

LIGHT SIGNAL TRANSDUCTION IN HIGHER PLANTS

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■ **Abstract** Plants utilize several families of photoreceptors to fine-tune growth and development over a large range of environmental conditions. The UV-A/blue light sensing phototropins mediate several light responses enabling optimization of photosynthetic yields. The initial event occurring upon photon capture is a conformational change of the photoreceptor that activates its protein kinase activity. The UV-A/blue light sensing cryptochromes and the red/far-red sensing phytochromes coordinately control seedling establishment, entrainment of the circadian clock, and the transition from vegetative to reproductive growth. In addition, the phytochromes control seed germination and shade-avoidance responses. The molecular mechanisms involved include light-regulated subcellular localization of the photoreceptors, a large reorganization of the transcriptional program, and light-regulated proteolytic degradation of several photoreceptors and signaling components.

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GENERAL INTRODUCTION

The survival of single-cell or multicellular organisms depends on their ability to accurately sense and respond to their extracellular environment. Light is a very important environmental factor and many species have evolved sophisticated photosensory systems enabling them to respond appropriately. Being sessile and photoautotrophic, plants are particularly sensitive to this crucial external signal (48, 147). In this review we focus on light responses in higher plants and emphasize recent progress in our understanding of the molecular events occurring upon photon capture. Most of this work has been performed in the small weed *Arabidopsis thaliana*, which has become the favorite subject of study for molecular genetics. Classic photobiological studies have determined that plants are most sensitive to UV-B, UV-A/blue, red, and far-red light (48, 147). The molecular nature of the UV-B photoreceptors is still elusive. Two families of UV-A/blue light receptors, the phototropins and the cryptochromes, have been identified (14, 111). More recently, a third family of putative blue light photoreceptors has been uncovered (85). The phytochromes were the first family of plant photoreceptors to be discovered; they are most sensitive to the red and far-red region of the visible spectrum (148). Although these photoreceptors specifically affect individual light responses, in most instances there is also abundant crosstalk between the different photosensory systems (19).

UV-B

In plants, UV-B light triggers developmental responses (10, 17, 95). In *Arabidopsis* seedlings, UV-B responses include inhibition of hypocotyl elongation and transcriptional regulation of a large number of genes (10, 95, 173, 183). Genome-wide analysis of gene expression suggests the possible involvement of more than one UV-B sensing mechanism (183). UV-B photoreceptor(s) are still unknown; however, these UV-B responses are clearly not triggered by the known photoreceptors, phototropins, cryptochromes, or phytochromes (173, 183). Two signaling components required for normal UV-B responses have been identified. ULI3 is specifically involved in UV-B responses. *uli3* mutants have no obvious phenotype when grown under any other light condition. The *ULI3* gene codes for a protein of unknown function with putative heme and diacylglycerol binding sites. ULI3-GFP fusions are localized in the cytoplasm and at the plasma membrane (173). The second known component of UV-B signaling is the bZIP transcription factor HY5. In contrast to ULI3, HY5 is required for normal development under all light conditions (183).

PHOTOTROPINS

Physiological Responses Mediated by the Phototropins

Phototropic responses were described more than a century ago. Typically, plant stems bend toward a unilateral source of light. In contrast, roots grow away (negative phototropism) from a unilateral light source. Action spectra of this response have shown that in higher plants this is a blue light response with maximal response around 450 nm (14, 16). The photoreceptor responsible for directional growth was identified just 7 years ago (79). In this short time, enormous progress has been made concerning the structure and the functions of this family of photoreceptors. We invite the readers to consult several recent and excellent reviews for a more in-depth coverage of the field (14, 15, 33, 114, 186). Phot1 (originally *nph1* for non phototropic hypocotyl) was identified based on the inability of *phot1* mutant hypocotyls to bend towards unilateral blue light (113). Phot2, a second phototropin, is present in *Arabidopsis* (13). This pair of photoreceptors is extremely important for a number of light responses that ultimately allow optimal photosynthesis, including phototropism, chloroplast movements, and stomatal opening (14, 186). This is consistent with photobiological studies that have shown that all these responses have similar action spectra. Phot1 is specialized for low blue light fluence rates; in contrast, phot2 is more important for high light responses (14). This can be observed for phototropism where phot1 alone is required under low light, but phot1 and phot2 have a redundant activity under higher light intensities (113, 150).

Higher plants display two types of chloroplast relocalization responses: a chloroplast accumulation response that maximizes light capture in low light, and a chloroplast avoidance response that minimizes chloroplast photodamage in high light (186). Phot2 is responsible for the chloroplast avoidance response, whereas phot1 acts redundantly with phot2 to achieve the accumulation response (89, 92, 150). The phot2-mediated chloroplast-avoidance response is of critical importance for plant survival in high light conditions (93).

Blue light-driven stomatal opening is also a phototropin-mediated response (98). However, this light response is also controlled by other photosensory systems including a UV-B and a blue-green light receptor (44, 177). A more in-depth analysis of *phot* mutants has shown that these photoreceptors are required for additional light responses. Phot1 transiently controls light-mediated inhibition of hypocotyl growth (54). The phototropins play a modest role in the blue light-induced remodeling of the transcriptional program; however, phot1 is essential for the high blue light-induced destabilization of the *LHCB* and *RBCL* transcripts (51, 141). In addition, both phototropins redundantly mediate cotyledon and leaf expansion (141, 152).

Phototropin Structure, Regulation, and Mode of Action

The phototropins are composed of an amino-terminal photosensory domain and a carboxy-terminal Ser/Thr protein kinase domain (14) (Figure 1A). A FMN (flavin

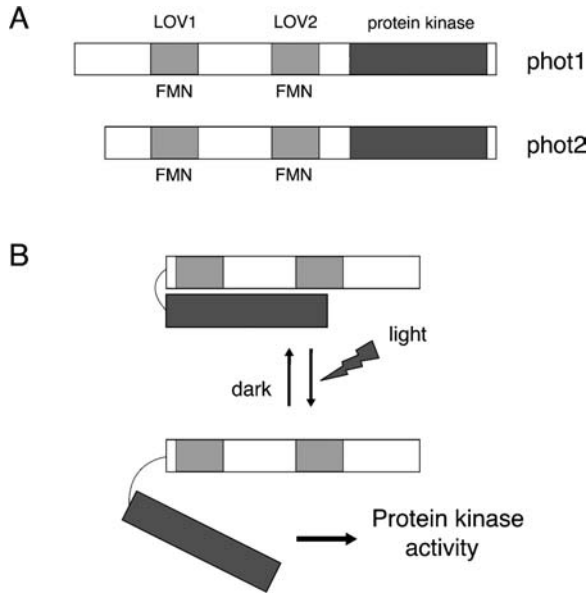


Figure 1 Structure and proposed mechanism of light activation of the phototropins. (A) Schematic representation of the phototropin structure. The phototropins contain two FMN binding LOV domains and a canonical Ser/Thr protein kinase domain at the C terminus. (B) Schematic mechanism of light activation according to the model proposed by (69).

mononucleotide) molecule tightly bound to the so-called LOV (Light, Oxygen, Voltage) domains allows light sensing. LOV domains are structurally related to PAS (Per, Arnt, Sim) domains (14). LOV domains are encountered in numerous photoreceptors from plants, fungi, and bacteria; they are coupled with a wide variety of signaling domains (33). An exceptional feature of phototropins is that they contain two LOV domains, called LOV1 and LOV2 (33). Photochemical and functional analyses have clearly demonstrated that these two domains play distinct functions (29).

