Phototropin Blue Light Receptors and Light-Induced Movement Responses in Plants

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Plants do not generally move around. However, as noticed by Charles Darwin in his book *The Power of Movement in Plants*, they are not entirely immobile (*1*). Environmental stimuli, such as light, can trigger various movement responses in plants, including phototropic curvature, chloroplast movement, and stomatal opening (Fig. 1). For instance, plant stems bend toward light to maximize photosynthesis in leaves, whereas roots bend away from light to ensure that they stay in soil for absorption of water and mineral nutrients. Chloroplasts move toward relatively weak light to maximize photon capture; however, these organelles "swim" away from high-intensity light to avoid photodamage. Stomata, which are pores that are formed by two surrounding guard cells in the epidermis, also adjust their aperture in response to light, opening during the day to allow gas exchange but closing at night to minimize water loss. For unknown reasons, blue light is usually the most effective wavelength of light to induce these responses. The blue light receptors mediating plant movement responses have remained elusive until recently. On the basis of a series of molecular genetic studies using the model plant *Arabidopsis thaliana*, it has become clear that the phototropin family of flavin-containing blue light receptors regulates these three movement responses (*2*-*6*).

Phototropin was first identified as an ~120-kilodalton (kD) plasma membrane protein that undergoes blue light-dependent phosphorylation (*7*). The gene encoding phototropin was cloned from an *Arabidopsis* mutant, *nph1* (nonphototropic hypocotyl 1) that is impaired in the blue light-induced hypocotyl and root curvature responses (*2*, *8*). The *NPH1* gene, *nph1* mutant, NPH1 apoprotein, and NPH1 holoprotein have been renamed as *PHOT1*, *phot1*, PHOT1, and phot1 (for phototropin 1), respectively (*9*). *Arabidopsis PHOT1* encodes a 996-residue polypeptide that contains two Per-Arnt-Sim (PAS) domains at the NH_2-terminus and a serine-threonine kinase domain at the COOH-terminus. Because the two PAS domains of PHOT1 are more closely related to those found in a subset of PAS domain-containing proteins that are regulated by light, oxygen, and voltage changes, they were collectively referred to as LOV domains (*2*). Recombinant PHOT1 expressed in insect cells binds noncovalently to flavin mononucleotide (FMN) and undergoes blue light-dependent autophosphorylation in vitro (*10*). The absorption and fluorescence excitation spectra of the recombinant PHOT1 are similar to the action spectrum of phototropism in *Arabidopsis* in vivo (*10*). PHOT1 LOV domains expressed in *Escherichia coli* bind FMN stoichiometrically, indicating that LOV domains are the FMN chromophore-binding sites of phototropins and that each holophototropin (phot1) contains two FMN molecules (*11*). Together with the genetic evidence, these results demonstrated that phot1 is a flavin-containing photoreceptor mediating blue light-induced phototropism.

