Journal of Plant Biology, June 2007, 50(3) : 230-240 **REVIEW**

Phytochrome-Mediated Photomorphogenesis in Plants

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Photomorphogenesis is the process by which plants grow and develop in response to light signals. This process is mediated by a sophisticated network of photoreceptors among which phytochromes play a key role. Phytochrome-mediated photomorphogenic responses are characterized by the complex variety of relationships between light input and physiological outputs, including germination, de-etiolation, shade avoidance, circadian rhythm, and flowering. Recent studies have resulted in several important advances, and have revealed the major consequences of phytochrome activity in terms of controlling protein subcellular localization, transcription, protein stability, and protein phosphorylation. In addition, many downstream components in the phytochrome signaling have now been identified, and a complex, highly regulated signaling network is envisaged. Here, we review the current knowledge about red/far-red photoreceptor phytochromes and provide a comprehensive summary of the phytochrome-mediated photomorphogenesis signaling network.

Keywords: light, photomorphogenesis, photoreceptor, phytochrome, signaling

PHOTOMORPHOGENESIS IN PLANTS

Light is essential for plant growth and development, serving as an energy source for photosynthesis and as an environmental signal for photomorphogenesis (Chen et al., 2004). Such light-mediated responses, known as photomorphogenesis, are most obvious in germinating seedlings, i.e., the period between seed germination and the formation of the first true leaves. However, light affects plants in many ways throughout all stages of life, ranging from germination, stem growth, chloroplast development, biosynthesis of chlorophylls and other pigments, circadian rhythm, and flowering. Growth habits also differ between dark and light-illuminated conditions. Under the former, plants have elongated stems (hypocotyls), undifferentiated chloroplasts, and closed, unexpanded leaves (cotyledons) protected by an apical hook. This "dark phenotype" is called skotomorphogenesis. In contrast, photomorphogenesis involves the inhibition of stem elongation, differentiation of chloroplasts, the accumulation of chlorophylls, and leaf expansion. In natural environments, the conversion between etiolated and de-etiolated development allows buried seed to emerge through the soil in search of light, and switch to a pattern optimal for photosynthesis. Because none of those aspects of photomorphogenesis occur in the absence of a light signal, photomorphogenesis can be considered as a phenomenon that constantly modulates a plant's ability to harness light energy most efficiently. The mechanism for photomorphogenesis is not yet elucidated fully; therefore, it is critical that researchers gain further understanding of the characteristics of photoreceptors, their action mechanisms, and signal transduction pathways.

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PHOTORECEPTORS

Sophisticated light-sensing systems in plants are used for responding to wavelengths, intensity, duration, and direction (Sullivan and Deng, 2003; Franklin et al., 2005; Wang, 2005). Three well-known classes of plant photoreceptors exist, namely the phytochromes, cryptochromes, and phototropins (Fig. 1). Phytochromes are a widespread family of red/far-red responsive photoreceptors that constitute the most important regulator of photomorphogenesis (Møller et al., 2002; Casal et al., 2003; Rockwell et al., 2006). This small family comprises, for example, five isoforms (Phytochromes A to E) in *Arabidopsis thaliana* (Mathews and Sharrock, 1997). They all utilize covalently attached bilin chromophores (Fig. 1A), and control growth and development in response to environmental cues. Red and far-red light (600 to 750 nm) are the most efficient wavebands for inducing conformational changes in phytochromes, consequently modifying their photochemical kinetics, nuclear/cytoplasmic partitioning, ability to phosphorylate substrates, and physical interactions with downstream components for photomorphogenesis.

Cryptochromes are UV-A/blue light receptors (320 to 500 nm) that mediate various light-induced responses in both plants and animals (Lin and Shalitin, 2003; Lin and Todo, 2005). Most plant cryptochromes have a chromophore-binding domain with a structure similar to DNA photolyase (Photolyase Homology Region, PHR), as well as a carboxyl terminal extension that contains a DQXVP-acidic-STAES (DAS) domain conserved from mosses to angiosperms (Fig. 1B). *Arabidopsis* has two cryptochromes, CRY1 and CRY2, that mediate light control of stem elongation, leaf expansion, photoperiodic flowering, and the circadian clock. Cryptochromes may function by interacting with proteins such as phytochromes, COP1 (constitutive photomorphogenesis 1), clock proteins, and/or chromatin and DNA. They possibly undergo blue light-dependent phosphorylation that

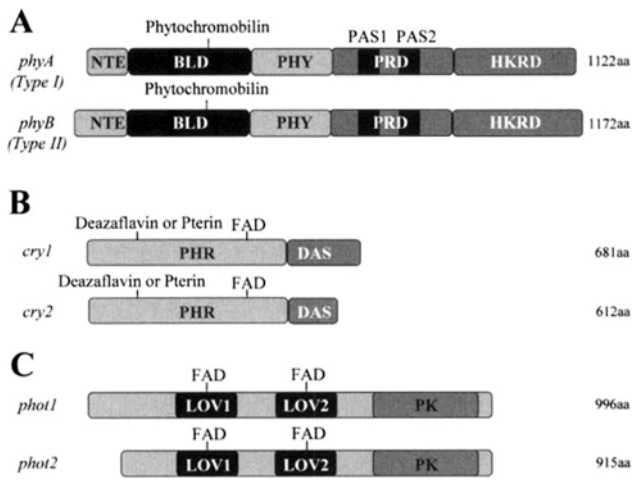

Figure 1. Protein structures of photoreceptors. (**A**) Phytochromes. NTE, N-Terminal Extension; BLD, Bilin Lyase Domain; PHY, PHYtochrome specific domain; PRD, PAS(Per/Arnt/Sim)-Related Domain; PAS1 & PAS2, two PAS repeats; HKRD, Histidine Kinase Domain (HKD)-Related Domain. Among 5 *Arabidopsis* phytochromes, phyA and phyB are shown as representatives of Type I and Type II phytochromes, respectively, because they play major roles in plants. With regard to protein structure, phyC (1111aa) and phyE (1112aa) are similar to phyA (1122aa), while phyD (1164aa) is similar to phyB (1172aa). (**B**) Cryptochromes. PHR, Photolyase Homology Region; DAS, DQXVP-acidic-STAES domain; FAD, Flavin Adenine Dinucleotide. (**C**) Phototropins. LOV, Light/Oxygen/Voltage domain; PK, Protein Kinase.

affects the conformation, intermolecular interactions, physiological activities, and protein abundance of the photoreceptors (Shalitin et al., 2002, 2003). Cryptochromes work together with red/far-red light receptor phytochromes to regulate various responses, including the control of cell elongation and photoperiodic flowering. They also act together with the blue light-receptor phototropins to mediate blue-light regulation of stomatal opening (Sullivan and Deng, 2003).

