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Phytochromes and photomorphogenesis in *Arabidopsis*

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Plants have evolved exquisite sensory systems for monitoring their light environment. The intensity, quality, direction and duration of light are continuously monitored by the plant and the information gained is used to modulate all aspects of plant development. Several classes of distinct photoreceptors, sensitive to different regions of the light spectrum, mediate the developmental responses of plants to light signals. The red–far-red light-absorbing, reversibly photochromic phytochromes are perhaps the best characterized of these. Higher plants possess a family of phytochromes, the apoproteins of which are encoded by a small, divergent gene family. *Arabidopsis* has five apophytochrome-encoding genes, *PHYA–PHYE*. Different phytochromes have discrete biochemical and physiological properties, are differentially expressed and are involved in the perception of different light signals. Photoreceptor and signal transduction mutants of *Arabidopsis* are proving to be valuable tools in the molecular dissection of photomorphogenesis. Mutants deficient in four of the five phytochromes have now been isolated. Their analysis indicates considerable overlap in the physiological functions of different phytochromes. In addition, mutants defining components acting downstream of the phytochromes have provided evidence that different members of the family use different signalling pathways.

Keywords: phytochrome; *Arabidopsis*; mutant; photomorphogenesis; light

1. INTRODUCTION

Plants possess a range of sensory systems that monitor the surrounding environment so enabling them to initiate appropriate modifications to their development. Being photoautotrophic, plants are especially sensitive to the light environment and plant morphogenesis is dramatically affected by variations in the intensity, quality, direction and duration of light. Information gained about these factors is used to modulate all aspects of plant development, from seed germination, through seedling de-etiolation and establishment, to the control of later events in vegetative development, the architecture of the plant and the transition to reproductive development.

The various developmental responses of plants to signals from the light environment are collectively referred to as photomorphogenesis. Photomorphogenesis is a complex process that depends upon the actions of several signal-transducing photoreceptor systems that interact with endogenous developmental programmes and with the endogenous circadian oscillator. These photoreceptor systems include the phytochromes, which absorb mainly in the red (R) and far-red (FR) regions of the spectrum, the cryptochromes, which absorb in the blue–UV-A region of the spectrum, and distinct UV-A and UV-B light-absorbing photoreceptors. The phytochromes are the most extensively characterized of these photoreceptors. Recently, some of the blue–UV-A photoreceptors have been identified and characterized at the molecular level (Cashmore 1997). The identity of any of the UV-A or UV-B photoreceptors remains elusive.

2. PHOTOCHROME PROPERTIES

The phytochromes are reversibly photochromic, soluble bilin-linked chromoproteins. Typically, phytochromes consist of a dimer of identical approximately 124 kDa polypeptides, each of which carries a single, covalently linked linear tetrapyrrole chromophore (phytochromobilin), attached via a thioether bond to a conserved cysteine residue in the N-terminal globular domain of the protein (Furuya & Song 1994). The more elongated, non-chromophorylated, C-terminal domain of the protein is involved in dimerization (Edgerton & Jones 1992). Phytochromes can exist in either of two relatively stable isoforms: an R light-absorbing form, Pr, with an absorption maximum at about 660 nm, or an FR light-absorbing form, Pfr, with an absorption maximum at about 730 nm. Light-induced interconversions between Pr and Pfr involve a Z-E isomerization of the linear tetrapyrrole chromophore about the C_{15} double bond that links the C- and D-rings of the tetrapyrrole (see Terry *et al*. 1993). The photoisomerization of the chromophore leads to reversible conformational changes throughout the protein moiety of phytochrome (Quail 1991). The altered protein conformations stabilize the chromophore isomers. Based on physiological and genetic studies, the Pfr form of phytochrome is generally considered to be biologically active, and Pr is considered to be inactive. It is assumed that the light-induced protein conformational changes account for the differences in activity between Pr and Pfr.

The absorption spectra of Pr and Pfr show considerable overlap throughout the visible light spectrum. As a consequence, the phytochromes are present in an

equilibrium mixture of the two forms under almost all irradiation conditions.

All higher plants studied so far, as well as some lower plants, possess multiple discrete phytochrome species which make up a family of closely related photoreceptors, the apoproteins of which are encoded by a small family of divergent genes (Quail 1994). The size of the phytochrome family varies among different plant species. In *Arabidopsis thaliana*, five apophytochrome encoding genes (*PHYA–PHYE*) have been characterized (Sharrock & Quail 1989; Clack *et al*. 1994). The *PHYA* gene has been demonstrated to encode the apoprotein of the well-characterized, light-labile phytochrome that predominates in etiolated seedlings, phytochrome A (phyA). Phytochrome A is rapidly depleted when etiolated tissues are exposed to light as a consequence of Pfr-induced proteolysis (Quail 1994). Light exposure also induces a reduction in *PHYA* transcript abundance (Quail 1994). The other *PHY* (*B–E*) genes encode the apoproteins of lower abundance, which appear to be more light-stable phytochromes, previously referred to as type 2 phytochromes (see Kendrick & Kronenberg 1994). The *Arabidopsis* PHYB and PHYD polypeptides are about 80% identical (Mathews & Sharrock 1997) and are somewhat more related to PHYE than to either PHYA or PHYC (about 50% identity). Furthermore, the PHYB, PHYD and PHYE polypeptides are the most recently evolved members of the phytochrome family. Counterparts of *PHYA*, *PHYB* and other *PHY* genes are present in most, if not all, higher plants (Mathews & Sharrock 1997).

