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Decoding of Light Signals by Plant Phytochromes and Their Interacting Proteins

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Key Words

phytochrome-interacting protein, protein degradation, photosensory, light signaling, PIF3, PIL5

Abstract

Phytochromes are red/far-red light photoreceptors that convert the information contained in external light into biological signals. The decoding process starts with the perception of red light, which occurs through photoisomerization of a chromophore located within the phytochrome, leading to structural changes that include the disruption of intramolecular interactions between the N- and C-terminal domains of the phytochrome. This disruption exposes surfaces required for interactions with other proteins. In contrast, the perception of far-red light reverses the photoisomerization, restores the intramolecular interaction, and closes the interacting surfaces. Light information represented by the concentration of opened interacting surfaces is converted into biological signals through the modulating activity of interacting proteins. This review summarizes plant phytochromes, phytochrome-interacting proteins, and signal transmission from phytochromes to their interacting proteins.

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Contents

INTRODUCTION

Although light is ubiquitous, the light in a given locale may vary in terms of its wavelength, irradiance, direction, and periodicity (114). Plants acquire energy solely from light, and plant survival depends on the availability of external light. Therefore, it is not surprising that plants are equipped with sophisticated photoreceptor systems capable of monitoring external light conditions and continuously make light-specific adjustments to physiological and developmental processes (75). Because the absorption spectra of chlorophyll molecules cover blue and red light, plants have evolved to detect these spectra. At least four different types of photoreceptors have been identified in *Arabidopsis*, including the three classical photoreceptors (phytochromes, cryptochromes, and phototropins) and a newly recognized set of blue light photoreceptors (zeitlupes), F-box proteins containing a light, oxygen, and voltage (LOV) domain and kelch repeats. These different photoreceptors play shared but distinct roles in the induction of light responses upon the perception of blue or red/far-red light. Among these photoreceptors, the phytochromes, which are red and far-red light photoreceptors, are encoded by five different genes (*PHYA* to *PHYE*) in *Arabidopsis*, and are responsible for regulating various red light responses, including seed germination, seedling photomorphogenesis, shade avoidance, flowering, and many other adaptive responses (20). This review focuses on how light signals are perceived by phytochromes and their interacting proteins. A brief summary of other photoreceptors and relevant references can be found in the **Supplemental Material**. Follow the **Supplemental Material** link from the Annual Reviews home page at **http://www.annualreviews.org**.

PLANT PHYTOCHROMES

Perception of Light by Plant Phytochromes

Since the seminal work by Borthwick and coworkers (10) on the role of red/far-red light on lettuce seed germination, phytochromes have been the protein of interest among plant scientists. Plant phytochromes are dimeric proteins typically consisting of two identical apoproteins covalently linked with phytochromobilin, a linear tetrapyrrole bilin compound that acts as a chromophore

*(1) should read (Figure 1). View Erratum at http://arjournals.annualreviews.org/doi/abs/10.1146/annurev.PP.59.080506.200001

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(51, 63, 113). The ability of a given phytochrome to absorb red and far-red light stems from its bound phytochromobilin, which undergoes a reversible photoisomerization at the C15-C16 double bond in response to red light (666 nm) and far-red light (730 nm) (1).* After initial assembly of the phytochrome, the phytochromobilin assumes the C15-*Z*,*anti* conformation and is ready to absorb red light. This form of phytochrome is called the Pr form and is considered the biologically inactive form. Upon the absorption of red light, the C15-*Z*,*anti* conformation is converted to the C15-*E*,*anti* conformation. This form of the phytochrome is called Pfr. The Pfr form interacts with other proteins either in the cytosol or inside the nucleus (after translocation into the nucleus) and regulates their functions to induce light responses. A more detailed description of a proposed photoconversion process can be found in a recent review by Rockwell and colleagues (100). The conversion between Pr and Pfr by red and far-red light is reversible, allowing the phytochrome to act as a switch that is turned on by red light and turned off by far-red light (9).

The Phytochrome Gene Family

The plant phytochromes are encoded by a small gene family in most plant species; there are five *PHY* genes (*PHYA* to *PHYE*) in *Arabidopsis*, three *PHY* genes (*PHYA* to *PHYC*) in rice, four *PHY* genes (*PHYP1*, *PHYP2*, *PHYN*, and *PHYO*) in *Pinus*, and three *PHY* genes (*PHYP*, *PHYN*, and *PHYO*) in *Ginkgo*. Phylogenetic analysis has shown that an ancestral phytochrome bifurcated before the divergence of seed plants (75). Thus, all phytochromes found in modern plant species can be classified into two groups, namely the PHYA branch (including PHYA, PHYC, PHYN, and PHYO) and the PHYB branch (including PHYB, PHYD, PHYE, and PHYP). However, the phylogenetic dichotomy of plant phytochromes is not directly correlated with their molecular properties and functions.

The various phytochromes show similar but different molecular properties. First, PHYA is light labile, whereas all the other phytochromes are light stable (1, 36, 124). Owing to this difference in light stability, PHYA is the predominant phytochrome in etiolated seedlings, whereas PHYB and the others predominate in light-grown plants. Curiously, the stability of PHYC is dramatically decreased in *phyB* mutants in both *Arabidopsis* and rice, suggesting that PHYB controls the activity of PHYC in these species by regulating its stability (79, 121). Second, *Arabidopsis* PHYA dimerizes only with itself, whereas all the other *Arabidopsis* PHYs can form dimers with each other (106). The functional significance of heterodimerization is not yet fully understood.

The various phytochromes differ largely with respect to their spectral specificities. For the majority of light responses in *Arabidopsis*, PHYA is responsible for the very low fluence response (VLFR) and the far-red high irradiance response (FR-HIR) (23, 81, 112, 138), whereas the other phytochromes are responsible for the red/far-red reversible low fluence response (LFR) (97, 98). However, PHYA can mediate red light signaling under very high irradiance red light and during dark-to-light transitions (32, 122), whereas PHYE can mediate FR-HIR for seed germination (39). In rice, FR-HIR is mediated by both PHYA and PHYC, whereas LFR is mediated by both PHYA and PHYB (121).

The various phytochromes play overlapping but distinct roles. In rice, all three phytochromes promote de-etiolation and delay flowering in long-day (LD) conditions, whereas in short-day (SD) conditions PHYB delays flowering and PHYA promotes flowering, especially in the absence of PHYB (121). In *Arabidopsis*, both PHYA and PHYB promote seed germination and de-etiolation in response to far-red (FR) and red (R) light, respectively. PHYB inhibits shade avoidance responses under a high ratio of R:FR light, whereas PHYA inhibits excessive shade avoidance responses under a low ratio of R:FR light;

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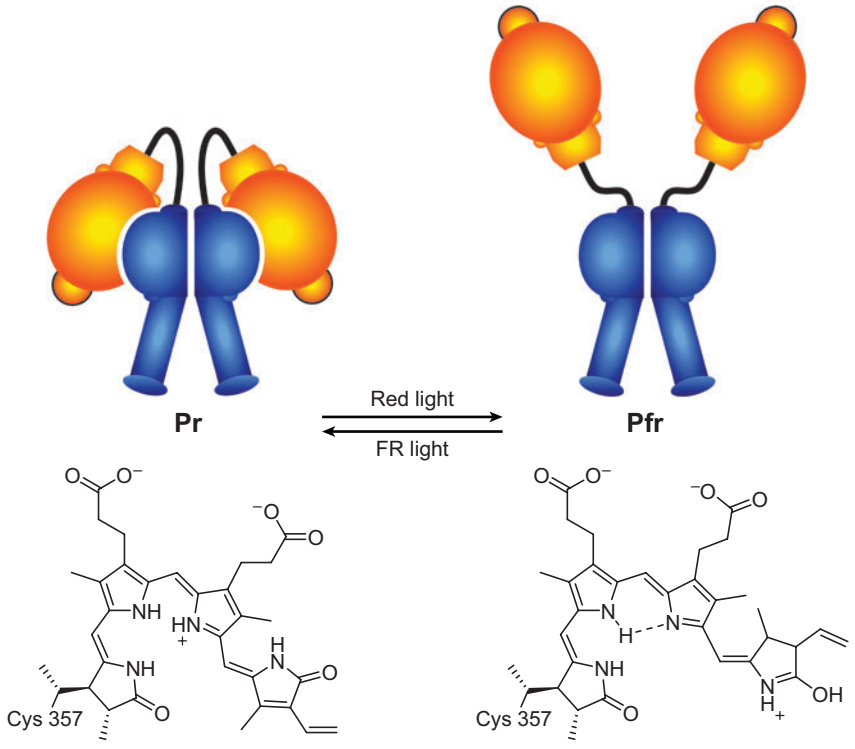

Figure 1

The photoisomerization of phytochromobilin and the accompanying structural change in phytochrome. Pr, C15-*Z*,*anti* conformation; Pfr, C15-*E*,*anti* conformation; FR light, far-red light.

PHYA promotes flowering, whereas PHYB delays flowering (34, 97). In *Arabidopsis*, the three other phytochromes also have overlapping but distinct functions. PHYC promotes seedling de-etiolation and primary leaf expansion in response to red light and delays flowering (4, 33, 79). Similarly, PHYD and PHYE promote seedling de-etiolation and suppress shade avoidance responses (3, 27, 28). Curiously, for seed germination, PHYE can promote seed germination under both LFR and FR-HIR conditions (39). The functional differences among the *Arabidopsis* phytochromes are partly due to their intrinsic properties (107). When PHYB, PHYD, and PHYE are overexpressed in the *phyB* mutant under control of the *PHYB* promoter, all three phytochromes rescue the seedling and leaf morphology phenotypes of the *phyB* mutant either partially (PHYD and PHYE) or fully (PHYB). In contrast, PHYB and PHYE rescue the flowering phenotype, but PHYD does not. Taken together, the characterizations of various phytochromes from the same or different species indicate that phytochromes share similar functions but have diverged to adopt various roles irrespective of their phylogenetic origins.

Functional Domains of Plant Phytochromes

All plant phytochromes can be divided into an N-terminal photosensory domain and a C-terminal dimerization domain. The N-terminal photosensory domain may be further divided into four consecutive subdomains called P1, P2/PAS, P3/GAF, and P4/PHY (named sequentially from the N terminus), whereas the C-terminal domain may be

divided into two subdomains, the PAS-A and PAS-B domains and the histidine kinase–related domain (HKRD) (139) (**Figure 2**). The PAS domain is named after three proteins in which it occurs: Per (period circadian protein), Arn (Ah receptor nuclear translocator protein), and Sim (single-minded protein). The HKRD lacks a critical histidine residue, and thus may be an evolutionary remnant rather than an active histidine kinase (10). Among the N-terminal subdomains, the P1 domain is uniquely present in plant phytochromes, whereas the P2/PAS, P3/GAF, and P4/PHY domains are also found in phytochrome-like proteins of various origins. Among the C-terminal subdomains, the PAS-A and PAS-B domains are unique to plant phytochromes, whereas HKRDs are also found in phytochrome-like proteins.

The P1 domain is not essential for the function of PHYB. Deletion of amino acids 1–57 of *Arabidopsis* PHYB yields a protein with full activity (131). Even proteins with a deletion of the N-terminal 103 amino acids retain the ability to inhibit hypocotyl elongation in red light, although to a reduced degree (131) (**Figure 3**). In contrast, the function of the P1 domain is more complicated in PHYA. Deletion of amino acids 25–33 or 50–62 from oat PHYA destabilizes the Pfr conformation in vitro and severely reduces the activity of the protein when expressed in tobacco (17). In contrast, deletion of amino acids 6–12 of oat PHYA confers hypersensitivity to far-red light in both tobacco and *Arabidopsis* (12). However, an *Arabidopsis* PHYA protein harboring the same deletion mediates normal VLFR, but not FR-HIR, when expressed under the native PHYA promoter in *Arabidopsis* (125). These findings seem to suggest that the P1 domains of different PHYA proteins play varied roles in different plant species. This complex role of the P1 domain may suggest that its regulatory role evolved after the divergence of these species. Biochemically, serine 7 of oat PHYA is phosphorylated by unidentified kinases, whereas serine 17 of the same protein is autophosphorylated (67). The dephosphorylation of these serine residues by PHYTOCHROME-ASSOCIATED PHOSPHATASE 5 (PAPP5) stabilizes PHYA in *Arabidopsis* (101). The stabilizing effect of dephosphorylation is further supported by the hypersensitivity of oat PHYA proteins with alanines substituted in place of these serines (116). However, because deletion of the same region from *Arabidopsis* PHYA destabilizes rather than stabilizes the protein, the precise role of these phosphorylation events warrants careful investigation. Collectively, the existing data suggest that the P1 domain of PHYA regulates the stability of both the phytochrome and its Pfr conformation, but the specific roles of this domain are variable across different phytochromes and plant species.