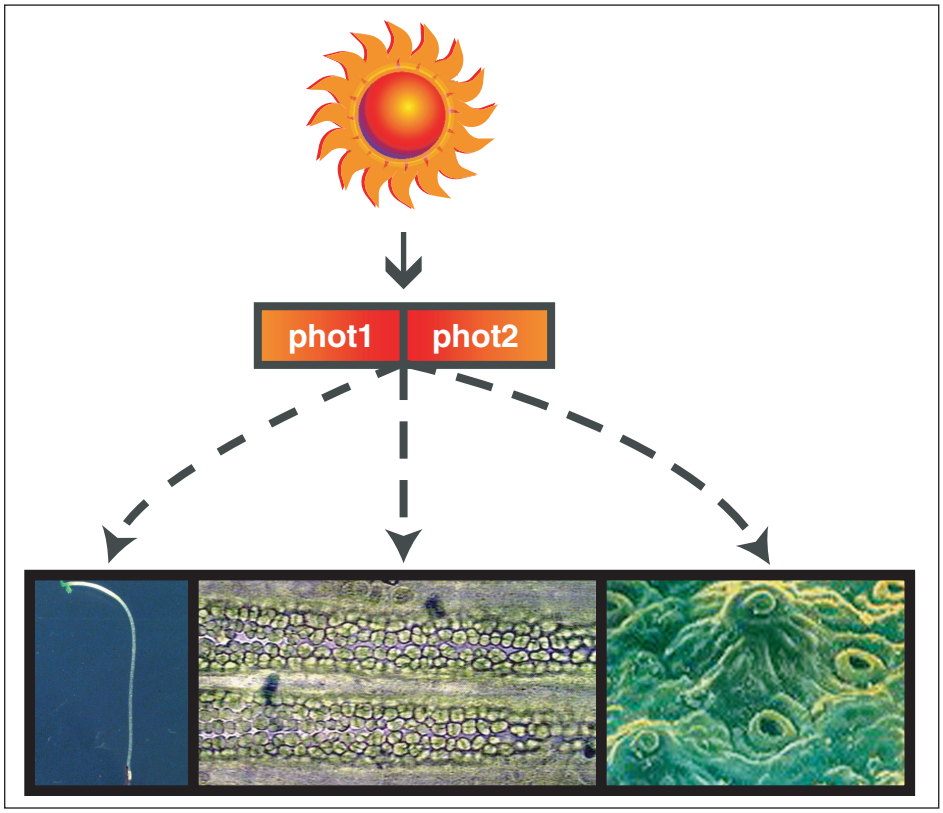

Fig. 1. Phototropins (phot1 and phot2) mediate movement responses in plants, including phototropic curvature (**left**), chloroplast movement (**middle**), and stomata opening (**right**).

However, phot1 is not the sole photoreceptor capable of mediating phototropic responses. In a study of *Arabidopsis rpt* (root phototropism) mutants impaired in negative curvature

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(bending away from a light source) of roots in response to blue light, one of the mutants, *rpt1*, was allelic to—that is, the mutation mapped to the same chromosomal locus as—*nph1/phot1*. Although *nph1/phot1* mutants are completely insensitive to both high and low intensities of blue light with respect to root phototropism, they curved normally toward blue light at fluence rates that were much higher than those previously seen (*12*). Because the *phot1* mutants showed normal hypocotyl curvature under conditions of high-intensity blue light, this indicated the existence of another photoreceptor that mediates hypocotyl phototropism.

Before the identification of the second photoreceptor that mediates phototropism, the story took a turn and brought us to the blue light receptors mediating chloroplast movement. In two independent studies, an *NPH1/PHOT1* homolog, termed *NPL1*, was found to function as a photoreceptor that regulates the chloroplasts' avoidance response in intense light (*4*, *13*). The *NPL1* gene, which was isolated on the basis of its sequence similarity to *NPH1/PHOT1*, encodes a protein that is ~58% identical to that of PHOT1 (*14*). An *npl1* mutant was isolated, which showed little abnormality in several different blue light responses tested, including phototropic curvature of hypocotyls (*4*). When assessed with a leaf light-transmittance assay, which measures light transmission changes resulting from chloroplast relocation, *npl1* was found to be defective in chloroplast movement in intense light but not in low light, which suggested a role for the *NPL1* gene in chloroplast avoidance but not in chloroplast accumulation. In the other study, *NPL1* mediated the chloroplast avoidance response (*15*). The chloroplasts in wild-type *Arabidopsis* moved toward a microbeam of blue light of relatively low intensity (chloroplast accumulation), but they moved away from a microbeam of high-intensity blue light (chloroplast avoidance) (*3*, *15*). However, an *Arabidopsis* mutant, termed *cav1* (for chloroplast avoidance), was isolated that exhibited impaired chloroplast avoidance in response to high-intensity blue light. The gene corresponding to the *cav1* mutant was found to be *NPL1* (*13*). Additionally, mutation of *NPL1* did not affect chloroplast accumulation around a microbeam of low-intensity blue light (*13*). Therefore, these studies identified *NPL1*, which is now renamed *PHOT2* (for phototropin 2), as another blue light receptor that mediates physiological responses to blue light (*9*). Indeed, like phot1, the flavoprotein phot2 undergoes blue light-induced autophosphorylation (*6*).

