Minireview

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Blue Light Sensing in Higher Plants*

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Plants have evolved a range of sophisticated mechanisms to adapt and respond to their natural habitat. For example, plants rely heavily upon the surrounding light environment to direct their growth and development. Several different photoreceptor families are known to mediate the effects of light on plant development (1–3). These include the phytochrome (phy) family of photoreceptors, which monitor the red (600–700 nm) and far-red (700–750 nm) regions of the solar spectrum (4). In addition to the phytochromes, many important aspects of plant development are regulated by specific blue (390–500 nm) and/or UV-A (320–390 nm) light-absorbing receptors (5–7). Currently two classes of blue light receptors have been identified in plants: the cryptochromes and the phototropins. Here we briefly review the most recent advances in our understanding of blue light perception and signaling with an emphasis on the cryptochrome and phototropin photosensory systems.

Cloning of the HY4 Gene Encoding Cryptochrome 1

Our present knowledge of blue light perception would not be possible without the isolation of photoregulatory mutants. The rationale behind the genetic approach has been to screen for mutants with altered responses to light. For example, when dark-grown *Arabidopsis* seedlings are transferred to light, hypocotyl elongation is dramatically suppressed (Fig. 1*A*). This response is mediated by blue, red, and far-red light in *Arabidopsis* (8). Screening for an elongated hypocotyl phenotype in white light has resulted in the isolation of both phytochrome and blue light regulatory mutants. One of these long hypocotyl mutants, *cry1* (originally designated *hy4*), is specifically impaired in its ability to respond to blue light (Fig. 1*A*).

The *HY4* gene was isolated through the use of a *hy4* mutant allele tagged with a T-DNA insertion (9). Examination of the deduced amino acid sequence revealed that the *HY4* gene encodes a 75-kDa protein with significant sequence homology to microbial DNA photolyases (Fig. 2). Sequence homology is highest in the regions associated with chromophore binding. The *HY4* gene was therefore proposed to encode a photoreceptor that mediates the blue light inhibition of hypocotyl elongation (9). Based on further characterization, the hy4 protein was named cryptochrome 1 (cry1), a term previously introduced to describe the enigmatic nature of plant blue/UV-A photoreceptors and their presumed prevalance in lower plants and fungi (cryptogams) (10).

Photolyases as a Model for Blue Light Sensing

DNA photolyases are now considered to be the evolutionary precursors for the cryptochromes (1, 6). Photolyases are blue light-activated enzymes, found in both prokaryotes and eukaryotes, that catalyze the light-dependent repair of damaged DNA produced from exposure to UV-B irradiation (280–320 nm) (11). Type I and type II photolyases mediate the repair of cyclobutane pyrimidine dimers, whereas (6-4) photolyases catalyze the repair of pyrimidine (6-4) pyrimidone photoproducts. In each case, the enzymes bind two blue/UV-A light-absorbing chromophores (Fig. 2). The primary chromophore, FAD, is bound noncovalently at the C-terminal region of the enzyme and functions to catalyze the cleavage of the pyrimidine dimer via electron transfer to the damaged DNA. The second chromophore, either a pterin or a deazaflavin, is bound at the N-terminal region of the protein and serves as an antenna, transferring harvested light energy to the FAD chromophore.

Cryptochrome Blue Light Receptors

Like the photolyases, cry1 binds FAD but lacks detectable photolyase activity (12, 13). The cry1 protein also contains a distinctive C-terminal extension that is absent in the photolyases (Fig. 2). Under certain redox conditions, the FAD chromophore bound to cry1 forms a stable semiquinone intermediate that absorbs green light (12). The occurrence of this flavin species is consistent with the observation that light from this region contributes to cry1 action (14). In addition, cry1 has been shown to bind a second pterin chromophore, 5,10-methenyltetrahydrofolate (MTHF)[1] when expressed in *Escherichia coli* (13).

Mutants at the *CRY1* (*HY4*) locus are impaired in a number of extension growth responses, including cotyledon expansion (15). The cry1 photoreceptor also functions to control the blue light induction of anthocyanin formation by regulating the transcription of chalcone synthase and other flavonoid biosynthetic enzymes (16).

