Phytochrome Evolution in Green and Nongreen Plants

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Abstract

Photoreceptors are critical molecules that function at the interface between organism and environment. Plants use specific light signals to determine their place in time and space, allowing them to synchronize their growth, metabolism, and development to the environments in which they occur. Thus, innovation in light sensing mechanisms is expected to coincide with adaptation and diversification. Three studies involving the well-characterized phytochrome photoreceptor system in plants indicate that much work is yet needed to test this expectation. In early diverging flowering plants, episodic positive selection influenced the evolution of phytochrome A, but little of the functional data needed to link molecular adaptation with a change in gene function are available. In the model plant Arabidopsis thaliana, known functional differences between a recently duplicated gene pair remain difficult to characterize at the sequence level. In parasitic plants, patterns of development that in autotrophs are under the control of light signals are highly modified, suggesting that phytochromes and other photoreceptors function differently in nonphotosynthetic plants. Analyses of phytochrome A coding sequences indicate that they are evolving under relaxed constraints in nonphotosynthetic Orobanchaceae, consistent with the expectation of functional change. Further work is needed to determine which of the processes mediated by phyA may have been altered, a line of investigation that may improve our understanding of divergence points in downstream signaling pathways.

Photoreceptors, Plants, and the Environment

Photoreceptors are critical molecules that function at the interface between organism and environment. Variable responses to light indicate that plants use specific light signals to determine their place in time and space, allowing them to synchronize their growth, metabolism, and developmental transitions to the environments in which they occur. In autotrophic plants, light cues provide circadian and seasonal information, used to mediate the induction and inhibition of flowering, the induction and breaking of bud dormancy, the opening and closing of stomata and flowers, and the cycling between sleep and waking movements. Light cues also provide positional information, used to induce and inhibit germination, control the pattern of seedling development, induce directional growth, determine adult architecture, and detect and avoid neighbors (Figure 1).

Based on characterization of the known photoreceptor systems, plants use only a subset of the pigments harbored in their cells to monitor the light environment, relying heavily on pigments that absorb maximally in the blue (400–500 nm) and in the red and far-red (600–800 nm) regions of the visible spectrum. This may reflect the utility of these particular pigments to serve as reliable indicators of ecologically significant fluctuations in the light environment. Shorter wavelengths in the visible spectrum are less attenuated by nonselective diffusing filters, such as clouds, than are longer wavelengths (Smith 1982, Figure 1a). Light scattering by clouds may in fact lead to a slight increase in blue light (Smith 1982). Thus, blue light receptors might have particular utility for fundamental processes, such as early seedling development and the perception of time and season. In Arabidopsis thaliana, the blue light receptors, cryptochromes and phototropins, have significant or unique roles in these processes (Casal 2000; Sullivan and Deng 2003). Conversely, light scattering within a stem will be greater for short wavelengths, and there will be steeper gradients of blue than of red light in a stem irradiated with unilateral light (Hart 1988). Phototropism relies on light gradients as well as light absorption (Iino 1990), and this may be one reason why blue light receptors have been employed to mediate phototropic responses. In Arabidopsis, and perhaps in most angiosperms, the sensitivity of the cryptochrome and phototropin systems is enhanced by the possession of at least two copies of each pigment, one that is sensitive to weak light signals and one that is sensitive to strong light signals (Briggs and Huala 1999; Galen et al. 2004). This could ensure that processes
mediated by blue light signals would be relatively robust to the variation in light quantity between open and dimly lit environments, between clear and overcast skies, and below and above the soil surface. However, blue light receptors are inadequate for neighbor detection, for which a dual sensing system is needed.