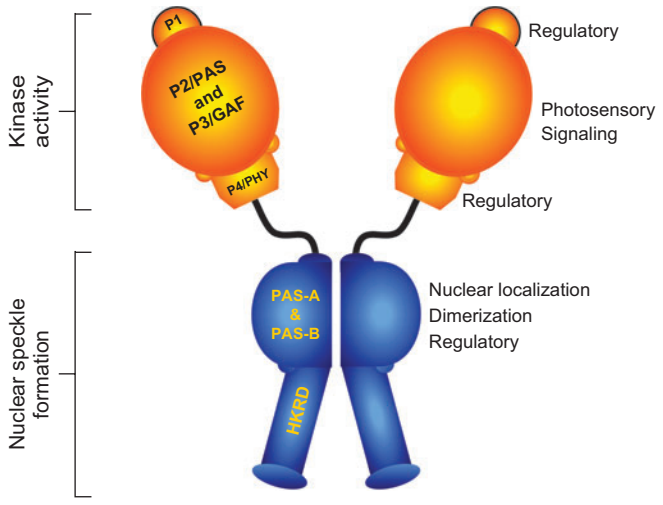

Figure 2

Domain structures of phytochrome and their associated functions. PAS, Per (period circadian protein) Arn (Ah receptor nuclear translocator protein), and Sim (single-minded protein); PHY, phytochrome; HKRD, histidine kinase–related domain; GAF, cGMP-stimulated phosphodiesterase, *Anabena* adenylate cyclases, and *Escherichia coli* FhlA.

In contrast, the P2/PAS and P3/GAF domains form a core photosensory domain and are conserved in most phytochromes and phytochrome-related proteins. These domains contain bilin lyase activity, which is responsible for ligating the chromophore to a cysteine residue either in the P2/PAS domain

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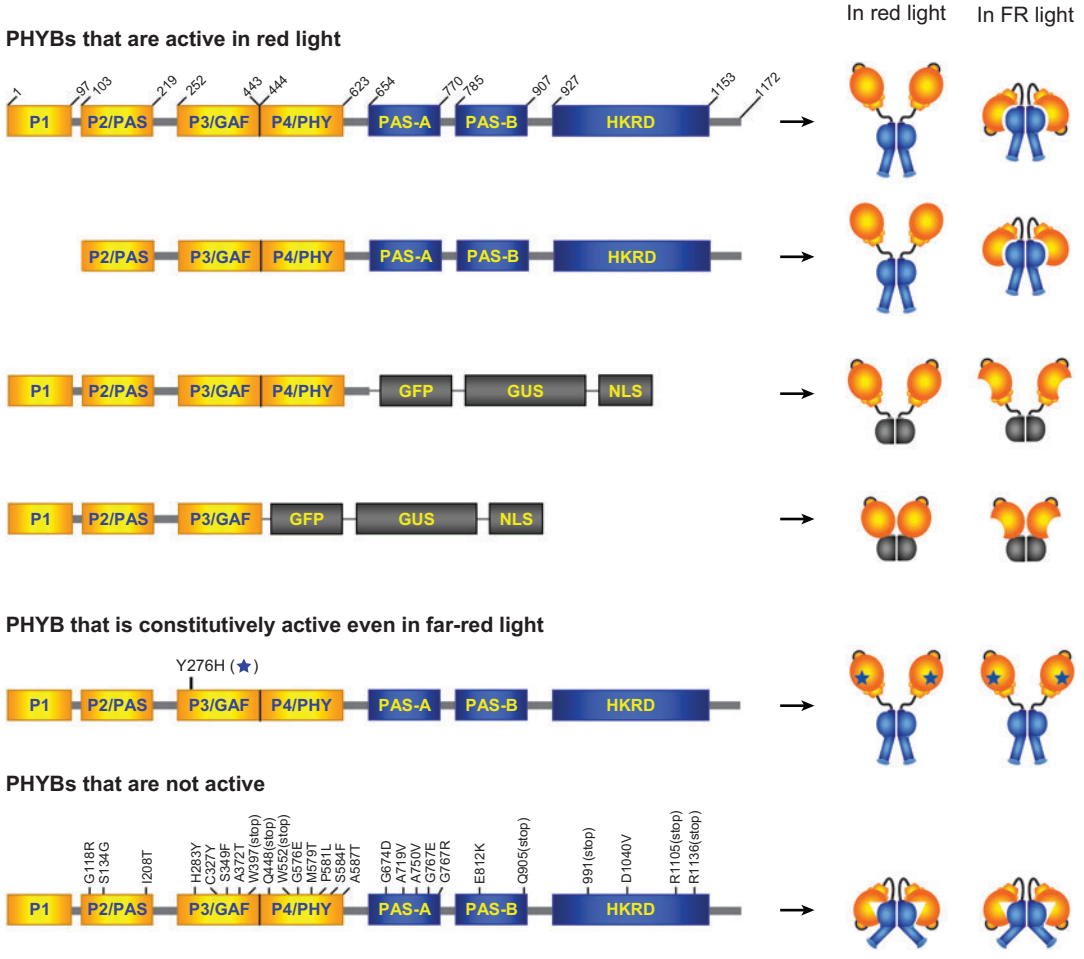

Figure 3

Phytochrome B mutants and their hypothetical conformations in red and far-red light. PHY, phytochrome; PAS, Per (period circadian protein), Arn (Ah receptor nuclear translocator protein), and Sim (single-minded protein); GAF, cGMP-stimulated phosphodiesterase, *Anabena* adenylate cyclases and *Escherichia coli* FhlA; HKRD, histidine kinase–related domain; GFP, green fluorescent protein; GUS, β-glucuronidase; NLS, nuclear localization signal; FR, far-red.

(in bacteriophytochromes) or in the P3/GAF domain (in plant phytochromes) (64, 66, 139). Accordingly, many point mutations in these domains affect either chromophore assembly or the spectral properties of the mutant proteins (**Figure 3**). Further functional separation of the two domains has proven difficult, however, because deletion of either domain impairs chromophore incorporation, resulting in a grossly nonfunctional protein. The crystal structure of the *Deinococcus* bacteriophytochrome shows that the two domains are tightly linked not only by the peptide backbone of the protein, but also by a trefoil knot (133, 134). The functional significance of this knot is unknown, but many loss-of-function missense mutations in these domains map to the knot region, suggesting that the knot formed between the P2/PAS and P3/GAF domains plays an important functional role.

A recent analysis of another mutation in the P3/GAF domain suggests that this

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domain plays a critical role in light signaling (117). Substitution of PHYB tyrosine 276 with histidine (Y276H) causes the loss of red light–induced photoisomerization, meaning that the mutant PHYB^{Y276H} behaves like the Pr form (at least from the spectral standpoint). However, when the mutant protein is overexpressed in *Arabidopsis*, the chromophore-assembled PHYB^{Y276H} is localized in the nucleus and is constitutively active, yielding constitutive photomorphogenic phenotypes even in the dark (**Figure 3**). Consistent with the mutant's loss of photoisomerization ability, the constitutive photomorphogenic phenotypes are not reversed by far-red light. A similar mutation in PHYA (PHYA^{Y242H}) is less active than PHYB^{Y276H}, but still yields constitutively photomorphogenic phenotypes in the dark. Taken together, these results indicate that the P2/PAS and P3/GAF domains play critical roles in both photosensing and light signaling (**Figure 2**). The constitutively active status of PHYB^{Y276H} and PHYA^{Y242H} further suggests that structural changes, rather than photoisomerization per se, are critically important for signaling.

The P4/PHY domain, which is conserved in all phytochromes and their related proteins, is necessary for fine tuning phytochrome activity. Deletion of the P4/PHY domain increases the dark reversion rate (i.e., the instability of the Pfr conformation) and causes a blue shift in absorption by both Pr and Pfr (90). Three missense mutations found in the P4/PHY domain are especially informative. First, an *Arabidopsis* PHYB harboring a missense mutation (G564E) is hyperactive, due at least in part to its decreased dark reversion rate (62). Second, a natural variation of PHYA (M548T; identified from the Lm-2 accession) shows a significant reduction in PHYA activity, a 6-nm blue shift for Pfr absorption, and reduced kinase activity (72). Third, a missense PHYB mutation (A587T) disrupts the nuclear localization of PHYB (15). Collectively, these data suggest that the P4/PHY domain is necessary for fine tuning the stability of the Pfr conformation and ensuring proper spectral properties, nuclear localization, and kinase activity.

A truncated PHYB comprising the N-terminal 651 amino acids of PHYB (including the P1, P2/PAS, P3/GAF, and P4/PHY domains) is functional when fused to a dimerization motif provided by β-glucuronidase (GUS) and the SV40 nuclear localization signal (NLS) (N651G-GUS-NLS) (99) (**Figure 3**). The activity of N651G-GUS-NLS is higher than wild-type activity, indicating that the N-terminal domain has all the activities necessary for phytochrome function except for the dimerization and nuclear localization activities. The higher activity of N651G-GUS-NLS compared with the full-length PHYB further implies that the C-terminal domain has a negative regulatory function. A truncated PHYB comprising the N-terminal 450 amino acids of PHYB is also functional when fused to GUS and the SV40 nuclear localization signal (N450G-GUS-NLS, including P1, P2/PAS, and P3/GAF), but shows an increased dark reversion rate owing to the lack of the P4/PHY domain (90) (**Figure 3**). Owing to its increased dark reversion rate, N450G-GUS-NLS becomes less active following exposure to red pulses with longer intermittent periods. Because the P1 domain of PHYB is dispensable (as discussed above), P2/PAS and P3/GAF are the minimal domains necessary for PHYB activity (90).

Unlike fusions involving the N-terminal domain of PHYB, fusion of the N-terminal domain of PHYA to GUS and NLS (PHYA-65-GFP-GUS-NLS) causes weak but constitutive photomorphogenic phenotypes, including shorter hypocotyls and cotyledon opening in the dark (74). PHYA-65-GFP-GUS-NLS shows an effective VLFR, but no FR-HIR. This suggests that, unlike the N-terminal domain of PHYB, the N-terminal domain of PHYA is sufficient for VLFR, but not for FR-HIR. Why the N-terminal domains of PHYA and PHYB behave differently remains unclear, but this result is consistent with the differences observed between PHYB^{Y276H} and PHYA^{Y242H} (117).

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Although the N-terminal domains of phytochromes contain the essential photochemical and photobiological activities, the C-terminal domain also plays important roles for the proper function of the intact proteins, as indicated by the phenotypes described for numerous nonsense and missense mutations in the C-terminal domain (99, 140). The two most obvious functional motifs in the C-terminal domain are a dimerization motif and a nuclear localization signal (**Figure 2**). The dimerization domain has been roughly mapped to a region that includes parts of the PAS-A and PAS-B domains (30, 51). However, bacterial phytochromes without PAS-A and PAS-B domains still dimerize (65), suggesting that the HKRD may also contribute to the dimerization of intact phytochromes. The nuclear localization signal has been roughly mapped to a region that also includes parts of the PAS-A and PAS-B domains. The GFP-fused C-terminal domain of PHYB constitutively localizes to the nucleus and constitutively forms nuclear speckles, bright fluorescent spots in the nucleus that can also be seen in light-activated GFP-fused full-length phytohcromes (103, 126). A region that includes the PAS-A and PAS-B domains (amino acids 594–917) showed robust nuclear localization when fused to YFP (16). However, this region is not sufficient for the formation of nuclear speckles, suggesting that the formation of nuclear speckles requires both the PAS-A, PAS-B, and the HKRD domains. A few loss-of-function missense mutations found in the PAS domain also failed to show nuclear localization (G674D, A719V, and G767R of PHYB), further supporting the notion that the two PAS domains are important for proper nuclear localization (16, 76). Collectively, these data show that the PAS-A and PAS-B domains of PHYB are necessary for dimerization and nuclear localization, whereas PAS-A, PAS-B, and the HKRD domains are necessary for nuclear speckle formation. Unlike the PHYB C-terminal domain, the C-terminal domain of PHYA provides the dimerization motif but not the nuclear localization signal, which is provided instead by its interacting proteins, FHY1 (FAR-RED ELONGATED HYPOCOTYL 1) and FHL (FHY1-LIKE) (40, 41).

Finally, at least one domain must be responsible for the serine/threonine kinase activity that governs phytochrome autophosphorylation and phytochrome-directed phosphorylation of other proteins, such as PHYTOCHROME-INTERACTING FACTOR 3 (PIF3) (55, 147). The functional significance of this kinase activity remains unknown, but it may play a key role in signaling. HKRD was initially suggested to be a kinase domain because of its relatedness to bacterial histidine kinase. Deletion analysis, however, shows that the N-terminal domain has full kinase activity toward itself and PIF3, indicating that the kinase domain resides in the N-terminal domain (J. Kim, unpublished data) (**Figure 2**). Future work will be required to determine the exact location and functional significance of the kinase domain.

