

Decoding of Light Signals by Plant Phytochromes and Their Interacting Proteins

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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.

Contents	
INTRODUCTION.....	282
PLANT PHYTOCHROMES.....	282
Perception of Light by Plant	
Phytochromes.....	282
The Phytochrome Gene Family... ..	283
Functional Domains of Plant	
Phytochromes.....	284
Light-Induced Structural Changes	
in Plant Phytochromes.....	288
Nuclear Translocation	
of Plant Phytochromes.....	289
LIGHT INFORMATION	
PERCEIVED BY PLANT	
PHYTOCHROMES.....	290
Wavelength and Irradiance	
Information.....	290
Directional Light Information....	291
Photoperiod Information.....	292
PHYTOCHROME-	
INTERACTING	
PROTEINS.....	292
Phytochrome-Interacting Proteins	
That Regulate the Nuclear	
Localization of Phytochromes..	293
Phytochrome-Interacting Proteins	
That Modulate the Output	
Activity of Phytochromes.....	295
Phytochrome-Interacting Proteins	
Whose Activities are Modulated	
by Phytochromes.....	296
THE FLOW OF LIGHT	
INFORMATION DURING	
SEED GERMINATION.....	300

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 capa-

ble 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 phytychromobilin, a linear tetrapyrrole bilin compound that acts as a chromophore

(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 (*PHY1*, *PHY2*, *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;

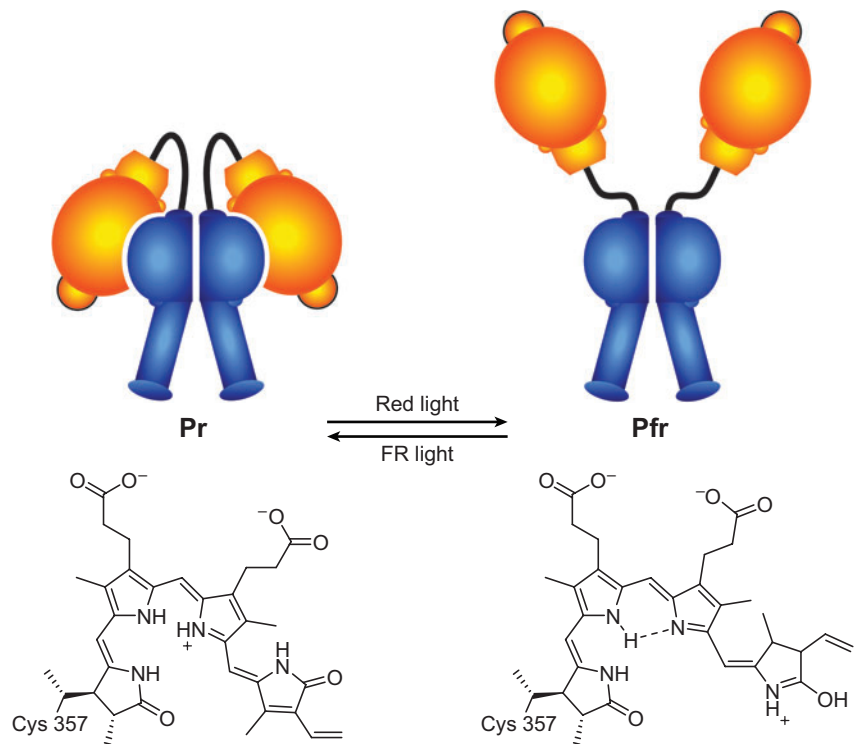


Figure 1

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 autophos-

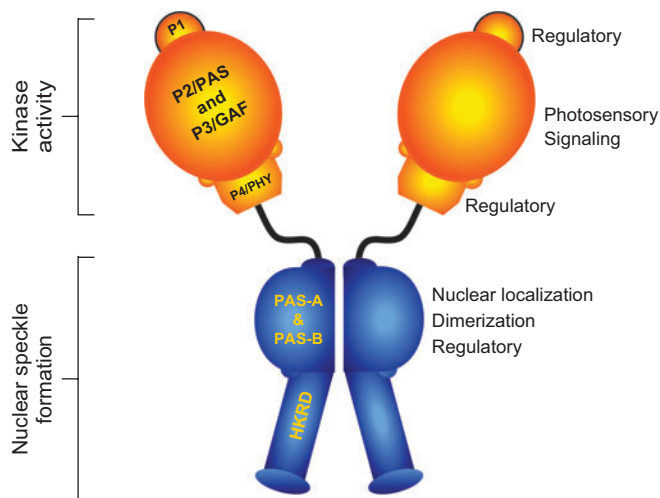


Figure 2

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.

phorylated (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.