The spatial and temporal expression patterns of the *Arabidopsis PHYA*, *PHYB*, *PHYD* and *PHYE* genes have been studied by introducing into *Arabidopsis* fusions between *PHY* promoter regions and the *Escherichia coli GUS* gene. Staining for GUS indicated detectable activity of all of the promoters throughout seedling development (Somers & Quail 1995; Goosey *et al*. 1997). The *PHYA* and *PHYB* promoters showed similar spatial patterns of activity, being expressed in most parts of the plant. Thus, differences in photoreceptor apoprotein gene expression cannot account for the different physiological roles of these two phytochromes (see following paragraphs). The *PHYD* and *PHYE* promoters were observed to drive much lower GUS expression than the *PHYA* or *PHYB* promoter and were found to show a more restricted pattern of expression, notably in cotyledons, leaves and sepals (Goosey *et al*. 1997). In the shoots of young seedlings, the activities of the *PHYA*, *PHYB* and *PHYD* promoters were found to be higher for dark-grown seedlings than for light-grown seedlings, whereas the opposite was the case for *PHYE* (Goosey *et al*. 1997). The *PHYA* promoter shows the greatest down-regulation by light (Somers & Quail 1995).

3. PHYTOCHROME FUNCTIONS

The most revealing approaches to the identification of the roles of individual photoreceptors have been those involving the analysis of mutants that are deficient in one or more photoreceptors. Additional insights into photoreceptor functions have also come from the study of transgenic plants that overexpress photoreceptor genes.

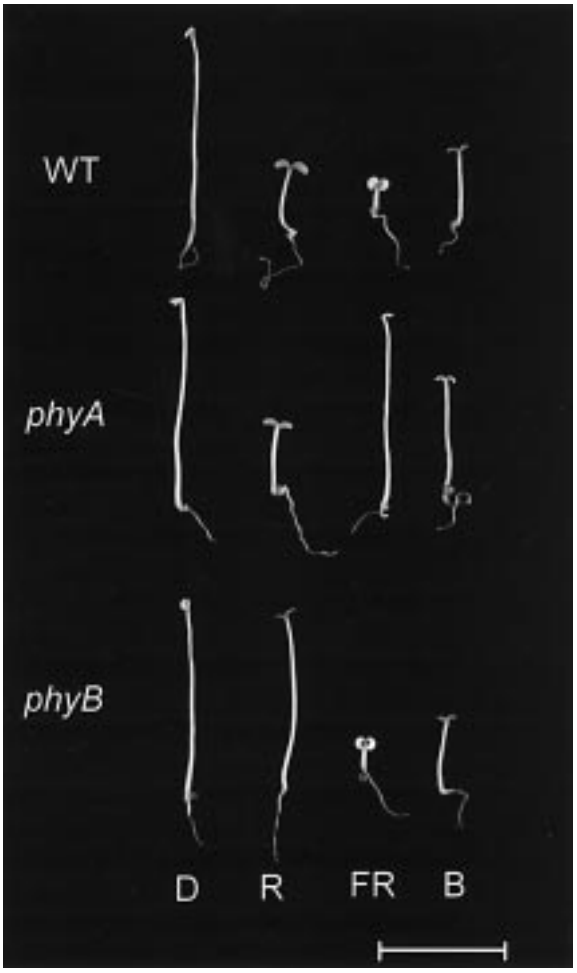

Figure 1. Phytochrome photoreceptor mutants of *Arabidopsis*. Wild-type (WT), *phyA* and *phyB* seedlings were grown in the dark (D), under continuous red light (R), continuous far-red light (FR), or continuous blue light (B) for four days. Scale bar, 1 cm.

Arabidopsis has been a focus for both approaches. Dark-grown *Arabidopsis* seedlings display a typical etiolated phenotype, characterized by extreme hypocotyl elongation growth, a hypocotyl hook, small folded cotyledons, absence of chlorophyll and lack of plastid development. In response to R, FR or blue-light signals, a series of growth and developmental changes are initiated that lead to de-etiolation and photomorphogenic seedling development (see figure 1). Genetic screens for mutants that are insensitive to light with regard to photomorphogenic seedling development have led to the identification of mutations in the *PHYA* and *PHYB* genes, as well as in two genes (*HY1* and *HY2*) that encode proteins involved in tetrapyrrole chromophore synthesis. This screen also identified mutations in cryptochrome apoprotein. The physiological characterization of photoreceptor mutants and transgenic plants is enabling us to form a picture of the roles of, and interactions among, the phytochromes.