Light-Induced Structural Changes in Plant Phytochromes

Several lines of evidence indicate that the conversion between Pr and Pfr is accompanied by protein structural changes. Circular dichroism (CD) analysis shows that the α-helix content increases by 5% when Pr is converted to Pfr (22). Diffusion coefficient measurements show that the surface for intermolecular hydrogen bonding increases markedly during the conversion to Pfr (31). More directly, probing with tryptophan-modifying 2-hydroxy-5-nitrobenzyl bromide (HNB-Br) shows that two tryptophan residues (W773 and W777) in the C-terminal domain of oat PHYA are modified preferentially in the Pfr form; probing with cysteine-modifying iodoacetamide shows that a cysteine residue (C311) in the N-terminal domain of oat PHYA is also selectively modified in the Pfr form (68, 136). These findings indicate that the Pr to Pfr conversion is accompanied by exposure of the N-terminal P3/GAF domain

(C311) and the C-terminal PAS-A and PAS-B domains (W773 and W777). The simultaneous exposure of these relatively distant amino acids in response to red light may indicate the presence of light-dependent intramolecular interactions between these two regions. Indeed, yeast two-hybrid experiments and in vitro binding assays show that the N-terminal domain (227–651) and the C-terminal domain (594–917) of PHYB interact with each other in the dark, but dissociate upon irradiation with red light (16) (**Figure 1**). Although the chemical probing experiments involved oat PHYA and the intramolecular binding experiments involved *Arabidopsis* PHYB, the two data sets are consistent in supporting the notion that red light induces structural changes that lead to the dissociation and subsequent opening of the P3/GAF domain and the PAS-A and PAS-B domains (**Figure 1**).

Dissociation of the N- and C-terminal domains may expose the nuclear localization signal, but it does not confer phytochrome activity per se. When the N-terminal domain of PHYB or PHYA is fused to a dimerization motif and a nuclear localization motif, the chimeric photoreceptor is constitutively localized to the nucleus. Unlike $PHYB^{Y276H}$, however, N651G-GUS-NLS (or N450G-GUS-NLS) is not constitutively active, but rather requires red light for activation (76, 90, 117) (**Figure 3**). This suggests that photoisomerization causes structural changes that not only expose the C-terminal NLS, but also change the N-terminal domain in some way to allow signaling. Interestingly, some phytochrome-interacting proteins preferentially bind to the Pfr form (see **Supplemental Table 1** in **Supplemental Material**), suggesting that the structural changes associated with photoisomerization may also provide the interacting surfaces for partner proteins.

Nuclear Translocation of Plant Phytochromes

The presumed structural changes required to generate the Pfr form, including exposure of the P3/GAF, PAS-A, and PAS-B domains, initiate signaling events that induce various light responses in plants. These signaling events could occur in the cytosol for the regulation of various light responses, such as cytoplasmic motility and chloroplast movement, or the light-dependent structural changes could induce translocation of the Pfr form into the nucleus, where it could initiate other signaling events. The C-terminal domain of PHYB constitutively localizes to the nucleus irrespective of light conditions and forms nuclear speckles (126), whereas full-length PHYA or PHYB localize to the nucleus and form nuclear speckles upon light irradiation (58, 141). In the absence of light, the nuclear localization activity of the C-terminal domain is blocked by the N-terminal P3/GAF and P4/PHY domains, as indicated by the observation that a truncated PHYB protein lacking the P1 and P2/PAS domains still shows light-dependent nuclear localization (16). Because these N- and C-terminal regions interact with each other in the dark, and amino acids in these regions are exposed upon the perception of red light, structural changes in these regions likely cause the translocation of phytochromes into the nucleus.

Detailed analysis of such nuclear translocation indicates, however, that the various phytochromes behave differently in response to different light spectra. For example, PHYB-GFP moves into the nucleus and forms nuclear speckles (also called nuclear bodies) in response to red light but not far-red light (58). However, nuclear speckle formation is not strictly dependent on light. Analysis of PHYB localization in seedlings grown under light/dark cycles reveals that the number of PHYB nuclear speckles increases at dawn in anticipation of incoming light, suggesting that nuclear translocation or nuclear speckle formation is regulated not only by light, but also by circadian rhythm (57, 141). Unlike PHYB-GFP, PHYA-GFP moves into the nucleus and forms nuclear speckles in response to both white/red and far-red light (58). Under white/red light, however, PHYA is rapidly

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degraded, meaning that the PHYA-GFP signal decreases under red light and PHYA-GFP nuclear speckles persist in the long-term only under far-red light. The nuclear localization behavior of PHYA-GFP is consistent with the functional properties of PHYA, which is activated by all spectra of light including far-red light. In contrast, PHYC, PHYD, and PHYE fusions to GFP constitutively localize to the nucleus in light/dark grown seedlings, but their nuclear speckle formations are either light-dependent (PHYC-GFP and PHYE-GFP) or light-independent (PHYD-GFP) (57). As with PHYB-GFP, other PHY-GFPs (except for PHYD-GFP) show increased nuclear speckle formation at dawn in anticipation of incoming light. Consistent with these differences among the various PHYs, their nuclear localization kinetics also differ. PHYA-GFP signals are apparent after 15 min of light irradiation, whereas the PHYB-GFP signal appears after 2 h. These complex patterns of nuclear localization and speckle formation by different phytochromes suggest that light-dependent structural changes might not be identical among the various phytochromes. Alternatively, translocation and speckle formation may be regulated not only by light-induced structural changes, but also by other specific factors.

The precise nature of the above-described nuclear speckle is unknown, but speckle sizes and numbers may vary. In animal cells, similar nuclear speckles are associated with transcription, RNA processing, and protein degradation (37). In *Arabidopsis*, nuclear speckles are formed by a few other light signaling-related proteins, including cryptochromes, CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), and PIF3, suggesting that at least a part of the light signaling process occurs in nuclear speckles (7, 60, 130). However, whether speckle formation is a prerequisite for phytochrome function is unclear. Many missense mutants fail to form speckles, including the loss-of-function mutations E777K and G788E of PHYA and A776V and E838K of PHYB, suggesting that nuclear speckle formation may be associated with phytochrome function (15, 145). In contrast, no speckles are formed by N651G-GUS-NLS and N450G-GUS-NLS fusion proteins, which are more active than full-length PHYB, or by PHYA-65-GFP-GUS-NLS, which is functional for VLFR (74, 76, 90). Similarly, the C-terminal domain of PHYB, which does not affect PHYB-mediated light signaling, still forms nuclear speckles (126). Thus, future identification of protein components associated with the PHY-based speckles will be necessary to help determine the precise function of nuclear speckles.

LIGHT INFORMATION PERCEIVED BY PLANT PHYTOCHROMES

Wavelength and Irradiance Information

Light information is consistent in wavelength, irradiance, direction, and periodicity. Among these values, wavelength and irradiance information is represented by the concentration of the biologically active Pfr form available in a given plant cell. This property can be more easily understood by calculating the change of Pfr concentration at the photoequilibrium state. The concentration of Pfr in vivo can be expressed as the following (see **Supplemental Material** for the kinetics calculation):

$$Pfr = \frac{\sum_\lambda \varepsilon_{r\lambda}\phi_{r\lambda}I_\lambda}{\sum_\lambda \varepsilon_{r\lambda}\phi_{r\lambda}I_\lambda + \sum_\lambda \varepsilon_{f\lambda}\phi_{f\lambda}I_\lambda + k_d + k_r} Pt \quad (1)$$

where *Pr* and *Pfr* indicate the concentrations of the Pr and Pfr forms, P_t is the concentration of total phytochrome ($Pr + Pfr$), $\varepsilon_{r\lambda}$ and $\varepsilon_{f\lambda}$ are the extinction coefficients of Pr and Pfr, respectively, for a given wavelength λ, $\phi_{r\lambda}$ and $\phi_{f\lambda}$ are the quantum yields of the Pr-to-Pfr and Pfr-to-Pr conversions, respectively, for a given wavelength λ, I_λ is the irradiance of light for a given wavelength λ,

k_d is the rate constant for Pfr protein degradation, and k_r is the rate constant for dark reversion.

Equation 1 indicates that the concentration of Pfr is the function of total phytochrome concentration (P_t), wavelength (λ), and irradiance (I). First, the concentration of Pfr is linearly proportional to P_t in a given cell, indicating that plants that contain higher amounts of total phytochromes have correspondingly higher amounts of Pfr under a given light condition. This explains why phytochrome overexpression causes stronger light responses in transgenic plants compared with wild-type plants under the same light conditions (11, 132). Second, the extinction coefficients and quantum yields vary depending on the wavelength, meaning that the concentration of Pfr varies by wavelength even under identical light irradiance and total phytochrome amounts. Owing to these varying extinction coefficients and quantum yields, phytochromes can extract wavelength information from light and convert it into the concentration of biologically active Pfr in a cell. Third, the concentration of Pfr is a rational function with irradiance as a variable, indicating that the concentration of Pfr increases with increasing irradiance. However, the concentration of Pfr does not increase linearly; instead, the value approaches an asymptotic value, which explains why the light response reaches a plateau as light irradiance increases. *Pt* also varies depending on irradiance in some plant species, meaning that the exact relationship between Pfr concentration and irradiance is more complicated in planta. Nevertheless, this simplified kinetic calculation shows how phytochromes convert wavelength and irradiance information into a concentration of the biologically active Pfr form.

Directional Light Information

Plants perceive information regarding light direction and adjust their physiological and developmental processes accordingly (137). Phototropism and chloroplast movement are two well-known examples of such responses. Blue light is the major light spectrum that provides directional information to plants. Among the blue light photoreceptors, phototropins are responsible for recognizing blue light directional information (18, 56). Directional information of red light has also been implicated in phototropism and chloroplast movement. Most research regarding the perception of red light directional information has been carried out in ferns and filamentous alga, which perceive red light directional information through neochromes rather than canonical phytochromes (52, 119). In *Physcomitrella*, however, a canonical phytochrome perceives the directional information of red light and regulates chloroplast movement (78). Other reports show that red light–induced root phototropism is impaired in *Arabidopsis phyA* and *phyB* mutants (21, 59), and the FR-induced negative shoot phototropism is defective in the cucumber *phyB* mutant (*lh*) (70), suggesting that canonical phytochromes can perceive light directional information in plants.

However, we do not yet fully understand how phytochromes perceive red light directional information. Because chlorophylls contained within chloroplasts strongly absorb red and blue light, phytochromes located behind chloroplasts are less likely to be converted to Pfr. Direct measurement shows that the irradiance of red light drops to 15% of the initial value within the first half of the palisade cells in *Medicago sativa* leaves (128), suggesting that a Pfr concentration gradient may be formed along the light directional axis, even in a single palisade cell. At the tissue level, the absorption of red light by the chloroplasts of outer layer cells could greatly reduce the red light irradiance that reaches inner layer cells. Therefore, red light directional information is converted into a Pfr concentration gradient among cells, which in turn generates gradients of both nuclear and cytoplasmic light signaling events along the axis of light. Further research is warranted to determine whether the formation of a Pfr concentration gradient within a cell or

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among cells has any physiological significance in higher plants.

Photoperiod Information

Plants perceive photoperiod information through phytochromes, cryptochromes, and zeitlupes, and regulate various physiological and developmental processes accordingly. Flowering is one of the best-studied photoperiod-regulated developmental processes. Molecular analysis of photoperiodic flowering responses in model species suggests that the external coincidence between a circadian phase and light is used for photoperiod perception (45). In *Arabidopsis*, two floral integrator genes, *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF CONSTANS* (*CO*) *1* (*SOC1*), integrate various flowering signals from the autonomous, gibberellin, photoperiod, and vernalization pathways (94). CO, a B-box zinc finger protein, plays a critical role in translating photoreceptor-perceived light signals into expression of the *FT* and *SOC1* genes. The expression of *CO* is under control of the circadian rhythm; expression reaches a broad peak between 12:00 hours and dawn in LD conditions (118). Day length has a mild but significant impact on the expression pattern of *CO*. In SD conditions, the expression peak at 12:00 hours disappears. This earlier expression peak of *CO* is induced by the targeted degradation of CDF1 by FKF1 in the presence of blue light; the expression of FKF1 is in turn regulated by a circadian clock (46, 47, 82). Therefore, the earlier *CO* expression peak in the LD condition is the product of coincidence between external light and the afternoon phase of the circadian clock.

The protein stability of CO is also regulated by light. CO is destabilized by the action of PHYB in the morning, stabilized by PHYA and CRYs in the afternoon, and destabilized by the SUPPRESSOR OF PHYA proteins (SPA1, SPA3, and SPA4) in the night (69, 127). This transcriptional and posttranscriptional regulation produces a peak of CO protein levels in the late afternoon only under LD conditions when external light and the dusk phase coincide, but not under SD conditions. In response to the differential accumulation of CO protein in LD and SD, *FT* is expressed at high levels only in LD, resulting in flowering of *Arabidopsis*. A similar external coincidence between circadian clock and external light is proposed to regulate photoperiodic flowering responses not only in other LD plants such as *Populus* (8), but also in SD plants such as rice (38, 48). In rice, the external coincidence between the circadian clock and light represses *Hd6*, the rice homolog of *FT*, inhibiting flowering under LD conditions. It will be interesting to determine if any of the rice zeitlupes also participate in the perception of photoperiod information.

