flowering of SD and LD plants responds oppositely to daylength. One

interpretation of this observation is that phyB action may suppress floral initiation regardless of photoperiods, but the signal transduction or cell's responsiveness to phyB signaling is gated by the action of the circadian clock, resulting in different daylength responses in the flowering time of different plants.

Finally, the effect of cry2 on floral initiation may be best interpreted by the gating hypothesis. As described previously, the function of cry2 is clearly involved in photoperiodic flowering, because both mutation and overexpression of the *CRY2* gene result in reduced responsiveness of floral initiation to photoperiods. However, unlike phyA, phyB, and cry1, cry2 is not obviously involved in light entrainment of the circadian clock. Therefore, the effect of cry2 on photoperiodic flowering is more likely to result from its signal transduction (or the plant's response to cry2 signaling) being differentially affected by an output of the circadian clock in different daylength conditions.

PERSPECTIVE

Recent molecular genetic studies of plant photoreceptors have demonstrated that the action of individual phytochromes and cryptochromes can either suppress or promote floral initiation, and that a photoreceptor may function within the nucleus to affect transcription of the flowering-time genes. It remains unclear how photoreceptors control photoperiodic flowering. A photoreceptor may regulate flowering time in response to different photoperiods via its regulation of the circadian clock. Alternatively, the direct effect of a photoreceptor on floral initiation may be gated by the circadian clock, resulting in different responses in different photoperiods. It is conceivable that the expression level or activity of a photoreceptor signaling molecule may oscillate with distinct cycling phases in different photoperiods, and as such may serve as the hypothesized gating factor that determines the signal transduction of a photoreceptor (and thus the flowering time) in different photoperiods. The identification of such factors and investigation of how the expression or activity of these factors affects the function of photoreceptors may shed more light on the mechanism of photoreceptors in the control of flowering time.

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