Phototropins are blue-light receptors that modulate a range of responses for optimizing photosynthetic efficiency. These include phototropism, light-induced stomatal opening, and chloroplast movements in response to changes in light intensity (Christie, 2007). *Arabidopsis* contains two phototropins, PHOT1 and PHOT2, that exhibit overlapping functions while also having unique physiological roles (Fig. 1C). Phototropins are light-activated serine/threonine protein kinases. Light-sensing is mediated by the LOV domain at the N-terminal region. Photoexcitation of that domain results in receptor autophosphorylation and activation of the C-terminal kinase domain, thereby initiating phototropin signaling.

Photomorphogenesis can be induced by red, far-red, or blue light. At low light intensities, plant development is primarily under the control of phytochrome A (phyA). As seedlings become exposed to light, phyA is degraded so that control though phytochrome B (phyB) and the cryptochromes becomes dominant (Sullivan and Deng, 2003). Under experimental conditions, phyA perceives continuous far-red (FR) light and phyB perceives red (R) light, while cryptochromes function in the perception of blue (B) light during de-etiolation. Many other factors also influence the response of *Arabidopsis* to light during early development, e.g., circadian rhythm and hormonal regulation via auxins, cytokinins, brassinosteroids, abscisic acid, and ethylene (Choi et al., 2005; Franklin et al., 2005; Kwon and Choe, 2005). Here, we focus on studies of phytochrome-mediated photomorphogenesis, including various signaling steps and mechanisms.

PHYTOCHROMES

Phytochrome signaling has pleiotropic effects on gene expression and plant development. Phytochromes recognize different light information, including intensity, wavelength, duration, and direction, so that these signals are transduced to develop almost every step in the life cycle, such as germination, de-etiolation, chloroplast development, biosynthesis of chlorophyll and other pigments, circadian rhythm, and flowering (Fig. 2). Here, we review the plant phytochromes, from biosynthesis to signal transduction.

Biosynthesis of phytochromes

Phytochromes, meaning "plant color", are dimeric chromopeptides (monomer sizes of 120~130 kDa). Their chromophore moiety is phytochromobilin (PΦB), which is covalently linked to the apo-phytochrome via a thioester linkage to a cysteine residue (Rockwell et al., 2006). This phytochrome chromophore is synthesized in the chloroplasts. The biosynthetic pathway for PΦB is common to chlorophyll synthesis, from 5-aminolevulinic acid (ALA) to protoporphyrin IX. The pathway for PΦB biosynthesis then branches from chlorophyll biosynthesis at the point of conversion of protoporphyrin IX to heme. From the heme, the first committed step in chromophore synthesis is cleavage of the tetrapyrrole ring of heme (Fig. 3). This reaction is catalyzed by a heme oxygenase encoded by the *HY1* gene in

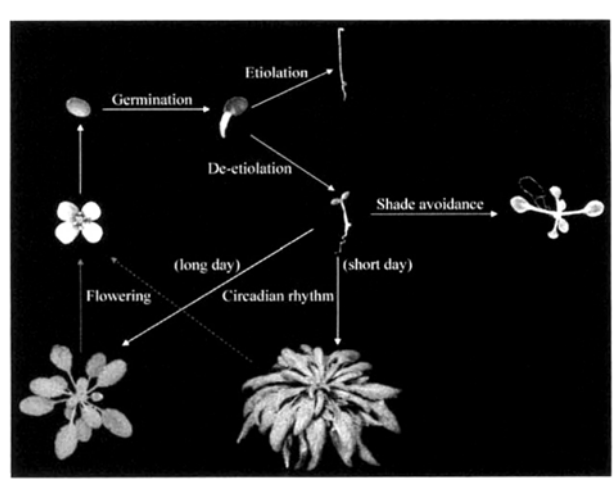

Figure 2. Phytochrome-mediated development in plants. Phytochromes perceive diverse light signals that modulate growth and development throughout plant life cycle, including germination, de-etiolation (also chloroplast development and chlorophyll biosynthesis, etc.), shade avoidance, circadian rhythm, and flowering.

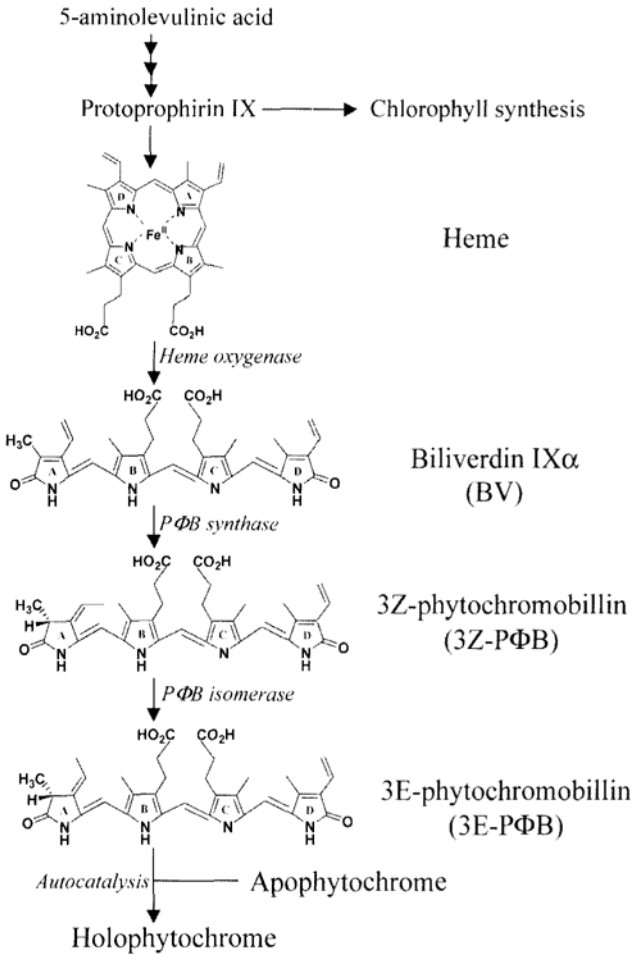

Figure 3. Biosynthesis of phytochromobilin (PΦB) and holophytochrome in plants. Apo-phytochrome autocatalytically assembles with 3E-phytochromobilin and becomes holo-phytochrome via bilin lyase activity in N-terminal domain of phytochrome molecule.