Arabidopsis mutants deficient in phyA (*phyA*) were isolated independently by several laboratories in screens for a long hypocotyl phenotype following seedling growth of prolonged FR light (Nagatani *et al*. 1993; Parks & Quail 1993; Whitelam *et al*. 1993). Seedlings that are null for phyA display a complete insensitivity to

prolonged FR light with respect to all aspects of seedling photomorphogenesis, including inhibition of hypocotyl elongation, the opening and expansion of cotyledons (figure 1), the synthesis of anthocyanin and the regulation of expression of several genes (see, for example, Whitelam *et al.* 1993; Johnson *et al.* 1994; Barnes *et al.* 1996). As other apophytochrome mutants are not impaired in their responses to prolonged FR light, phyA is implicated as the sole photoreceptor mediating seedling responses to this waveband (see Quail *et al.* 1995). This view is supported by the finding that transgenic overexpression of an oat *PHYA* cDNA in *Arabidopsis* leads to exaggerated light responses, particularly responses to FR light (Boylan & Quail 1991; Whitelam *et al.* 1992). Sensitivity to R light in *phyA* seedlings is more or less normal, indicating that phyA plays only a small role in seedling responses to this waveband.

There are two distinct modes of phytochrome action which have been recognized for the responses of etiolated seedlings to FR light (Smith & Whitelam 1990). In the FR high irradiance response (FR-HIR), prolonged irradiation with continuous FR light of a relatively high fluence rate ($0.1-50\ \mu mol\ m^{-2}\ s^{-1}$) is required for display of responses, and the extent of the HIR is dependent on the fluence rate of FR light (see Smith & Whitelam 1990). In contrast, very low fluence responses (VLFR) are initiated by fluences of light as low as $10^{-9}\ mol\ m^{-2}$ and are fully saturated by very low concentrations of Pfr. As even a single pulse of broad band FR light can saturate the VLFR, this response mode does not obviously display prototypical R–FR reversibility (see Smith & Whitelam 1990). Both HIR and VLFR (e.g. for the inhibition of hypocotyl elongation and the promotion of cotyledon expansion) modes are absent in *phyA* mutant seedlings (Casal *et al.* 1997).

Although seedlings growing in the natural environment would never be exposed to prolonged FR light, there is evidence that FR-HIR is of fundamental importance in seedling establishment under natural canopy shade conditions. Yanovsky and co-workers (1995) have shown that, for *Arabidopsis* seedlings growing under dense vegetational shade, an FR light-rich light environment, *phyA* mutants display severely impaired de-etiolation and extreme hypocotyl elongation. As a consequence, significant numbers of *phyA* seedlings fail to become established and die. Wild-type seedlings are less elongated under these conditions and are so more able to become established.

When grown in white light, or under non-shaded conditions in the greenhouse, *phyA* mutants develop normally and display a vegetative phenotype that is indistinguishable from that of wild-type seedlings (Whitelam *et al.* 1993). This might be taken to indicate that phyA plays only a restricted or specialized role in early seedling photomorphogenesis. However, despite being light-labile, phyA is one of the most abundant phytochromes in the fully de-etiolated light-grown plant (Clack *et al.* 1994) and there is abundant evidence that phyA continue to regulate development throughout the life of the plant. For example, Johnson and co-workers (1994) showed that *Arabidopsis phyA* mutant seedlings flower later than wild-type seedlings following growth under 8-h photoperiods. *Arabidopsis* is a facultative long-day plant, and flowering is promoted as daylength increases. As expected, the flowering of wild-type seedlings is very markedly promoted when short photoperiods are extended to 16 h by low fluence rate incandescent light. However, the flowering of *phyA* mutants is hardly promoted by this type of day extension (Johnson *et al.* 1994). Furthermore, for seedlings grown under long days in the greenhouse, *phyA* mutants typically flower significantly later than wild-type seedlings (G. C. Whitelam, unpublished observations). The phytochrome A-deficient *fun* mutant of another long day plant, garden pea (*Pisum sativum*), also has an impaired ability to perceive long days and is late flowering under such conditions (Weller *et al.* 1997). In contrast to the late flowering of *phyA* mutants, transgenic *Arabidopsis* seedlings that overexpress an oat *PHYA* cDNA are markedly early flowering, particularly following growth under short photoperiods (Bagnall *et al.* 1995). Thus, the transgenic overexpressors grown under short photoperiods, respond in a manner similar to that of wild-type seedlings grown under long photoperiods. A similar phenomenon has recently been reported for transgenic hybrid aspen overexpressing oat *PHYA* cDNA (Olsen *et al.* 1997). In wild-type aspen trees, short-day conditions induce apical growth cessation and cold acclimatization; transgenic trees show a marked insensitivity to short photoperiods.

As well as playing a key role in seedling establishment and daylength perception, phyA is also involved in the photocontrol of *Arabidopsis* seed germination (Johnson *et al.* 1994; Reed *et al.* 1994; Shinomura *et al.* 1994; Botto *et al.* 1996; Whitelam & Devlin 1996). For dormant wild-type seeds, germination is significantly promoted by brief or prolonged exposure to broad band FR light. This effect of FR light is not seen in seeds of the *phyA* mutant (Johnson *et al.* 1994). The germination promoting effect of single pulses of FR light indicates that phyA is operating in the VLFR mode (Shinomura *et al.* 1996). Botto *et al.* (1996) have shown that, in the field, the promotion of seed germination by single pulses of deep canopy shade–light also reflects a phyA-mediated VLFR.