The characterization of recombinant phot1 demonstrated that it is indeed the primary photoreceptor for phototropism (27, 28). In the dark state, phot1 binds to FMN noncovalently. The absorption spectrum of this recombinant protein closely matches the action spectra for phot1-mediated responses (27, 28). Phosphorylation of a 120-kD membrane protein is an early marker for phototropism (15, 16). The identification of phot1 showed that the phosphorylated protein is the photoreceptor itself (79). Since recombinant phot1 undergoes this light-dependent reaction in the absence of any other plant proteins, it can be concluded that phot1 is necessary and sufficient for light perception and light-regulated protein kinase activity (27). Recombinant phot2 has very similar spectral and protein kinase properties (150).

The structures of several LOV domains have confirmed the similarity between LOV and PAS domains (31, 33). Both spectroscopic and structural analyses have

uncovered a self-contained light cycle that this photoreceptor undergoes upon photon capture (32, 154). In the ground state, FMN is noncovalently bound to the LOV domain. Light absorption triggers a transient covalent binding of the FMN molecule to an invariant Cys residue in the core of the LOV domain (32, 154). The return to the ground state is a relatively slow process, suggesting that this light-activated (signaling) state is long-lived (33). The functional importance of the transient covalent attachment of the FMN to the Cys has been demonstrated both for phot1 and phot2 (29). Moreover, the LOV2 domain is clearly more important for biological activity than the LOV1 domain (29). Consistent with this finding, spectroscopic analysis indicates that the LOV2 domain is the predominant photosensing domain of phototropins (29, 33).

Modest structural rearrangements are observed during the photocycle of isolated LOV domains (32, 155, 175). However, a recent NMR study of an oat phototropin construct containing the LOV2 domain with a 40 amino-acid carboxy-terminal extension (the linker helix between the LOV2 domain and the protein kinase domain) indicates a large light-driven structural rearrangement (70). In the dark, the helix is associated with the LOV2 domain, but light disrupts this interaction. This suggests that in the dark the protein kinase domain is closely associated with the amino-terminal photosensory domain (closed conformation). Upon light absorption, this interaction is broken, liberating the protein kinase domain and presumably allowing protein kinase activity (Figure 1B) (70).

Phot1 and phot2 are highly similar proteins; they are both plasma membrane associated, but the mechanism of membrane binding is currently unknown (67, 152). Upon light stimulation, a fraction of phot1 is released into the cytoplasm (152). In etiolated seedlings, phot1 expression is strongest in the elongation zones of both the hypocotyl and the root. These are the regions of the seedling where the light signal for phototropism is perceived (152). phot1 is evenly distributed at the plasma membrane of epidermal cells but largely confined to the plasma membrane close to the transverse cell walls in cortical cells. This asymmetric distribution might be relevant for the asymmetric growth response initiated by unilateral light. The fairly good overlap between phot1 localization and the localization of members of the PIN family of auxin efflux carriers is quite tantalizing given that phototropin activation ultimately leads to an asymmetric auxin distribution to allow oriented growth (see below) (57, 152). In leaves, phot1 is uniformly expressed at the plasma membrane of epidermal, mesophyll, and guard cells (152). It is also noteworthy that phot1 protein levels decrease when seedlings are exposed to extended periods of light, a possible explanation for the greater importance of phot1 under lower fluence rates (152). *PHOT2* mRNA levels are very low in the dark but are light-induced, a possible explanation for the more prominent role of phot2 in high light-mediated processes (89).

Phototropin-Mediated Signaling

Light-regulated phot1 autophosphorylation appears to be the initial event in the transmission of the light signal. It has long been recognized that this biochemical response requires significantly more light than some of the phototropin-mediated

responses (15). The role for autophosphorylation is, therefore, still not fully resolved. Phot1 autophosphorylates at multiple Ser residues (156). Phosphorylation of some of these sites already occurs in response to low fluences of blue light (sufficient to trigger phototropism); in contrast, other sites require much higher fluences (156). As such, it is plausible that phosphorylation of some of the residues is required for the signaling state. In contrast, other phosphorylation events may be involved in a desensitization mechanism (156). Another possibility is that phosphorylation of phot1 is required for phot1-mediated inhibition of hypocotyl growth, rather than phototropism, because the fluence rate requirements for inhibition of hypocotyl growth are much higher (52). After phototropin autophosphorylation, a 14-3-3-type protein binds rapidly to the activated photoreceptor (99). Such proteins are often involved in signaling and upon binding they can alter enzymatic activities, modify subcellular localization, or serve as a landing platform for additional interactions (159). The functional significance of this finding remains to be elucidated but the temporal correlation between light-activated autophosphorylation and 14-3-3-binding is striking. A second unresolved point is whether phototropins also phosphorylate other proteins in addition to themselves. So far no phototropin substrate has been reported, although after phot1 autophosphorylation, a plasma membrane H⁺ ATPase also becomes phosphorylated. Both phosphorylation events are inhibited by flavoprotein inhibitors, suggesting the requirement of a phototropin to phosphorylate the plasma membrane H⁺ ATPase (99). Although this study does not prove that the H⁺ ATPase is a phototropin substrate, activation of this enzyme makes sense since activation of the H⁺ ATPase is a very early event allowing stomatal guard cell opening (99). Consistent with this idea, epidermal cell strips of *phot1 phot2* double mutants fail to extrude protons and open stomata in response to blue light—again emphasizing the importance of the phototropins for H⁺ ATPase activation (98).

The analysis of mutants with nonphototropic hypocotyls has led to the identification of two classes of proteins that are involved in phototropin signaling. NPH3 is a member of the first class. Similar to phot1, NPH3 is associated with the plasma membrane by an unknown mechanism. The biochemical function of NPH3 is not known, but it can interact directly with phot1 (131). NPH3 is a member of a large plant-specific gene family with more than 30 members in *Arabidopsis* (131, 151). RPT2, another member of this family, binds to phot1 and is also required for phototropism (86, 151). RPT2, but not NPH3, is also required for stomatal opening, indicating that several members of this gene family have distinct functions in phototropin signaling (86). This idea is consistent with the finding that NPH3 is also dispensable for the phot1-mediated inhibition of hypocotyl elongation and indicates early branching during phot1-mediated signal transduction (54).