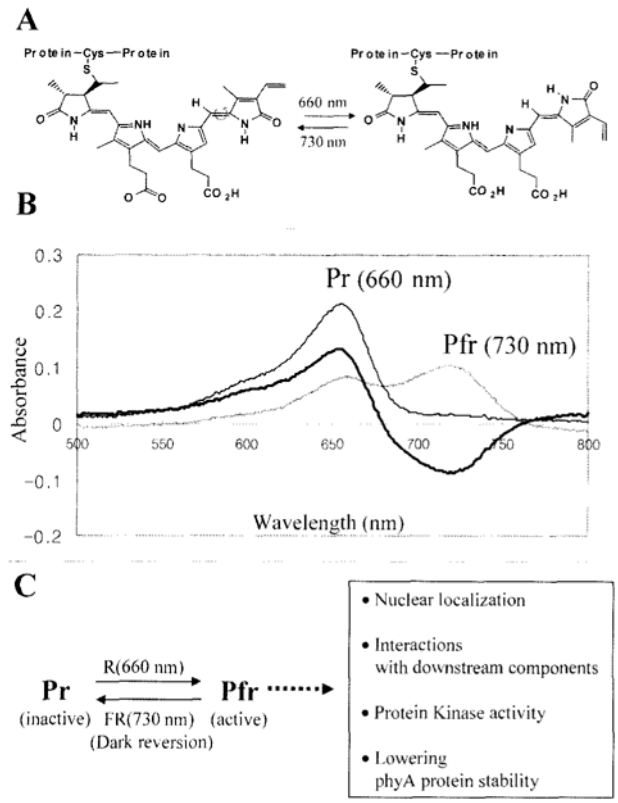

Figure 4. Photochromism of phytochromes. (**A**) Photoisomerization of phytochromobilin in Pr-to-Pfr photochromic transformation in phytochromes. Arrow shows rotation of D ring during phototransformation. (**B**) Absorption spectra of light-interconvertible Pr and Pfr forms of phytochromes. A difference spectrum (bold line) can be obtained by subtracting Pr spectrum from Pfr spectrum. (**C**) Functions of active form of phytochrome Pfr. Photoactivated Pfr is localized into nucleus and interacts with downstream components to initiate signaling, probably exerting protein kinase activity. Protein stability of phyA is very much reduced in its Pfr.

Arabidopsis (Emborg et al., 2006). Afterward, 3E-PΦB is synthesized by PΦB synthase and PΦB isomerase. Phytochrome apoprotein binds to the 3E-PΦB in the cytoplasm to yield holo-phytochromes. This occurs autocatalytically through bilin lyase activity that resides within the bilin lyase domain (BLD) of the phytochromes (Fig. 1A).

Photochromism of phytochrome

The most striking characteristic of phytochromes is their reversible photochromism, i.e., the ability to change color upon photon absorption as well as reversion to the original form following the absorption of another photon (Fig. 4). Phytochromes are synthesized in the red light-absorbing form (Pr, λmax = 660 nm) which can be phototransformed into the far-red light-absorbing form (Pfr, λmax = 730 nm) upon exposure to red light. The absorption of red light triggers a "Z" to "E" isomerization of the chromophore in the C-15 double bond between the C and D rings of the linear tetrapyrrole (Fig. 4A), resulting in the Pfr form. From the Pr and Pfr absorption spectra, a difference spectrum can be obtained by subtracting one spectrum from the other (Fig. 4B); this indicates the photochemical property of the phytochromes. Conformational protein changes accompany with this photochromism (Kim et al., 2002b; Kim and Song, 2005). Pfr can be converted to Pr either by a slow non-photoinduced reaction (dark reversion) or through much faster photoreversion via the absorption of far-red light. Due to the promotive effect of red light on most physiological responses, Pfr is considered the active form and Pr the inactive form. Once the Pfr form is obtained, phytochrome moves into the nucleus and then interacts with downstream components, probably exerting their protein kinase activity (Fig. 4C). Therefore, the molecular mechanism for this on/off switching is driven by photochromic phototransformation between these two forms, in which the photo-activated Pfr signals are transduced by interacting with a wide array of downstream signaling components and regulating the genes involved in photomorphogenesis and photosynthesis.

Phytochrome species

Comparisons of phytochrome sequences indicate that multiple isoforms exist within the same plant. For example, five isoforms (phytochromes A to E) have been isolated from the model plant *A. thaliana* (Mathews and Sharrock, 1997). These different isoforms often have greater homology to

paralogs from other plant species than to other phytochromes within the same species. For example, phyA from *Arabidopsis* is 65 to 80% identical to phyA sequences from other monocots and dicots, but is only 48 to 52% identical to self-encoded phyB-E sequences. Despite these differences, regions of high amino acid sequence homology exist among all isoforms, suggesting that all phytochromes have a similar biochemical mode of action. However, phenotypic analyses of phytochrome-deficient mutants or transgenic plants overexpressing different phytochromes have shown that specific isoforms control distinct facets of photomorphogenesis. For example, phyA regulates seed germination and seedling growth in response to continuous far-red light (FRc). In contrast, phyB controls germination in continuous red light (Rc), plant growth in response to the R/FR ratio and end-of-day FR, and flowering time (Quail et al., 1995; Casal et al., 2003; Chen et al., 2004).