PHYTOCHROME-INTERACTING PROTEINS

Phytochromes convert perceived light information into absolute concentrations and/or concentration gradients of the Pfr form, leading to the regulation of various physiological and developmental processes in plants. How is the concentration of the Pfr form translated into other biological signals? Because phytochromes do not possess any known biochemical activities other than serine/threonine kinase activity, phytochromes are believed to regulate their downstream processes by interacting with other proteins. Consistent with this notion, phytochrome-interacting proteins have been identified by several different approaches. The most widely used approach is yeast two-hybrid screening; the C-terminal domain is typically used as a bait, but the N-terminal domain or the full-length phytochrome are also sometimes used. Once a putative interacting protein is identified by screening or other methods such as targeted testing, the interaction between phytochromes and the candidate interacting proteins is generally confirmed by an in vitro binding assay, in vivo pull-down assay, or in vivo colocalization test. The functional

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significances of many interacting proteins have been further confirmed by genetic analysis. To date, more than 20 phytochrome-interacting proteins have been reported in the literature (see **Supplemental Table 1** in the **Supplemental Material** for the identities and functions of all phytochrome-interacting proteins). Here we focus on a few representative interacting proteins and use them to delineate how phytochrome-perceived light information is transmitted to downstream components.

Phytochrome-Interacting Proteins That Regulate the Nuclear Localization of Phytochromes

Some phytochrome-interacting proteins are needed for the nuclear/cytoplasmic partitioning of phytochromes. In eukaryotes, proteins larger than ~40 kD must be actively transported into or out of the nucleus through nuclear pore complexes, with the help of transport proteins such as importins and exportins (115). Because phytochrome dimers are approximately 240 kD in size, they require interacting proteins for their nuclear localization. Among the identified phytochrome-interacting proteins, FHY1 and FHL, which interact with PHYA but not with PHYB and promote the translocation of PHYA to the nucleus, fit the definition of this class of phytochrome-interacting proteins (**Figure 4**).

The history of FHY1 essentially parallels the history of the molecular genetic analysis of PHYA signaling in *Arabidopsis*. The *fhy1* mutant was the first *Arabidopsis* PHYA signaling mutant isolated; *fhy1* was first reported in 1993 together with *fhy2* (*phyA* mutant) and *fhy3* (138). *fhy1* mutant plants contain a normal amount of PHYA, but are partially defective in far-red-induced de-etiolation processes (FR-HIR) (138). Numerous light responses are affected in the *fhy1* mutant, including seed germination (50), hypocotyl elongation under a low R/FR ratio (50), far-red–induced inhibition of greening (5), induction of the *CHALCONE SYNTHASE* (*CHS*) gene (6), functional interactions between PHYA- and PHYB signaling (13), and the far-red-induced phase shift (146). In contrast, a few processes remain unaffected in these mutants, including flowering under low R/FR and extended short day conditions (50) and induction of the *CHLOROPHYLL a/b BINDING PROTEIN* (*CAB*) gene (6). These phenotypes indicate that FHY1 is responsible for mediating a branch of PHYA signaling. Microarray analysis of *fhy1*, however, shows that all genes affected by the *phyA* mutation are also affected by the *fhy1* mutation, but to a lesser degree (135). This result suggests that the incompleteness of the phenotypic defects in the *fhy1* mutant is likely due to redundancy. More recently, researchers identified an *FHY1* homolog called *FHL*; the double loss-of-function mutant (*fhy1 fhl*) is indistinguishable from the *phyA* mutant, indicating that PHYA requires FHY1 and FHL for complete function (150).

FHY1 and *FHL* encode 202-amino-acid and 181-amino-acid proteins, respectively, both of which contain a NLS and a nuclear exclusion signal (NES) at their N termini and a septin-related domain (SRD) at their C termini (26, 150). In vitro binding assays show that the two proteins are capable of both homo- and heterodimerization through their C-terminal domains (150). The NLS and SRD are functionally important, because the removal of those domains disrupts the function of FHY1 (149).

FHY1 and FHL are required for the nuclear localization of PHYA-GFP; this nuclear localization is significantly reduced in the *fhy1* mutant and is virtually absent in *fhy1 FHL* RNA interference (RNAi) lines under both high irradiance response (HIR) and VLFR conditions (40, 41). The nuclear localization of PHYB, however, is not affected in these mutants, indicating that FHY1 and FHL are needed for the nuclear localization of PHYA, but not PHYB. The role of FHY1 in the nuclear localization of PHYA is associated with its ability to interact with the

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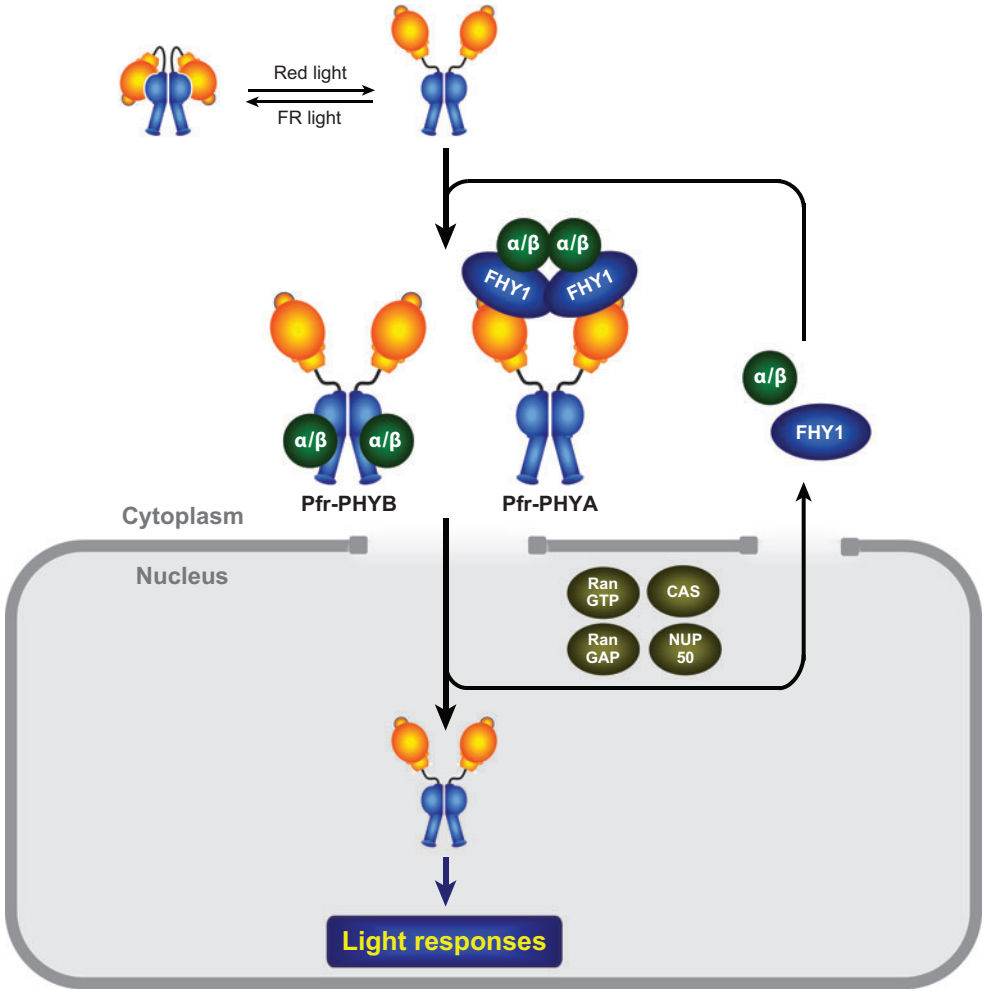

Figure 4

Regulation of phytochrome nuclear localization by two phytochrome-interacting proteins, FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) and FHY1-LIKE (FHL). The involvement of importins (α/β) and other general components [RanGTP, nucleoporin 50 (NUP50), cellular apoptosis susceptibility (CAS), and RanGAP] has not been proven yet. FHL is not shown in the figure. FR, far-red; Pfr, C15-*E*,*anti* conformation of phytochrome.

Pfr form of this phytochrome. The interaction between the Pfr form of PHYA and FHY1/FHL occurs through the N-terminal domain of PHYA (amino acids 1–406) and the SRD of FHY1/FHL, as shown by yeast two-hybrid screening and in vitro binding assays (40). The interaction is further corroborated by the colocalization of YFP-FHY1/FHL and PHYA-CFP in nuclear speckles.

These findings indicate that the following sequence of events occurs in the nuclear translocation of PHYA (**Figure 4**): absorption of light by phytochromobilin → photoisomerization and accompanying structural changes that expose the N-terminal domain → binding of FHY1/FHL to the exposed N-terminal domain → translocation of the PHYA-FHY1/FHL complex into the

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nucleus via the NLS of FHY1/FHL → formation of nuclear speckles. As noted, the above-described sequence holds true only for PHYA, because the nuclear translocation of PHYB does not require the function of FHY1 and FHL (41). However, photoisomerization also likely exposes the N- and C-terminal domains of PHYB (16). Because the exposed C-terminal domain of PHYB contains a functional NLS (16, 103), the Pfr form of PHYB might be functionally equivalent to the PHYA-FHY1/FHL complex.

A few additional results indicate the need for further functional characterization of FHY1 and FHL. First, YFP-FHY1 and YFP-FHL both form nuclear speckles with PHYA-CFP in a manner that suggests the formation of stable, not temporary, complexes inside the nucleus (41). If FHY1 and FHL merely act to carry PHYA into the nucleus, they should dissociate from PHYA in the nucleus and return to the cytosol for another round of translocation. Second, PHYA promotes the degradation of the FHY1 protein through the 26S proteasome (109). This degradation could be a part of a negative feedback loop, but additional studies are required to examine why PHYA would help to degrade its carrier. Overexpression of constitutively nuclear-localized PHYA in the *fhy1 fhl* double mutant will be informative as to whether the function of FHY1/FHL is limited to the translocation of PHYA into the nucleus.

Phytochrome-Interacting Proteins That Modulate the Output Activity of Phytochromes

Some phytochrome-interacting proteins modulate the signaling output of phytochromes under a given light condition (**Figure 5**). Because the Pfr form is the biologically active form, some proteins are expected to interact with the Pfr form and regulate its output activity by either altering the concentration of Pfr or modulating its ability to transmit signals to downstream components. The phytochrome output activity can also be modulated either by altering its affinity for its downstream component or by changing its transmitting activity. Functionally, the expression levels or activities of these interacting proteins in a given plant cell will determine the signal output by phytochromes, allowing phytochrome signaling to be fine tuned in accordance with the plant's developmental and physiological status.

ARR4, which binds to PHYB and stabilizes the Pfr form, was the first identified phytochrome-interacting protein that modulates the output activity of phytochromes (120). *Arabidopsis* contains 10 type-A *Arabidopsis* response regulators (ARRs), which act as negative regulators of cytokinin signaling (123). Inspired by the histidine kinase activity of cyanobacterial phytochrome (CphI) (148), ARR4 was selected as a candidate for a signaling protein because the ARR4 protein is accumulated by red light in a PHYB-dependent manner. ARR4 binds to the N-terminal end of PHYB (amino acids 1–137), as proven by in vitro binding assays, in vivo coimmunoprecipitation analysis, and yeast two-hybrid experiments. The binding of ARR4 inhibits the dark reversion of PHYB in both yeast and plants. An aspartate residue of ARR4 is phosphorylated by cytokinin receptors. Interestingly, a mutated ARR4 (ARR^{D95N}) cannot inhibit dark reversion (77), suggesting that the ability to inhibit dark reversion is dependent on the phosphorylation of an aspartate residue. Consistent with their molecular characteristics, overexpression of ARR4 but not ARR^{D95N} is associated with shorter hypocotyl length under red light. Taken together, these studies show that ARR4 binds to the Pfr form of PHYB and increases its output activity by inhibiting the dark reversion rate of PHYB.