It has long been suspected that asymmetric growth (the basis for growth toward or away from a light source) requires a gradient of the plant hormone auxin (57). This hypothesis has received strong genetic support with the isolation of a number of mutants defective for phototropism (58, 69, 179). The establishment of such an auxin gradient requires the action of auxin efflux carriers that transport the hormone out of the cell (57). The *PIN* gene family in *Arabidopsis* (57) encodes

these efflux carriers. PIN3 appears to be particularly important to establish auxin gradients in response to changes in the gravity vector and phototropism (58). Upon light stimulation, indirect measurements indicate that an auxin gradient is rapidly established (58). Characterization of the *pin3* mutant suggests that other members of the PIN family act in concert with PIN3 to control tropic growth. Normal localization of PIN1 is required for normal phototropism (8, 140). The ARF (Auxin Response Factor) transcriptional activator, NPH4, and the IAA (Indole-3-Acetic Acid) transcriptional repressor protein, MSG2, represent two other clear connections between auxin-mediated asymmetric growth and phototropism (69, 179). *Msg2* gain-of-function mutants have very similar phenotypes to *nph4* loss-of-function mutants, indicating that an auxin-regulated transcriptional response is required for normal phototropism.

Blue light stimulation leads to a number of very rapid electrophysiological responses. Most notably, Ca^{2+} concentration rapidly rises in the cytoplasm in a phototropin-dependent manner. Ca^{2+} uptake from the apoplast is mediated by phot1 and phot2. Phot2 has also been implicated in Ca^{2+} release from intracellular stores (4, 6, 67, 171). A recent publication provides a first functional implication for these phot1-mediated changes in intracellular Ca^{2+} concentrations (52). When the phot1-mediated change in Ca^{2+} concentration is inhibited with a Ca^{2+} chelator, the rapid blue light-mediated hypocotyl growth inhibition is prevented (52). The same chelator did not affect phototropism (52). These data are consistent with phot1 eliciting distinct signaling mechanisms. Only some of these signaling branches involve changes in cytoplasmic Ca^{2+} levels (52). Changes in Ca^{2+} concentrations are also functionally important for the regulation of stomatal opening. In this case as well, the phot1-mediated changes in Ca^{2+} concentration could be functionally relevant.

Other LOV Domain Photoreceptors in Plants?

In addition to the phototropins, a few other *Arabidopsis* proteins have LOV domains (33). Three have a similar domain organization with an amino-terminal LOV domain, followed by an F-box and several kelch repeats (137, 169). They are known as ZTL (Zeitlupe), LKP2 (LOV Kelch repeat Protein 2), and FKF1 (Flavin-binding, Kelch repeat, F-box). Gain- and loss-of-function experiments have indicated that these proteins are required to sustain normal circadian clock function and photoperiod-dependent flowering in *Arabidopsis* (85, 198). A detailed description of their involvement in circadian biology is beyond the scope of this review, and we refer the readers to the following publication (198). However, this class of proteins may represent a fourth class of photoreceptors in *Arabidopsis* (85). The LOV domain of FKF1, LKP2, and ZTL displays similar photochemistry to the LOV domain of phototropins, with the exception of a very slow dark-reversion rate (85). FKF1 likely regulates the waveform of the circadian expression of the floral inducer *CO*, thereby controlling long day-induced flowering in *Arabidopsis* (85). Despite its close similarity to FKF1, ZTL appears to work in a different way. It interacts with the circadian clock central oscillator component TOC1 via its LOV

domain (119). This interaction appears to mediate dark-dependent degradation of TOC1 protein in a ZTL- and proteasome-dependent manner (119).

CRYPTOCHROMES

Physiological Responses Mediated by the Cryptochromes

The cryptochrome family of UV-A/blue light photoreceptors mediate a number of specific light responses in plants (109, 114). These photoreceptors are very important during de-etiolation, the transition of a dark grown seedling living from its seed reserves to a photoautotrophically competent seedling. This developmental transition includes a massive reorganization of the transcriptional program, inhibition of hypocotyl growth, promotion of cotyledon expansion, and synthesis of a number of pigments including chlorophyll and anthocyanins (109, 114). In addition, this class of photoreceptor is important for photoperiod-dependent flowering induction and in resetting the circadian oscillator (24, 198). It is important to point out that the cryptochromes act in coordination with the phytochromes (discussed below) in numerous instances (19).

Owing to space constraints, we only present a succinct summary of cryptochrome functions. For a more detailed description, we refer the readers to the following recent reviews (16, 109, 111, 114). *Arabidopsis* has two cryptochromes, cry1 and cry2, with known functions and a more divergent family member, cry3, for which there is no known function (102, 111). Both cry1 and cry2 are implicated in resetting the circadian clock (37, 167). cry1 and cry2 are also involved in de-etiolation responses, but cry1 is the primary photoreceptor under high blue light fluence rates, whereas cry2 is most important under low blue light fluence rates (1, 112). Both photoreceptors play partially redundant functions during de-etiolation (122, 126). Most of these physiological responses presumably require an extensive remodeling of the transcriptional program. In response to blue light, this is predominantly mediated by cry1 and cry2, with lesser contributions by the phototropins and phyA (53, 90, 141). Interestingly, the microarray study by Folta and colleagues indicates that blue light-induced inhibition of hypocotyl elongation is a cry1 response occurring by suppressing the levels and/or sensitivity of two phytohormones (gibberellins and auxin) (53).

Flowering time of numerous plants is determined by daylength. *Arabidopsis* is a facultative long-day plant, indicating that it flowers more rapidly when grown in long days than in short days. cry2 mutants flower late in long days specifically (64). Cry1 has a more modest contribution to flowering-time control in *Arabidopsis* (125, 126). It was recently shown that the cryptochromes are directly involved in the light-dependent stabilization of the floral-inducing transcription factor CO (184, 197).

Cryptochrome Structure, Regulation, and Mode of Action

The cryptochromes are structurally related to DNA photolyases, but they do not possess DNA photolyase activity (158). DNA photolyases are a class of UV-A/blue

light-induced enzymes that repair UV-B-induced damage on DNA (158). Although originally identified in *Arabidopsis*, the cryptochromes have now been found in bacteria, plants, and animals (1, 18, 24). Cryptochromes have an amino-terminal photolyase homology region (PHR) noncovalently binding a primary/catalytic FAD chromophore (Flavin Adenine Dinucleotide) and a second light-harvesting chromophore, a pterin or deazaflavin (110, 158). In addition to the PHR domain, most plant cryptochromes have a distinctive carboxy-terminal domain (111). At first glance, the carboxy-terminal extensions of plant cryptochromes have little in common. They are of variable length but they share short stretches of homology (111). Going from the amino-terminal to the carboxy-terminal end of this extension, one finds a DQXVP motif, a stretch of acidic residues, STAES, and finally GGXVP. Following the nomenclature from C. Lin, we refer to these sequence motifs as DAS (Figure 2) (111). Cry3 differs significantly from cry1 and cry2 and is most closely related to the recently identified cryptochrome from cyanobacteria, dubbed cry-DASH (*Drosophila*, *Arabidopsis*, *Synechocystis*, *Homo*) (18, 102). It has no carboxy-terminal extension but has a transient peptide sequence targeting it to both chloroplasts and mitochondria (102).

