Photoreceptors and Regulation of Flowering Time[1]

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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

[1] This work was supported by the National Institutes of Health (grant no. GM–56265) and by the U.S. Department of Agriculture (grant no. 99–35304–8085).

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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 *CRY*s, 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 photosignal 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*, *COP1*, and *DET1*) have functions in both hypocotyl inhibition and flowering time (Levy and Dean, 1998, and refs. therein). These observations 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

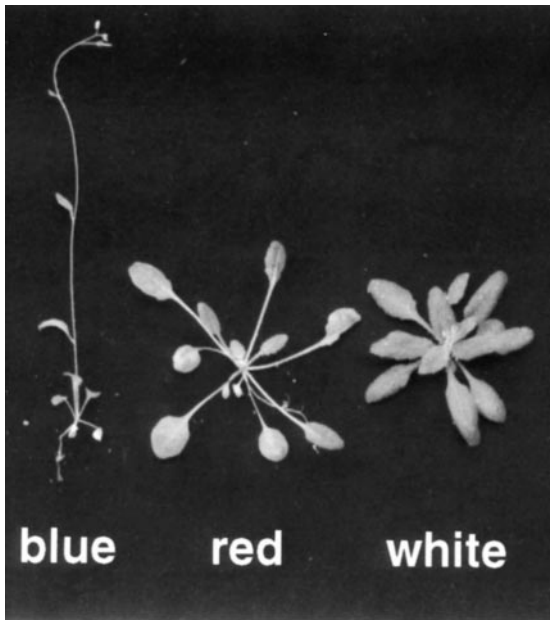

Figure 1. Effect of red and blue light on Arabidopsis flowering time. The 21-d-old Arabidopsis (ecotype Columbia) plants shown were imbibed at 4°C for 4 d in the dark and then grown under continuous blue, red, or white light (all approximately 100 μmol s^{-1} m^{-2}). The plant grown in blue light had 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-transmittable 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 (Ma_3^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 Wassilewskija 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

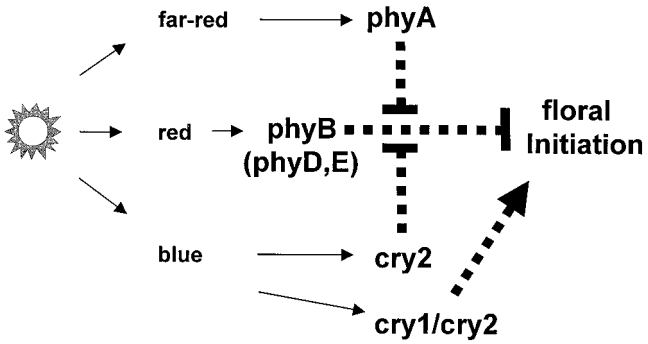

Figure 2. Antagonistic or redundant functions of photoreceptors in regulating floral initiation of Arabidopsis. The broken lines represent signal transduction pathways of photoreceptors; arrows denote the positive effects; and lines terminated with a bar denote inhibitory effects.

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 background) 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 *fha* mutant, although the *cry2* alleles (in the Columbia ecotype background) had stronger phenotype than the *fha* 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. Similar 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).

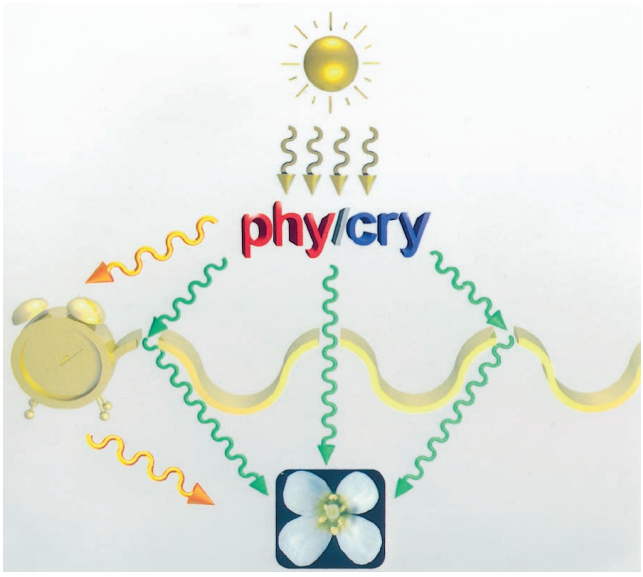

Figure 3. How photoreceptors may regulate flowering time in response to different photoperiods. The daylength signal may be transmitted by photoreceptors, through the circadian clock, to affect floral initiation (orange arrows). Alternatively, the circadian clock may regulate (gate) the signal transduction 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.