**The Phytochrome System**

Among the most critical light cues used by plants are those that indicate where they are in relation to neighbors that might impinge on their access to photosynthetically active radiation. Because the pigments in stems and leaves absorb wavelengths below about 700 nm, reflected light and shade are enriched for wavelengths in the far-red region but depleted for wavelengths in the blue to red regions of the spectrum (Smith 1982, Figure 1e). This suggests that a pair of photoreceptors with contrasting absorption maxima above and below about 700 nm would serve as a useful indicator of changes in the relative proportions of short and long wavelengths that accompany the encroachment by neighbors. What plants actually have is an elegant system relying on a single type of pigment, phytochrome, with two photointerconvertible forms, one absorbing maximally in the red (660 nm) and the other absorbing maximally in the far-red (730 nm). Absorption of red light (R) by the red-absorbing form (Pr) induces conversion of the protein to the far-red-absorbing form (Pfr); likewise, absorption of far-red light (FR) by Pfr induces conversion to Pr. Thus at any one time, Pr and Pfr are in a dynamic equilibrium that reflects the relative proportions of R and FR in ambient light. Furthermore, phytochromes are very sensitive indicators of increasing or decreasing shade because small changes in the R:FR ratio of ambient light lead to large changes in the ratio of Pfr to Ptotal (Smith 1982). Thus, even very small changes are detectable, such as those that occur in the light reflected from stems of small neighbors (Ballare´ et al. 1990). In response to the detection of neighbors, shade-intolerant plants increase extension growth, suppress branches, make thinner leaves with less chlorophyll, flower early, and increase allocation to storage organs—a set of processes collectively referred to as the shade avoidance syndrome (Smith and Whitelam 1997). In similar conditions, an animal might fight or take flight.

Shade avoidance is adaptive in flowering plants (Aphalo et al. 1999; Schmitt et al. 1995) and it is clear that one critical function of phytochrome is to detect changes in R:FR (Smith 1982). In *A. thaliana*, phytochrome B (phyB) is the principal mediator of shade avoidance (Aukerman et al. 1997; Devlin et al. 1998, 1999; Franklin et al. 2003b; Sharrock et al. 2003a; Smith and Whitelam 1997). PhyB also is the primary mediator of developmental responses to saturating pulses of R that are reversible with pulses of FR (Whitelam and Devlin 1997). These responses are important for the control in open habitats of light-regulated seed germination and early seedling development, a critical stage of life when the young

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**Figure 1.** The roles of different photoreceptors in the development of *Arabidopsis* (Sullivan and Deng 2003). R, red light; FR, far-red light; B, blue light; PHY, phytochromes; CRY, cryptochromes; PHOT, phototropins.
seedling must make the transition from dependence on maternal resources stored in the seed to photosynthetic independence (Figure 1). An emerging seedling may enter one of two alternative pathways of development: skotomorphogenesis or photomorphogenesis. Germinating seedlings that receive a light signal switch from skotomorphogenetic to photomorphogenetic development, a process known as de-etiolation, inhibit extension growth and develop leaves and a photosynthetic apparatus (Figure 1). Germinating seedlings that do not receive a light signal remain skotomorphogenetic; they delay the production of leaves and photosynthetic development and use their capacity for elongation to seek light.

In the event that the seedling fails to reach the light, it may outgrow its resources and die. During this stage of development, when greening and the inhibition of extension growth are critical, shade avoidance reactions could be counterproductive to seedling survival (Smith et al. 1997). Thus, a potential cost of shade avoidance may be a decrease in seedling establishment in shady habitats or in dense stands. If so, seedling mortality in dense stands would be avoided because phyB inhibits germination in these conditions. Only when a canopy gap of sufficient size is detected will the activity of phyB promote germination (Smith 1995). This inhibitory influence on germination is important for shade-intolerant species, but if present in shade-tolerant species, could limit their ability to establish under canopies.