A mechanism of light activation was proposed for cryptochromes based on the well-described light activation of DNA photolyases (111, 190, 195, 196). In DNA photolyases, an electron is transiently transferred from the FAD chromophore to the damaged DNA (158). A laser flash spectroscopy study of recombinant *Arabidopsis* cry1 is consistent with the existence of such an electron-transfer reaction involving FAD, Trp, and Tyr residues of the cry1 protein (60). This electron-transfer reaction is hypothesized to trigger a conformational change of cry1 that has been proposed to initiate signaling reactions (see below, Figure 2B). No direct proof of such a light-induced conformational change is currently available, but this scenario is consistent with a study showing that the carboxy-terminal domain of cry1 and cry2 can adopt a constitutively activated conformation when fused to the GUS (β -glucuronidase) protein (196). Excellent recent reviews expose the cryptochrome structure-function relationship in detail (23, 111, 114).

The cryptochromes undergo light-regulated photochemistry that is beginning to be unraveled. Based on the homology with DNA photolyases, one might have expected that they also bind DNA. This has actually been demonstrated for *Arabidopsis* cry3 and cry-DASH, its *Synechocystis* homolog (18, 102). In *Synechocystis*, cry-DASH is directly involved in gene regulation; such a function has not been demonstrated yet for cry3 (18). Direct DNA binding of cry1 and cry2 has not been reported; however, a cry2 carboxy-terminal extension-GFP fusion is chromatin associated (34).

An additional enzymatic activity has recently been found for cry1. The recombinant protein binds ATP; this binding is stoichiometric and depends on FAD binding (12). In addition, recombinant cry1 autophosphorylates in a light-regulated manner, but no other substrate has been found (12, 163). Blue light triggers cry1 and cry2 phosphorylation at multiple sites in vivo (162, 163). Some of these sites are within the carboxy-terminal extension of cry2 (162). This reaction is blue light specific and fluence rate dependent (162, 163). Taken together with the in vitro

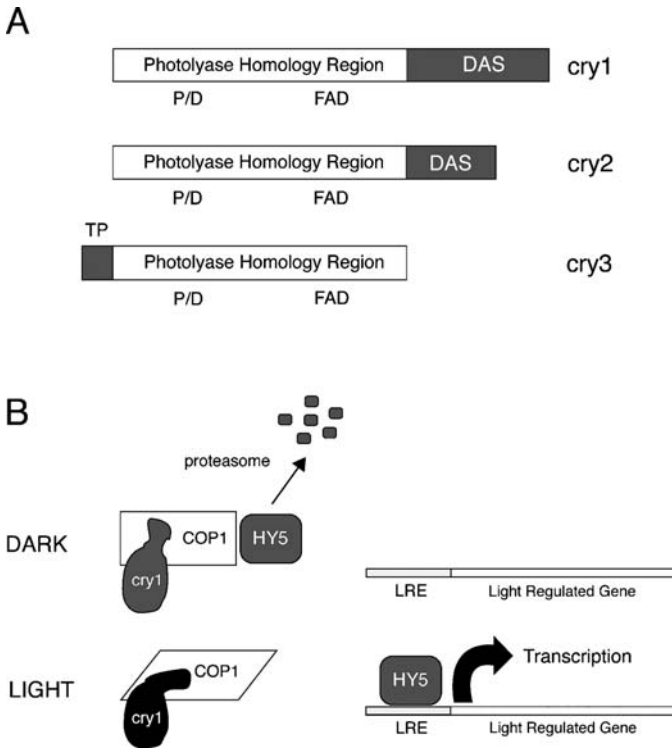


Figure 2 Structure and proposed mechanism of light activation of the cryptochromes. (A) Schematic representation of the cryptochrome structure. The cryptochromes have a photolyase homology region that binds to FAD and a pterin or deazaflavin (P/T). cry1 and cry2 have short carboxy-terminal extensions with little conservation except for short stretches of homology (DAS) according to the nomenclature by Lin & Shalitin (111). cry3 has a transient peptide (TP) required for localization in the chloroplast and mitochondria. (B) Schematic mechanism of light activation according to the model proposed by Cashmore (23). Upon light perception the conformation of cry1 is modified, leading to a conformational change of COP1. The change of COP1 conformation releases the transcription factor HY5 that can activate light-induced genes.

characterization of cry1, one might propose that this is the result of autophosphorylation. An earlier report has shown that phytochrome A can phosphorylate the cryptochromes in vitro (2). However, the phosphorylation state of both cry1 and cry2 does not appear to depend on the phytochromes in vivo (162, 163). Given that a *phyA-phyE* quintuple mutant is currently not available, the role of the phytochromes in cryptochrome phosphorylation cannot be fully excluded. In the case of cry2, phosphorylation is associated with proteolytic degradation (162). This degradation is in part mediated by the E3 ubiquitin ligase COP1. In addition, phosphorylation of both cry1 and cry2 appears to be closely linked to function.

When the carboxy-terminal domain of *cry2* is fused to GUS, it results in constitutive signaling activity (even in the dark) and constitutive phosphorylation (162). Several missense mutants severely affecting *cry1* function in vivo also fail to undergo light-dependent phosphorylation in vivo, again suggesting a link between phosphorylation and function (163).

When fused to either GUS or GFP, *cry1* and *cry2* are nuclear (24, 62, 103, 196). However, *cry2* is constitutively in the nucleus in contrast to *cry1*, which is mainly nuclear in the dark but predominantly cytoplasmic in the light (196). Subcellular fractionation experiments are consistent with this idea (62). The subcellular localization of *cry3* is distinct; this cryptochrome is present in both the mitochondria and the chloroplasts (102).

In young seedlings, *CRY1* and *CRY2* have somewhat different expression profiles. *CRY2* expression is highest in the root and shoot primordia and lower levels are found in the cotyledons, the hypocotyl, and the root (182). *CRY1* is strongly expressed in all aerial parts but absent in the root (182). In addition, both *CRY2* and *CRY1* expression are under circadian control but their phase of expression is different (182). In the case of *CRY2*, this is translated at the protein level. *Cry2* protein levels fluctuate diurnally in short days but remain stable in long days (45, 125). This regulation at the protein level is due to the light-mediated instability of the protein (112, 125). In contrast, *cry1* protein levels do not fluctuate diurnally, presumably because of higher protein stability (112, 125). *Cry2* and *phyA* have similar expression profiles and are light labile. This is interesting in view of their importance in promoting flowering in long days (168, 182, 184, 197, 198).