A few other interacting proteins that modulate phytochrome output activity have been identified (**Figure 5**). COP1 binds to PHYA and decreases PHYA output activity by decreasing the total PHYA concentration (P_t) (104). PAPP5 binds to both PHYA

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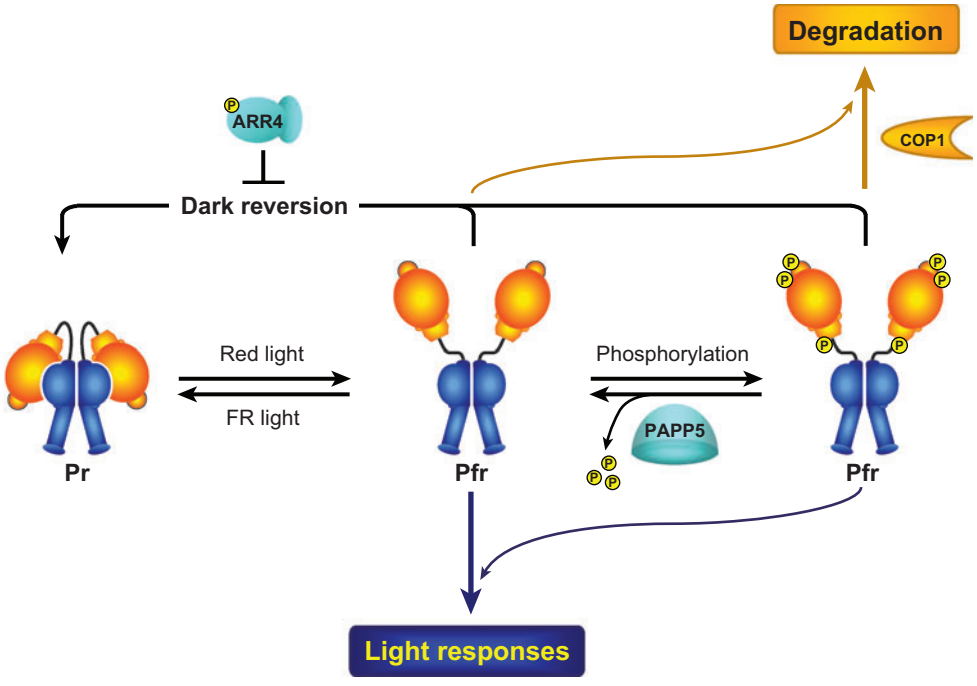

Figure 5

Modulation of phytochrome (PHY) output activity by three representative phytochrome-interacting proteins, *ARABIDOPSIS* RESPONSE REGULATOR 4 (ARR4), CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), and PHYTOCHROME ASSOCIATED PHOSPHATASE 5 (PAPP5). ARR4 specifically inhibits the dark reversion of PHYB, whereas COP1 ubiquitinates the Pfr form of PHYA. Whether the Pfr form (C15-*E*,*anti* conformation) of PHYB is also ubiquitinated by COP1 is unknown. FR, far-red; Pr, C15-*Z*,*anti* conformation of PHY.

and PHYB and preferentially dephosphorylates the Pfr form (101). The dephosphorylation of phytochromes by PAPP5 increases their affinity for the interacting proteins nucleoside diphosphate kinase 2 (NDPK2) and PIF3, increases the stability of PHYA, and increases the stability of the Pfr form, suggesting that PAPP5 is a versatile regulator that enhances phytochrome output activity.

Phytochrome-Interacting Proteins Whose Activities are Modulated by Phytochromes

Some phytochrome-interacting proteins directly regulate the light responses, allowing the phytochromes to indirectly regulate various light responses by binding to these proteins and modulating their activities. This class of interacting proteins includes basic helix-loop-helix transcription factors such as PIF3 and PIF3-like 5 (PIL5), as well as other proteins such as COP1 (**Figure 6**).

PIF3 was the first phytochrome-interacting protein to be identified, and its characterization provides a framework for understanding how phytochromes regulate their downstream components. PIF3 was originally identified by yeast two-hybrid screening that used the C-terminal domain (amino acids 645–1210) of PHYB as bait (83). The binding between PIF3 and phytochromes (PHYA and PHYB) was further confirmed by numerous in vitro binding

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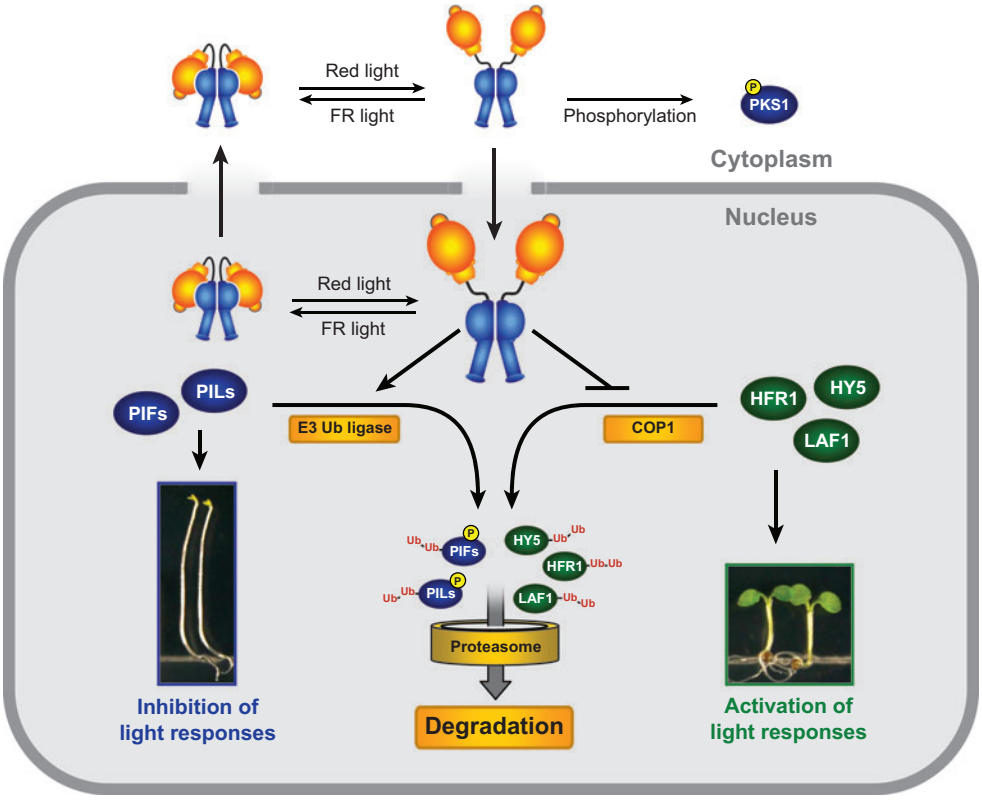

Figure 6

Activation of light responses by phytochromes (PHY) and their interacting proteins. Light responses are repressed in the dark, because negative components such as phytochrome-interacting factors (PIFs)/PIF3-like proteins (PILs) inhibit light responses, whereas positive components such as LONG HYPOCOTYL IN FAR-RED 1 (HFR1), LONG HYPOCOTYL 5 (HY5), and LONG AFTER FAR-RED LIGHT 1 (LAF1) are degraded by the nuclear-localized CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1). Upon irradiation, the Pfr forms (C15-*E*,*anti* conformation) of phytochromes initiate cytosolic light responses by binding cytosolic interacting proteins such as PHYTOCHROME KINASE SUBSTRATE (PKS1) or enter the nucleus with (PHYA) or without (PHYB) the help of FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) or FHY1-LIKE (FHL). In the nucleus, the Pfr forms activate the degradation of PIFs/PILs through an unidentified E3 ubiquitin (Ub) ligase and inhibit COP1 by excluding it from the nucleus. Owing to decreasing levels of negative components and increasing levels of positive components, light responses are initiated. FR, far-red.

assays and in vivo colocalization analysis (7, 73, 151). Although PIF3 was identified using the C-terminal domain of PHYB, the mapping of the interacting domains shows that PIF3-PHYB binding is not confined to the C-terminal domain of PHYB. In vitro binding assays and yeast two-hybrid analysis supplemented with chromophore show that both the PHYB C-terminal domain alone and the N-terminal domain alone are sufficient for PIF3 binding (84, 110, 151). However, the exact location of this binding activity remains unclear. Deletion of the N-terminal 90 amino acids and the C-terminal 50 amino acids of PHYB virtually eliminates the interaction (151). Loss-of-function missense mutations in the PAS-A and PAS-B domain of PHYB (A776V, G793R, and E838K) also inhibit the

interaction between PIF3 and PHYB (83, 84). Taken together, the results suggest that although N- and C-terminal fragments of PHYB can bind to PIF3 individually, overall structural integrity of PHYB is required for proper binding.

The binding domains of PIF3 also display complicated features. Updated mapping of binding domains shows that a part of the PIL domain of PIF3 (amino acids 13–59), dubbed the APB (for active phytochrome binding motif; amino acids 27–39), but not the putative PAS domain, is necessary and sufficient for the binding between PHYB and PIF3 (53). Binding assays show that the APB of PIF3 binds specifically to PHYB but not to other phytochromes (PHYA, PHYC, PHYD, or PHYE). Further yeast two-hybrid experiments supplemented with chromophore show that PHYA does not bind to the APB, but instead binds to another motif called the APA (for active PHYA binding motif; amino acids 193–210 of PIF3) (2). Alanine substitution of two phenylalanine residues within the APA (F203A and F209A) eliminates the binding. It should be noted that the APA motif contining two phenylalanines is not present in other PIF/PIL proteins except for a distantly related amino acid sequence found in PIL5, suggesting that the APA motif and the functionality of its two phenylalanine residues could be specific to PIF3. Thus, although both PHYA and PHYB bind to PIF3, the precise interacting domains or motifs have not yet been clearly resolved.

The functional significance of this binding is the degradation of PIF3. Red light irradiation causes rapid degradation of PIF3, as shown by the disappearance of both endogenous PIF3 and overexpressed PIF3 tagged with GFP or myc upon irradiation with red or far-red light (7, 95). PHYA is responsible for the rapid degradation of PIF3 in response to far-red light, whereas PHYA, PHYB, and PHYD are responsible for this degradation in response to red light. The degradation of PIF3 is partly associated with the nuclear speckles seen in GFP-tagged phytochromes, as shown by the rapid colocalization of PHY-YFP and PIF3-CFP and the subsequent disappearance of the PIF3-CFP signal upon irradiation. Early nuclear speckle formation (2 min after the red pulse) by PHYB is disrupted in the *pif3* mutant *photocurrent 1* (*poc1*); however, late nuclear speckle formation (6 h after a red pulse) is not disrupted, indicating that only the early PHYB nuclear speckles are dependent on PIF3. Because N651G-GUS-NLS does not form nuclear speckles (76), it will be interesting to see if N651G-GUS-NLS can still activate the degradation of PIF3 without forming nuclear speckles.

Treatment with 26S proteasome inhibitors blocks the degradation of PIF3 following irradiation (95). Because the 26S proteasome mainly degrades ubiquitinated proteins, this observation suggests that phytochromes activate the ubquitination and subsequent 26S proteasome-mediated degradation of PIF3. This hypothesis is supported by the appearance of very high molecular weight PIF3-immunoreactive bands after light irradiation, and these bands cross-react with an antiubiquitin antibody. Similar to PIF3, a few other phytochrome-interacting bHLH proteins, including PIL2/PIF6, PIF4, PIL5/PIF1, and PIL6/PIF5, are also degraded through the 26S proteasome by light (85, 89, 108), indicating that phytochromes activate degradation of PIFs/PILs by promoting ubiquitination.

The molecular mechanisms by which phytochromes activate the degradation of PIF3 are not clearly understood. The light-mediated activation of phytochromes causes the rapid appearance of higher molecular weight PIF3 bands (2 min after red pulse) in sodium dodecyl sulfate (SDS) gels (2). These band shifts are abolished in the *phyA phyB* double mutant, which also shows significantly reduced PIF3 degradation. Similarly, deletion of the phytochrome-binding motifs of PIF3 abrogates both the band shifts and the degradation, suggesting that the band shifts are correlated with PIF3 degradation. Phosphatase treatment causes the shifted bands to

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disappear, suggesting that the band shift is caused by phosphorylation. The experimental evidence present in the literature, however, makes it difficult to distinguish whether the phosphatase treatment converts the shifted bands to the lower band versus selectively degrading the shifted bands. In addition, the shifted bands are not a single molecular weight band, but rather form a multiple band continuum (95). Within 30 minutes after light treatment, PIF3 can be detected as a high molecular weight smear, suggesting that the band shift is not a single event. The initial small band shift may be caused by phosphorylation and the later higher molecular weight bands could be caused by other modifications, such as ubiquitination. Because phytochromes can phosphorylate PIF3 in vitro, it is tempting to postulate that activated phytochromes bind and phosphorylate PIF3, which may then be ubiquitinated by an E3 ubiquitin (Ub) ligase, leading to degradation by the 26S proteasome. Future characterization of the various shifted bands will help clarify the sequence of molecular events that leads to degradation of PIF3.

Irrespective of the underlying molecular mechanism, the degradation of PIF3 and other PIFs/PILs by light likely inhibits the function of PIFs/PILs. PIF3 acts as a negative component in both PHYA- and PHYB-mediated seedling de-etiolation processes such as hook opening, whereas it selectively acts as a negative component in PHYB-mediated inhibition of hypocotyl elongation (54). In adult plants, overexpression of PIF3 causes elongated petioles, pale green leaves, and early flowering, which is also observed in the *phyB* mutant. Two potential exceptions are anthocyanin biosynthesis under far-red light and chloroplast development during the dark-light transition. PIF3 positively regulates anthocyanin biosynthesis under far-red light by directly binding to the promoters of anthocyanin biosynthetic genes via G-box elements, and activating their transcription in the presence of HY5 (LONG HYPOCOTYL 5), a basic leucine zipper (bZIP) transcription factor (111). However, because the level of PIF3 protein in continuous far-red light is similar to that in the dark, it is difficult to infer the precise functional relationship between PIF3 and phytochromes during the expression of anthocyanin biosynthetic genes. In chloroplast development, PIF3 is suggested to act as a positive component, especially when etiolated seedlings are transferred to light (80). More careful examination, however, indicates that the seemingly retarded chloroplast development in the *pif3* mutant is due to light-induced bleaching rather than retarded chloroplast development (G. Choi, unpublished data). Thus, PIF3 inhibits all tested light responses in the dark and phytochromes release this inhibition by removing PIF3. Other PIFs/PILs that are degraded by light also mainly act to inhibit light responses in the dark (35, 43, 44, 87). Consistent with their roles in the dark, a *pif3 pif4 pil5 pil6* quadruple mutant is constitutively photomorphogenic even in the dark (G. Choi, unpublished data). Collectively, the apparently negative roles played by the PIFs/PILs suggest that phytochromes induce light responses by degrading negative light signaling components such as PIF3 (**Figure 6**).

