Cryptogam blue-light photoreceptors
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Blue light regulates various aspects of plant development. Extensive research using Arabidopsis thaliana has advanced our understanding of blue-light photoreceptors and signal transduction pathways in flowering plants, but our knowledge of blue-light signaling in other plant systems, particularly in cryptogams (i.e. ferns, mosses, algae and so on) has lagged behind. The recent cloning and characterization of the blue-light photoreceptors of a fern (Adiantum capillus-veneris), a moss (Physcomitrella patens) and two algae (Chlamydomonas reinhardtii and Euglena gracilis) have provided new insights into blue-light signaling in plants.

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Abbreviations
Chop1 Channel rhodopsin1
Cop chlamyopsin
CRY cryptochrome
CSRA/B Chlamydomonas sensory rhodopsin A/B
GUS [γ-glucuronidase
LOV light, oxygen, voltage
M molecular mass
NAA 1-naphthylene acetic acid
PAC photoactivated adenyl cyclase
PFB paraflagellar body
PHOT phototropin
PHY3 PHYTOCHROME3
Vop volvoxopsin

Introduction
Plants utilize light not only as a source of energy for photosynthesis but also as an environmental cue to modulate their growth and development. Blue light regulates many developmental and physiological responses, including de-etiolation, flowering, circadian rhythm, gene expression, phototropism, chloroplast movement and stomatal movement [1]. Molecular genetic analysis in the model flowering plant Arabidopsis thaliana has identified two types of blue-light photoreceptor: cryptochrome (CRY) and phototropin (PHOT). Arabidopsis has two cryptochrome (CRY1 and CRY2) and two phototropin (PHOT1 and PHOT2) genes. The two Arabidopsis CRY genes are involved in regulating de-etiolation, gene expression, flowering time, circadian rhythm and so on. Not only plants but also animals (i.e. mouse, zebra fish, and Drosophila etc.) have one or more CRYs that have an important role in regulating the circadian clock [2,3]. The CRY proteins have an amino-terminal domain that is similar to the microbial type-I photolyases but has no photolyase activity [2]. The carboxy-terminal domain of the CRY proteins has no homology to any other protein (Figure 1). The two Arabidopsis PHOT genes redundantly regulate phototropism, chloroplast movement and stomatal opening [4,5]. The PHOT proteins are blue-light autophosphorylated kinases that contain two tandem LOV (light, oxygen, voltage) domains at their amino-terminus and a carboxy-terminal serine/threonine kinase domain (Figure 1; [4]).

Although the blue-light photoreceptors and their signal transduction pathways have been well characterized in flowering plants, those in cryptogams (i.e. ferns, mosses and algae) are not well understood. Because of their simple architecture and life cycle, however, cryptogams are amenable to the physiological and photobiological analysis of responses that are regulated by blue light. A large amount of data on the blue-light responses of cryptogams has therefore accumulated [6–8]. In this commentary, we report recent progress from studies on blue-light photoreceptors from the fern Adiantum capillus-veneris, the moss Physcomitrella patens and the algae Chlamydomonas reinhardtii and Euglena gracilis (Figure 2). Although the term ‘cryptogam’ includes fungi, we have not included fungal blue-light photoreceptors in this paper.

Blue-light photoreceptors in ferns: cryptochromes, phototropins and PHYTOCHROME3

The gametophytes of the fern Adiantum capillus-veneris have many physiological responses that are induced by blue light (Figure 2a). For example, blue light activates apical swelling [9] and subsequent cell division [10], and inhibits red-light-induced spore germination [11] and tip growth [12] in these gametophytes. Phototropism [13] and chloroplast movement [14] are regulated by both red and blue light. The genes encoding the blue-light photoreceptors from Adiantum have been cloned and characterized, providing insights into how these photoreceptors mediate the various responses of this fern to blue light.

Five Adiantum cryptochrome genes (CRY1–5) have been cloned using the Arabidopsis CRY1 cDNA as a probe [15,16]. All of the proteins encoded by these genes lack photolyase activity [16]. During light-induced spore
Schematic illustrations of the blue-light photoreceptors of cryptogams. (a) Cryptochrome (CRY) has an amino-terminal photolyase-like domain (blue) in which pterin and flavin adenine dinucleotide (FAD) are used as chromophores. The carboxy-terminal domain of CRY (yellow) functions as a transducer of light signals. (b) Phototropin (PHOT) is a kinase that is autophosphorylated by blue light. It has two tandem LOV domains, which serve as flavin-binding sites, and a serine/threonine kinase domain (orange). (c) PHY3 has a photochrome chromophore-binding region (red) and a complete PHOT domain. (d) PAC is a blue-light activated adenyl cyclase that consists of two flavin-binding domains (F1 and F2) and two adenyl cyclase catalytic domains (C1 and C2). (e) CSRA and CSRB are similar to microbial opsin-related proteins. Their retinal-binding domain (light green and dark green) contains seven transmembrane regions (dark green). (f) Cop and Vop are similar to invertebrate opsins but they have only four transmembrane segments.