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

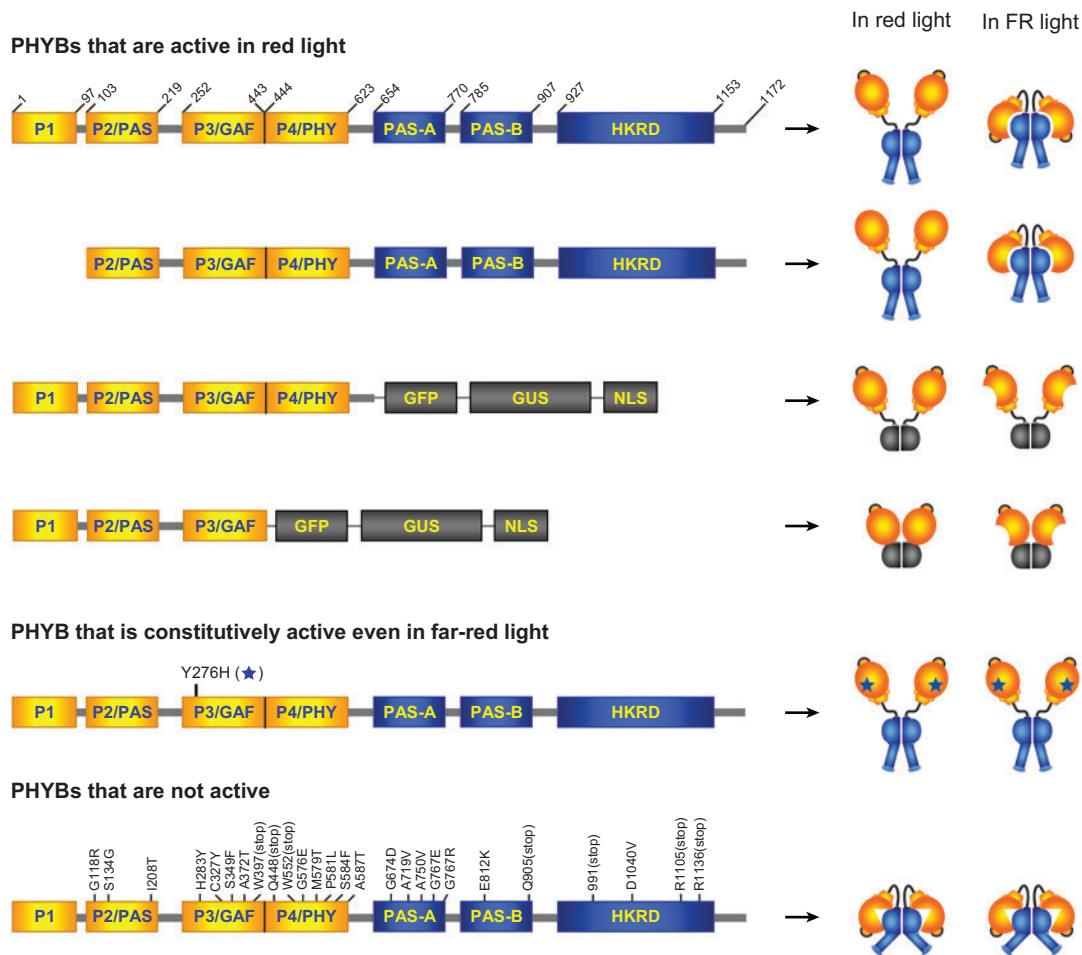


Figure 3

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* bacterio-

phytochrome 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

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 over-expressed 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).