The puzzle of which photoreceptor mediates hypocotyl phototropism under high-intensity light or chloroplast accumulation under low-intensity light was solved by a study of the *phot1phot2* double mutant, which is completely insensitive to both low- and high-intensity light in both hypocotyl phototropism and chloroplast movement responses (*6*). Thus, it became clear that these two phototropins mediate similar blue light responses, but they have different photosensitivities (*6*). In *Arabidopsis*, phot1 mediates the negative root curvature throughout a wide range of light intensities, and it can act alone to effect the positive hypocotyl curvature response in low light. Under high-intensity light, phot1 and phot2 act redundantly in mediating hypocotyl phototropism. On the other hand, phot2 is the major photoreceptor mediating chloroplast avoidance under high-intensity light, whereas phot1 and phot2 act redundantly in mediating chloroplast accumulation under low-intensity light.

An old lesson relearned from the phototropin story is that genes (and proteins) often act in a redundant manner, which leads to an obvious question: Do phototropins mediate blue light responses other than phototropism and chloroplast relocation? When *phot1* or *phot2* mutants were investigated for blue light responses such as de-etiolation (whereby plants resynthesize chlorophyll and grow larger leaves in response to sufficient light), photoperiodic flowering, and stomatal opening, the monogenic mutants showed little defect in these responses (*4*, *8*, *16*). The lack of function of phototropins in de-etiolation and photoperiodism may not be surprising, because these photomorphogenic responses are controlled by a different type of blue light receptors, termed "cryptochromes," as well as red and far-red light receptors called phytochromes (*17*). The blue light-induced stomatal opening, however, turns out to be mediated redundantly by phot1 and phot2 (*5*). A study that compared stomatal opening in *Arabidopsis* mutants exposed to both blue and red light found that stomata in the *phot1phot2* double mutant fail to open (as compared to the wild type) under these conditions (*5*). Because stomata opened much wider in response to the combination of blue and red light than to red light alone, these investigators concluded that stomatal opening was a blue light-mediated response controlled by phot1 and phot2.

Although the photoreceptors that affect stomatal opening have only recently been identified, we know more about the downstream molecular mechanism associated with stomatal opening than about that associated with either phototropic curvature or chloroplast relocation (*18*-*20*). The size of the stomatal aperture is controlled by the shape of the guard cells. In response to light, the salt concentration increases in the guard cells, causing an inflow of water, expansion of the guard cells, and opening of the stomatal aperture. Blue light might induce phosphorylation of a plasma membrane proton adenosine triphosphatase (H^+-ATPase), which increases the inside negative electrical potential gradient across the plasma membrane (*21*). This electrical potential gradient drives a voltage-gated K^+ channel, resulting in an accumulation of potassium inside guard cells and, eventually, the opening of the stomata (*19*). Because phot1 is a plasma membrane-associated light-dependent protein kinase, it is likely to be responsible for the blue light-induced phosphorylation and activation of the H^+-ATPase. Indeed, the blue light-induced increase of H^+-ATPase activity was abolished in the *phot1phot2* double mutant (*5*). This result narrows the gap in our understanding of how light promotes stomatal opening. In addition, our knowledge of blue light-induced stomata opening provides new clues about where to look for the signaling components downstream from phototropins concerning phototropic and chloroplast movement responses. Phototropins may regulate ion transporters in the plasma membrane in leaf cells. This could change ion homeostasis in leaf cells, which may alter the network of the cytoskeleton, change the status of the cytoplasmic stream, and eventually change the location of chloroplasts. It is also conceivable that phototropins may interact with and phosphorylate auxin transporters in the plasma membrane to alter the signaling process of this phytohormone, resulting in differential growth. These and other interesting hypotheses could be tested using the excellent genetic resource now available for *Arabidopsis* (http://www.arabidopsis.org).

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