germination, CRY1–4 mRNAs (but not CRY5 mRNA) accumulated in dark-imbibed spores [16]. When these spores were irradiated with blue or red light after incubation in darkness, the accumulation of CRY4 mRNA was reduced whereas CRY5 mRNA was increased. The effect of red light is reversed by far-red light, suggesting that phytochrome is involved in the expression of CRY4 and CRY5 mRNAs. The effect of blue light on CRY5 gene expression is also reversible by far-red light, whereas the effect of blue light on CRY4 expression is not. It appears, therefore, that the expression of the CRY4 gene is repressed by both phytochrome and the blue-light photoreceptor, whereas CRY5 gene expression is regulated only by phytochrome. In Arabidopsis, the phosphorylation and subsequent degradation of CRY2 are regulated by blue light [17]. The mechanism by which blue-light regulates CRYs in Adiantum remains to be elucidated.

The predominant localization of β-glucuronidase (GUS): CRY3 and GUS::CRY4 fusion proteins in the nucleus has been shown by transient expression assays in Adiantum protonemal cells and in onion epidermal cells under various light conditions (the exception being GUS::CRY3 under blue light). However, the GUS activities of GUS::CRY1, GUS::CRY2 and GUS::CRY5 were observed in the cytosol. The carboxy-terminal region of CRY3 or CRY4 was sufficient for the nuclear localization of the respective GUS fusion proteins. GUS::CRY3 showed no clear nuclear localization under blue light, being found primarily in the cytosol under these conditions, whereas the localization pattern of the other four CRYs was independent of the light conditions. Arabidopsis CRY1 localized in the nucleus in darkness but was depleted from nucleus under white light conditions [18]. Both Arabidopsis CRY2 and Adiantum CRY4 showed constitutive localization in the nucleus [19,20]. Physiological analysis has indicated that the blue-light photoreceptor that is involved in the inhibition of spore germination in Adiantum is localized in or close to the nuclear compartment before irradiation with blue light [21]. The accumulation of CRY3 and CRY4 mRNAs in dark-imbibed spores, and the localization of the CRY3 and CRY4 proteins in the nucleus in darkness suggest that CRY3 and/or CRY4 may control the germination of fern spores.

Adiantum also has two PHOT genes, AcPHOT1 and AcPHOT2 [22]. The AcPHOT2 protein has greater
Developmental processes that are regulated by light in a fern (Adiantum capillus-veneris), a moss (Physcomitrella patens) and two algae (Chlamydomonas reinhardtii and Euglena gracilis). (a) When Adiantum spores germinate under red light, single-celled protonemal cells grow towards the light source. (ii) When the protonemal cells are transferred to white or blue light they show apical swelling followed by cell division, finally resulting in the formation of heart-shaped protonemal cells. (b) In Physcomitrella patens, formation of the side branches of protonemal cells and (ii) stem elongation and leaf expansion of the gametophore are regulated by two cryptochromes. (c) Opsin-like photoreceptors perceive light and activate the movement of two flagella in Chlamydomonas reinhardtii. Phototaxis or photophobic movement of this alga is induced according to the ambient light condition. (d) (i) Fluorescence image under excitation by blue-violet light and (ii) bright-field image of Euglena gracilis. Although PFBs and eyespots are located very close to each other within these cells, they are not the same structure. Photographs were kindly provided by (a,b) A Kadota, (c) A Nagatani, and (d) M Watanabe and M Iseki.

similarity to seed plant PHOT2s than to PHOT1s. Both *Arabidopsis* PHOT2 (T. Kagawa, personal communication) and *Arabidopsis* PHOT2 [23,24] mediate the chloroplast response for avoidance of strong light to prevent photodamage. The PHOT-related gene PHYTOCHROME3 (PHY3) has been cloned [25]; the peptide that it encodes has a chromophore-binding domain of phytochrome at its amino-terminus and a complete phototropin domain at its carboxyl terminus (Figure 1). A recombinant PHY3 protein that was reconstituted with the chromophore phycocyanobilin had an absorption spectrum that was similar to those of conventional phytochromes [25]. Moreover, when the two LOV domains of the PHY3 carboxy-terminal phototropin domain were expressed in *Escherichia coli*, they bound flavin mononucleotide and had absorption and fluorescence spectra similar to those of the LOV domains in seed plant PHOTs [26]. PHY3 protein therefore has an ability to absorb both red and blue light. A red- and blue-light photoreceptor(s) at or close to the plasmamembrane is known to regulate phototropism and chloroplast movement in *Adiantum* [27,28], and PHY3 may be a candidate for this photoreceptor.