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 phytochromes (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 lo-

calization 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/PAS 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

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):

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

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. P_t also varies depending on irradiance in some plant species, meaning that the exact relationship between Pfr concentration and irradiance is more complicated in plants. 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 *Phycomitrella*, 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 (*lb*) (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

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 LOCUST (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 pro-

tein 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

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

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 activ-

ity 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

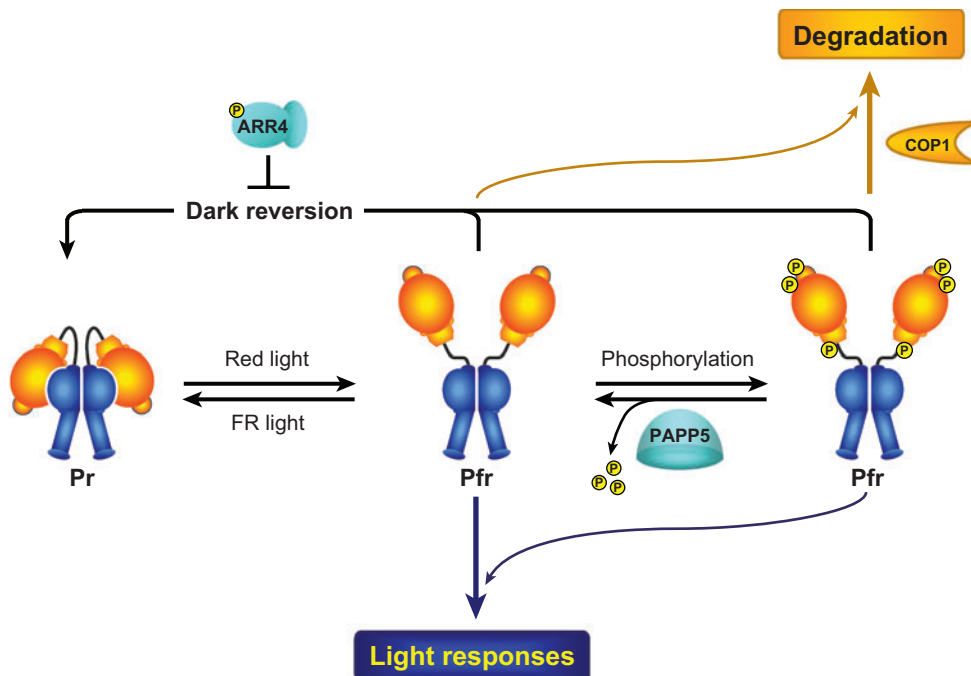


Figure 5

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

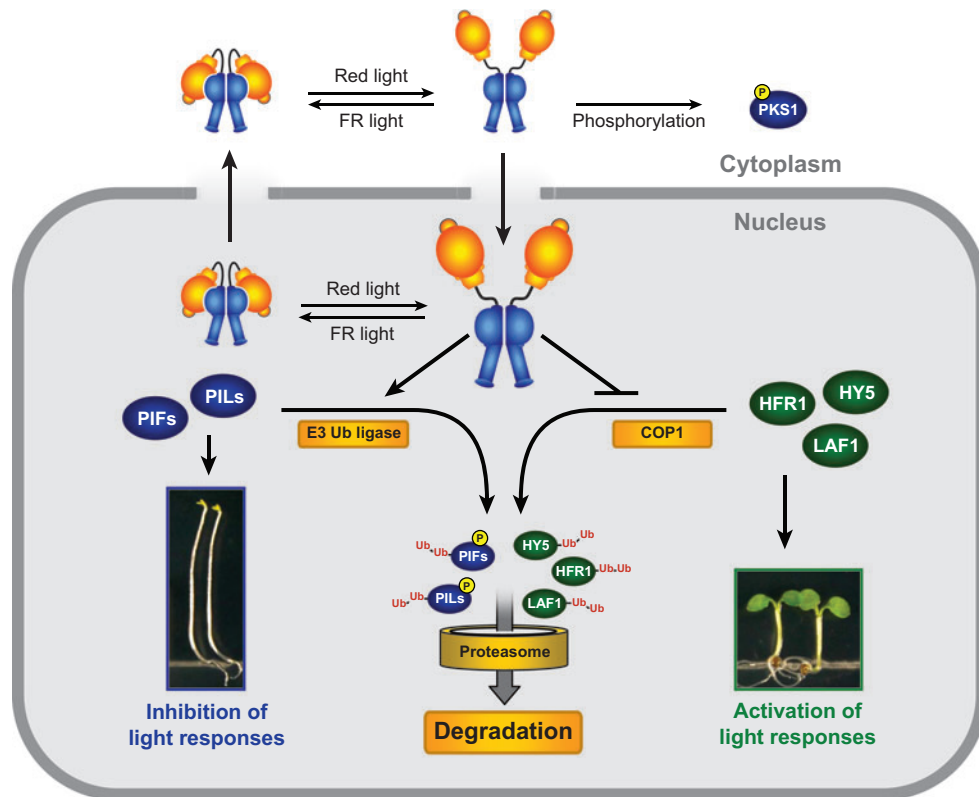


Figure 6

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 containing 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 ubiquitination 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

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 fac-

tor (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 photomorphogenic mutant together with other *cop1/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 [*GIBBERELIC 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

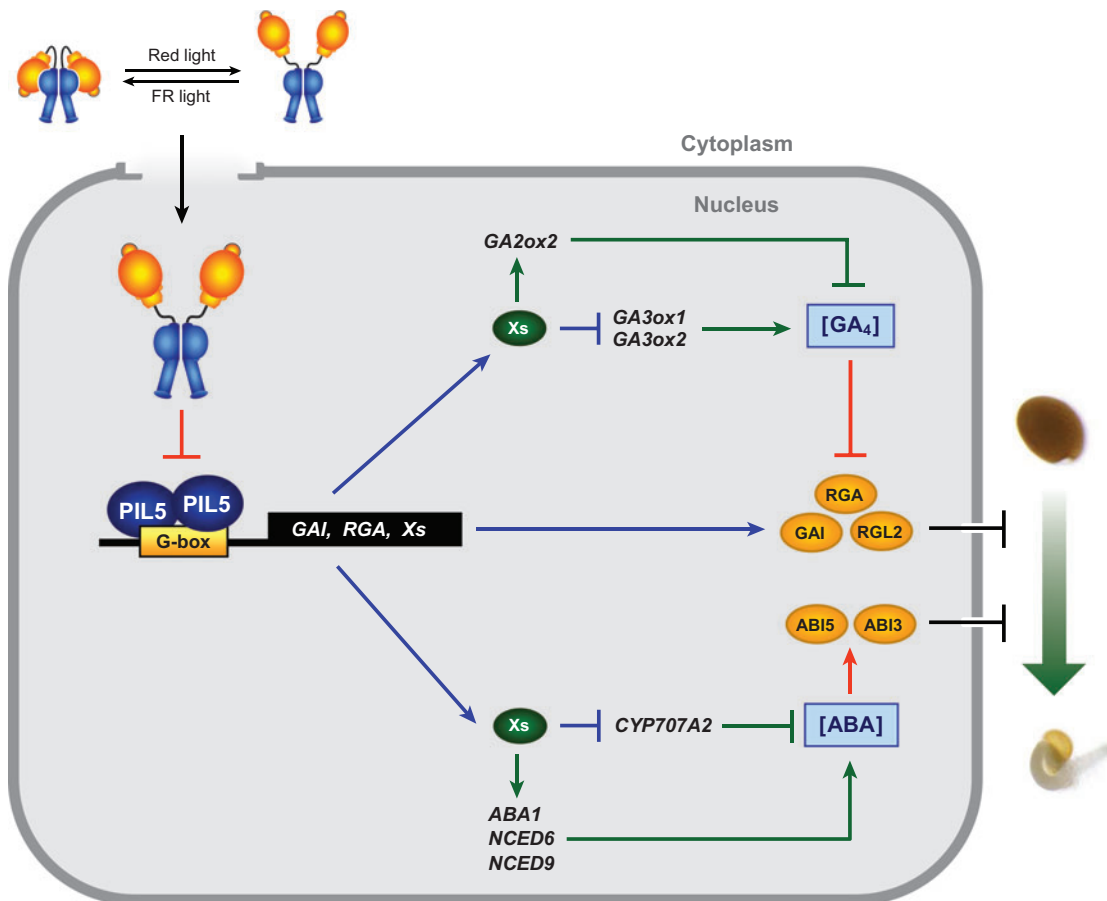


Figure 7

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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Contents