Cryptochrome-Mediated Signaling

Light-regulated protein degradation appears to be central to cryptochrome signaling. Such a mechanism is well described for animal cryptochromes and also occurs for both *cry1* and *cry2* in *Arabidopsis* (23). Both cryptochromes interact with the E3 ubiquitin ligase COP1 (190, 195). The COP1 protein is required for the light-regulated degradation of several transcription factors involved in light-regulated transcription (77, 142, 161). In the dark, COP1 degrades these transcription factors including the bZIP protein HY5, but upon light perception this degradation is prevented (77, 142, 161). The constitutively de-etiolated phenotype of *cop1* mutants is consistent with this model, since in those mutants a number of transcription factors (and presumably other COP1 targets) can accumulate in the absence of a light signal (161). Similarly, the light-hyposensitive phenotype of *hy5* mutants can also be reconciled with this model (77, 142, 161). COP1 interacts with the cryptochromes both in the light and the dark, indicating that the light-driven electron-transfer reaction that was postulated to induce a conformation change in the cryptochromes does not disrupt this interaction (190, 195, 196). It was proposed that the light-driven conformational modification of the cryptochromes induces a structural modification of COP1 (190, 195). Light-induced alteration of COP1 structure would release HY5 that was bound to COP1 in the dark. HY5 (and other COP1-regulated

transcription factors) can then accumulate and bind to light-regulated promoter elements to initiate de-etiolation (23, 111, 114) (Figure 2B).

The cryptochromes also interact with a number of other proteins, but the functional implications of these interactions are still unclear. The direct interaction between the cryptochromes and the phytochromes is perhaps most exciting, given the large literature indicating coaction between these two families of photoreceptors (2, 118). Photoreceptor interactions might even be more extensive since cry1 can also interact with the putative photoreceptor ZTL in vitro (88).

A limited number of cryptochrome-signaling components have been identified. As already discussed, several transcription factors are required for normal development in response to blue light. They include HY5, which is required for de-etiolation under all light conditions; the HY5 homolog, HYH (HY5 Homologue), which is mainly required in blue light; and the bHLH protein HFR1 (long Hypocotyl in FR light), a component of both cryptochrome and phyA signaling (40, 77, 183). Of note, a negative regulator of cryptochrome-signaling SUB1 (Short Under Blue light) also modulates phyA signaling (63). This gene codes for a cytoplasmic protein with Ca²⁺ binding domains. The identification of this mutant indicates that certain cryptochrome-signaling events occur in the cytoplasm. We have already discussed the blue light-dependent changes in cytosolic Ca²⁺ levels that are mediated by the phototropins and not the cryptochromes. However, the cryptochromes rapidly activate anion channels, resulting in plasma-membrane depolarization and also demonstrating nongenomic functions of the cryptochromes (144). The PP7 protein phosphatase is actually the only positive regulator that appears to be specifically required for cryptochrome signaling. Seedlings with a reduced level of this protein are defective for all tested de-etiolation responses (127). It is currently difficult to make a simple model including all elements involved in cryptochrome signaling. It is probably naive to view these events as a linear pathway. We have a fairly good understanding of some signaling branches (for example, the COP1 branch) but know little about the way in which other signaling events are linked to cryptochrome photoactivation.

PHYTOCHROMES

The discovery of physiological responses, such as the germination of lettuce seeds that is promoted by red (R) light and repressed by subsequent far-red (FR) light, led to the identification of phytochromes (94). It has been suggested that phytochromes evolved from bacterial bilinsensory proteins, a hypothesis that is supported by the discovery of phytochrome-like proteins in photosynthetic bacteria, nonphotosynthetic eubacteria, and fungi (130). *Arabidopsis* phytochromes are encoded by five genes designated *PHYA* to *PHYE* (165). Based on their stability in the light, phytochromes have been classified into two types. Type I phytochromes (photo-labile) accumulate in etiolated seedlings and degrade rapidly upon light exposure, whereas

type II phytochromes (photo-stable) are relatively stable in the light (59). In *Arabidopsis*, phyA is the only type I phytochrome; phyB-E are type II phytochromes (146, 164).

Physiological Responses Mediated by the Phytochromes

Phytochrome responses have been subdivided into different classes based on the radiation energy of light that is required to obtain the response. These include low fluence responses (LFRs), very low fluence responses (VLFRs), and high irradiance responses (HIRs). LFRs are the classical phytochrome responses with R/FR reversibility. VLFRs are not reversible and are sensitive to a broad spectrum of light between 300 nm and 780 nm. HIRs require prolonged or high-frequency intermittent illumination and usually are dependent on the fluence rate of light (20, 21, 134, 166). Genetic studies of *Arabidopsis* phytochrome mutants demonstrate that type I phytochrome phyA is responsible for the VLFR and the FR-HIR, and that phyB is the prominent type II phytochrome responsible for the LFR and R-HIR during photomorphogenesis (134, 148).

Extensive physiological and genetic studies on individual and combinations of phytochrome mutants have unraveled distinct, redundant, antagonistic, and synergistic roles among different phytochromes in *Arabidopsis* development and growth (55, 129; for reviews, see 19, 56). With the exception of seed germination and the shade-avoidance response, which are controlled solely by phytochromes in *Arabidopsis* (22, 136), other physiological processes, including seedling development and floral induction, are controlled by interconnected networks of both phytochromes and cryptochromes (72, 123, 126, 135). The current understanding of the function of each phytochrome in seed germination, seedling establishment, shade-avoidance response, and floral induction has been extensively reviewed recently (56). Here we highlight recent evidence demonstrating that both ambient temperature and photoperiod significantly modulate the function and interaction of photoreceptors.

The effects of temperature and photoperiod are best characterized in the control of *Arabidopsis* flowering time. The roles of each phytochrome and cryptochrome in flowering initiation vary dramatically with a small temperature change from 23°C to 16°C. Null phyB mutants flower early at 23°C but flower at the same time as wild type at 16°C (65). Genetic studies further revealed that phyA/D/E play prominent roles in flowering control at lower temperatures (66). Similar temperature-dependent alterations in flowering time have been observed for *cry1* and *cry2* mutants (9). Besides flowering time, the control of *Arabidopsis* rosette habit (or internode elongation) by phytochromes and cryptochromes is also temperature sensitive (66, 121). Temperature-dependent hypocotyl elongation has also been reported and shown to be related to an increase in auxin levels at elevated temperatures (61). It remains unclear whether temperature-dependent internode elongation is related to auxin levels. Photoperiod modulates the relative contributions of different photoreceptors as well (56, 66). Taken together, these studies have broadened our views on the function of each individual phytochrome, and

elucidated a plastic and sophisticated photosensory network that monitors and responds to a wide range of ambient light and temperature changes.