Cryptochrome photoreceptors have been identified in several plant species, including ferns (17) and algae (18), and appear to be ubiquitous throughout the plant kingdom. A second member of the *Arabidopsis* cryptochrome family, cry2, shows considerable homology to cry1 (Fig. 2). Like *Arabidopsis* cry1, a cry2 homologue from mustard, originally designated SA-PHH1, binds FAD and MTHF chromophores and lacks photolyase activity (13, 19). In addition to cry1, cry2 has also been shown to regulate blue light-mediated inhibition of hypocotyl elongation and anthocyanin formation (20, 21). However, in contrast to cry1, cry2 is rapidly degraded in response to high intensity blue light (20–22). The rapid, light-dependent decrease in cry2 protein levels corresponds to the observation that cry2 functions under low light intensities, whereas cry1 functions mainly under high light intensities to regulate the blue light inhibition of hypocotyl elongation (20, 21).

Cryptochromes and Flowering

For many plant species, the transition of the apical meristem from vegetative to reproductive development is regulated by day length. In long day plants such as *Arabidopsis*, the switch to flowering is accelerated by long photoperiods. Mutations at the *CRY2* locus exhibit a delayed flowering phenotype under continuous white light and are allelic to the late flowering mutant *fha*, suggesting that cry2 is involved in measuring the photoperiod (23).

In *Arabidopsis*, continuous illumination with either far-red or blue light promotes flowering whereas continuous illumination with red light has an inhibitory effect (24). Far-red light promotes flowering through the function of phyA whereas red light mediates its inhibitory effect through the action of phyB (24). Because blue light promotes flowering, one might expect the response to be delayed in the *cry2* mutant. However, the *cry2* mutant flowers at the same time as wild-type plants under continuous blue or red light alone (23). Nevertheless, the delayed phenotype of *cry2* mutants originally observed under white light can be phenocopied by illumination with both blue and red light (23, 25). As a result, cry2 has been proposed to promote flowering by repressing phyB function in response to blue light (25).

The *cry1*/*cry2* double mutant exhibits a delayed flowering time

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[1] The abbreviations used are: MTHF, 5,10-methenyltetrahydrofolate; PAS, PER/ARNT/SIM.

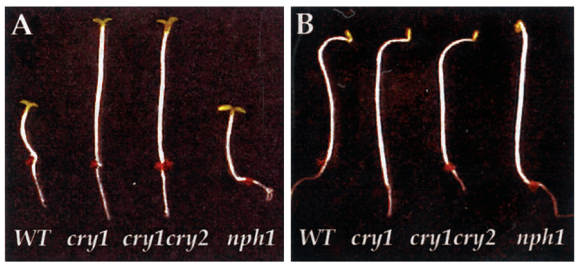

FIG. 1. **Physiological characteristics of cryptochrome and phototropin mutants.** *A*, blue light-dependent inhibition of hypocotyl elongation in wild-type (WT), *cry1* (*hy4*) mutant, *cry1/cry2* double mutant, and *nph1* mutant *Arabidopsis* seedlings. Seedlings were grown for 3 days in continuous blue light from above (20 μmol m^{-2} s^{-1}). *B*, hypocotyl phototropism in 3-day-old wild-type and mutant seedlings exposed to 6 h of unilateral blue light (1 μmol m^{-2} s^{-1}).

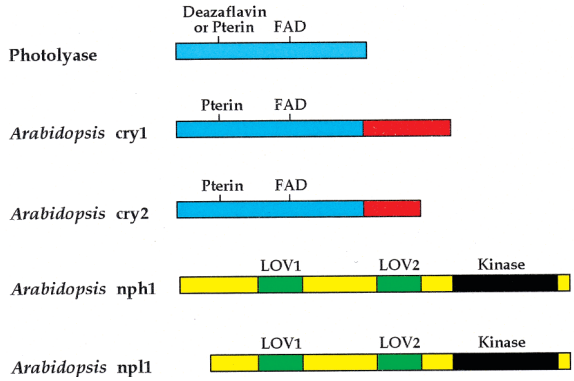

FIG. 2. **Protein structures of *E. coli* type I photolyase (472 amino acids), *Arabidopsis* cry1 (681 amino acids), *Arabidopsis* cry2 (612 amino acids), *Arabidopsis* nph1 (996 amino acids), and *Arabidopsis* npl1 (915 amino acids).** C-terminal extensions of cry1 and cry2 are shown in *red*.