Our Work with Cyanogenic Plants <i>Eric E. Conn</i>	1
New Insights into Nitric Oxide Signaling in Plants <i>Angélique Besson-Bard, Alain Pugin, and David Wendebenne</i>	21
Plant Immunity to Insect Herbivores <i>Gregg A. Howe and Georg Jander</i>	41
Patterning and Polarity in Seed Plant Shoots <i>John L. Bowman and Sandra K. Floyd</i>	67
Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo <i>Neil R. Baker</i>	89
Seed Storage Oil Mobilization <i>Ian A. Graham</i>	115
The Role of Glutathione in Photosynthetic Organisms: Emerging Functions for Glutaredoxins and Glutathionylation <i>Nicolas Rouhier, Stéphane D. Lemaire, and Jean-Pierre Jacquot</i>	143
Algal Sensory Photoreceptors <i>Peter Hegemann</i>	167
Plant Proteases: From Phenotypes to Molecular Mechanisms <i>Renier A.L. van der Hoorn</i>	191
Gibberellin Metabolism and its Regulation <i>Shinjiro Yamaguchi</i>	225
Molecular Basis of Plant Architecture <i>Yonghong Wang and Jiayang Li</i>	253
Decoding of Light Signals by Plant Phytochromes and Their Interacting Proteins <i>Gabyong Bae and Giltso Choi</i>	281
Flooding Stress: Acclimations and Genetic Diversity <i>J. Bailey-Serres and L.A.C.J. Voeseek</i>	313

Roots, Nitrogen Transformations, and Ecosystem Services <i>Louise E. Jackson, Martin Burger, and Timothy R. Cavagnaro</i>	341
A Genetic Regulatory Network in the Development of Trichomes and Root Hairs <i>Tetsuya Ishida, Tetsuya Kurata, Kiyotaka Okada, and Takuji Wada</i>	365
Molecular Aspects of Seed Dormancy <i>Ruth Finkelstein, Wendy Reeves, Tobru Ariizumi, and Camille Steber</i>	387
Trehalose Metabolism and Signaling <i>Matthew J. Paul, Lucia F. Primavesi, Deveraj Jhurreea, and Yubua Zhang</i>	417
Auxin: The Looping Star in Plant Development <i>René Benjamins and Ben Scheres</i>	443
Regulation of Cullin RING Ligases <i>Sara K. Hottton and Judy Callis</i>	467
Plastid Evolution <i>Sven B. Gould, Ross F. Waller, and Geoffrey I. McFadden</i>	491
Coordinating Nodule Morphogenesis with Rhizobial Infection in Legumes <i>Giles E.D. Oldroyd and J. Allan Downie</i>	519
Structural and Signaling Networks for the Polar Cell Growth Machinery in Pollen Tubes <i>Alice Y. Cheung and Hen-ming Wu</i>	547
Regulation and Identity of Florigen: FLOWERING LOCUS T Moves Center Stage <i>Franziska Turck, Fabio Fornara, and George Coupland</i>	573
Plant Aquaporins: Membrane Channels with Multiple Integrated Functions <i>Christophe Maurel, Lionel Verdoucq, Doan-Trung Luu, and Véronique Santoni</i> ...	595
Metabolic Flux Analysis in Plants: From Intelligent Design to Rational Engineering <i>Igor G.L. Libourel and Yair Shachar-Hill</i>	625
Mechanisms of Salinity Tolerance <i>Rana Munns and Mark Tester</i>	651
Sealing Plant Surfaces: Cuticular Wax Formation by Epidermal Cells <i>Lacey Samuels, Ljerka Kunst, and Reinhard Jetter</i>	683
Ionomics and the Study of the Plant Ionome <i>David E. Salt, Ivan Baxter, and Brett Labner</i>	709

Alkaloid Biosynthesis: Metabolism and Trafficking <i>Jörg Ziegler and Peter J. Facchini</i>	735
Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part I) <i>Peggy G. Lemaux</i>	771

Indexes

Cumulative Index of Contributing Authors, Volumes 49–59	813
Cumulative Index of Chapter Titles, Volumes 49–59	818

Errata

An online log of corrections to *Annual Review of Plant Biology* articles may be found at <http://plant.annualreviews.org/>