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Photoresponses of Light-Grown *phyA* Mutants of *Arabidopsis*[1]

Phytochrome A Is Required for the Perception of Daylength Extensions

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Several aspects of the photophysiology of wild-type *Arabidopsis thaliana* seedlings were compared with those of a phytochrome A null mutant, *phyA-1*, and a mutant, *fhy1*, that is putatively involved in the transduction of light signals from phytochrome A. Although *phyA* seedlings display a near wild-type phenotype when grown in white light (W), they nevertheless display several photomorphogenic abnormalities. Thus, whereas the germination of wild-type and *fhy1* seeds is almost fully promoted by a pulse of red light (R) or by continuous far-red light (FR), *phyA* seed germination is responsive only to R. Following growth under day/night cycles, but not under continuous W, the hypocotyls of light-grown *phyA* and *fhy1* seedlings are more elongated than those of wild-type seedlings. For seedlings grown under low red/far-red (R/FR) ratio light conditions, *phyA* and *fhy1* seedlings display a more marked promotion of hypocotyl elongation than wild-type seedlings. Similarly, seedlings that are doubly null for phytochrome A and phytochrome B (*phyA phyB*) also have more elongated hypocotyls under low R/FR ratio conditions than *phyB* seedlings. This indicates that phytochrome A action in light-grown seedlings is antagonistic to the action of phytochrome B. Although wild-type, *fhy1*, and *phyA* seedlings flower at essentially the same time under both short-day and long-day conditions, an obvious consequence of phytochrome A deficiency is a pronounced late flowering under conditions where a short day of 8 h of fluorescent W is extended by 8 h of low-fluence-rate incandescent light. The evidence thus indicates that phytochrome A plays a role in seed germination, in the control of elongation growth of light-grown seedlings, and in the perception of daylength.

Phytochrome is the best characterized of the photoreceptors that regulate plant photomorphogenesis. Plants possess multiple, discrete molecular species of phytochrome, the apoproteins of which are encoded by a small family of divergent genes (Quail, 1991; Furuya, 1993). In *Arabidopsis thaliana*, five distinct apophytochrome-encoding genes have been identified, and full-length cDNAs representing three of these, *PHYA*, *PHYB*, and *PHYC*, have been cloned and sequenced (Sharrock and Quail, 1989). The apoprotein moiety of the well-characterized, light-labile phytochrome species that predominates in etiolated plant tissues is encoded by the *PHYA* gene (Quail, 1991; Furuya, 1993). This light-labile phytochrome is referred to as phytochrome A. The *PHYB* and *PHYC* genes encode the apoproteins of phytochromes B and C, which are low-abundance, light-stable species of the photoreceptor (Quail, 1991; Somers et al., 1991). That members of the phytochrome family display a marked diversity among their amino acid sequences as well as being differentially expressed is consistent with the suggestion that each phytochrome may play a discrete role in photomorphogenesis (Smith and Whitelam, 1990).

The characterization of mutants that carry lesions in individual *PHY* genes or that exhibit specific loss of function of a single phytochrome species is perhaps the best approach to assigning functions to individual phytochromes (Kendrick and Nagatani, 1991; Reed et al., 1992). The long hypocotyl mutant *hy3* of *Arabidopsis* is selectively deficient in immunochemically detectable phytochrome B (Nagatani et al., 1991; Somers et al., 1991) and sequence analysis has established that *hy3* alleles carry mutations within the *PHYB* structural gene (Reed et al., 1993). Therefore, it has been proposed that *hy3* be referred to as *phyB*. The phenotype of *hy3* (*phyB*) mutants is remarkably similar to that of the phytochrome B-deficient long hypocotyl, *lh*, mutant of cucumber and the elongated internode, *ein*, mutant of *Brassica rapa* (Devlin et al., 1992; Lopez-Juez et al., 1992). Compared with their respective isogenic wild types, all have elongated hypocotyls when grown in W or R but display near normal inhibition of hypocotyl elongation when etiolated seedlings are exposed to FR (Koornneef et al., 1980; Adamse et al., 1987; Devlin et al., 1992). In addition to elongated hypocotyls, light-grown seedlings of all three mutants display additional signs of impaired de-etiolation. Thus, the cotyledons of all three mutants display reduced expansion, and the *hy3* and *ein* mutants show reduced pigmentation (Adamse et al., 1987; Chory, 1992; Devlin et al., 1992). These observations

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Abbreviations: EOD FR, end-of-day far-red light; FR, far-red light; HIR, high-irradiance response; R, red light; R/FR ratio, the photon fluence rate ratio of red to far-red light in 10-nm bandwidths centered on 660 nm and 730 nm; W, white light.

indicate that phytochrome B plays a role in at least some aspects of de-etiolation.