Phytochromes also bind to COP1, a master repressor of photomorphogenesis, and negatively regulate COP1 activity in the light (142). The *cop1* mutant was identified as a constitutively photomorphogenetic mutant together with other *cop*/*de-etiolated* (*det*)/*fusca* (*fus*) mutants (24). Molecular characterization shows that *COP1* encodes a protein with a N-terminal RING finger domain followed by a coiled-coil motif and a WD-40 repeat domain (25). COP1 acts as an E3 Ub ligase that ubiquitinates at least three positive light-signaling transcription factors, HY5, LAF1 (LONG AFTER FAR-RED LIGHT 1), and HFR1 (LONG HYPOCOTYL IN FAR-RED 1) (49, 102, 105, 144). Because phytochromes inhibit COP1 activity partially by excluding COP1 from the nucleus (91, 129), three positive light signaling transcription factors are selectively degraded in the

dark and accumulated in the light (29, 42, 93, 105, 144). Apparently, COP1 ubiquitinates and degrades these factors in conjunction with other COP/DET/FUS proteins which are components of the CDD complex (consisting of COP10, DET1, and DDB1) or the COP9 signalosome (CSN) complex (consisting of CSN1 to CSN8) and other RING finger proteins, such as SPA1 (14, 102, 143). The inhibition of COP1 activity by phytochromes and the subsequent accumulation of positive light signaling transcription factors play important roles in the induction of light responses in the light (29, 49, 92, 105). Microarray analysis shows that COP1-regulated genes largely overlap with light-regulated genes (71), further suggesting that COP1 is a master repressor of photomorphogenesis and phytochromes promote photomorphogenesis partly by inhibiting COP1 activity. Thus, phytochromes induce light responses partly by removing negative light signaling transcription factors such as PIFs/PILs through protein degradation and partly by accumulating positive light signaling transcription factors such as HFR1, HY5, and LAF1 by nuclear exclusion of COP1 (**Figure 6**).

THE FLOW OF LIGHT INFORMATION DURING SEED GERMINATION

The overall flow of light information through phytochromes and their interacting proteins to the final light responses can be better exemplified by the regulation of PIL5 (also known as PIF1 and bHLH015) by phytochromes during seed germination (**Figure 7**). Phytochromes promote seed germination partly by increasing bioactive gibberellic acid (GA) levels in seeds (61, 86). The increased GA levels are caused by transcriptional activation of GA biosynthetic genes and transcriptional repression of GA catabolic genes (86). Because phytochromes are not transcription factors per se, it was expected that some phytochrome-interacting proteins may mediate light signaling to modulate GA biosynthesis. PIL5 serves this role.

PIL5 negatively regulates seed germination by inhibiting GA biosynthesis and GA signaling while simultaneously activating abscisic acid (ABA) biosynthesis. PIL5 inhibits GA biosynthesis by repressing two GA synthetic genes (*GA3ox1* and *GA3ox2*) and activating a GA catabolic gene (*GA2ox2*), resulting in lower GA levels in seeds (89, 96). Similarly, PIL5 activates ABA biosynthesis by activating ABA biosynthetic genes (*ABA1*, *NCED6*, and *NCED9*) and repressing an ABA catabolic gene (*CYP707A2*), increasing the ABA levels in seeds (88). In addition, PIL5 also activates the expression of two DELLA genes [*GIBBERELLIC ACID INSENSITIVE* (*GAI*) and *REPRESSOR OF GA 1-3* (*RGA*)], which are key negative GA signaling components (88). Chromatin immunoprecipitation analysis shows that of all the PIL5-regulated genes, PIL5 binds directly to the promoters of only the two DELLA genes, *GAI* and *RGA*, suggesting that PIL5 regulates *GAI* and *RGA* directly, whereas it regulates other biosynthetic genes indirectly. Owing to decreased GA levels, increased DELLA protein levels, and increased ABA levels, seeds do not germinate in the dark.

Phytochromes promote seed germination by inhibiting PIL5 activity. The expression levels of all the abovementioned genes are regulated oppositely by phytochromes in seeds, and this regulation is not present in the *pil5* mutant, suggesting that phytochromes regulate these genes by inhibiting PIL5 activity (88). How do phytochromes inhibit PIL5 activity? Upon light irradiation, the Pfr forms of both PHYA and PHYB enter the nucleus, bind to PIL5, and activate its degradation by the 26S proteasome (43, 87, 89, 108). The effect of PIL5 degradation by phytochromes can be seen in the regulation of PIL5 direct target genes (88). The expression levels of *GAI* and *RGA* genes are high in the

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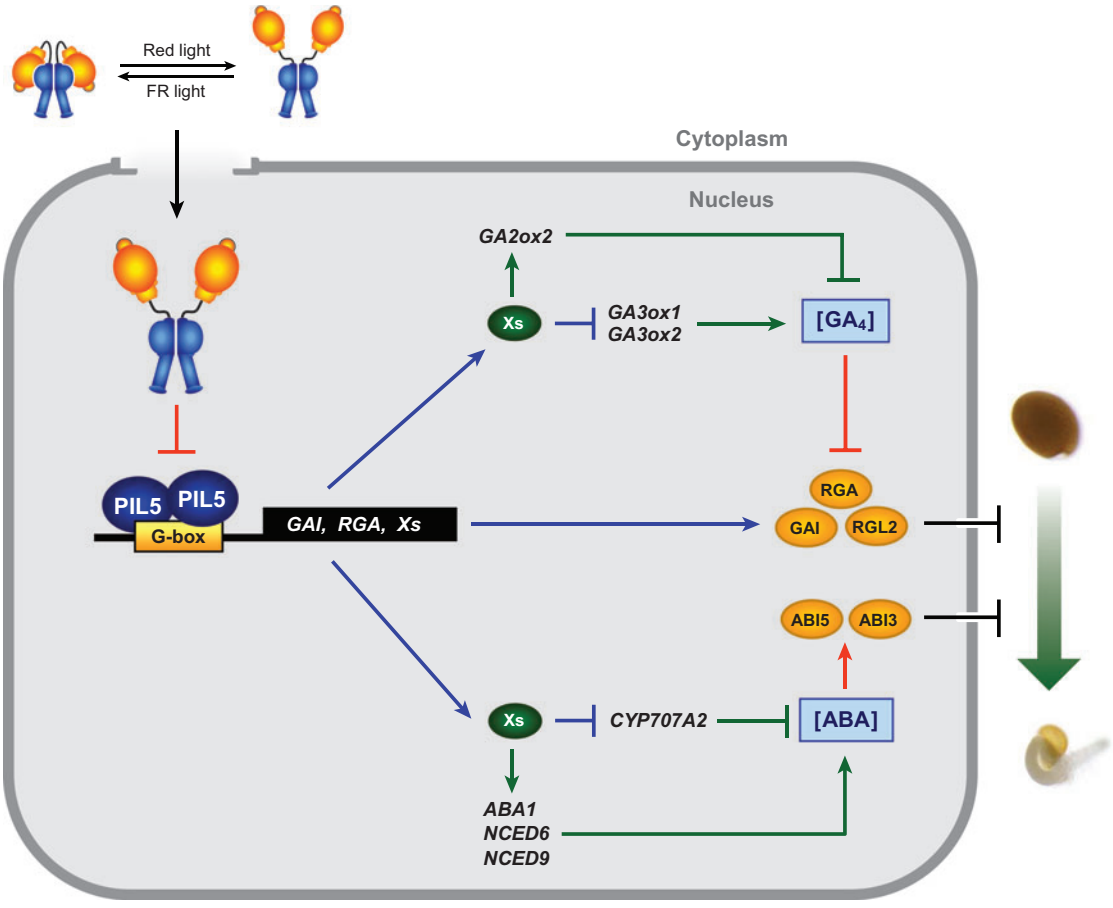

Figure 7

The flow of light information during seed germination. In the dark, PHYTOCHROME-INTERACTING FACTOR 3 (PIF3)-LIKE 5 (PIL5) activates the expression of *GIBBERELLIC ACID INSENSITIVE* (*GAI*), *REPRESSOR OF GA 1-3* (*RGA*), and other unknown factors (*Xs*), by binding directly to their promoters through G-box elements. The unknown factors repress gibberellic acid (GA) biosynthetic and abscisic acid (ABA) catabolic genes and activate GA catabolic and ABA biosynthetic genes, resulting in decreased GA levels and increased ABA levels. The decrease in GA levels further stabilizes GAI and RGA proteins, leading to the suppression of GA responses. The increase in ABA increases the levels of ABA insensitive 3 (ABI3) and ABI5, leading to the activation of ABA responses. Upon light irradiation, the Pfr form of phytochrome (C15-*E,anti* conformation) binds PIL5 and activates the degradation of PIL5. The decreased level of PIL5 translates to decreased levels of GAI, RGA, and X factors, resulting in increased GA levels and decreased ABA levels. Owing to changes in hormone levels and signaling components, seeds start to germinate. Red lines signify events occurring at the protein level, blue lines show events occurring at the transcriptional level, and green lines show events occurring via enzyme activities. RGL, RGA-like. Adapted from Reference 88.

dark; upon light irradiation, PIL5 is rapidly degraded and consequently the expression levels of *GAI* and *RGA* genes decrease. Degradation of PIL5 is further accompanied by altered expressions of GA and ABA biosynthetic genes resulting in increased GA levels and decreased ABA levels. Owing to increased GA levels, decreased DELLA protein levels, and ABA levels, seeds start to germinate in the light.

Taken together, the overall flow of light information during seed germination can be summarized as follows:

1. Light causes the photoisomerization of phytochromobilin.
2. Photoisomerization causes structural changes in phytochromes.
3. Light information is represented by the concentration of Pfr.
4. Pfr enters the nucleus either alone (PHYB) or with the help of FHY1/FHL (PHYA).
5. In the nucleus, Pfr removes PIL5 by initiating its degradation, thus the concentration of Pfr is translated into the level of PIL5.
6. The level of PIL5 is translated into the levels of two plant hormones, GA and ABA, and levels of their signaling components.
7. In response to changing hormonal levels and levels of signaling components, seeds germinate.

FUTURE ISSUES

1. Which biochemical/molecular activities of phytochromes are sufficient to induce light responses? This issue is closely associated with the question of how phytochromes activate the degradation of phytochrome interacting factors (PIFs)/PIF3-like proteins (PILs). Does this occur through kinase activity, or do phytochromes act as adaptor molecules linking the PIFs/PILs to the protein degradation machinery?
2. How are PIFs/PILs degraded? Because the degradation of PIFs/PILs is an important mechanism through which light information is converted to biological signals, it is essential to elucidate the molecular mechanism of PIF/PIL degradation.
3. What are the functional relationships between phytochrome-interacting proteins and other genetically identified light signaling components? Light information processed by phytochromes and phytochrome-interacting proteins must go through a plethora of genetic networks to induce the final responses. Can we define a specific genetic network for each light response?