ACKNOWLEDGMENTS

The author thanks Todd Mockler and other colleagues for critical reading of the manuscript, Hongyun Yang and Timothy Riley for figure preparation, and Dr. Detlef Weigel for permission to use the photograph of an Arabidopsis flower (Fig. 3) from his web site.

Received November 11, 1999; accepted January 27, 2000.

LITERATURE CITED

Adam SA (1999) Transport pathways of macromolecules between the nucleus and the cytoplasm. Curr Opin Cell Biol **11:** 402–406

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

Ahmad M, Cashmore AR (1996) The pef mutants of *Arabidopsis thaliana* define lesions early in the phytochrome signaling pathway. Plant J **10:** 1103–1110

Ahmad M, Jarillo JA, Smirnova O, Cashmore AR (1998) The CRY1 blue light photoreceptor of Arabidopsis interacts with phytochrome A in vitro. Mol Cell **1:** 939–948

Araki T, Komeda Y (1993) Flowering in darkness in *Arabidopsis thaliana*. Plant J **4:** 801–811

Aukerman MJ, Hirschfeld M, Wester L, Weaver M, Clack T, Amasino RM, Sharrock RA (1997) A deletion in the PHYD gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. Plant Cell **9:** 1317–1326

Bagnall DJ, King RW, Hangarter RP (1996) Blue-light promotion of flowering is absent in hy4 mutants of Arabidopsis. Planta **200:** 278–280

Bagnall DJ, King RW, Whitelam GC, Boylan MT, Wagner D, Quail PH (1995) Flowering responses to altered expression of phytochrome in mutants and transgenic lines of *Arabidopsis thaliana* (L.) Heynh. Plant Physiol **108:** 1495–1503

Blazquez MA, Soowal LN, Lee I, Weigel D (1997) LEAFY expression and flower initiation in Arabidopsis. Development **124:** 3835–3844

Blazquez MA, Weigel D (1999) Independent regulation of flowering by phytochrome B and gibberellins in Arabidopsis. Plant Physiol **120:** 1025–1032

Carre IA (1998) Genetic dissection of the photoperiod-sensing mechanism in the long-day plant *Arabidopsis thaliana. In* PJ Lumsden, AJ Millar, eds, Biological Rhythms and Photoperiodism in Plants. BIOS Scientific Publishers, Oxford, pp 257–269

Cashmore AR (1998) Higher-plant phytochrome: "I used to date histidine, but now I prefer serine." Proc Natl Acad Sci USA **95:** 13358–13360

Cashmore AR, Jarillo JA, Wu YJ, Liu D (1999) Cryptochromes: blue light receptors for plants and animals. Science **284:** 760–765

Ceriani MF, Darlington TK, Staknis D, Mas P, Petti AA, Weitz CJ, Kay SA (1999) Light-dependent sequestration of TIMELESS by CRYPTOCHROME. Science **285:** 553–556

Childs KL, Miller FR, Cordonnier-Pratt MM, Pratt LH, Morgan PW, Mullet JE (1997) The sorghum photoperiod sensitivity gene, Ma3, encodes a phytochrome B. Plant Physiol **113:** 611–619

Choi G, Yi H, Lee J, Kwon Y-K, Soh MS, Shin B, Luka Z, Hahn T-R, Song P-S (1999) Phytochrome signaling is mediated through nucleoside diphosphate kinase 2. Nature **401:** 610–613

Clough RC, Jordan-Beebe ET, Lohman KN, Marita JM, Walker JM, Gatz C, Vierstra RD (1999) Sequences

within the N- and C-terminal domains of phytochrome A are required for PFR ubiquitination and degradation. Plant J **17:** 155–167

Devlin PF, Halliday KJ, Harberd NP, Whitelam GC (1996) The rosette habit of *Arabidopsis thaliana* is dependent upon phytochrome action: novel phytochromes control internode elongation and flowering time. Plant J **10:** 1127–1134

Devlin PF, Patel SR, Whitelam GC (1999a) Phytochrome E influences internode elongation and flowering time in Arabidopsis. Plant Cell **10:** 1479–1488

Devlin PF, Robson PR, Patel SR, Goosey L, Sharrock RA, Whitelam GC (1999b) Phytochrome D acts in the shade-avoidance syndrome in Arabidopsis by controlling elongation growth and flowering time. Plant Physiol **119:** 909–915

Dunlap JC (1999) Molecular bases for circadian clocks. Cell **96:** 271–290

Elich TD, Chory J (1997) Phytochrome: if it looks and smells like a histidine kinase, it is a histidine kinase? Cell **91:** 713–716