Phytochrome B-deficient *Arabidopsis* mutants were isolated many years ago on the basis of their long hypocotyl phenotype following growth under white light (Koornneef *et al.* 1980; Reed *et al.* 1993). Etiolated *phyB* seedlings display a marked insensitivity to brief or prolonged R light with respect to almost all aspects of seedling de-etiolation, including inhibition of the hypocotyl elongation, cotyledon expansion and chlorophyll synthesis. However, *phyB* seedlings de-etiolate normally following growth under prolonged FR or blue light (Koornneef *et al.* 1980; Reed *et al.* 1993). This indicates that phyB plays a principal role in seedling responses to R light but plays no role in responses to prolonged FR light. Thus, phyB appears to control seedling de-etiolation under R light and phyA controls the same processes under FR light.

Phytochrome B also plays a principal role in the photoregulation of *Arabidopsis* seed germination (Shinomura *et al.* 1994, 1996; Reed *et al.* 1994). The germination of dormant wild-type seeds is markedly promoted by a single brief pulse of R light. Furthermore, the effect of the R light pulse can be nullified by a subsequent pulse of FR light. This R–FR reversible response, the classical

hallmark of phytochrome action, reflects the low fluence response mode of phytochrome action, and is largely (although not completely) absent in seeds from phyB-null mutants (Shinomura *et al.* 1994, 1996; Reed *et al.* 1994). The Pfr form of phyB, present in the dry seed, can also promote the germination of seeds that have been allowed to imbibe in darkness (McCormac *et al.* 1993).

Phytochrome B plays a predominant role in controlling the shade avoidance syndrome of responses (Smith 1995; Smith & Whitelam 1997). The perception of alterations in the relative amounts of R and FR light represents one of the most important aspects of photomorphogenesis in nature. Light that is reflected from, or transmitted through, vegetation is depleted in the R region relative to the FR region as a consequence of absorption by chlorophyll. This reduced ratio of R to FR light (R:FR ratio) is perceived by the phytochrome system as a change in the relative amounts of Pr and Pfr, and provides the plant with an unambiguous signal that potential competitors are nearby (see Smith 1995). Many plants, including *Arabidopsis*, in an attempt to avoid being shaded, respond to these low R:FR ratio signals by increasing elongation of internodes and/or petioles, reducing leaf growth, increasing apical dominance and accelerating flowering (Smith 1995; Smith & Whitelam 1997). This shade avoidance phenotype in constitutively displayed by *phyB* mutants (see, for example, Whitelam & Smith 1991; Devlin *et al.* 1996). Furthermore, seedlings that are null for phyB show greatly attenuated (although not completely absent) responses to low R:FR ratio signals (see, for example, Whitelam & Smith 1991; Halliday *et al.* 1994; Devlin *et al.* 1996). In wild-type plants, the shade avoidance syndrome of responses can be quite effectively induced by pulses of FR light given at the end of each photoperiod. These end-of-day (EOD) responses are also greatly attenuated in phyB-null seedlings (Nagatani *et al.* 1991; Devlin *et al.* 1996). Taken together, these findings implicate phyB as a main contributor to shade avoidance.

The early flowering of *phyB* mutants is displayed following seedling growth under either long or short photoperiods (see, for example, Goto *et al.* 1991; Halliday *et al.* 1994). This presumably reflects the constitutive shade avoidance phenotype. Nevertheless, *phyB* mutants are still responsive to daylength, flowering much earlier under long photoperiods than under short photoperiods (see, for example, Goto *et al.* 1991; Bagnall *et al.* 1995). This suggests that in *Arabidopsis* phyB may not be playing a significant role in the detection of daylength.

The retention of responses to R and FR light by mutants that are doubly null for phyA and phyB has provided some clues to the physiological functions of other members of the phytochrome family. For example, it has been shown that *phyAphyB* double mutants respond to low R:FR ratio signals and EOD FR light treatments by a promotion of elongation growth and earlier flowering (Halliday *et al.* 1994; Devlin *et al.* 1996). The responses of *phyAphyB* seedlings to EOD FR light pulses show R–FR reversibility, clearly implicating the action of one or more other phytochromes (Devlin *et al.* 1996). Similarly, seeds of the *phyAphyB* double mutant show an R–FR reversible promotion of germination (Whitelam & Devlin 1996; Poppe & Schäfer 1997). Also, the steady-state level of transcripts of the *ATHB-2* gene, which encodes a novel type of homeodomain protein, and the *CAB* gene, which encodes a structural component of the light-harvesting complex, are light-regulated in an R–FR reversible manner in *phyAphyB* mutants (Carabelli *et al.* 1996; Hamazato *et al.* 1997).