Phytochromes can be classified into two groups based on their expression patterns and their protein stability: Type I (phyA in *Arabidopsis*) is light-labile, whereas Type II (phyB through phyE in *Arabidopsis*) is light-stable (Table 1). In dark-grown tissues, phyA (Type I) is most abundant. The level of phyA mRNA decreases rapidly, to 1/100-fold, upon exposure to light, and phyA proteins are also quickly degraded (Sharrock and Clark, 2002, 2004). This degradation is light-dependent and requires selective recognition and ubiquitination (Seo et al., 2004). In light-grown plants, phyB becomes the most abundant Type II phytochrome and phyC through phyE are less common. Among these five family members, phyA (Type I) and phyB (Type II) have well-known differences in their functioning. For example, phyA is abundant and active in dark-grown etiolated plants, whereas phyB functions in light-grown green plants. PhyA induces germination of *A. thaliana* in response to a very low fluence rate and in a red/far-red irreversible manner while phyB induces germination in response to a low fluence rate and a high irradiance rate in a photo-reversible manner (Table 1). For the control of seedling de-etiolation, the effects of FRc are mediated exclusively by phyA while those of Rc are mediated predominantly by phyB. An *in vivo* functional assay of phyA and phyB can be accomplished by measuring seedling hypocotyl lengths under FRc and Rc, respectively (Fig. 5).

Table 1. Comparison of characteristics between Type I and Type II phytochromes

Property	Type I	Type II
Light stability Phytochrome	Light-labile phyA	Light-stable phyB-E
Apoprotein synthesis	Photo-inhibitory	Constitutive
Relative amount of protein (%)		
Etiolated seedlings	85	10:2:1.5:1.5
Green plants	5	40:15:15:25
Light response modes[a]	VLFR, FR-HIR	VFR, R-HIR
Pfr decay in the dark (= Dark reversion)	Very slow	Quick
Hypocotyl length in mutant[b] (Hypocotyl length)		
Continuous R	Normal	Elongated
Continuous FR	Elongated	Normal

[a]VLFR, very low fluence response; FR-HIR, far-red high irradiance response; LFR, low fluence response; R-HIR, red high irradiance response. [b]These characteristics are used for the *in vivo* functional assays of phyA and phyB (Fig. 5).

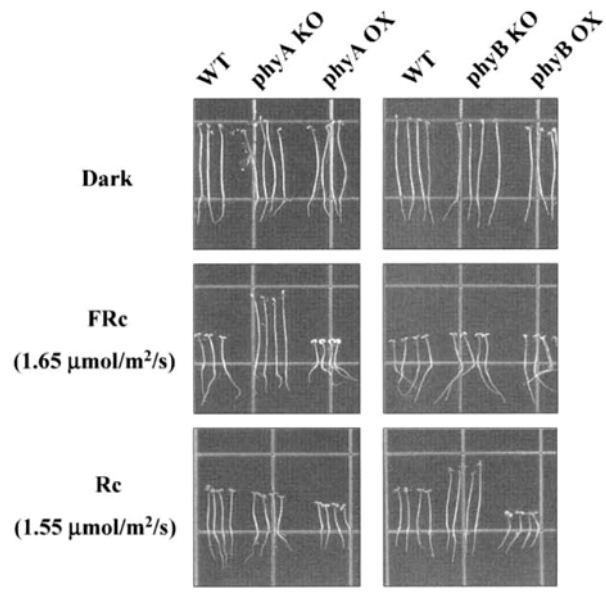

Figure 5. Seedling de-etiolation of phytochrome mutants under continuous far-red (FRc) and continuous red (Rc) light. WT, wild-type *Arabidopsis*; phyA KO and phyB KO, phyA knockout and phyB knockout *Arabidopsis*; phyA OX and phyB OX, *Arabidopsis* overexpressing phyA and phyB, respectively. Because de-etiolation under FRc is mediated exclusively by phyA, and that under Rc is mediated predominantly by phyB, phyA KO shows etiolated phenotypes under FRc similar to dark-grown seedlings while phyB KO shows etiolated phenotypes under Rc.

Structural motifs and domains in phytochromes

The phytochrome molecule consists of two major structural domains -- the globular N-terminal chromophore-binding domain (~65 kDa) and the conformationally open or extended C-terminal domain (~55 kDa). These two are connected via a flexible hinge region. The N-terminal light-sensing domain has a few sub-domains (Kim et al., 2005; Rockwell et al., 2006): N-terminal extension (NTE), bilin lyase domain (BLD), and phytochrome-specific domain (PHY). NTE is dispensable for chromophore binding, but is necessary for biological activity. A domain swap experiment has demonstrated that the chromophore-bearing N-terminal domains of phyA and phyB determine their photosensory specificity and differential light lability. The difference in NTEs between phyA and phyB might explain their photosensing specificities. For example, the longer NTE of phyB could induce phyB-specific inter-domain crosstalk with the C-terminal domain. The bilin lyase domain (BLD), a chromophore-binding GAF domain found on phytochromes, is sufficient for bilin attachment (Wagner et al., 2005; Mateos et al., 2006). Finally, the PHY domain is a distinct and GAF-related domain on eukaryotic phytochromes adjacent to the BLD, and might interact with the D ring of the chromophore to stabilize the Pfr form (Kim et al., 2005; Rockwell et al., 2006).

The importance of the C-terminal half is illustrated by numerous missense mutations that affect this part of the protein (Quail et al., 1995; Krall and Reed, 2000). The C-terminal domain contains the Per-Arnt-Sim (PAS)-related domain (PRD), which consists of a pair of PAS repeats (PAS1 and PAS2), as well as the histidine kinase-related domain (HKRD) (Fig. 1A). Phytochrome PRD has a regulatory core domain for phytochrome dimerization (Kim et al., 2006), nuclear localization (Chen et al., 2005), and the protein-protein interaction of phytochromes with their downstream components (Shen et al., 2005). HKRD is homologous to a histidine kinase domain, but may not be a functional kinase domain because the key conserved residues within the histidine kinase domain (HKD) are absent in the phytochrome HKRD (Kim et al., 2005); this domain is necessary but dispensable for phyB signaling (Krall and Reed, 2000). Thus, it is likely that the HKRD domain plays a regulatory role in phytochrome signaling because the domain interacts with downstream signaling components such as PKS1 (Phytochrome Kinase Substrate 1; Fankhauser et al., 1999) and PAPP5 (Phytochrome-Associated Protein Phosphatase 5; Ryu et al., 2005). By itself, the phyB N-terminal domain is functional *in vivo* when it can exist as dimers and be localized in the nucleus (Matsushita et al., 2003; Oka et al., 2004). This suggests a model that describes the C-terminal domain as being dispensable for phytochrome functions except dimerization and nuclear localization. Considering that the N-terminal domain is apparently necessary and sufficient for phytochrome activity, it is difficult to explain why many of the missense phytochrome mutants are due to mutations in the C-terminal regulatory domain (Quail et al., 1995). It is also puzzling why many downstream components, such as NDPK2 (Nucleoside Di-Phosphate Kinase 2; Choi et al., 1999), PKS1, ELF3 (EARLY FLOWERING 3; Liu et al., 2001), and Ado1/ZTL (ZEITLUPE; Jarillo et al., 2001), can be obtained by yeast two-hybrid screens that use the C-terminal domain as bait. It is likely that this domain may be necessary for the fine-tuning of phytochrome signaling events via signal attenuation and amplification.