under continuous blue light, whereas no late flowering phenotype is observed with either *cry1* or *cry2* single mutants under these conditions (25). Thus, cry2, in addition to repressing phyB activity, must also function to promote flowering in response to blue light. In the absence of functional cry2, the latter process is redundantly mediated by cry1. Indeed, functional redundancy among photoreceptors has been shown to exist for a number of plant responses and is known to occur between members of the same and different photoreceptor families (26).

Cryptochromes and the Circadian Clock

Circadian clocks are ubiquitous biological timing mechanisms that function to coordinate a wide variety of physiological and developmental processes with the daily light/dark cycle. The clock consists of three major components: a central oscillator that generates the 24-h oscillation, an input pathway that entrains the oscillator in response to environmental cues such as light, and an output pathway that couples the oscillator to various circadian responses (27). Recently, cry1 has been shown to mediate photoentrainment of the circadian oscillator. Transgenic *Arabidopsis* plants expressing the firefly luciferase gene fused to the clock-responsive chlorophyll *a/b*-binding protein (*CAB2*) promoter exhibit a circadian rhythm of bioluminescence (28). This reporter system provides a powerful tool to dissect the role of plant photoreceptors in regulating the circadian clock. In the absence of cry1, *CAB2* promoter activity oscillates at a slower pace in the *cry1* mutant when plants are transferred to continuous blue light (29). Likewise, overexpression of cry1 shortens the period length. Loss of cry2, on the other hand, has little effect on the circadian period length, implying that cry2 plays a minor role in regulating this response. This result is surprising considering the role of cry2 in the regulation of flowering time (23, 25). However, the effect of cry2 on flowering time may not directly involve the regulation of the circadian clock. Instead, output from the circadian oscillator may influence photoperiodic flowering indirectly by acting on cry2 signaling (7, 24). Alternatively, cry1 and cry2 may function in a redundant manner to regulate the circadian clock. Further detailed studies with the *cry1/cry2* double mutant will help resolve this issue.

Cryptochrome homologues have been identified in mice, humans, and *Drosophila* (6). Like the plant cryptochromes, mammalian cryptochromes bind FAD and MTHF chromophores but lack photolyase activity (30). Mouse and *Drosophila* cryptochromes have also been shown to play a role in light regulation of the circadian clock (31–33). Interestingly, sequence analysis reveals that mammalian cryptochromes resemble the (6-4) photolyases (30, 31, 34), whereas plant cryptochromes are more closely related to the type I photolyases (9). It is therefore proposed that plant and mammalian cryptochromes have arisen independently during the course of evolution from separate photolyase ancestors (1, 6).

Cryptochrome Signaling

From their homology to photolyases, one might expect the cryptochromes to initiate signal transduction by light-driven electron transfer to a specific redox-sensitive partner. To date, no such interacting protein for either cry1 or cry2 has been identified. Recently, cry1 and cry2 from *Arabidopsis* have been shown to accumulate in the nucleus (1, 22, 35). Although no effect of light was observed on cry2 nuclear localization (22), these findings raise the intriguing possibility that cryptochromes may regulate blue light-induced gene expression directly by interacting with DNA or DNA-binding proteins. However, only a small fraction of cry1 was found in the nuclei of light-grown plants (22). Therefore, cry1, and possibly cry2, may function to regulate blue light-regulated processes associated with cellular compartments other than the nucleus.