Fankhauser C, Chory J (1999) Light receptor kinases in plants! Curr Biol **9:** R123–R126

Fankhauser C, Yeh KC, Lagarias JC, Zhang H, Elich TD, Chory J (1999) PKS1, a substrate phosphorylated by phytochrome that modulates light signaling in Arabidopsis. Science **284:** 1539–1541

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. EMBO J **18:** 4679–4688

Goto N, Kumagai T, Koornneef M (1991) Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. Physiol Plant **83:** 209–215

Green RM, Tobin EM (1999) Loss of the circadian clock associated 1 (CCA1) protein causes altered clock-regulated gene expression. Proc Natl Acad Sci USA **96:** 4176–4179

Guo H, Duong H, Ma N, Lin C (1999) The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. Plant J **19:** 279–287

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

Halliday KJ, Hudson M, Ni M, Qin M, Quail PH (1999) poc1: an Arabidopsis mutant perturbed in phytochrome signaling because of a T DNA insertion in the promoter of PIF3, a gene encoding a phytochrome-interacting bHLH protein. Proc Natl Acad Sci USA **96:** 5832–5837

Halliday KJ, Koornneef M, Whitelam GC (1994) Phytochrome B and at least one other phytochrome mediate the accelerated flowering response of *Arabidopsis thaliana* (L.) to low red/far red ratio. Plant Physiol **104:** 1311–1315

Hicks KA, Millar AJ, Carre IA, Somers DE, Straume M, Meeks-Wagner DR, Kay SA (1996) Conditional circadian dysfunction of the Arabidopsis early-flowering 3 mutant. Science **274:** 790–792

Hoecker U, Tepperman JM, Quail PH (1999) SPA1, a WD-repeat protein specific to phytochrome A signal transduction. Science **284:** 496–499

Hoecker U, Xu Y, Quail PH (1998) SPA1: a new genetic locus involved in phytochrome A-specific signal transduction. Plant Cell **10:** 19–33

Hughes J (1999) Prokaryotes and phytochrome. Plant Physiol **121:** 1059–1068

Johnson E, Bradley M, Harberd NP, Whitelam GC (1994) Photoresponses of light-grown *phyA* mutants of Arabidopsis. Plant Physiol **105:** 141–149

Kendrick RE, Kronenberg GHM (1994) Photomorphogenesis in Plants, Ed 2. Kluwer Academic Publishers, Dordrecht, The Netherlands

Kircher S, Kozma-Bognar L, Kim L, Adam E, Harter K, Schafer E, Nagy F (1999) Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. Plant Cell **11:** 1445–1456

Kleiner O, Kircher S, Harter K, Batschauer A (1999) Nuclear localization of the Arabidopsis blue light receptor cryptochrome 2. Plant J **19:** 289–296

Koornneef M, Alonso-Blanco C, Peeters AJM, Soppe W (1998) Genetic control of flowering time in Arabidopsis. Annu Rev Plant Physiol Plant Mol Biol **49:** 345–370

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

Koornneef M, Rolff E, Spruit CJP (1980) Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenphysiol Bd **100:** 147–160

Levy YY, Dean C (1998) The transition to flowering. Plant Cell **10:** 1973–1990

Lin C, Ahmad M, Cashmore AR (1996) Arabidopsis cryptochrome 1 is a soluble protein mediating blue light-dependent regulation of plant growth and development. Plant J **10:** 893–902

Lin C, Ahmad M, Gordon D, Cashmore AR (1995a) Expression of an Arabidopsis cryptochrome gene in transgenic tobacco results in hypersensitivity to blue, UV-A, and green light. Proc Natl Acad Sci USA **92:** 8423–8427

Lin C, Robertson DE, Ahmad M, Raibekas AA, Jorns MS, Dutton PL, Cashmore AR (1995b) Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science **269:** 968–970

Lin C, Yang H, Guo H, Mockler T, Chen J, Cashmore AR (1998) Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. Proc Natl Acad Sci USA **95:** 2686–2690

Millar AJ, Kay SA (1996) Integration of circadian and phototransduction pathways in the network controlling CAB gene transcription in Arabidopsis. Proc Natl Acad Sci USA **93:** 15491–15496

Millar AJ, Straume M, Chory J, Chua NH, Kay SA (1995) The regulation of circadian period by phototransduction pathways in Arabidopsis. Science **267:** 1163–1166

Mockler TC, Guo H, Yang H, Duong H, Lin C (1999) Antagonistic actions of Arabidopsis cryptochromes and

phytochrome B in the regulation of floral induction. Development **126:** 2073–2082