Phytochrome signal transduction pathways in plants

Since their discovery in the 1950s, phytochromes have been studied intensively through a broad range of experimental approaches. However, there is no definitive picture for how they transduce light signals into physiological responses. Nevertheless, recent examinations have resulted in several important advances in our understanding of this signaling. Four major consequences of phytochrome activity have been described for the control of 1) protein subcellular localization, 2) transcription, 3) protein stability, and 4) protein phosphorylation. First, the photoconversion of cytoplasmic Pr to Pfr causes the translocation of phytochromes into the nucleus (Kircher et al., 2002; Nagatani, 2004). Thus, activation of phytochrome signaling brings that phytochrome into the vicinity of the genes that it regulates. Because phytochromes interact with transcriptional regulators such as PIF3 (Phytochrome-Interacting Factor 3), these findings have allowed researchers to devise a general model for action, whereby phytochromes perceive light, enter the nucleus, interact with transcriptional regulators, and thus regulate gene transcription (Chen et al., 2004; Lorrain et al., 2006).

The photoconversion of phyA to Pfr stimulates its protein degradation via ubiquitination and 26S proteasome (Sullivan et al., 2003; Seo et al., 2004; Hoecker, 2005). Many other light signaling components, such as PIF3, HFR1 (long hypocotyl in far-red 1), LAF1 (long after far-red light 1), and HY5, are also regulated by ubiquitin-dependent proteolysis (Hardtke et al., 2000; Seo et al., 2003; Duek et al., 2004; Park et al., 2004; Al-Sady et al., 2006). It has been suggested that phytochromes regulate this protein degradation in plants (Bauer et al., 2004). Thus, it is possible that phytochrome mediates photomorphogenic responses, in part, by controlling protein degradation signaling. Nevertheless, one must still elucidate how these phytochromes regulate the ubiquitin-dependent proteolysis signaling.

It has been reported that phosphorylation in the hinge region prevents phyA from interacting with its downstream components (Kim et al., 2004). Likewise, a phytochrome-specific protein phosphatase, PAPP5, positively regulates phytochrome interaction with NDPK2 and increases protein stability (Ryu et al., 2005). Therefore, phytochrome phosphorylation and dephosphorylation are also important for its signaling. Because phytochromes are known as serine/threonine kinases (Yeh and Lagarias, 1998), it is possible that they exert their kinase activity to control those downstream components. Collectively, phytochrome signaling comprises a highly regulated network that is, in turn, regulated by various mechanisms. To elucidate these signaling complexities, further studies should investigate, for example, how phytochrome-interacting proteins and their relationship with phytochromes trigger the downstream signal transduction cascade in plant photomorphogenesis, and how these various control mechanisms cross talk.

STEPS IN PHYTOCHROME-MEDIATED PHOTOMORPHOGENESIS

Many nuclear and cytoplasmic factors that are known to be phytochrome signaling components have been identified by genetic screens; their signaling branches include positive and negative regulations (Table 2). Such screening has identified two classes of components -- those acting downstream of a single photoreceptor and those that function downstream of multiple photoreceptors. This presumably is based on the fact that light signals perceived by different photoreceptors must be integrated, e.g., positively acting factors (i.e., HY5 and HYH) and a large group of negative regulators of photomorphogenesis (DET/COP/FUS). The study of these mutants and phytochrome-interacting proteins has revealed a complex signaling network (Fig. 6). Here, we divide this network into four major steps.

Step I. Conformational changes upon light absorption

The most likely first step in phytochrome-mediated photomorphogenesis is the conformational change following the absorption of light. Because nuclear localization of the phytochrome is important for interactions with downstream components in the nucleus, and because this localization

Table 2. Signaling components in phytochrome-mediated photomorphogenesis.

Component	Characteristics	Phenotype of mutant	Signaling/Interaction
HFR1	bHLH transcription factor	Long hypocotyl in FR	phyA signaling
LAF1	R2R3 MYB-like transcription factor	Impaired response to FR	phyA signaling
FHY1	Novel light regulated protein	Impaired response to FR	phyA signaling
FHY3	Not cloned	Impaired response to FR	phyA signaling
FIN2	Not cloned	Impaired response to FR	phyA signaling
FAR1	Putative coiled-coil domain	Impaired response to FR	phyA signaling
PAT1	VHIID/GRAS protein	Insensitive to FR	phyA signaling
FIN219	Auxin-inducible GH3 protein	Impaired response to FR	phyA signaling
SPA1	WD repeat protein	Suppressor of a weak phyA mutation	phyA signaling/COP1 in Y2H[a]
EID1	Increased sensitivity to far-red	F-box protein with leucine zipper motif	phyA signaling AKS1 and AKS2 in Y2H
SUB1	Ca^{2+}-binding protein	Hypersensitive responses to B and FR	phyA and CRY signaling Negative regulation of HY5
PIF3	bHLH transcription factor Phytochrome Interacting Factor 3	Hypersensitive for R--induced de-etiolation	phyA & phyB in Y2H
NDPK2	Nucleotide diphosphate kinase 2	Impaired response to red and far-red	phyA & phyB in Y2H
PEF1	Not cloned	Impaired response to red and far-red	phyA & phyB signaling
PKS1	Phytochrome kinase substrate 1	Impaired response to R in overexpressor	phyB signaling phyA & phyB in Y2H
PSI2	Not cloned	Hypersensitive to red and far-red	phyA & phyB signaling
GI	Circadian clock-controlled gene GIGANTEA	Impaired response to red	phyB signaling
ELF3	Circadian clock-regulated protein	Early flowering	phyB signaling
ARR4	*Arabidopsis* response regulator 4	Hypersensitivity to R in overexpressor	phyB signaling
PEF2/PEF3	Not cloned	Impaired response to red	phyB signaling
RED1	Not cloned	Suppressor of a phyB overexpressor phenotype. Impaired response to R	phyB signaling
PIF4/SRL2	bHLH transcription factor Phytochrome-interacting factor 4	Short under red-light	phyB signaling & binding
SRL1	Not cloned	short hypocotyl in red light enhanced responsiveness to cR	phyB signaling
COP/DET/FUS	COP1, RING finger/coiled-coil/WD40 DET1, novel nuclear localized protein	Photomorphogenic phenotype in the dark	phyA, phyB and CRY signaling
HY5	bZIP transcription factor	Impaired responses to far-red, red and blue light	COP1 in Y2H