A phytochrome D-deficient mutant has recently been obtained following the discovery that certain accessions of the Ws ecotype of *Arabidopsis* carry a 14-bp deletion, beginning at amino acid 29, of the coding region of the *PHYD* gene (Aukerman *et al.* 1997). The deletion leads to translation termination at a nonsense codon 138 nucleotides downstream of the end point of the deletion. This *phyD* mutation has been introgressed into the La *er* ecotype and has been combined with other *phy* mutations. Analyses of the various mutants indicates that phyD plays a role similar to that played by phyB in initiating shade avoidance responses. In both the Ws and La *er* ecotypes, *phyD* mutant seedlings grown under R light have very slightly elongated hypocotyls compared with wild-type seedlings (Aukerman *et al.* 1997). This effect of phyD-deficiency is more marked in a *phyB* mutant background. Furthermore, phyD-deficiency eliminates the small EOD FR light-induced hypocotyl elongation response displayed by Ws *phyB* seedlings. Phytochrome D-deficiency causes several defects in adult plant development, some of which are only readily detected in a *phyB* background. In particular, in both the Ws and La *er* ecotypes, mutants deficient in both *phyB* and *phyD* flower earlier, and have noticeably longer petioles than mutants lacking only *phyB* (Aukerman *et al.* 1997; see figure 2).

The overlapping functions of the closely related phyB and phyD indicates that there is significant redundancy within the phytochrome family with regard to R:FR ratio signal perception and the initiation of shade avoidance reactions. This idea is further reinforced by our recent isolation and analysis of a *phyE* mutant in *Arabidopsis* which suggests that phyE also plays a role in mediating shade avoidance responses. The *phyE* mutant was isolated in a screen involving M_2 seedlings derived from mutagenesis of the *phyAphyB* mutant. Previously, Devlin and co-workers (1996) had shown that *phyAphyB* seedlings respond to EOD FR light treatments by elongation of the internodes between 'rosette' leaves and by early flowering (see figure 2). Significantly, both of these responses are also displayed by *phyAphyBphyD* triple mutants (figure 2), suggesting that either phyC and/or phyE mediates these responses. In the screen, M_2 seedlings were grown for several weeks under white light and elongated–early-flowering individuals were selected. The selected plants were selfed and the progeny re-screened. Several elongated and/or early flowering mutants were isolated. One of the mutants displaying both elongated rosette internodes and early flowering was found to have a 1-bp deletion within the coding region of the *PHYE* gene at position equivalent to amino acid 784. The resulting frame shift creates a stop codon 42-bp downstream of the deletion. The deletion in *PHYE* disrupts a *Hin*fI restriction enzyme site which provided a diagnostic test used in the isolation of the *phyE* mutant following backcrosses to *phyAphyB* to the wild-type. One of the most striking features of the *phyAphyBphyE* triple mutant is that, in contrast with *phyAphyB* or *phyAphyBphyD* seedlings, this mutant shows little or no response to EOD FR light