Together the potential cost of early shade avoidance and the inhibitory actions of phyB on germination in shaded environments could limit the ability of plants to establish under canopies, decreasing their ecological amplitude. These costs could be a general factor affecting the capacity of seed plants to colonize shady habitats because both gymnosperms and angiosperms have shade avoidance responses and FR-inhibition of germination (e.g., Toole et al. 1961; Warrington et al. 1988). However, the degree to which any group of species is limited by these costs would be influenced by other factors. For example, counterproductive early shade avoidance is most likely to affect angiosperms because of their rapid growth rates, especially when combined with the possession of small seeds. Conversely, cycads, an anciently derived group of seed plants, would be less affected because they have relatively large seeds and their seedlings grow very slowly; they can exist for very long periods without light (Mathews S and Tremonte D unpublished data). Ginkgo also has large seeds and relatively slow growth. Many conifers have small seeds and growth rates intermediate between cycads and angiosperms. However, in many conifers, seedling de-etiolation is uncoupled from light signals so that they are constitutively photomorphogenic, or green in the dark (e.g., Bogorad 1950; Mukai et al. 1992). For these species, early shade avoidance would less likely be counterproductive.

As noted, angiosperms are particularly at risk because they evolved rapid growth rates. This characteristic is coupled with the widespread occurrence of small seeds, including in the earliest diverging lineages (Feild et al. 2004) and skotomorphogenetic seedling development. Angiosperms then may have a special need for a mechanism to counteract early shade avoidance, and also the inhibitory actions of phyB on germination and seedling development in deep shade. A candidate for such a mechanism is one of the other angiosperm phytochromes, phyA (Smith et al. 1997), which functions to promote germination and seedling photomorphogenesis in continuous FR and in deep shade (Casal et al. 1997; Yanovsky et al. 1995). Additionally, phyA induces germination in response to millisecond pulses of broad spectrum light, promoting germination after brief soil disturbances (Casal et al. 1997) and possibly after exposure to sunflecks.

In flowering plants, phyA and phyB represent the principal mediators, respectively, of FR- and R-mediated development (Whitelam and Devlin 1997), and null mutants of each have severe phenotypes. Three additional phytochromes occur in A. thaliana, phyC, phyD, and phyE. Each of these apparently has a lesser role in photomorphogenesis (Figure 2), and phenotypes of the nulls are most apparent in mutants with lesions in multiple phytochromes (Aukerman et al. 1997; Devlin et al. 1998; Franklin et al. 2003a,b; Hennig et al. 2002; Monte et al. 2003; Sharrock et al. 2003a,b). PhyC is widely distributed in flowering plants, diverging from

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<th>Gene</th>
<th>Light Cue</th>
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<tr>
<td>PHYA</td>
<td>Far-red light, Millisecond pulse</td>
<td>Germination, Deetiolation</td>
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<td>PHYC</td>
<td>Red light</td>
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<td>PHYE</td>
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<td>PHYD</td>
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Figure 2. The relationships of phytochrome genes of A. thaliana, where phyA and phyB have been demonstrated to be the principal mediators of responses to far-red and red light, respectively.
phyA prior to their origin (Mathews and Sharrock 1997; Sharrock and Quail 1989). PhyE also is widely distributed in flowering plants, diverging from phyB very early in their history (Mathews S unpublished data), although sporadic losses of phyE have occurred (Mathews and Sharrock 1997). PhyD is restricted in distribution to Arabidopsis and its relatives, diverging from phyB near the origin of the mustard family (Brassicaceae; McBreen K and Mathews S unpublished data).

The Evolution of phyA in Early Angiosperms

The possibilities that the some or all of the functions of phyA might help plants establish in shady habitats and that the origin of one or more of these functions might have coincided with the origin of flowering plants suggest that they might have provided an adaptive advantage to early angiosperms during their colonization of the forest dominated by ferns and gymnosperms. The radiation of the angiosperms is one of the more spectacular events in the history of land plants. They number ~260,000 species, about six times more than all other land plants combined (~38,000 species), and they far outnumber other seed plants (~1,000 species). Although these numbers represent current diversity, no other group of land plants previously achieved comparable levels of diversity, and the rise of flowering plants to ecological dominance was similarly remarkable (Bond 1989; Crane et al. 1995; Wing and Boucher 1998).

Multiple factors likely account for the success of flowering plants, but in the attempt simply to gain a foothold, photoreceptor evolution may have played a role.