[a]Y2H, yeast two-hybrid.

occurs only with the Pfr forms, the conformational changes from Pr to Pfr are critical in phytochrome signaling. For example, the extreme N-terminal extension (NTE) is altered from a random coil in Pr to an amphiphilic a-helix in Pfr (Kim and Song, 2005). Two tryptophan residues near the core regulatory region of oat phyA become preferentially exposed in the Pfr form, as demonstrated by fluorescence quenching. Based on these results, we have proposed a scenario in which the NTE covers the regulatory C-terminal domain in the Pr form by interacting with the regulatory core region (Kim and Song, 2005). After the Pr-to-Pfr phototransformation, the NTE is withdrawn by its interaction with the chromophore, exposing that core region to interactions with downstream components for generating phytochrome signaling (we designate this model as "interdomain crosstalk"). A surface plasmon resonance study of oat phyA with monoclonal antibodies has provided conclusive evidence for Pr-to-Pfr dependent topographic changes in the NTE and the C-terminal subdomain (Natori et al., 2007). Regulation of phyB nuclear localization is also controlled by this interdomain crosstalk (Chen et al., 2005). Therefore, this step is important for enhancing our understanding of phytochrome signaling in plants, and for explaining how phytochromes interact with multiple partners and regulate cytoplasmic/nuclear localization for photomorphogenesis.

Besides nuclear localization and interactions with downstream components, our studies have demonstrated the importance of Pr-to-Pfr phototransformation in regulating phytochrome signaling through phosphorylation and dephosphorylation. For example, phosphorylation at Ser-598 of oat phyA in the hinge region has been identified as an attenuating mechanism for phytochrome signaling that prevents phyA interaction with its downstream components, NDPK2 and PIF3 (Kim et al., 2004). These results indicate that Ser-598 phosphorylation has an inhibitory role in photomorphogenesis. We have also reported that two phytochrome-specific protein phosphatases -- FyPP (flower-specific, Phytochrome-associated Protein phosphatase) and PAPP5 -- positively modulate phy-

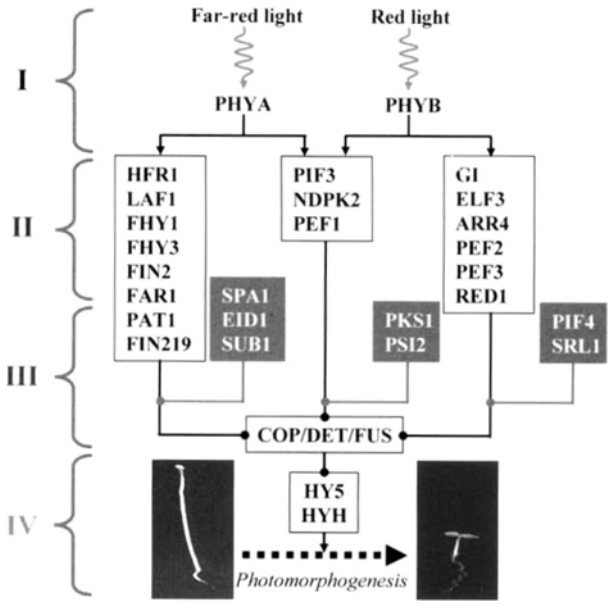

Figure 6. Simplified signaling network for phytochrome-mediated photomorphogenesis. PhyA and phyB have both separate and shared signaling components, with COP/DET/FUS serving as signal integration point in phytochrome signaling network. Arrowheads indicate positive signaling, and close circles indicate negative signaling. Downstream components are also summarized in Table 2. Phytochrome-mediated photomorphogenesis signaling network is divided into four major steps. Step I is light absorption by phytochrome and subsequent conformational changes; Step II is interaction of phytochromes with various downstream components directly or indirectly, and initiation of signaling cascade; Step III is integration of signaling through ubiquitin-dependent proteolysis; and Step IV is regulation of genes for photomorphogenesis.

tochrome signaling (Kim et al., 2002a; Ryu et al., 2005). The latter is co-localized with phytochromes in the nucleus in a light-dependent manner, promoting their interaction with downstream signal transducers such as NDPK2. Moreover, phytochrome stability is increased by PAPP5. These results are consistent with other reports of the negative regulation of phytochrome signaling in plants through protein phosphorylation. Thus, two roles are possible: namely, the control of phytochrome interactions with downstream components and protein stability. Therefore, early phytochrome-mediated signal transduction is modulated by protein phosphorylation and dephosphorylation; the former blocks interaction with its signal transducers, while the latter enforces that interaction. In addition, phosphorylation destabilizes phytochromes while dephosphorylation enhancing their protein stability.