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Abe H, Yamamoto K, Nagatani A, Furuya M. 1985. Characterization of green tissue-specific phytochrome isolated immunologically from pea seedlings. *Plant Cell Physiol.* 26:1387–99

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2. Al-Sady B, Ni W, Kircher S, Schäfer E, Quail PH. 2006. Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. *Mol. Cell* 23:439–46
3. Aukerman MJ, Hirschfeld M, Wester L, Weaver M, Clack T, et al. 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* 9:1317–26
4. Balasubramanian S, Sureshkumar S, Agrawal M, Michael TP, Wessinger C, et al. 2006. The *PHYTOCHROME C* photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*. *Nat. Genet.* 38:711–15
5. Barnes SA, Nishizawa NK, Quaggio RB, Whitelam GC, Chua NH. 1996. Far-red light blocks greening of *Arabidopsis* seedlings via a phytochrome A-mediated change in plastid development. *Plant Cell* 8:601–15
6. Barnes SA, Quaggio RB, Whitelam GC, Chua NH. 1996. fhy1 defines a branch point in phytochrome A signal transduction pathways for gene expression. *Plant J.* 10:1155–61
7. Bauer D, Viczian A, Kircher S, Nobis T, Nitschke R, et al. 2004. Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in *Arabidopsis*. *Plant Cell* 16:1433–45
8. Bohlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, et al. 2006. CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. *Science* 312:1040–43
9. Borthwick HA, Hendricks SB, Parker MW, Toole EH, Toole VK. 1952. A reversible photoreaction controlling seed germination. *Proc. Natl. Acad. Sci. USA* 38:662–63
10. Boylan M, Quail PH. 1996. Are the phytochromes protein kinase? *Protoplasma* 195:12–17
11. Boylan MT, Quail PH. 1991. Phytochrome A overexpression inhibits hypocotyl elongation in transgenic *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 88:10806–10
12. Casal JJ, Davis SJ, Kirchenbauer D, Viczian A, Yanovsky MJ, et al. 2002. The serine-rich N-terminal domain of oat phytochrome A helps regulate light responses and subnuclear localization of the photoreceptor. *Plant Physiol.* 129:1127–37
13. Cerdan PD, Yanovsky MJ, Reymundo FC, Nagatani A, Staneloni RJ, et al. 1999. Regulation of phytochrome B signaling by phytochrome A and FHY1 in *Arabidopsis thaliana*. *Plant J.* 18:499–507
14. Chen H, Shen Y, Tang X, Yu L, Wang J, et al. 2006. *Arabidopsis* CULLIN4 forms an E3 ubiquitin ligase with RBX1 and the CDD complex in mediating light control of development. *Plant Cell* 18:1991–2004
15. Chen M, Schwab R, Chory J. 2003. Characterization of the requirements for localization of phytochrome B to nuclear bodies. *Proc. Natl. Acad. Sci. USA* 100:14493–98
16. Chen M, Tao Y, Lim J, Shaw A, Chory J. 2005. Regulation of phytochrome B nuclear localization through light-dependent unmasking of nuclear-localization signals. *Curr. Biol.* 15:637–42
17. Cherry JR, Hondred D, Walker JM, Vierstra RD. 1992. Phytochrome requires the 6-kDa N-terminal domain for full biological activity. *Proc. Natl. Acad. Sci. USA* 89:5039–43
18. Christie JM. 2007. Phototropin blue-light receptors. *Annu. Rev. Plant Biol.* 58:21–45
19. Christie JM, Reymond P, Powell GK, Bernasconi P, Raibekas AA, et al. 1998. *Arabidopsis* NPH1: a flavoprotein with the properties of a photoreceptor for phototropism. *Science* 282:1698–701

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20. Clack T, Mathews S, Sharrock RA. 1994. The phytochrome apoprotein family in *Arabidopsis* is encoded by five genes: the sequences and expression of *PHYD* and *PHYE*. *Plant. Mol. Biol.* 25:413–27
21. Correll MJ, Kiss JZ. 2005. The roles of phytochromes in elongation and gravitropism of roots. *Plant Cell Physiol.* 46:317–23
22. Deforce L, Tokutomi S, Song PS. 1994. Phototransformation of pea phytochrome A induces an increase in α-helical folding of the apoprotein: comparison with a monocot phytochrome A and CD analysis by different methods. *Biochemistry* 33:4918–22
23. Dehesh K, Franci C, Parks BM, Seeley KA, Short TW, et al. 1993. *Arabidopsis* HY8 locus encodes phytochrome A. *Plant Cell* 5:1081–88
24. Deng XW, Caspar T, Quail PH. 1991. *cop1*: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. *Genes Dev.* 5:1172–82
25. Deng XW, Matsui M, Wei N, Wagner D, Chu AM, et al. 1992. *COP1*, an *Arabidopsis* regulatory gene, encodes a protein with both a zinc-binding motif and a G β homologous domain. *Cell* 71:791–801
26. Desnos T, Puente P, Whitelam GC, Harberd NP. 2001. FHY1: a phytochrome A-specific signal transducer. *Genes Dev.* 15:2980–90
27. Devlin PF, Patel SR, Whitelam GC. 1998. Phytochrome E influences internode elongation and flowering time in *Arabidopsis*. *Plant Cell* 10:1479–87
28. Devlin PF, Robson PR, Patel SR, Goosey L, Sharrock RA, Whitelam GC. 1999. Phytochrome D acts in the shade-avoidance syndrome in *Arabidopsis* by controlling elongation growth and flowering time. *Plant Physiol.* 119:909–15
29. Duek PD, Elmer MV, van Oosten VR, Fankhauser C. 2004. The degradation of HFR1, a putative bHLH class transcription factor involved in light signaling, is regulated by phosphorylation and requires COP1. *Curr. Biol.* 14:2296–301
30. Edgerton MD, Jones AM. 1992. Localization of protein-protein interactions between subunits of phytochrome. *Plant Cell* 4:161–71
31. Eitoku T, Zarate X, Kozhukh GV, Kim JI, Song PS, Terazima M. 2006. Time-resolved detection of conformational changes in oat phytochrome A: time-dependent diffusion. *Biophys. J.* 91:3797–804
32. Franklin KA, Allen T, Whitelam GC. 2007. Phytochrome A is an irradiance-dependent red light sensor. *Plant J.* 50:108–17
33. Franklin KA, Davis SJ, Stoddart WM, Vierstra RD, Whitelam GC. 2003. Mutant analyses define multiple roles for phytochrome C in *Arabidopsis* photomorphogenesis. *Plant Cell* 15:1981–89
34. Franklin KA, Whitelam G. 2007. Red:far-red ratio perception and shade avoidance. In *Light and Plant Development*, ed. G Whitelam, K Halliday, pp. 211–34. Oxford: Blackwell
35. Fujimori T, Yamashino T, Kato T, Mizuno T. 2004. Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in *Arabidopsis thaliana*. *Plant Cell Physiol.* 45:1078–86
36. Furuya M. 1989. Molecular properties and biogenesis of phytochrome I and II. *Adv. Biophys.* 25:133–67
37. Handwerger KE, Gall JG. 2006. Subnuclear organelles: new insights into form and function. *Trends Cell Biol.* 16:19–26
38. Hayama R, Yokoi S, Tamaki S, Yano M, Shimamoto K. 2003. Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature* 422:719–22
39. Hennig L, Stoddart WM, Dieterle M, Whitelam GC, Schafer E. 2002. Phytochrome E controls light-induced germination of *Arabidopsis*. *Plant Physiol.* 128:194–200

40. Hiltbrunner A, Tscheuschler A, Viczian A, Kunkel T, Kircher S, Schafer E. 2006. FHY1 and FHL act together to mediate nuclear accumulation of the phytochrome A photoreceptor. *Plant Cell Physiol.* 47:1023–34
41. Hiltbrunner A, Viczian A, Bury E, Tscheuschler A, Kircher S, et al. 2005. Nuclear accumulation of the phytochrome A photoreceptor requires FHY1. *Curr. Biol.* 15:2125–30
42. Holm M, Ma LG, Qu LJ, Deng XW. 2002. Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in *Arabidopsis*. *Genes Dev.* 16:1247–59
43. Huq E, Al-Sady B, Hudson M, Kim C, Apel K, Quail PH. 2004. Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. *Science* 305:1937–41
44. Huq E, Quail PH. 2002. PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in *Arabidopsis*. *EMBO J.* 21:2441–50
45. Imaizumi T, Kay SA. 2006. Photoperiodic control of flowering: not only by coincidence. *Trends Plant Sci.* 11:550–58
46. Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA. 2005. FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in *Arabidopsis*. *Science* 309:293–97
47. Imaizumi T, Tran HG, Swartz TE, Briggs WR, Kay SA. 2003. FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. *Nature* 426:302–6
48. Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K. 2002. Phytochrome mediates the external light signal to repress FT orthologs in photoperiodic flowering of rice. *Genes Dev.* 16:2006–20
49. Jang IC, Yang JY, Seo HS, Chua NH. 2005. HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. *Genes Dev.* 19:593–602
50. Johnson E, Bradley M, Harberd NP, Whitelam GC. 1994. Photoresponses of light-grown *phyA* mutants of *Arabidopsis* (phytochrome A is required for the perception of daylength extensions). *Plant Physiol.* 105:141–49
51. Jones AM, Quail PH. 1986. Quaternary structure of 124-kDa phytochrome from *Avena sativa* L. *Biochemistry* 25:2987–95
52. Kawai H, Kanegae T, Christensen S, Kiyosue T, Sato Y, et al. 2003. Responses of ferns to red light are mediated by an unconventional photoreceptor. *Nature* 421:287–90
53. Khanna R, Huq E, Kikis EA, Al-Sady B, Lanzatella C, Quail PH. 2004. A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix-loop-helix transcription factors. *Plant Cell* 16:3033–44
54. Kim J, Yi H, Choi G, Shin B, Song PS, Choi G. 2003. Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. *Plant Cell* 15:2399–407
55. Kim JI, Shen Y, Han YJ, Park JE, Kirchenbauer D, et al. 2004. Phytochrome phosphorylation modulates light signaling by influencing the protein-protein interaction. *Plant Cell* 16:2629–40
56. Kimura M, Kagawa T. 2006. Phototropin and light-signaling in phototropism. *Curr. Opin. Plant Biol.* 9:503–8
57. Kircher S, Gil P, Kozma-Bognar L, Fejes E, Speth V, et al. 2002. Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D, and E is regulated differentially by light and exhibits a diurnal rhythm. *Plant Cell* 14:1541–55
58. Kircher S, Kozma-Bognar L, Kim L, Adam E, Harter K, et al. 1999. Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. *Plant Cell* 11:1445–56

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59. Kiss JZ, Mullen JL, Correll MJ, Hangarter RP. 2003. Phytochromes A and B mediate red-light-induced positive phototropism in roots. *Plant Physiol.* 131:1411–17
60. Kleiner O, Kircher S, Harter K, Batschauer A. 1999. Nuclear localization of the *Arabidopsis* blue light receptor cryptochrome 2. *Plant J.* 19:289–96
61. Koornneef M, van der Veen JH. 1980. Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana*. *Theor. Appl. Genet* 58:257–63
62. Kretsch T, Poppe C, Schäfer E. 2000. A new type of mutation in the plant photoreceptor phytochrome B causes loss of photoreversibility and an extremely enhanced light sensitivity. *Plant J.* 22:177–86
63. Lagarias JC, Mercurio FM. 1985. Structure function studies on phytochrome. Identification of light-induced conformational changes in 124-kDa Avena phytochrome in vitro. *J. Biol. Chem.* 260:2415–23
64. Lamparter T, Carrascal M, Michael N, Martinez E, Rottwinkel G, Abian J. 2004. The biliverdin chromophore binds covalently to a conserved cysteine residue in the N-terminus of *Agrobacterium* phytochrome Agp1. *Biochemistry* 43:3659–69
65. Lamparter T, Esteban B, Hughes J. 2001. Phytochrome Cph1 from the cyanobacterium *Synechocystis* PCC6803. Purification, assembly, and quaternary structure. *Eur. J. Biochem.* 268:4720–30
66. Lamparter T, Mittmann F, Gartner W, Borner T, Hartmann E, Hughes J. 1997. Characterization of recombinant phytochrome from the cyanobacterium *Synechocystis*. *Proc. Natl. Acad. Sci. USA* 94:11792–97
67. Lapko VN, Jiang XY, Smith DL, Song PS. 1997. Posttranslational modification of oat phytochrome A: phosphorylation of a specific serine in a multiple serine cluster. *Biochemistry* 36:10595–99
68. Lapko VN, Jiang XY, Smith DL, Song PS. 1998. Surface topography of phytochrome A deduced from specific chemical modification with iodoacetamide. *Biochemistry* 37:12526–35
69. Laubinger S, Marchal V, Le Gourrierec J, Wenkel S, Adrian J, et al. 2006. *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. *Development* 133:3213–22
70. Lopez-Juez E, Nagatani A, Tomizawa K, Deak M, Kern R, et al. 1992. The cucumber long hypocotyl mutant lacks a light-stable PHYB-like phytochrome. *Plant Cell* 4:241–51
71. Ma L, Gao Y, Qu L, Chen Z, Li J, et al. 2002. Genomic evidence for COP1 as a repressor of light-regulated gene expression and development in *Arabidopsis*. *Plant Cell* 14:2383–98
72. Maloof JN, Borevitz JO, Dabi T, Lutes J, Nehring RB, et al. 2001. Natural variation in light sensitivity of *Arabidopsis*. *Nat. Genet.* 29:441–46
73. Martínez-Garcia JF, Huq E, Quail PH. 2000. Direct targeting of light signals to a promoter element-bound transcription factor. *Science* 288:859–63
74. Mateos JL, Luppi JP, Ogorodnikova OB, Sineshchekov VA, Yanovsky MJ, et al. 2006. Functional and biochemical analysis of the N-terminal domain of phytochrome A. *J. Biol. Chem.* 281:34421–29
75. Mathews S. 2006. Phytochrome-mediated development in land plants: red light sensing evolves to meet the challenges of changing light environments. *Mol. Ecol.* 15:3483–503
76. Matsushita T, Mochizuki N, Nagatani A. 2003. Dimers of the N-terminal domain of phytochrome B are functional in the nucleus. *Nature* 424:571–74
77. Mira-Rodado V, Sweere U, Grefen C, Kunkel T, Fejes E, et al. 2007. Functional cross-talk between two-component and phytochrome B signal transduction in *Arabidopsis*. *J. Exp. Bot.* 58:2595–607