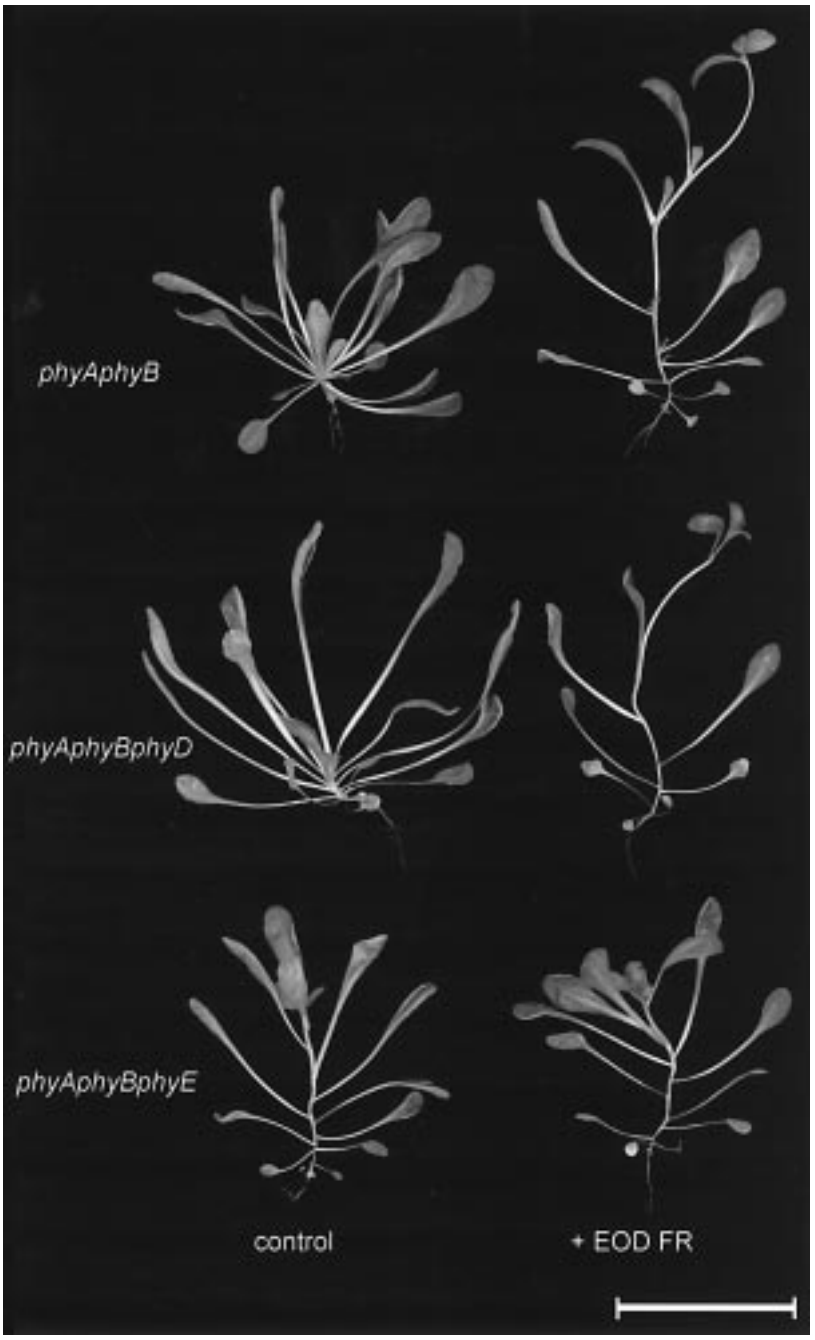

Figure 2. Shade avoidance responses of phytochrome-deficient mutants. Plants were grown for 60 days under 8 h light : 16 h dark photoperiods (control), or under the same conditions with a 10 min pulse of FR light given immediately prior to transfer to darkness (+EOD FR). Scale bar, 5 cm.

treatments (figure 2). Monogenic *phyE* mutant seedlings appear indistinguishable from wild-type seedlings at both the seedling and rosette stage. However, phyE-deficiency is detectable in a *phyB* background, where the *phyBphyE* double mutant has more elongated petioles and is earlier flowering than the monogenic *phyB* mutant (G. C. Whitelam, unpublished observations). This is similar to the situation observed for the *phyD* mutant (Aukerman *et al*. 1997; figure 2).

Mutants that are deficient in phyC have so far proved elusive. We have isolated a mutant, tentatively designated *gn7*, that carries a large deletion (spanning *PHYC*) on the top arm of chromosome 5 (G. C. Whitelam, unpublished data). The mutant is diminutive with small leaves and reduced elongation growth, shows reduced apical dominance, aberrant flower development and is male sterile. However, the size of the deletion (at least 14-kbp) precludes attempts to assign aspects of the phenotype to phyC-deficiency. The introduction of a *PHYC* transgene, under the control of the cauliflower mosaic virus 35S promoter, into *gn7* failed to rescue any features of the *gn7* mutant phenotype (G. C. Whitelam, unpublished data).

Some clues to the possible photoregulatory roles of phyC have come from experiments in which *PHYC* has been overexpressed in wild-type plants (Qin *et al*. 1997; Halliday *et al*. 1997). *Arabidopsis* plants overexpressing *Arabidopsis PHYC* display a slightly enhanced hypocotyl elongation inhibition response to prolonged R light and an increase in primary leaf expansion following growth in white light (Qin *et al*. 1997). The overexpression of *Arabidopsis PHYC* in tobacco plants does not alter responsivity of the hypocotyls to R light, but it does lead to

significant increases in both cotyledon and leaf expansion, as well as increasing overall plant size (Halliday *et al.* 1997). The effects on leaf expansion are specific to *PHYC* overexpression because in both *Arabidopsis* and tobacco, leaf expansion is not enhanced by the overexpression of either *PHYA* or *PHYB* (Qin *et al.* 1997; Halliday *et al.* 1997). This may indicate that phyC has discrete functions, not directly comparable to the functions of the other members of the family.

4. PHYTOCHROME SIGNAL TRANSDUCTION

It is obvious that to bring about photomorphogenesis, the absorption of light by the phytochromes has to be coupled to the modulation of gene expression or other changes in cell physiology. Genetic and transgenic approaches are being used to elucidate the pathways of phytochrome signal transduction in *Arabidopsis*. Because of their importance in seedling de-etiolation, particularly the photoregulation of hypocotyl elongation, and the obvious phenotype associated with their absence, phytochrome A and phytochrome B have been the main focus of these studies.