If the origin of any of the functions of phyA provided an adaptive advantage for early diverging angiosperms, we might expect (1) to find evidence of episodic selection in the sequences of species belonging to the remnants of these lineages, (2) to find that phyA functions occur in these species, and (3) that these functions are not found outside angiosperms. PhyA may be unique to flowering plants because it arose by gene duplication prior to their origin (Mathews and Sharrock 1997; Sharrock and Quail 1989). However, its relationships with phytochromes in gymnosperms remain ambiguous in the absence of data that can resolve whether separate gene duplications occurred in angiosperms and gymnosperms or whether a single duplication occurred prior to the divergence of angiosperms from gymnosperms (Sharrock and Mathews in press). Whether the protein is ancient or was more recently derived, phyA sequences provide unambiguous evidence that innovation at the molecular level occurred very early in the history of angiosperms. An apparent episode of positive selection and a high proportion of radical amino acids map to the branch leading to the angiosperm phyA clade (Mathews et al. 2003; Mathews 2004), consistent with an adaptive role for phyA. However, linking this episode of molecular adaptation with changes in photoreceptor function requires characterization of responses to continuous FR (FRc) in gymnosperms and in the representatives of the earliest diverging angiosperm lineages. If the capacity to establish successfully in FRc or deep shade is not unique to angiosperms, or if it arose late in their history, it is unlikely that it is a special feature that aided early angiosperms.

Previous studies have suggested that the seedlings of Ginkgo and conifers have a limited ability to de-etiolate in FRc; seedlings of both groups exhibited greater cotyledon or leaf development in FRc than in the dark, but extension growth was similar in both treatments, suggesting that FRc and perhaps deep shade cannot fully induce early seedling development (Burgin et al. 1999; Christensen et al. 2002). This work needs to be extended. There are no data from Gnetales, a distinctive group of seed plants (Gnetum, Ephedra, and Welwitschia) that are related to conifers, or from most conifers, where only shade-intolerant members of the pine family have been examined. No previous studies have examined cycads, but preliminary data suggest that they lack even the rudimentary phyA-like responses observed in Pinaceae and Ginkgo (Mathews S and Tremonte D unpublished data). There are also no data from most groups of flowering plants, and none at all from species that diverged early in their history. However, similar responses to FRc in rice (a monocot; Takano et al. 2000), Arabidopsis (a eudicot), and Calycanthus (Mathews S and Tremonte D unpublished data) (a woody shrub that diverged before the eudicot radiation) suggest that phyA responses were established relatively early. It also will be important to establish the ecological relevance of seedling responses to FRc. The mortality of Arabidopsis phyA-null mutants in shade suggests that this function of phyA is critical to seedling establishment under canopies (Yanovsky et al. 1995), but this remains to be tested in a wider range of species.

Finding that molecular adaptation and the origin of FRc-mediated seedling development are correlated would be consistent with the hypothesis that innovation in phyA function was adaptive in early angiosperms. An important next test of the link between molecular adaptation and functional innovation is under way. It relies on mutagenesis experiments to determine the phenotypic effects of changing to their ancestral states the amino acid residues that changed along the branch to all phyA sequences and were then fixed. If the spectral activity of the resulting photoreceptor molecule is changed and/or if expression of the sequences in phyA-null mutants of Arabidopsis cannot fully restore phyA function, it would be consistent with a role for these sites in the evolution of angiosperm phyA function.