The role of the C-terminal extension of cry1 and cry2 in signaling is still unclear. This region is lacking in photolyases and appears to be essential for cryptochrome function (36). Although the C-terminal extensions of cry1 and cry2 differ in size and sequence, these regions are functionally interchangeable, suggesting that both photoreceptors operate via the same signaling mechanism (20). The C-terminal region of human cry2 interacts with and modulates the activity of a nuclear serine/threonine phosphatase *in vitro* (37). Whether a similar interacting partner exists for higher plant cryptochromes remains to be determined. Additional information regarding the role of the C-terminal extension in cryptochrome signaling has come from recent overexpression studies. When fused to β-glucuronidase, the C-terminal domain of both cry1 and cry2 mediates a constitutive photomorphogenic phenotype in dark-grown *Arabidopsis* seedlings (38). Overexpression of the cry1 and cry2 C-terminal fusions also affects a number of light-regulated processes, including anthocyanin formation and the onset of flowering. Thus, it appears that the C-terminal regions of cry1 and cry2 are sufficient to initiate signaling, implying that the cryptochromes function through a light-mediated derepression of the C-terminal domain.

Further studies have also shown that *Arabidopsis* cry1 and cry2 interact with phyA *in vitro* and act as substrates for phyA-mediated phosphorylation (39). More recently, cry2 has been shown to functionally interact with phyB in a light-dependent manner using fluorescence resonance energy transfer microscopy (40). Taken together, these findings imply that cross-talk between separate photosensory systems can occur directly between different photoreceptor proteins.

Blue light induces a rapid (within 30 s), transient depolarization of the plasma membrane in hypocotyl cells of several plant species, including *Arabidopsis* (41). The depolarization event immediately precedes the blue light inhibition of hypocotyl elongation and is proposed to reflect a signaling step associated with this response. The blue light-induced change in membrane potential involves activation of a plasma membrane anion channel (42). Mutants lacking cry1 exhibit a reduced magnitude of depolarization compared with wild-type seedlings, indicating that plasma membrane depolarization is mediated, at least in part, by cry1 (41, 43). Moreover, the anion channel inhibitor, 5-nitro-2-(3-phenylpropylamino) benzoic acid, suppresses the effect of blue light on both membrane depolarization and hypocotyl elongation (42). However, the blue light inhibition of hypocotyl growth can be separated into two phases in *Arabidopsis*: a rapid inhibition occurring within a few minutes and a slow inhibition that occurs hours after blue light

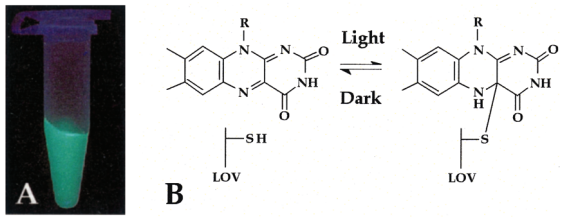

FIG. 3. **Light sensing by the LOV domains of phototropin.** *A*, purified LOV2 from oat phototropin viewed under UV light. *B*, schematic representation illustrating the proposed light-induced formation of a C(4a)-thiol adduct between the FMN chromophore and a conserved cysteine residue within the LOV domain.

treatment (43). Anion channel activation by cry1 appears to be involved in the slow inhibition of hypocotyl growth (41). Rapid growth inhibition in response to blue light is unaffected in the *cry1* mutant and appears to be 5-nitro-2-(3-phenylpropylamino)benzoic acid-insensitive (43), suggesting that this response is mediated by a separate, as yet unidentified, photosensory system. Given the known functional redundancy between cry1 and cry2, it will be interesting to examine both anion channel activation and rapid growth inhibition in the *cry1/cry2* double mutant.

Taken together, cry1 and cry2 function to regulate a number of responses in higher plants. However, *cry1/cry2* double mutants retain a substantial degree of blue light responsiveness (for example, phototropism, Fig. 1*B*), indicating the presence of additional blue/UV-A photoreceptors in *Arabidopsis*.

Cloning of the NPH1 Gene Encoding Phototropin

Studies on phototropism have led to the identification of a new family of blue light receptors. Phototropism is the adaptive process whereby plants bend toward a light source to maximize light capture for photosynthesis. Blue and UV-A light are the most effective wavelengths for inducing phototropic curvature in higher plants. Screening for an altered curvature response to unilateral blue light has resulted in the isolation of a number of phototropism mutants (3, 5, 44). One of these mutants, non-phototropic hypocotyl 1 (*nph1*), lacks phototropic responsiveness to low fluence rates of unilateral blue light (Fig. 1*B*). Mutants at the *NPH1* locus also lack the blue light-induced phosphorylation of a 120-kDa plasma membrane-associated protein (45). Indeed, extensive biochemical characterization has shown that the 120-kDa phosphoprotein is directly involved in the phototropic response (5, 46). The encoded protein, nph1, was therefore proposed to represent a phototropic receptor that undergoes autophosphorylation in response to blue light (45).