Phytochrome Structure, Localization, and Function

PHYTOCHROME STRUCTURE Phytochromes are homodimers in solution. Each monomer is a ~125-kDa polypeptide with a covalently attached linear tetrapyrrole chromophore, phytochromobilin, which is synthesized in the chloroplasts from heme (35, 104, 134, 145, 146). The phytochrome protein can be divided into two domains: an amino-terminal photosensory (signal input) domain and a carboxy-terminal domain that has been traditionally regarded as a regulatory, dimerization and signal output domain (146). The N-terminal domain comprises four subdomains: P1 (N-terminal extension, NTE), P2, P3 (bilin lyase domain, BLD), and P4 (Figure 3A) (130, 192). The P3 domain contains a conserved cysteine residue

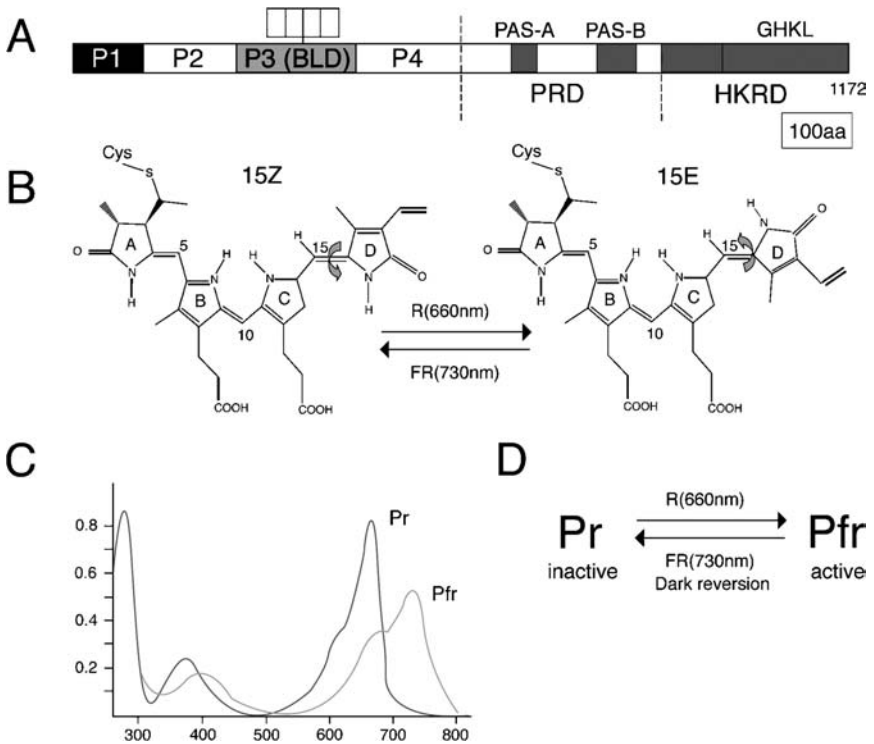


Figure 3 Domain structure and photochemical property of phytochromes. (A) Domain structure of phytochromes using *Arabidopsis* phyB as a model. (B) Two isomers of phytochromobilin, 15Z (Pr chromophore) and 15E (Pfr chromophore). (C) Absorption spectra of Pr and Pfr forms of phytochrome. (D) Photoconversion and dark reversion between Pr (inactive) and Pfr (active) form of phytochrome.

that forms a thioether linkage with the A ring of phytochromobilin and also autocatalyzes the bilin ligation reaction (Figure 3B) (94, 192). The P4 domain has been suggested to directly interact with the D ring of the chromophore to maintain its extended linear conformation in the Pr form and to stabilize the Pfr form (130). The carboxy-terminal half of phytochrome contains two subdomains: a PAS-related domain (PRD) containing two PAS domains (PAS-A and PAS-B) (11) and a histidine kinase-related domain (HKRD), which belongs to the ATPase/kinase GHKL (gyrase, Hsp90, histidine kinase, MutL) superfamily (Figure 3A) (42, 130, 200).

Phytochromes have two relatively stable, spectrally distinct, and interconvertible conformers: an R-absorbing Pr form and a FR-absorbing Pfr form (146). The Pfr form is considered to be the active form because many physiological responses are promoted by R light. Photoconversion between Pr and Pfr, which is triggered by a configuration change between 15Z and 15E isomers of phytochromobilin, occurs upon FR or R absorption, respectively (Figure 3C,D) (94). The Pfr form also converts to Pr thermally, which is called dark reversion (94). Dark reversion rate is a biophysical property of a phytochrome molecule in vitro; however, it can be modulated in vivo (134). For example, ARR4 (*Arabidopsis* response regulator 4) binds preferentially to and stabilizes the Pfr form of phyB (176). The dark reversion rates vary for different phytochromes. *Arabidopsis* phyB has a fast dark reversion rate, which is suggested to be the reason for the fluence rate-dependency of phyB R responses (26, 43, 73). This contrasts with phyA, which is very stable in the Pfr conformation (43, 73). Biochemical studies on oat phyA indicate that the phytochromes undergo substantial structural rearrangements upon phototransformation (143). Some of the significant changes noted include: (a) The P1 domain is relatively exposed in the Pr and forms an alpha-helical conformation shielding the chromophore in the Pfr. (b) The hinge region between amino- and carboxy-halves is more exposed in the Pfr. (c) PAS-B is more exposed in the Pfr than in the Pr form (143). Similar light-regulated structural rearrangements probably hold true for all phytochromes.

Higher plant phytochromes are Ser/Thr kinases (47, 200). The autophosphorylation of oat phyA is down-regulated by chromophore attachment and enhanced by R light (200). Recombinant phyA also phosphorylates a number of proteins in vitro, including PKS1 (protein kinase substrate 1) (50), Aux/IAA (30), cry1, and cry2 (2). However, the physiological significance of these phosphorylation events remains unknown. PhyA from the *Arabidopsis* natural accession Lm-2 has reduced autophosphorylation activity and is less sensitive to FR, suggesting that phyA kinase activity might be important to its function (116). Two in vivo phosphorylation sites in oat phyA have been identified. Ser-7 is phosphorylated in both the Pr and Pfr forms, whereas Ser-598 is preferentially phosphorylated in the Pfr form (105, 106). Phosphorylation on both Ser-7 and Ser-598 has been suggested to desensitize oat phyA activity. Oat phyA with a S598A mutation (143) or deletion of the very amino-terminal serine rich region including Ser-7 (20) expressed in *Arabidopsis* phyA mutant plants are hypersensitive to FR. Recently, the catalytic subunit of a protein phosphatase 2A has been implicated in dephosphorylation

of oat phyA in a light-dependent manner, which could serve as a mechanism to regulate phyA activity (96).

PHYTOCHROME LOCALIZATION One of the major breakthroughs in phytochrome research in recent years is the discovery of light-regulated translocation of phytochromes from the cytoplasm to the nucleus. In *Arabidopsis*, all five phytochromes accumulate in the cytoplasm in the dark and translocate into the nucleus in a light-dependent manner (100, 153, 193). A Pr to Pfr conformational change is required for nuclear import (101). The light quality requirements and nuclear import kinetics are different for each of the different phytochromes (134). PhyA translocates to the nucleus in FR (100, 134), while all five phytochromes accumulate in the nucleus in R or white light (100). The nuclear import of phyA is much faster than that of phyB/C/D/E (100, 134). Only a fraction of phytochrome molecules localize to the nucleus in the light (134).