### Cryptochromes modulate auxin-mediated developmental pathways in a moss

Mosses, particularly *Funaria* and *Physcomitrella*, have been valuable systems in which to study hormone (i.e. auxin and cytokinin)-mediated development [29]. Nevertheless, until recently, the effects of light in controlling the growth and development of these mosses was not well understood. Homologous recombination in *Physcomitrella patens* (Figure 2b) has, however, been used recently to shed light on morphogenesis in this species [30].

There are two *CRY* genes (*CRY1a* and *CRY1b*) in the *Physcomitrella* genome [31]. The nucleotide sequence of both introns and exons within these genes is almost identical, and the encoded proteins differ by just one amino acid. This level of homology indicates that these *CRY* genes were recently duplicated [31]. Like *Adiantum* CRY3 and CRY4, CRY1a and CRY1b have a monopartite nuclear localization signal in their carboxy-terminal region [16]. Proteins in which GUS was fused with CRY1a or CRY1b were nuclear localized under all of the light conditions tested, as were *Arabidopsis* CRY2 [19,20] and *Adiantum* CRY4 [16].

Single (*cry1a* or *cry1b*) and double (*cry1a cry1b*) cryptochrome disruptants were generated by homologous recombination, and the growth of protonemal colonies of wildtype and *cry* disruptants was analyzed under white-, blue- and red-light. Under blue light, the diameters of *cry* disruptant colonies were greater than those of wildtype colonies, but their densities were thinner. No such differences were observed under white or red light. This colony phenotype of the *cry* disruptants resulted from a
defect in their blue-light-induced formation of side branches. In the cry disruptants, the side-branch initials were able to differentiate but stopped growing. Gametophore development and leaf size were also regulated by the CRYs.

Developmental stages that are controlled by cryptochromes in Physcomitrella were also regulated by plant hormones, such as auxin and cytokinin. Consequently, the auxin sensitivity of the growth and development of wildtype mosses and cry disruptants has also been examined. Colonies of cry disruptants that were cultivated on an agar medium that was supplemented with the auxin analog 1-naphthalene acetic acid (NAA) had a concentration-dependent auxin hypersensitive phenotype when grown under either white- or blue-light but not under red light. Auxin hypersensitivity resulted in cry colonies that had larger diameter and thinner density than those of wildtype moss. Furthermore, the transient expression of an auxin-responsive fusion gene comprising the soybean GH3 promoter::GUS has been assayed in moss protoplasts. NAA-dependent GUS-activity was greater in cry disruptants than in the wildtype under white and blue light but not under red light. Moreover, the transcripts of native auxin-responsive genes were accumulated to higher levels in the absence of NAA, and were induced more rapidly following NAA application, in cry1a cry1b disruptants than in wildtype Physcomitrella. These results suggest that a cryptochrome signal modulates developmental pathways in Physcomitrella by suppressing auxin signaling.

New photoreceptors for photomovement in flagellates

Like land plants, the green alga Chlamydomonas reinhardtii (Figure 2c) has both CRY [32] and PHOT genes [33–35]. The Chlamydomonas PHOT has properties that are similar to those of seed plant PHOTs, including two LOV domains [33,34] and membrane localization [35]. Chlamydomonas does not show phototropism, chloroplast movement or stomatal opening, however, so the function of Chlamydomonas PHOT remains unknown.

It is well known that Chlamydomonas algae show phototaxis (i.e. light-oriented movement) and photophobic movement (i.e. an abrupt change in the area in which these algae swim and/or in swimming direction in response to change in light fluence rate). It has been suggested that the photoreceptors for these responses are retinal-based rhodopsin-like proteins [36]. Chlamyopsin (Cop) was identified as a candidate photoreceptor in Chlamydomonas ([37]; Figure 1), and subsequently a Cop homologue, volvoxopsin (Vop), was identified in the splanhoidal green alga Volvox carteri ([38]; Figure 1). Cop has now been ruled out as the photoreceptor for the photomotility responses of Chlamydomonas [39], but Vop is probably the photoreceptor for phototaxis [38].