In seedling de-etiolation, phytochromes A and B control a large number of common responses, including inhibition of hypocotyl growth, promotion of hypocotyl hook opening and cotyledon expansion, and the photoinduction of *CAB* gene expression. Of course, although the 'outputs' of phyA and phyB action may be common, these two photoreceptors display discrete photosensory specificities (see here; Quail 1997). Furthermore, phyA and phyB appear to have quite discrete regulatory functions in the adult plants. Nevertheless, in de-etiolating seedlings it seems likely that the phyA and phyB signalling pathways share some common components. This notion is supported by the finding that these phytochromes have a common spatial pattern of expression and so both will be present in the same cells. Circumstantial evidence from the analysis of transgenic plants that overexpress *PHY* gene sequences provides support for the idea of shared intermediates in phyA and phyB signalling. Wagner *et al.* (1996*a*) have done reciprocal domain swap experiments in which the chromophore-bearing N-terminal domain of phyA fused with the C-terminal domain of phyB (phyA–B), and the converse fusion (phyB–A), have been overexpressed in transgenic plants. Overexpression of either chimera leads to a light-hypersensitive phenotype. Analyses of the photoresponses of transformants to R and FR light showed that the phyA–B chimera mimicked the effects of phyA overexpression, whereas the phyB–A chimera mimicked the effects of phyB overexpression. This indicates that the photosensory specificities of phyA and phyB reside in the N-terminal domains (Wagner *et al.* 1996*a*). Furthermore, because the overexpression of either N-terminal domain alone does not cause light-hypersensitivity, it seems that the C-terminal domain provides at least some of the determinants necessary for the transmission of the light signal. The interchangeability of the C-terminal domains suggests that these determinants are common to phyA and phyB (Wagner *et al.* 1996*a*). Although the role of the C-terminal domain may be structural, it is possible that it may be involved in mediating an interaction with a component of the signalling pathway. The observation that overexpression of *PHYB* leads to a dominant negative interference with the action of endogenous phyA in the FR-HIR controlling the inhibition of hypocotyl elongation provides additional circumstantial evidence that phyA and phyB share a common reaction partner (Wagner *et al.* 1996*b*). To reconcile this proposal with the specificity of signal perception determined by the N-terminal domains of phyA and phyB, a two-point contact model for phytochrome action has been proposed (Quail 1997; Wagner *et al.* 1997). In this model, the N-terminal domains of phyA and phyB carry determinants that allow each to recognize its own, discrete cognate reaction partner. The common, C-terminal determinants, required for regulatory activity, are then proposed to mediate an identical modification of the reaction partners, for example, a phosphorylation of a key residue. In this model, the subsequent events in the signalling pathway could converge immediately or remain separate until the then 'end points' (see Quail 1997). This latter idea is supported by the genetic analysis of phyA and phyB signal transduction (see this paper).

It has been suggested that phyA and phyB are differentially localized within the cell (Sakamoto & Nagatani 1996) and so may have discrete primary actions. Immunochemical methods have previously established that phyA is a soluble, cytoplasmic protein. Sakamoto & Nagatani (1996) present evidence suggesting that *Arabidopsis* phyB is a nuclear protein. Following the expression of translational fusions between the non-chromophorylated C-terminus of PHYB and GUS in transgenic *Arabidopsis* plants, GUS activity was observed to be localized to the nucleus in both cotyledon protoplasts and epidermal peels. In addition, PHYB protein was detected immunochemically in preparations of isolated nuclei and anti-PHYB monoclonal antibodies were found to stain nuclei in immunocytochemical tests (Sakamoto & Nagatani 1996). The derived amino-acid sequence of PHYB (and other apophytochromes), is known to contain sequences with some homology to nuclear localization signals. However, these proposals are not very readily reconciled with observations on the activities of chimeric phytochromes and with the dominant negative effects of transgenic phyB on endogenous phyA activity (Wagner *et al.* 1996*a*,*b*).

Genetic approaches to the dissection phytochrome signal transduction pathways in *Arabidopsis* have tended to focus on the identification of mutants that show gross defects in de-etiolation. Almost all mutants isolated on the basis of an insensitivity to light, for example, long hypocotyl mutants, have defined genes encoding the apoproteins of photoreceptors or proteins necessary for photoreceptor chromophore synthesis. However, there are a small number of exceptions, and the genes defined by these mutants are thought to identify positive regulators of phytochrome signalling.

The analysis of putative signal transduction mutants tends to indicate that the phyA and phyB signalling pathways are discrete. Mutants selectively impaired in de-etiolation responses to prolonged FR light have been described (Whitelam *et al.* 1993). These mutants define components specific to the phyA signalling pathway. The *fhy1* and *fhy3* mutants resemble *phyA* mutants in their

insensitivity to prolonged FR light and a normal sensitivity to prolonged R light. However, neither of the mutations is linked to the *PHYA* gene and both mutants have normal levels of immunochemically and spectrally detectable phyA (Whitelam *et al.* 1993). Additional physiological and molecular analyses of the *fhy1* mutant have revealed that it is defective in a subset of phyA-mediated responses, suggesting that the phyA signalling pathway may be branched (Johnson *et al.* 1994; Barnes *et al.* 1996). Thus, although the inhibition of hypocotyl elongation under FR irradiation is impaired in *fhy1*, the mutant displays normal germination responses to FR light (Johnson *et al.* 1994). Similarly, whereas the FR light-mediated induction of *CHS* gene expression is deficient in *fhy1*, the induction of *CAB* and *NR* gene expression is relatively unaffected by the mutation (Barnes *et al.* 1996). The induction of all of these genes by FR light is deficient in *phyA* seedlings. The *CHS* and *CAB* genes had previously been proposed to define distinct branches of the phyA signalling pathway based on biochemical analyses done in microinjected tomato hypocotyl cells (see Barnes *et al.* 1997).

Several mutants that show a selective defect in de-etiolation responses to R light, but which are unlinked to *PHYB*, have been isolated. For example, the *pef2* and *pef3* mutants, which were selected on the basis of their early flowering, display a long hypocotyl phenotype following growth under R light, but a normal inhibition of hypocotyl elongation in response to prolonged FR light (Ahmad & Cashmore 1996). As *phyB* mutants are characterized by a long hypocotyl phenotype selectively under R light and by early flowering, it is possible that *PEF2* and *PEF3* may encode proteins that act early in the phyB signalling pathway. The *red1* mutant also displays a selective defect in seedling responses to R light (Wagner *et al.* 1997). This mutant was selected from the M_2 population derived following mutagenesis of a transgenic *Arabidopsis* line that overexpresses *PHYB* and so is hypersensitive to R light. The *red1* mutation segregates independently of the *PHYB* transgene and the mutant phenotype is displayed in non-transgenic background. In addition to their long hypocotyls, *red1* mutants show attenuated responses to EOD FR light treatments (Wagner *et al.* 1997). This suggests that *RED1* encodes a protein that functions as an intermediate in a phyB signalling pathway that regulates elongation growth. Significantly, Wagner *et al.* (1997) have reported that *red1* seedlings do not show early flowering, suggesting that elongation growth regulation and flowering time represent separate branches of phyB signalling.