The *NPH1* gene was isolated by a chromosome walk and found to encode a protein of 996 amino acids (47). Although nph1 is found to be associated with the plasma membrane upon isolation from *Arabidopsis* and several other plant species (48), hydrophobicity analysis reveals that nph1 is a soluble protein with no membrane-spanning domains (47). Thus, nph1 may undergo post-translational modification or bind a protein anchor to facilitate interaction with the plasma membrane. The C-terminal region of nph1 contains the 11 signature domains found in serine/threonine protein kinases (49) (Fig. 2). The N-terminal region of the protein contains a repeated motif of 110 amino acids that belongs to the PAS domain superfamily. PAS domains are found in a variety of proteins and are reported to mediate protein-protein interactions and to function as internal sensors of oxygen, redox potential, and light (50). The PAS domains of nph1 are more closely related to a subset of proteins within the PAS domain superfamily that are regulated by light, oxygen, or voltage. Hence, the PAS domains of nph1 were designated LOV1 and LOV2 (47).

The sensing nature of a particular PAS domain is determined by the binding of a specific cofactor (50). The LOV domains of nph1 are highly fluorescent and bind a blue light-absorbing chromophore, FMN (Fig. 3*A*). When expressed in insect cells, recombinant nph1 noncovalently binds FMN and undergoes autophosphorylation in response to blue light irradiation (51). Moreover, the fluorescence excitation spectrum of recombinant nph1 (51) and the absorption spectra of the isolated LOV domains (52) are similar to the action spectrum for phototropism, with fine structure between 400 and 500 nm and a broad peak at 370 nm. The nph1 protein was therefore named phototropin after its functional role in phototropism (52).

Phototropin Homologue NPL1

Phototropin represents a new class of flavoprotein photoreceptors, unrelated to the cryptochromes or photolyases. A second member of the *Arabidopsis* phototropin family, designated nph1-like (npl1), shows considerable homology to phototropin (53) (Fig. 1). Homologues of phototropin have also been identified in several plant species, including rice (54), ferns (55), and more recently *Chlamydomonas*.[2] In addition, a novel protein, phy3, isolated from the fern *Adiantum* contains features of both phytochrome and phototropin photoreceptors (52, 56). It is therefore possible that the effects of red and blue light on phototropism of *Adiantum* protonemata are mediated by a single photoreceptor.

Like phototropin, the LOV domains of *Arabidopsis* npl1 noncovalently bind FMN.[3] Similarly, npl1 undergoes light-dependent autophosphorylation when expressed in insect cells, indicating that npl1 also functions as a photoreceptor kinase[4] as does the phototropin homologue from *Chlamydomonas*.[5] A recent study has shown that a second photoreceptor is required to mediate phototropic curvature in *Arabidopsis* (57). Although null mutants of phototropin (*nph1*) lack phototropism in response to low fluence rates of unilateral blue light (1 μmol m^{-2} s^{-1}), hypocotyl curvature is normal under high fluence rates (100 μmol m^{-2} s^{-1}). Although mutants at the *NPL1* locus exhibit normal phototropism (57), it will be important to examine the curvature response of a *nph1/npl1* double mutant to determine whether npl1 functions as an additional photoreceptor for phototropism. It seems unlikely that the cryptochromes play a major role in regulating phototropic curvature, as recently suggested (58), because *cry1/cry2* double mutants retain phototropic responsiveness to blue light (59) (Fig. 1*B*). Instead, it is possible that the cryptochromes, like the phytochromes, function to modulate the phototropic response output under certain light conditions (60).