In the nucleus, phytochromes compartmentalize to discrete small dot-like subnuclear foci (134). Similar localization patterns of both endogenous pea phyA and *Arabidopsis* phyB have been observed by immunolocalization, suggesting that the formation of subnuclear foci is not an artifact of overexpression of phytochrome::GFP fusion proteins (74, 100). Subnuclear compartments have been studied extensively in animal systems. Some of the best-studied are the nucleolus, the speckles [also called the splicing-factor compartments (SFCs) or IGCs (interchromatin granule clusters)], the Cajal body (CB), the promyelocytic leukemia oncoprotein (PML) body, and many other small dot-like nuclear bodies (41, 170). With the exception of the nucleolus, the precise functions of the nuclear bodies remain elusive (41, 170). Since the nature of phytochrome subnuclear foci is still unknown, we adopt a more general term, nuclear body (NB), to describe phytochrome subnuclear domains in this review.

The steady-state pattern of subnuclear phytochrome localization has been most studied for *Arabidopsis* phyB, whose localization to NBs is dependent on the percentage of phytochrome in the Pfr form (26). Nuclear import is not sufficient for phyB NB association, and these two processes have different requirements for the amount of Pfr to total phyB. PhyB nuclear import occurs at very low fluence rates of R light, conditions in which phytochromes are most likely PfrPr heterodimers. PhyB NB formation requires higher fluence rates of R, conditions in which PfrPfr are likely to be present (26). This observation suggests that a PfrPr heterodimer is sufficient for nuclear import and that PfrPfr homodimers favor localization to phyB NBs (Figure 4). The association of phytochrome to NBs also displays a diurnal rhythm (100).

PHYTOCHROME STRUCTURE, LOCALIZATION, AND FUNCTION RELATIONSHIP Recent efforts have focused on the elucidation of the domains of phytochrome responsible for its signaling function and regulated subcellular localization. Localization studies using phyB truncated proteins have revealed that the carboxy-terminal domain localizes to discrete subnuclear foci even in the dark, whereas the

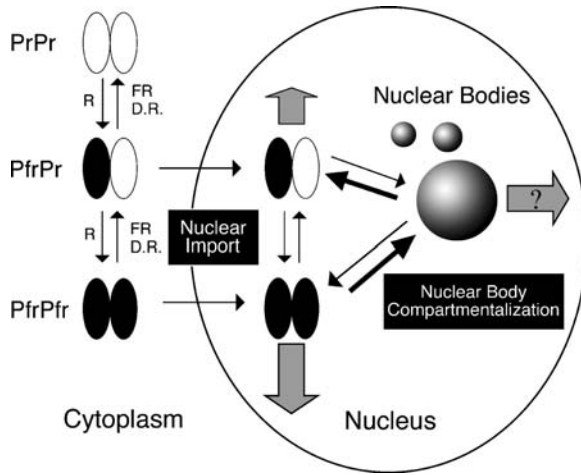


Figure 4 A schematic illustration of phytochrome localization using phyB as a model. There are two steps involved in phytochrome translocation after light activation, nuclear import and localization to nuclear bodies. Nuclear import requires at least one phytochrome molecule in the Pfr form in a phytochrome dimer. In the nucleus, PfrPfr homodimers are more likely to compartmentalize to nuclear bodies. Shaded arrows represent phyB signaling function. D.R., dark reversion.

amino-terminal domain remains mostly in the cytoplasm (120, 133). This suggests that phyB's carboxy-terminus contains the structural requirements for both nuclear import and NB localization, and that the amino-terminal domain regulates both of these two localization signals in a light-dependent manner. As such, both the amino- and carboxy-termini contribute to the subcellular localization of phytochromes. This notion is consistent with studies on the localization of phytochrome mutant proteins. Localization studies on *phyA* and *phyB* PRD mutants have shown that most of them localize to the nucleus but fail to form NBs (26, 100, 199). One notable exception is phyB G767R, which is impaired in nuclear import (120). An unbiased genetic screen for phyB::GFP mislocalization mutants has demonstrated that mutations in the P2, P3, and P4 domains also affect phyB's localization to NBs, suggesting that in full-length phyB the integrity of the holoprotein is crucial for its localization (26). Moreover, deletion of either the N-terminal serine-rich region of oat phyA or most of the HKRD domain of phyB affects its NB patterns as well (20, 26).

The function and localization studies of phyB mutants also suggest that both nuclear localization and association to the NBs are crucial for the function of the full-length protein. A recent study using phyB::GR (glucocorticoid receptor) fusion proteins in transgenic *Arabidopsis* plants provides additional evidence supporting the conclusion that nuclear accumulation is required for the majority of phyB responses during seedling photomorphogenesis (83). Moreover, the formation of

large phyB NBs correlates positively with light responsiveness (26). However, the importance of NBs in phytochrome signaling has been questioned by a recent report. Matsushita et al. demonstrated that the N-terminal half of phyB, when fused to heterologous domains that allow dimerization and nuclear localization, can localize to the nucleoplasm without accumulation in NBs. Surprisingly, transgenic plants expressing such fusion proteins are hypersensitive to R light. Conversely, expression of the carboxy-terminal half of phyB localizes to subnuclear foci and has little function (120). The authors conclude that the amino-terminal domain of phyB is necessary and sufficient for both photosensory and signaling functions of phyB, whereas the function of the carboxy-terminal domain is simply in dimerization, localization, and regulation of the signaling function of the amino-terminal domain (120). As such, NBs may not be required for phyB function, leading to the proposal that localization of phyB to NBs is to desensitize phyB signaling in high intensities of R light (26, 120). On the other hand, many light-signaling components have been reported to localize to subnuclear foci, including cry2 (118), COP1 (185), HY5 (3), and LAF1 (long after far-red light) (5, 118). These data support an alternative hypothesis that full-length phyB localizes to NBs where it can function in a subset of responses in high fluence rates of light (26). Given the recent data showing the colocalization of phyA, LAF1, and COP1 in NBs and COP1-dependent phyA and LAF1 degradation, one possible function of NBs is as a site for protein degradation (5, 160, 161).

Phytochrome Signaling

Extensive genetic studies have identified both shared and separate downstream components for phyA and phyB pathways (81, 188). To uncover early phytochrome-signaling events, phytochrome-interacting proteins have been identified and characterized (134, 148). Here we concentrate only on emerging molecular mechanistic processes involved in phytochrome signaling. Owing to space constraints, we do not discuss the interconnected networks between phytochrome signaling and the circadian clock. We refer readers to recent reviews (49, 124, 198).