The chlorate-resistant mutant, *cr88*, also displays a long hypocotyl phenotype selectively under R light (Lin & Cheng 1997). Chlorate-resistance results from a defect in the light-dependent induction of expression of *NR2*, one of the two *Arabidopsis* genes that encode nitrate reductase. Light-mediated induction of expression of the *CAB* and *RBCS* genes is also impaired in *cr88*, although the inductions of *NR1* and *NiR* expression are normal. These observations suggest that *CR88* may encode a signalling component involved in a subset of phyB-mediated responses.

There are at least two putative phytochrome signalling mutants that display a long hypocotyl phenotype under both R and FR light, and as such possibly define common components of the phyA and phyB signalling pathways. The *hy5* mutant displays a light-insensitive phenotype characterized by a long hypocotyl for seedlings grown under R, FR, blue and UV-A light conditions, but not UV-B conditions (Koornneef *et al.* 1980). This suggests that the *HY5* gene product is required as a positive regulator in the transduction of light signals perceived by phyA, phyB and one or more of the cryptochromes. The *HY5* gene has recently been cloned and shown to encode a transcription factor of the b-zip class (see Barnes *et al.* 1997). The *pef1* mutant also displays an elongated hypocotyl following growth under either R or FR light (Ahmad & Cashmore 1996).

A large number of mutants that display photomorphogenic development in the absence of light, and so may define suppressers of photomorphogenesis, have been identified. These include the recessive *det–cop–fus* mutants (see von Arnim & Deng 1996). Severe alleles of ten of the *det–cop–fus* mutants lead to extremely pleiotropic photomorphogenic phenotypes and seedling lethality. This suggests that the products of these *DET–COP–FUS* genes are involved in essential cellular processes both in the light and the dark and not simply in repressing photomorphogenesis in the dark (see Castle & Meinke 1994; Mayer *et al.* 1996). The *DET1*, *COP1*, *COP9* and *FUS6* genes have been cloned and shown to encode novel nuclear-localized proteins (Deng *et al.* 1992; Pepper *et al.* 1994; Wei *et al.* 1994; Castle & Meinke 1994). The COP1 polypeptide has some homology with several regulatory proteins involved in the repression of transcription (see von Arnim & Deng 1996). Light has been reported to influence the subcellular location of the COP1 protein (von Arnim & Deng 1994). In etiolated tissues, COP1 appears to be predominantly nuclear, and light treatment is reported to cause a shift to a predominantly cytoplasmic localization. Furthermore, the nuclear localization of COP1 in the dark is reported to be dependent on its interaction with a nuclear complex of other COP–FUS proteins, the COP9 complex (Chamovitz & Deng 1996). It is proposed that COP1 interacts with the COP9 complex in the dark to repress photomorphogenesis and that light causes the dissociation of COP1 from the complex, followed by its export from the nucleus, so relieving the repression (Chamovitz & Deng 1996).

It has been argued that the *DET–COP–FUS* gene products may be general repressors of gene expression and development, rather than playing a specific role in photomorphogenesis. That severe alleles of several of the mutants are lethal argues that the products of the wild-type genes are necessary for general development in both the light and the dark (Castle & Meinke 1994). Furthermore, weak alleles of *cop1*, *det1* and *cop9* lead to inappropriate expression of several sets of genes, not only those that are normally light-regulated (Mayer *et al.* 1996).

5. CONCLUSIONS

Our understanding of the perception and transduction of regulatory light signals has advanced significantly in recent years, principally through the application of genetic and transgenic methods. *Arabidopsis* mutants that

define four of the five phytochrome structural genes have been identified and are being characterized. Although phytochromes A and B play the predominant role in seed germination and seedling de-etiolation, they are involved in the perception of quite discrete light signals. In the established plant, phyA is involved in the detection of daylength, whereas phytochromes B, D and E all play a role in the perception of R:FR ratio signals.

Several of the components that act downstream of the phytochromes are also being identified. Several mutants, such as *fhy1–fhy3*, *pef2–pef3* and *red1* define signalling components that are specific to the actions of particular phytochromes. The cloning of the genes defined by these mutations will be crucial in dissecting the early steps of light signalling. To date, there have been few genetic studies of the transduction of R:FR ratio signals. This is not surprising, given the limitations of doing genetic screens with adult plants, compared with the relative ease of screens involving seedling de-etiolation. However, R:FR ratio signal perception is a crucial function of the phytochromes and at least three members of the family are involved. A main challenge for the future will be to extend genetic analysis of phytochrome action to include the shade avoidance responses.

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