Morelli G (2000) Shade avoidance responses: driving auxin along lateral routes. Plant Physiol **122:** 621–626

Mozley D, Thomas B (1995) Developmental and photobiological factors affecting photoperiodic induction in *Arabidopsis thaliana* Heynh. Landsberg *erecta*. J Exp Botany **46:** 173–179

Neff MM, Chory J (1998) Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during Arabidopsis development. Plant Physiol **118:** 27–35

Ni M, Tepperman JM, Quail PH (1998) PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. Cell **95:** 657–667

Ni M, Tepperman JM, Quail PH (1999) Binding of phytochrome B to its nuclear signaling partner PIF3 is reversibly induced by light. Nature **400:** 781–784

Nilsson O, Lee I, Blazquez MA, Weigel D (1998) Flowering-time genes modulate the response to LEAFY activity. Genetics **150:** 403–410

Pao CI, Morgan PW (1986) Genetic regulation of development in *Sorghum bicolor*: I. Role of the maturity genes. Plant Physiol **82:** 575–580

Park DH, Somers DE, Kim YS, Choy YH, Lim HK, Soh MS, Kim HJ, Kay SA, Nam HG (1999) Control of circadian rhythms and photoperiodic flowering by the Arabidopsis GIGANTEA gene. Science **285**: 1579–1582

Pineiro M, Coupland G (1998) The control of flowering time and floral identity in Arabidopsis. Plant Physiol **117:** 1–8

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell **80:** 847–857

Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, Wagner D (1995) Phytochromes: photosensory perception and signal transduction. Science **268:** 675–680

Reed JW, Nagatani A, Elich TD, Fagan M, Chory J (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol **104:** 1139–1149

Sakamoto K, Nagatani A (1996) Nuclear localization activity of phytochrome B. Plant J **10:** 859–868

Sancar A (1994) Structure and function of DNA photolyase. Biochemistry **33:** 2–9

Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carre IA, Coupland G (1998) The *late elongated hypocotyl* mutation of Arabidopsis disrupts circadian rhythms and the photoperiodic control of flowering. Cell **93:** 1219–1229

Smith H (1999) Phytochromes: tripping the light fantastic. Nature **400:** 710–713

Smith H, Whitelam GC (1997) The shade avoidance syndrome: multiple responses mediated by multiple phytochromes. Plant Cell Environ **20:** 840–844

Somers DE (1999) The physiology and molecular bases of the plant circadian clock. Plant Physiol **121:** 9–20

Somers DE, Devlin PF, Kay SA (1998a) Phytochromes and cryptochromes in the entrainment of the Arabidopsis circadian clock. Science **282:** 1488–1490

Somers DE, Webb AA, Pearson M, Kay SA (1998b) The short-period mutant, toc1-1, alters circadian clock regulation of multiple outputs throughout development in *Arabidopsis thaliana*. Development **125:** 485–494

Sugano S, Andronis C, Ong MS, Green RM, Tobin EM (1999) The protein kinase CK2 is involved in regulation of circadian rhythms in Arabidopsis. Proc Natl Acad Sci USA **96:** 12362–12366

Thomas B, Vince-Prue D (1997) Photoperiodism in Plants. Academic Press, New York

Thresher RJ, Vitaterna MH, Miyamoto Y, Kazantsev A, Hsu DS, Petit C, Selby CP, Dawut L, Smithies O, Takahashi JS, Sancar A (1998) Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. Science **282:** 1490–1494

Wang ZY, Tobin EM (1998) Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. Cell **93:** 1207–1217

Weigel D, Nilsson O (1995) A developmental switch sufficient for flower initiation in diverse plants. Nature **377:** 495–500

Weller JL, Murfet IC, Reid JB (1997) Pea mutants with reduced sensitivity to far-red light define an important role for phytochrome A in day-length detection. Plant Physiol **114:** 1225–1236

Weller JL, Reid JB (1993) Photoperiodism and photocontrol of stem elongation in two photomorphogenic mutants of *Pisum sativum* L. Planta **189:** 15–23

Wong YS, Cheng HC, Walsh DA, Lagarias JC (1986) Phosphorylation of *Avena* phytochrome in vitro as a probe of light-induced conformational changes. J Biol Chem **261:** 12089–12097

Yeh KC, Lagarias JC (1998) Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. Proc Natl Acad Sci USA **95:** 13976–13981

Zagotta MT, Hicks KA, Jacobs CI, Young JC, Hangarter RP, Meeks-Wagner DR (1996) The Arabidopsis ELF3 gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. Plant J **10:** 691–702