Step II. Interaction of phytochrome with downstream components

Most current research on light-regulated plant development has focused on the signaling events downstream of photoreceptors, with a major breakthrough coming from the examination of de-etiolation in *Arabidopsis* seedlings (Møller et al., 2002; Jiao et al., 2007). Many downstream components of the phytochrome signaling pathways have been identified in studies of mutants defective in different aspects of de-etiolation (Table 2; Step II in Figure 6). Some components interact directly with phytochromes, while others do not, but, instead, mediate phytochrome signaling (Y2H *vs.* signaling in Table 2). In early examinations, yeast two-hybrid (Y2H) screens of cDNA libraries using the C-terminal domain of the phytochrome molecule revealed a few proteins capable of interacting with the photoreceptor, such PIF3 (Ni et al., 1998), NDPK2 (Choi et al., 1999), and PKS1 (Fankhauser et al., 1999). PIF3 is a basic helix-loop-helix (bHLH) transcription factor that exhibits phytochrome-mediated and light-dependent binding to the G-box promoter regions of various light-responsive genes, thereby providing a plausible mechanism for the direct photoregulation of gene expression by phytochromes (Martínez-Garcia et al., 2000). PIF3 can bind to both phyA and phyB, but its affinity is 10-fold lower in the former (Kim et al., 2003; Monte et al., 2004), suggesting that it is a transcriptional factor for phyB signaling in plants. NDPK2, activated in the presence of phyA, appears to play a role in both phytochrome and auxin signaling (Choi et al., 2005). The Pfr form of phyA specifically interacts with NDPK2, stimulating its γ-phosphate exchange activity *in vitro* by lowering the pKa value of active site H197 (Im et al., 2004; Shen et al., 2005). PKS1 is differentially phosphorylated under red-light conditions *in vivo*, and is a kinase substrate of phytochromes *in vitro*. It appears to be a negative regulator of phyB signaling, and forms a regulatory loop with its closest homologue in *Arabidopsis* (PKS2) to modulate phyA-mediated responses (Lariguet et al., 2003). PKS1 has also been suggested as providing a molecular link between phytochromes and phototropins (Lariguet et al., 2006). In addition, physical interactions with phytochromes have been shown for cryptochromes, the blue-light photoreceptors (Ahmad et al., 1998; Mas et al., 2000); as well as for Aux/IAA proteins (Colón-Carmona et al., 2000); two clock-input components, ELF3 (Liu et al., 2001) and ZTL (Jarillo et al., 2001); the response regulator ARR4 (Sweere et al., 2001); FyPP (Kim et al., 2002a); PIF4 (Huq and Quail, 2002); PAPP5 (Ryu et al., 2005); and more.

Because phytochrome-interacting proteins (PIPs) are numerous, we may question why phytochromes interact with so many downstream components and what their molecular mechanisms might be. In this regard, the most interesting group of such components comprise the bHLH class of transcriptional factors (Duek and Fankhauser, 2005; Qu and Zhu, 2006). These include HFR1, PIF3, and PIF3-like proteins (PILs). HFR1 does not interact with phytochromes but is involved in phyA signaling, whereas PIFs (or PILs) interact with phytochromes and are associated with distinct photomorphogenic processes. For example, PIF1/PIL5 acts in chlorophyll biosynthesis and seed germination (Huq et al., 2004; Oh et al., 2004), PIL2/PIF6 and PIL1 in shade avoidance (Salter et al., 2003), PIL6/PIF5 in circadian rhythms (Fujimori et al., 2004), and PIF3 and PIF4 in de-etiolation. These bHLH transcriptional factors are apparently most essential in phytochrome-mediated photomorphogenesis.

Another interesting group contains the protein phosphatases, e.g., FyPP, PAPP5, and PRP2 (Phee et al., 2006).

They all physically interact and dephosphorylate phytochromes. FyPP-overexpressing transgenic plants promote phytochrome activity in flowering and a decline in hypocotyl-lengthening, while the anti-sense repression of FyPP transgenic plants causes reduced phytochrome activity (Kim et al., 2002a). PAPP5 positively influences phytochrome stability and affinity for a downstream transducer, NDPK2, which is involved in the modulation of de-etiolation (Ryu et al., 2005). In the case of cryptochromes, a Type 7 protein phosphatase (PP7) is the only known positive regulator that appears to be specifically required for cryptochrome signaling (Møller et al., 2003). Seedlings with a reduced level of PP7 protein are defective in all de-etiolation responses tested. In addition, phytochrome phosphorylation has proven to be a controlling mechanism for phytochrome activity (Kim et al., 2004, 2005). Thus, the components related to this phosphorylation and dephosphorylation are particularly important in the regulation of phytochrome signaling.

Other interesting proteins are those phosphorylated by phytochromes. Because phytochromes are autophosphorylating serine/threonine kinases (Yeh and Lagarias, 1998; Kim et al., 2005), these components are probably their substrates. They include PKS1, Aux/IAA proteins, cryptochromes, and more (Kim et al., 2005). In addition, it has been reported that the Pfr form of phytochromes induced rapid *in vivo* phosphorylation of PIF3 preceding degradation (Al-Sady et al., 2006), which suggests that phytochrome kinase activity is important for photomorphogenesis. Another bHLH transcription factor, PIL5/PIF1, is also regulated by protein degration (Oh et al., 2006). Thus, the phytochrome-induced phosphorylation of proteins such as PIFs may indicate primary intermolecular signal transduction that tags the target protein for proteosomal degradation, possibly in nuclear speckles. However, the molecular mechanisms by which the phosphorylation of these proteins by phytochromes affects light signaling are still unknown. Further studies will be necessary to elucidate the signaling pathways related to phytochrome kinase activity.

There are also other factors in the cytosol, including PKS1, FIN219, SUB1 (Guo et al., 2001), and GRAS proteins such as PAT1 (Torres-Galea et al., 2006). One of the most rapid physiological actions by phytochrome is its effect on ion fluxes at the plasma membrane (Lee, 2006). Pharmacological studies have also identified cGMP (cyclic guanosine monophosphate) and calcium ions as early components of phytochrome signaling (Neuhaus et al., 1997). These secondary messengers induce chlorophyll and anthocyanin biosynthesis in addition to many light-regulated genes, such as CHS (chalcone synthase) and CAB (chlorophyll a/b-binding proteins). Thus, further investigation of these components could generate useful information for elucidating the cytosolic functioning of phytochromes.