The Evolution of phyB and phyD in Arabidopsis

Although it is clear that the divergence of phyA from phyC involved functional divergence, evolution in the phyB lineage has resulted in additional phytochromes that apparently make only minor contributions to processes principally mediated by phyB, at least in Arabidopsis (Figure 2). Neither phyD, which is restricted to Arabidopsis and its relatives, nor
phyE, which is found widely in angiosperms, can rescue the phyB-null mutant phenotype of *Arabidopsis* (Sharrock et al. 2003a), and both photoreceptors have phenotypes that are difficult to discern in single mutants (Aukerman et al. 1997; Devlin et al. 1998). Thus it is possible that these two additional phytochromes are evolving under relaxed constraints and are likely to be silenced. Consistent with this suggestion, phyE has been lost from monocots and from some dicot families (Mathews and Sharrock 1997; Sharrock and Mathews in press), and the phyD of the Ws (Wassilewskija) ecotype of *A. thaliana* is a pseudogene (Aukerman et al. 1997). However, the loci encoding both these phytochromes have been retained longer than the average half-life of duplicate genes of around 4 million years (Lynch and Conner 2000), and the *Arabidopsis* ecotypes commonly used in the lab retain a functional phyD (Aukerman et al. 1997). Moreover, in the case of phyD, there is evidence that purifying selection constrains its evolution (McBreen K and Mathews S unpublished data). This suggests that despite its apparently minor phenotype, phyD is important. The phyD coding sequence driven by the phyB promoter can fully complement the early flowering phenotype of the phyB null mutant (Sharrock et al. 2003a). It is possible that redundancy for flowering time is important enough for the retention of phyD, despite its disappearance from the Ws ecotype. It also is possible that subtle neofunctionalization or subfunctionalization may have occurred.

Experiments with chimeric proteins demonstrate that amino acids in the central regions of the phyB and phyD coding sequences are critical to their respective activities and that these two photoreceptors differ significantly in their abilities to activate signaling pathways (Sharrock et al. 2003b). This is consistent with either the origin of a new function, perhaps one that is not obvious in typical genetic screens, or with subdivision of ancestral phyB function at the level of downstream signaling pathways. A suggestion that may be worth exploring is that the role of phyD in some natural populations is more important than has been indicated by lab phenotypes. For example, Halliday and Whitelam (2003) have shown that both temperature and photoperiod affect the prominence of the role of phyD in control of flowering and leaf expansion. The natural light environment is considerably more complex than the laboratory environment, and it is becoming more apparent that temperature effects, photoperiod, and potentially other environmental factors influence phytochrome activity and the roles of specific phytochromes (Franklin and Whitelam 2004; Halliday and Whitelam 2003; Heschel MS and Donohue K personal communication).

**Figure 3.** Development in a nonphotosynthetic member of the parasitic Orobanchaceae, where aspects of development that are light-mediated in autotrophs (compare Figure 1) are modified or possibly absent.
The Evolution of Phytochromes in a Family of Parasitic Plants

Studies of phytochrome evolution in parasitic plants offer the opportunity to address the question of how photoreceptors evolve in plants in which development and light cues are to some extent unlinked (compare Figures 1 and 3). Several studies have provided insight into the evolution of plastid genomes in parasites, finding in many cases that photosynthetic genes are lost or nonfunctional (DePamphilis 1995). The plant family Orobanchaceae provides a useful system for characterization of phytochrome evolution in parasites because it comprises a single autotrophic lineage that is sister to the remaining family, which in turn is made up of sister pairs of lineages that either are photosynthetic root parasites (hemiparasites) or nonphotosynthetic root parasites (holoparasites). Germination in the holoparasites is promoted by signals from host plants, and both holoparasites and hemiparasites may have prolonged underground stages of skotomorphogenetic development during which they rely completely on the host plant. This developmental pathway contrasts markedly with development in the light-seeking seedlings of autotrophic plants (Figures 1, 3). On emergence, holoparasites produce only a nonphotosynthetic inflorescence (Figure 3), whereas hemiparasites develop a photosynthetic shoot system before flowering. The degree to which photoreceptors mediate gravitropism, phototropism, shade avoidance, stomatal opening, chloroplast movement, and flowering in hemiparasites remains unknown, but presumably the expression of these processes remains to a greater or lesser extent under the control of light signals. In contrast, some of these processes, such as phototropism and shade avoidance, are probably absent from holoparasites, and it remains unknown what controls stomatal opening and flowering (Figure 3). In at least one case, flowering of Orobanche minor appeared dependent on the flowering of the host in response to long days (Kuijt 1969). The mechanisms that underlie novel processes in the parasites, such as the inhibition of seedling emergence and of photosynthetic shoot development during emergence of the holoparasites, also remain unknown.