Recently, the Chlamydomonas sensory rhodopsin A (CSRA) [40]/Channel rhodopsin 1 (Chop1) [41] and CSRB [40] were identified as the photoreceptors for both phototaxis and photophobic movement (Figure 1). These proteins show homology to the sensory rhodopsin from the archea and the Neurospora rhodopsin NOP1 [42]. These three proteins each have an amino-terminal domain that contains seven transmembrane helices and a retinal-binding pocket, and an additional carboxy-terminal membrane-interaction domain. Sineshchekov et al. [40] generated cells that were enriched in CSRA or CSRB by RNA interference with the CSRB or CSRA gene, respectively. In both cell lines, they examined in the wavelength-dependence of photomovement and of the generation of a photoreceptor current, the two most immediately detected responses that are associated with photomovement. The properties of CSRA- or CSRB-generated currents precisely matched the light-sensitivity characteristics of the photomotility responses of CSRA- or CSRB-enriched cells, respectively, indicating that both CSRA and CSRB are photoreceptors for photomovement. When Nagel et al. [41] expressed Chop1/CSRA in Xenopus oocytes that contained all-trans retinal, irradiation with green light (but not with red light) induced inward currents at negative membrane potentials at pH 7.5. Lowering the pH shifted the reversal potential of the light-induced current to more a positive value, indicating that Chop1/CSRA is the light-gated proton channel. Consistent with results of the Sineshchekov et al. [40], the wavelength dependency of the inward current of Chop1/CSRA in Xenopus oocytes resembled that of both phototaxis and the photophobic responses.

The unicellular flagellate Euglena gracilis (Figure 2d) also shows photophobic responses to a sudden increase ('step-up') or decrease ('step-down') in ambient blue-light intensity, respectively [36]. The action spectra of these photophobic responses suggested that flavoproteins might be the photoreceptors for these photomovements. The parflagellar body (PFB; Figure 2d) near the base of the flagellum shows green autofluorescence, indicating that it contains flavoproteins. Iseki et al. [45] therefore hypothesized that flavoproteins that are localized in PFB act as the photoreceptor molecules in Euglena gracilis. They isolated PFBs and purified the flavoproteins by anion-exchange chromatography followed by gel filtration. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the fraction of apparent molecular mass ~400 000 (M , 400 000) indicated that the M , 400 000 protein may consist of two M , 105 000 subunits and two M , 90 000 subunits. Sequencing the amino acids of the M , 90 000 and the M , 105 000 subunits, and the subsequent isolation of the corresponding cDNAs, showed that these subunits are similar proteins. Each protein contains four domains in the order F1, C1, F2, and C2. F1 and F2 are similar to flavin adenine
dinucleotide (FAD)-binding domain from *Rhodobacter* AppA (activation of photopigment and puc expression A), which functions as a redox regulator during the formation of photosystems [44]. C1 and C2 share homology with catalytic domains from class III adenyl cyclase (Figure 1). Gel-filtration showed that the blue-light-dependent adenyl cyclase activity coincided precisely with the 530 nm fluorescence intensity and peaked at the $M_r$ 400 000 fraction. Iseki *et al.* [43] therefore named the flavoprotein ‘photovactivated adenyl cyclase’ (PAC) and designated the $M_r$ 105 000 and $M_r$ 90 000 subunits PACα and PACβ, respectively. When PACα or PACβ were depleted by RNA interference, the resulting PAC-depleted cells lacked the PFB and showed no step-up response, indicating that PAC is the PFB-localized blue-light photoreceptor for the step-up response. The step-down response of PAC-depleted cells was similar to that of wildtype cells, indicating that the step-down response is regulated by an unidentified blue-light photoreceptor(s).

Conclusions

Blue-light photoreceptors and signal transduction pathways have been studied mainly in *Arabidopsis*, and a large amount of important information has been provided by work on this model flowering plant. Research on the blue-light receptors of cryptogams has, however, provided many valuable insights that were not predicted by *Arabidopsis* research. New types of photoreceptors have been identified (Adiantum PHY3, Chlamydomonas CSRA/Chop1 and CSRB; and Euglena PAC) and new insights into blue-light signaling have been discovered (e.g., the suppression of auxin signaling by cryptochrome in *Physcomitrella*). Further investigation of the blue-light signaling of cryptogams will continue to shed light on the field of plant photomorphogenesis.

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References and recommended reading