Step III. Signal integration into protein degradation machinery

Protein degradation is emerging as a ubiquitous regulatory mechanism for many cellular processes, including light- and hormone-signaling, circadian rhythms, and flowering (Moon et al., 2004; Hoecker, 2005; Laubinger et al., 2006; Molas et al., 2006; Jiao et al., 2007). Genetic screens have identified mutants that exhibit light-grown phenotypes when grown under darkness, such as opened cotyledons, expanded leaves, and shorter hypocotyls. The genes identified in these screens are *COP* (constitutive photomorphogenesis), *DET* (de-etiolated), and *FUS* (fusca; for the red, or fuchsia, color of the anthocyanins that accumulate in light-grown seedlings) at 11 different loci (Wei and Deng, 2003; Yi and Deng, 2005). The recessive nature of these mutations suggests they encode proteins that operate as repressors of photomorphogenesis in the dark (i.e., the pattern of development observed in the light). Among the *COP/DET/FUS* genes, *COP1* encodes an E3 ubiquitin ligase that is essential for placing a small peptide tag (ubiquitin) on proteins. Once tagged, those proteins are transported into the proteasome where they are digested to their constituent amino acids. COP9 and several other COP/FUS proteins comprise the COP9 signalosome (CSN), which forms the lid of the proteasome complex, helping to determine which proteins enter. Thus, CSN is a protein complex paralogous to the lid subcomplex of the 26S proteasome (Wei and Deng, 2003), and is required for proteasome-mediated degradation of many proteins, such as HY5 (Hardtke et al., 2000). DET1 is a nuclear protein that interacts both biochemically and genetically with the plant homolog of UV-damaged DNA binding protein 1, which, in animal cells, interacts with histone acetyltransferase complexes. Because DET1 interacts with the amino-terminal tail of histone H2B, chromatin remodeling might be involved in light-controlled gene expression.

In dark-grown seedlings, COP1 is a nuclear protein that targets HY5 (long hypocotyl 5) for proteasome-mediated degradation in the nucleus. HY5 is a leucine-zipper transcription factor that directly binds to the promoters of genes whose expression is controlled by light illumination. The *hy5* mutant shows deficient photomorphogenesis in the light, and is epistatic to the *cop1* mutation under darkness. At least phyA, phyB, and CRY1 are able to induce subcellular re-localization of COP1 from the nucleus to the cytoplasm. Thus, light perceived by these photoreceptors causes COP1 migration to the cytosol, and the pool of HY5 is allowed to build up, thereby promoting the expression of light-induced genes. HY5 is only one of the proteins that interacts with COP1; others that are reportedly ubiquitinated by COP1 include PHYA (Seo et al., 2004), LAF1 (Seo et al., 2003), HFR1 (Duek et al., 2004; Jang et al., 2005), and more. Therefore, COP1 plays a key role in regulating photomorphogenic responses. Furthermore, CSN with COP9 is important in the integration of phytochrome signal transduction by connecting between light signals and photomorphogenesis in plants (Step III in Figure 6).

Step IV. Expression of photomorphogenic genes

Phytochromes regulate the transcription of numerous genes in the nucleus (Tepperman et al., 2004; Mazzella et al., 2005; Khanna et al., 2006). Many of those genes are involved in greening, such as chlorophyll a/b-binding proteins (CAB) in the light-harvesting complex and chalcone synthase (CHS). However, phytochromes can also repress transcription. Microarray analyses have indicated that the expression of thousands of genes changes in response to red or far-red light; this amounts to about 10% of the total

genome in *Arabidopsis*. Activation or repression of those genes is thought to be mediated by general transcription factors. In some cases, photoactivated phytochromes interact directly with these factors, e.g., PIF3 and PILs. In other cases, phytochromes do not interact directly but only influence the transcriptional activity of some factors via a signal cascade, e.g., HFR1 and HY5. Transcriptome analyses, ranging from skotomorphogenesis to photomorphogenesis, have revealed large differences in the expression patterns of genes involved in photosynthetic light reactions, photosynthetic carbon metabolism, starch and sucrose synthesis, photorespiration, cell wall synthesis, protein synthesis in chloroplasts, phenylpropanoid biosynthesis, chlorophyll and heme synthesis, transcription factors, and ubiquitin-proteasome pathways (Casal and Yanovsky, 2005). Thus, light (through its absorption by photoreceptors) profoundly affects the expression of many genes in photomorphogenesis.

From transcriptome analyses of phytochrome mutants, researchers have found that the largest functional classes responding to far-red light correspond to the genes involved in photosynthesis, chloroplast development, and cellular metabolism. Although the development of full photosynthetic capacity can take several days, some photosynthetic genes respond to the far-red light signal within the first hour of treatment. Transcription factors belonging to diverse classes, including zinc-finger, bZIP, homeodomain, MYB, APETALA 2 (AP2)-domain, WRKY, and bHLH proteins, dominate this group of early genes (Khanna et al., 2006). Therefore, these reports suggest that the massive change in gene expression induced by phyA activation is probably a result of a transcriptional cascade. Continuous red light also induces altrations in transcript levels that largely overlap those associated with continuous far-red light (Tepperman et al., 2004). The similarity is particularly striking when one considers those effects on early-responsive transcription factors. For example, although the *phyB* mutant has a clear morphological phenotype, under red light its transcriptome is just slightly different from the wild type. Only 14% of the genes that respond to red light exhibit a relatively robust dependence on phyB. The residual red-light effect observed in the *phyAphyB* double mutant is at least partially mediated by other phytochromes, phyC/phyD/phyE. Clearly, the various processes simultaneously controlled by red light have differential contributions from several members of the phytochrome family. Therefore, a future challenge will be to account for this complex signaling network in dynamic terms. Achieving this goal will require the identification of rate-limiting steps in signaling under specific contexts.

CONCLUDING REMARKS

Our understanding of phytochrome-mediated photomorphogenesis has improved dramatically in recent years. Phytochrome migration to the nucleus followed by Pfr-PIF/PIL-DNA element interaction presents a shortcut between light signals and the target genes. The control of COP1 localization by phytochrome, coupled to the role of COP1 in the proteolytic degradation of transcription factors that mediate photomorphogenesis, provides a second route for controlling this signaling. Regulation of phytochrome functioning by phosphorylation and dephosphorylation indicates yet another alternative for modulating photomorphogenesis. Therefore, phytochrome signaling is mediated by a network with multiple points of convergence and divergence via the control of protein subcellular localization, transcription, protein stability, and protein phosphorylation. Although dramatic progress has been made during the past decade in delineating the mechanism for phytochrome-signaling, largely through molecular genetics studies in the model plant *Arabidopsis*, it is also clear that information about the nature and extent of this phytochrome-mediated signaling network is still limited and fragmented. Much remains to be learned in order to build a connected network, with the ultimate goal of understanding how the various nodes in that network interact to transmit light signals in plants.

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