PHYTOCHROME AND TRANSCRIPTION REGULATION Global gene expression studies have shown that phytochrome responses are associated with massive alterations in gene expression (38, 115, 148, 180, 189). In phyA pathways, a number of transcription factors are either required for phyA signaling (HY5, LAF1, and HFR1) or are early targets of phyA responses, such as *CCA1* (circadian clock associated) and *LHY* (late elongated hypocotyl) (5, 25, 40, 180). Recently, FAR1 (far-red-impaired response) and FHY3 (far-red elongated hypocotyl), two additional phyA signaling components, have been suggested to be related to transposases regulating transcription as well (80, 82, 187).

Studies on PIF3 (phytochrome interacting factor) and PIF3-like basic helix-loop-helix (bHLH) transcription factors suggest a molecular mechanism directly bridging phytochromes to transcription regulation (148). PIF3, which was

identified as a phytochrome-interacting protein, binds preferentially to the Pfr form of phyB and, to a lesser extent, phyA (138, 139). PIF3 and phyB complexes have been shown to bind to a light-responsive G-box *cis*-element *in vitro* (117). PIF3 belongs to a large gene family of bHLH proteins in *Arabidopsis* (71, 181). A half-dozen bHLH proteins closely related to PIF3 have also been implicated in light responses, in particular in phytochrome responses (181, 194). Among those tested, PIF4 also binds to the Pfr form of phyB (84), whereas HFR1 does not interact directly with phytochromes (46). Genetic studies suggest that these bHLH transcription factors play positive or negative roles in overlapping or distinct branches of light-signaling pathways. For example, PIF3 has been suggested to be a negative regulator for both R and FR responses (97); HFR1 has been shown to be a positive component of FR and B responses (40); PIL1 (PIF3-like) and PIF4 are involved specifically in shade-avoidance and R responses, respectively (84, 157). The possibilities of heterodimer formation of these bHLH transcription factors add layers of complexity to their roles in light signaling (46, 181).

UBIQUITIN-MEDIATED PROTEIN DEGRADATION IN PHYTOCHROME SIGNALING Regulation of protein degradation is an integral part of the phytochrome signaling mechanism. First, phyA undergoes COP1-dependent ubiquitin-mediated degradation in the light, which serves as a desensitizing mechanism for phyA function (160, 164). The levels of phyB-E are also slightly down-regulated by light (87, 164). PhyB has been shown to interact with COP1 in the yeast two-hybrid system; however, it is still unknown whether this is related to phyB turnover (195). As mentioned above, downstream signaling components are subject to regulated protein degradation. HY5 is under the control of COP1-mediated ubiquitin-dependent degradation (77). LAF1, a phyA signaling component, is also a substrate of COP1 (149). Recently, a direct link has been established between phyA signaling components and COP1 function. SPA1 (suppressor of phytochrome A), a negative regulator of phyA signaling, directly interacts with COP1 and modulates its E3 ubiquitin ligase activities on LAF1 and HY5 (75, 149, 161). Moreover, two SPA1-related proteins, SPA3 and SPA4, interact with COP1 as well and act as negative regulators for FR, R, and B responses (108). These data suggest a model in which phyA signaling directly regulates COP1 activities through SPA1 and SPA1-like proteins. Other phyA signaling components may also play a role in protein degradation, including EID1 (Empfindlicher Im Dunkelroten licht 1) and AFR (attenuated FR response), two F-box proteins that play negative and positive roles in phyA signaling (39, 68).

PHYTOCHROME SIGNALING IN THE CYTOPLASM Despite recent data emphasizing phytochrome functions in the nucleus, it has long been thought that phytochromes also function in the cytoplasm. Pharmacological studies have suggested that heterotrimeric G proteins, cGMP, calcium, and calmodulin are early components in phytochrome signaling (132). Recent data further tested these possibilities. Genetic studies on mutants of alpha and beta subunits of the heterotrimeric G protein

indicate that heterotrimeric G protein is not directly involved in phytochrome-mediated seedling photomorphogenesis in *Arabidopsis* (91). On the other hand, the involvement of calcium is reinforced by the identification of a cytoplasmic-localized calcium binding protein SUB1 modulating phyA signaling (63).

A number of phyA signaling intermediates have been localized to the cytosol or in organelles (11, 78, 128). Perhaps the best-characterized cytoplasmic phytochrome signaling component is PKS1. PKS1 interacts with the HKRD of both phyA and phyB, can be phosphorylated by phyA *in vitro*, and is a phosphoprotein *in vivo* (50). The PKS1 primary sequence gives no clues concerning its biochemical activity. PKS1 is a member of a small gene family in *Arabidopsis* and to date only PKS1 and its closest homolog PKS2 have been characterized to some extent. Proper light regulation of PKS1 requires phyA. Moreover, phyA is involved in posttranslational regulation of PKS1 protein levels. PKS1-GFP fusion proteins are present in the cytoplasm and enriched at the plasma membrane. Both PKS1 and PKS2 are required for normal phyA signaling in particular during VLFR conditions (107). It is still unknown by what mechanism PKS1 and related proteins modulate phytochrome signaling.

SIGNAL INTEGRATION

A number of light responses are mediated by the coordinated action of several photoreceptors (19). For instance, phototropism and chloroplast movement are primarily controlled by the phototropins, but the amplitude of the response is modulated by both the phytochromes and the cryptochromes (36, 141, 172, 191). The underlying molecular mechanisms are not known. Seedling establishment, entrainment of the circadian clock, and photoperiod-induced flowering are controlled by the combined action of the phytochromes and the cryptochromes (19, 198). We are only beginning to understand how this is achieved. Different light colors that selectively activate different photoreceptors activate a highly overlapping set of genes, indicating the presence of shared signaling components (38, 53, 115, 148, 180, 189). These include the negative regulators of the DET/COP/FUS class and the positive regulator HY5 (148, 174). In particular, the E3 ligase COP1 is involved in the degradation of phyA, cry2, and HY5 (76, 160, 162). Other signaling components such as HFR1 and SUB1 are required for a subset of light responses. Since they act downstream of phyA and the cryptochromes, they might also represent elements of signal integration (40, 63). Physical interaction between the two families of photoreceptors has also been reported (2, 118). There will likely be several points of contact in this complex web of interactions.

Many important challenges lie ahead of us. We are beginning to understand events occurring during light-regulated transcriptional control; however, with the exception of light-regulated proteolysis, we still know very little about posttranslational mechanisms. The molecular function of numerous light-signaling components is not understood. Some might be involved in light-regulated subcellular

localization of the phytochromes, while others in rapid signaling events such as activation of various ion channels. Currently, the role of these photoreceptors outside the nucleus remains elusive. It is apparent that most light responses are coordinately controlled by several photoreceptors. Deciphering the complex architecture of these signaling networks will be difficult. Another field of investigation barely touched is the molecular basis for organ-specific light responses. How does light inhibit cell growth in the hypocotyl but promote it in the cotyledons? And, how are light responses communicated and coordinated between organs such as cotyledons (or leaves) and hypocotyls (or stems) (7, 178)? The answer to these questions presumably resides in the interplay between hormonal networks and light signaling.

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