These developmental differences between autotrophs and parasites suggest that the functions of photoreceptors differ among autotrophs, hemiparasites, and holoparasites. Changes in photoreceptor function may be required to maintain the differences in developmental pathways that characterize the three lifestyles. In some cases, origin of a novel function may be important. In others, aspects of photoreceptor function may become superfluous during the transition from autotrophic to parasitic habit. Functional changes may occur through gene loss, through altered expression patterns, by sequence-specific changes in coding regions of the genes, or through a combination of these factors. The holoparasite O. minor retains and expresses at least one cryptochrome gene (Okazawa et al. 2004), but other data regarding the distribution of cryptochromes and phototropins in the family are lacking. In contrast, there is evidence that the two major phytochromes, phyA and phyB, are widely retained in the family, as is phyE; fewer data are available about the distribution of phyC, but it has been detected in at least some species (Bennett JR and Mathews S unpublished data). PhyA is expressed in the holoparasite O. minor (Okazawa et al. 2004), but other expression data are lacking.

Because phytochromes participate directly in the transition from skotomorphogenetic to photomorphogenetic developmental pathways, it will be critical to compare patterns of gene expression among autotrophs and parasites during the transition from underground to above-ground phases. Characterization of changes in coding sequences also may provide insight. For example, if any one of the phytochromes is directly implicated in the transition to parasitism, convergent amino acid substitutions might be observed in multiple holoparasitic lineages, and there might be evidence that these positions have been influenced by positive selection (or more generally, that selective constraints have been altered at these sites). Conversely, if the function of any one of the phytochromes is less important in parasites, there should be evidence of relaxation in selective constraints. Preliminary analyses of phyA sequences provide no evidence of convergent change that would directly implicate changes in this photoreceptor in the transition to the parasitic habit. However, there is evidence that phyA is evolving under relaxed selective constraints in the holoparasites relative to both the hemiparasites and autotrophic species (Bennett JR and Mathews S unpublished data). These observations are consistent with a scenario in which at least some of the functions of phyA are less critical in holoparasites than they are in photosynthetic taxa. Ultimately, studies in this system may lead to the discrimination of divergence points in the downstream signaling pathways because some aspects of development differ between autotrophs and holoparasites whereas others do not (Figures 1, 3).

Concluding Remarks

These studies of the phytochrome system highlight the limits of our understanding of how plant photoreceptors might function in natural environments and outside model species. In Arabidopsis, the factors responsible for the functional differences between phyB and phyD remain elusive. Intriguing new data suggest that temperature has a profound effect on processes mediated by specific phytochromes (Halliday and Whitelam 2003; Heschel MS and Donohue K personal communication), a phenomenon certain to come into play outside the lab. In nonmodel systems, functional information generally is lacking. Nevertheless, it is clear that phytochrome-mediated processes differ in parasitic plants and outside angiosperms from those characterized in model systems. Limited knowledge of these systems hinders efforts to understand the roles that photoreceptor evolution might have in enhancing the capacity of plants to adapt to new environments during diversification events. It is important to develop strategies to take advantage of both natural environments and nonmodel species to increase understanding of how photoreceptor function evolves. Specifically,
functional models inferred from model systems should be tested in a wide variety of natural environments and in species with a wide range of morphologies and ecologies. Studies of *Arabidopsis* already are being extended to evaluate amounts of natural variation and to exploit natural variants (e.g., Borevitz et al. 2002; Botto and Smith 2002; Maloof et al. 2000, 2001; Mitchell-Olds 2001). A complimentary effort to exploit the rich resources of nonmodel systems, a more challenging task, is also needed.

**Acknowledgments**

This paper is based on a presentation given at the symposium entitled “Genomes and Evolution 2004,” cosponsored by the American Genetic Association and the International Society of Molecular Biology, and Evolution, at the Pennsylvania State University, State College, PA, USA, June 17–20, 2004.

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Corresponding Editor: Shozo Yokoyama