Annu. Rev. Plant Biol. 2008.59:281-311. Downloaded from www.annualreviews.org
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78. Mittmann F, Brucker G, Zeidler M, Repp A, Abts T, et al. 2004. Targeted knockout in *Physcomitrella* reveals direct actions of phytochrome in the cytoplasm. *Proc. Natl. Acad. Sci. USA* 101:13939–44
79. Monte E, Alonso JM, Ecker JR, Zhang Y, Li X, et al. 2003. Isolation and characterization of phyC mutants in *Arabidopsis* reveals complex crosstalk between phytochrome signaling pathways. *Plant Cell* 15:1962–80
80. Monte E, Tepperman JM, Al-Sady B, Kaczorowski KA, Alonso JM, et al. 2004. The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. *Proc. Natl. Acad. Sci. USA* 101:16091–98
81. Nagatani A, Reed JW, Chory J. 1993. Isolation and initial characterization of *Arabidopsis* mutants that are deficient in phytochrome A. *Plant Physiol.* 102:269–77
82. Nelson DC, Lasswell J, Rogg LE, Cohen MA, Bartel B. 2000. *FKF1*, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell* 101:331–40
83. 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* 95:657–67
84. Ni M, Tepperman JM, Quail PH. 1999. Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. *Nature* 400:781–84
85. Nozue K, Covington MF, Duek PD, Lorrain S, Fankhauser C, et al. 2007. Rhythmic growth explained by coincidence between internal and external cues. *Nature* 448:358–61
86. Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. 2003. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell* 15:1591–604
87. Oh E, Kim J, Park E, Kim JI, Kang C, Choi G. 2004. PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in *Arabidopsis thaliana*. *Plant Cell* 16:3045–58
88. Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, et al. 2007. PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the *GAI* and *RGA* promoters in *Arabidopsis* seeds. *Plant Cell* 19:1192–208
89. Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G. 2006. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in *Arabidopsis*. *Plant J.* 47:124–39
90. Oka Y, Matsushita T, Mochizuki N, Suzuki T, Tokutomi S, Nagatani A. 2004. Functional analysis of a 450-amino acid N-terminal fragment of phytochrome B in *Arabidopsis*. *Plant Cell* 16:2104–16
91. Osterlund MT, Deng XW. 1998. Multiple photoreceptors mediate the light-induced reduction of GUS-COP1 from *Arabidopsis* hypocotyl nuclei. *Plant J.* 16:201–8
92. Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* 405:462–66
93. Osterlund MT, Wei N, Deng XW. 2000. The roles of photoreceptor systems and the COP1-targeted destabilization of HY5 in light control of *Arabidopsis* seedling development. *Plant Physiol.* 124:1520–24
94. Parcy F. 2005. Flowering: a time for integration. *Int. J. Dev. Biol.* 49:585–93
95. Park E, Kim J, Lee Y, Shin J, Oh E, et al. 2004. Degradation of phytochrome interacting factor 3 in phytochrome-mediated light signaling. *Plant Cell Physiol.* 45:968–75
96. Penfield S, Josse EM, Kannangara R, Gilday AD, Halliday KJ, Graham IA. 2005. Cold and light control seed germination through the bHLH transcription factor SPATULA. *Curr. Biol.* 15:1998–2006

Annu. Rev. Plant Biol. 2008.59:281-311. Downloaded from www.annualreviews.org
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97. 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.* 104:1139–49
98. Reed JW, Nagpal P, Poole DS, Furuya M, Chory J. 1993. Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *Plant Cell* 5:147–57
99. Rockwell NC, Lagarias JC. 2006. The structure of phytochrome: a picture is worth a thousand spectra. *Plant Cell* 18:4–14
100. Rockwell NC, Su YS, Lagarias JC. 2006. Phytochrome structure and signaling mechanisms. *Annu. Rev. Plant Biol.* 57:837–58
101. Ryu JS, Kim JI, Kunkel T, Kim BC, Cho DS, et al. 2005. Phytochrome-specific type 5 phosphatase controls light signal flux by enhancing phytochrome stability and affinity for a signal transducer. *Cell* 120:395–406
102. Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, et al. 2003. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes Dev.* 17:2642–47
103. Sakamoto K, Nagatani A. 1996. Nuclear localization activity of phytochrome B. *Plant J.* 10:859–68
104. Seo HS, Watanabe E, Tokutomi S, Nagatani A, Chua NH. 2004. Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. *Genes Dev.* 18:617–22
105. Seo HS, Yang JY, Ishikawa M, Bolle C, Ballesteros ML, Chua NH. 2003. LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature* 423:995–99
106. Sharrock RA, Clack T. 2004. Heterodimerization of type II phytochromes in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 101:11500–5
107. Sharrock RA, Clack T, Goosey L. 2003. Differential activities of the *Arabidopsis* phyB/D/E phytochromes in complementing phyB mutant phenotypes. *Plant Mol. Biol.* 52:135–42
108. Shen H, Moon J, Huq E. 2005. PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in *Arabidopsis*. *Plant J.* 44:1023–35
109. Shen Y, Feng S, Ma L, Lin R, Qu LJ, et al. 2005. *Arabidopsis* FHY1 protein stability is regulated by light via phytochrome A and 26S proteasome. *Plant Physiol.* 139:1234–43
110. Shimizu-Sato S, Huq E, Tepperman JM, Quail PH. 2002. A light-switchable gene promoter system. *Nat. Biotechnol.* 20:1041–44
111. Shin J, Park E, Choi G. 2007. PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in *Arabidopsis*. *Plant J.* 49:981–94
112. Shinomura T, Nagatani A, Hanzawa H, Kubota M, Watanabe M, Furuya M. 1996. Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 93:8129–33
113. Siegelman HW, Turner BC, Hendricks SB. 1966. The chromophore of phytochrome. *Plant Physiol.* 41:1289–92
114. Smith H. 1983. The natural radiation environment: limitations on the biology of photoreceptors. Phytochrome as a case study. *Symp. Soc. Exp. Biol.* 36:1–18
115. Stewart M. 2007. Molecular mechanism of the nuclear protein import cycle. *Nat. Rev. Mol. Cell Biol.* 8:195–208

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116. Stockhaus J, Nagatani A, Halfter U, Kay S, Furuya M, Chua NH. 1992. Serine-to-alanine substitutions at the amino-terminal region of phytochrome A result in an increase in biological activity. *Genes Dev.* 6:2364–72
117. Su YS, Lagarias JC. 2007. Light-independent phytochrome signaling mediated by dominant GAF domain tyrosine mutants of *Arabidopsis* phytochromes in transgenic plants. *Plant Cell.* 19:2124–39
118. Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G. 2001. CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410:1116–20
119. Suetsugu N, Mittmann F, Wagner G, Hughes J, Wada M. 2005. A chimeric photoreceptor gene, *NEOCHROME*, has arisen twice during plant evolution. *Proc. Natl. Acad. Sci. USA* 102:13705–9
120. Sweere U, Eichenberg K, Lohrmann J, Mira-Rodado V, Baurle I, et al. 2001. Interaction of the response regulator ARR4 with phytochrome B in modulating red light signaling. *Science* 294:1108–11
121. Takano M, Inagaki N, Xie X, Yuzurihara N, Hihara F, et al. 2005. Distinct and cooperative functions of phytochromes A, B, and C in the control of de-etiolation and flowering in rice. *Plant Cell* 17:3311–25
122. Tepperman JM, Hwang YS, Quail PH. 2006. phyA dominates in transduction of red-light signals to rapidly responding genes at the initiation of *Arabidopsis* seedling de-etiolation. *Plant J.* 48:728–42
123. To JP, Haberer G, Ferreira FJ, Deruere J, Mason MG, et al. 2004. Type-A *Arabidopsis* response regulators are partially redundant negative regulators of cytokinin signaling. *Plant Cell* 16:658–71
124. Tokuhisa JG, Daniels SM, Quail PH. 1985. Phytochrome in green tissue: spectral and immunochemical evidence for two distinct molecular species of phytochrome in light-grown *Avena* sativa L. *Planta* 164:321–32
125. Trupkin SA, Debrieux D, Hiltbrunner A, Fankhauser C, Casal JJ. 2007. The serine-rich N-terminal region of *Arabidopsis* phytochrome A is required for protein stability. *Plant Mol. Biol.* 63:669–78
126. Usami T, Matsushita T, Oka Y, Mochizuki N, Nagatani A. 2007. Roles for the N- and C-terminal domains of phytochrome B in interactions between phytochrome B and cryptochrome signaling cascades. *Plant Cell Physiol.* 48:424–33
127. Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science* 303:1003–6
128. Vogelmann TC, Bornman JF, Josserand S. 1989. Photosynthetic light gradients and spectral regime within leaves of *Medicago sativa*. *Philos. Trans. R. Soc. London Ser. B* 323:411–21
129. von Arnim AG, Deng XW. 1994. Light inactivation of *Arabidopsis* photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell* 79:1035–45
130. von Arnim AG, Deng XW, Stacey MG. 1998. Cloning vectors for the expression of green fluorescent protein fusion proteins in transgenic plants. *Gene* 221:35–43
131. Wagner D, Koloszvari M, Quail PH. 1996. Two small spatially distinct regions of phytochrome B are required for efficient signaling rates. *Plant Cell* 8:859–71
132. Wagner D, Tepperman JM, Quail PH. 1991. Overexpression of phytochrome B induces a short hypocotyl phenotype in transgenic *Arabidopsis*. *Plant Cell* 3:1275–88

Annu. Rev. Plant Biol. 2008.59:281-311. Downloaded from www.annualreviews.org
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133. Wagner JR, Brunzelle JS, Forest KT, Vierstra RD. 2005. A light-sensing knot revealed by the structure of the chromophore-binding domain of phytochrome. *Nature* 438:325–31
134. Wagner JR, Zhang J, Brunzelle JS, Vierstra RD, Forest KT. 2007. High resolution structure of *Deinococcus* bacteriophytochrome yields new insights into phytochrome architecture and evolution. *J. Biol. Chem.* 282:12298–309
135. Wang H, Ma L, Habashi J, Li J, Zhao H, Deng XW. 2002. Analysis of far-red light-regulated genome expression profiles of phytochrome A pathway mutants in *Arabidopsis*. *Plant J.* 32:723–33
136. Wells TA, Nakazawa M, Manabe K, Song PS. 1994. A conformational change associated with the phototransformation of *Pisum* phytochrome A as probed by fluorescence quenching. *Biochemistry* 33:708–12
137. Whippo CW, Hangarter RP. 2006. Phototropism: bending towards enlightenment. *Plant Cell* 18:1110–19
138. Whitelam GC, Johnson E, Peng J, Carol P, Anderson ML, et al. 1993. Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. *Plant Cell* 5:757–68
139. Wu SH, Lagarias JC. 2000. Defining the bilin lyase domain: lessons from the extended phytochrome superfamily. *Biochemistry* 39:13487–95
140. Xu Y, Parks BM, Short TW, Quail PH. 1995. Missense mutations define a restricted segment in the C-terminal domain of phytochrome A critical to its regulatory activity. *Plant Cell* 7:1433–43
141. Yamaguchi R, Nakamura M, Mochizuki N, Kay SA, Nagatani A. 1999. Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic *Arabidopsis*. *J. Cell Biol.* 145:437–45
142. Yang HQ, Tang RH, Cashmore AR. 2001. The signaling mechanism of *Arabidopsis* CRY1 involves direct interaction with COP1. *Plant Cell* 13:2573–87
143. Yang J, Lin R, Hoecker U, Liu B, Xu L, Wang H. 2005. Repression of light signaling by *Arabidopsis* SPA1 involves post-translational regulation of HFR1 protein accumulation. *Plant J.* 43:131–41
144. Yang J, Lin R, Sullivan J, Hoecker U, Liu B, et al. 2005. Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in *Arabidopsis*. *Plant Cell* 17:804–21
145. Yanovsky MJ, Luppi JP, Kirchbauer D, Ogorodnikova OB, Sineshchekov VA, et al. 2002. Missense mutation in the PAS2 domain of phytochrome A impairs subnuclear localization and a subset of responses. *Plant Cell* 14:1591–603
146. Yanovsky MJ, Mazzella MA, Whitelam GC, Casal JJ. 2001. Resetting of the circadian clock by phytochromes and cryptochromes in *Arabidopsis*. *J. Biol. Rhythms* 16:523–30
147. Yeh KC, Lagarias JC. 1998. Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. *Proc. Natl. Acad. Sci. USA* 95:13976–81
148. Yeh KC, Wu SH, Murphy JT, Lagarias JC. 1997. A cyanobacterial phytochrome two-component light sensory system. *Science* 277:1505–8
149. Zeidler M, Zhou Q, Sarda X, Yau CP, Chua NH. 2004. The nuclear localization signal and the C-terminal region of FHY1 are required for transmission of phytochrome A signals. *Plant J.* 40:355–65

Annu. Rev. Plant Biol. 2008.59:281-311. Downloaded from www.annualreviews.org
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150. Zhou Q, Hare PD, Yang SW, Zeidler M, Huang LF, Chua NH. 2005. FHL is required for full phytochrome A signaling and shares overlapping functions with FHY1. *Plant J.* 43:356–70
151. Zhu Y, Tepperman JM, Fairchild CD, Quail PH. 2000. Phytochrome B binds with greater apparent affinity than phytochrome A to the basic helix-loop-helix factor PIF3 in a reaction requiring the PAS domain of PIF3. *Proc. Natl. Acad. Sci. USA* 97:13419–24

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