Light Sensing by Phototropin

The LOV domains can be expressed and purified from *E. coli* in amounts suitable for biochemical characterization (52) and provide an excellent system to study the photochemical properties of phototropin. Recent studies have shown that both LOV1 and LOV2 domains of phototropin function as light sensors and undergo a self-contained photocycle (61). The spectral properties of the photoproduct produced for LOV1 and LOV2 resemble those of a flavin C(4a)-cysteinyl adduct. Replacement of a highly conserved cysteine residue with an alanine or a serine abolishes the light-induced photochemical reaction of both LOV1 and LOV2 (61). Thus, light sensing by phototropin appears to occur via the formation of a stable adduct between the FMN chromophore and the conserved cysteine residue within the LOV domain (Fig. 3*B*). Moreover, the recently obtained crystal structure of *Adiantum* phy3 LOV2 is consistent with the formation of an adduct at the C(4a) position of the isoalloxazine ring of the FMN chromphore, which is bound tightly within the LOV domain (62). It is therefore hypothesized that the light-driven reactions of the LOV domains result in a conformational change of the phototropin protein, which in turn leads to activation of the receptor kinase (61). Interestingly, the isolated LOV domains exhibit a 10-fold difference in light sensitivity (61), suggesting that LOV1 and LOV2 may have distinct light-sensing roles. Further structure-function studies with the full-length protein will help to elucidate the individual roles of LOV1 and LOV2 in blue light sensing.

Phototropin Signaling

From their role as light-activated kinases, one might expect the phototropins to initiate signal transduction through a phosphorylation cascade. However, to date, no such substrate for phototropin phosphorylation has been identified. Given that FMN is tightly bound within the LOV domains, it is also unlikely that phototropin

[2] A. Nagatani, personal communication.
[3] M. Kasahara, T. E. Swartz, J. M. Christie, and W. R. Briggs, unpublished data.
[4] J. M. Christie, M. Kasahara, and W. R. Briggs, unpublished data.
[5] J. M. Christie, A. Onodera, A. Nagatani, and W. R. Briggs, unpublished data.

initiates signaling through intermolecular energy transfer to a moiety associated with a downstream reaction partner. Alternatively, phototropin may initiate signaling through a conformational change in response to light-driven autophosphorylation. Autophosphorylation of phototropin may also play a role in receptor adaptation. Clearly there are many questions to be addressed.

A phototropin-interacting protein, NPH3, was recently identified by the isolation of additional phototropism mutants (45, 63). NPH3 is a novel protein containing several protein-protein interaction motifs and interacts with phototropin *in vitro* (63). Like phototropin, NPH3 is associated with the plasma membrane and is proposed to function as an adapter or scaffold to bring together components of the phototropic signaling complex. A protein closely related to NPH3, designated root phototropism 2 (RPT2), was recently isolated from a separate genetic screen (57). In contrast to *NPH3*, *RPT2* gene expression is enhanced at increased light intensities. Thus, RPT2 may play a role in mediating phototropic curvature at high fluence rate conditions.

Further studies have shown that calcium is involved in phototropin signaling. Blue light induces a transient increase in cytosolic calcium in wild-type seedlings, which is severely impaired in the *nph1* mutant, suggesting that phototropin may act to regulate the activity of a putative plasma membrane calcium channel (64). A role for reversible protein phosphorylation in auxin transport has been reported in several plant systems (44). It is therefore possible that the generation of lateral auxin transport generally associated with tropic curvatures may result from the activity of a calcium-dependent protein kinase induced by a phototropin-stimulated increase in cytosolic calcium (44).

Conclusions

Recent progress has unveiled two distinct classes of blue/UV-A photoreceptors in higher plants: the cryptochromes and the phototropins. It is likely that other potential candidates await isolation. For example, the action spectrum for stomatal opening resembles the spectral properties of a flavoprotein (65). However, recent genetic and biochemical evidence suggests that a carotenoid-based chromophore may be involved in this response (66, 67). The isolation of additional blue/UV-A response mutants will facilitate the identification of additional blue/UV-A light receptors in higher plants. A more detailed analysis of cryptochrome- and phototropin-deficient mutants will also help to determine the role of these photoreceptors in regulating other blue light-activated processes.

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