Light-grown seedlings of the phytochrome B-deficient mutants display a number of common abnormalities in their photomorphogenic behavior. Thus, all of the mutants show increased stem and petiole elongation and increased apical dominance. Also, in contrast to their respective wild types, none of the mutants show growth promotions by EOD FR treatments (Lopez-Juez et al., 1990; Nagatani et al., 1991; Devlin et al., 1992), and all three mutants respond poorly to reductions in R/FR ratio (Whitelam and Smith, 1991; Devlin et al., 1992). This, and the similarity between the phenotypes of phytochrome-deficient mutants and their wild types following growth in low R/FR ratio conditions, has led to the suggestion that phytochrome B plays a major role in the shade-avoidance syndrome (Whitelam and Smith, 1991).

Phytochrome A is light labile and is relatively abundant in etiolated tissues, but is depleted in light-treated tissues (Quail, 1991; Furuya, 1993). This species of phytochrome, which can be readily isolated from etiolated seedlings, has been the subject of intense physico-chemical analysis (Whitelam, 1993). The relative abundance of phytochrome A in etiolated tissues is consistent with earlier suggestions that this phytochrome species plays a major role in the de-etiolation and greening processes (Smith and Whitelam, 1990; Kendrick and Nagatani, 1991). From physiological studies, a clearly predictable role for light-labile phytochrome A is the mediation of the FR-HIR (Smith and Whitelam, 1990). This response mode is displayed in a normal manner in phytochrome B-deficient mutants (Koornneef et al., 1980; Adamse et al., 1987; Devlin et al., 1992). *Arabidopsis* mutants that selectively lack the FR-HIR for the inhibition of hypocotyl elongation have recently been isolated by several laboratories (*fre1*, Nagatani et al., 1993; *hy8*, Parks and Quail, 1993; *fhy2*, Whitelam et al., 1993). Seedlings of these mutants are not elongated in R or W and have been shown to lack spectrophotometrically detectable phytochrome and to be deficient in immunochemically detectable phytochrome A polypeptide and *PHYA* transcripts (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). We have shown that the *fhy2-1* mutation is allelic with mutations at *hy8* and *fre1* (G.C. Whitelam and N.P. Harberd, unpublished data), and that *fhy2-1* mutants contain a structural rearrangement of the *PHYA* gene. Thus, these mutant alleles are now referred to as *phyA* alleles (for example, *fhy2-1* is now *phyA-1*; Whitelam et al., 1993). Although *Arabidopsis* seedlings that are null for phytochrome A display a very slight reduction in Chl synthesis (Nagatani et al., 1993), they do de-etiolate and display a near wild-type phenotype when grown in W (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993).

In the mutant screen that identified *phyA* mutants, mutations at two other loci, *fhy1* and *fhy3*, that also confer an elongated hypocotyl selectively in FR were identified (Whitelam et al., 1993). Plants that are homozygous for either of these mutations have normal levels of spectrally active phytochrome A, so the *FHY1* and *FHY3* gene products may be responsible for transducing the FR signal from phytochrome A. Both of these mutants display a wild-type phenotype when grown in W (Whitelam et al., 1993). This, and the wild-type phenotype of W-grown *phyA* mutants, suggests that phytochrome A may play a rather specialized role in the photomorphogenesis of *Arabidopsis*.

The most obvious phenotypic characteristic of *phyA* mutations is loss of the FR-HIR. This phytochrome response mode is generally considered to be transiently displayed by etiolated seedlings, with sensitivity to prolonged FR being lost upon de-etiolation, because phytochrome A levels are depleted (e.g. Beggs et al., 1980; Whitelam et al., 1992). It is known that transgenic seedlings that express introduced *PHYA* cDNAs under control of the 35S cauliflower mosaic virus promoter retain characteristics of the FR-HIR even following prolonged de-etiolation, further establishing the link between phytochrome A and the FR-HIR (McCormac et al., 1992b; Whitelam et al., 1992). Despite the fact that the FR-HIR is usually considered to be restricted in its display to etiolated seedlings, it has frequently been proposed that a FR-HIR operates in fully de-etiolated, light-grown plants. Thus, action spectra for inductive, long night-breaks in LDPs commonly show maxima near 710 to 720 nm (see Vince-Prue, 1986; Carr-Smith et al., 1989), which is characteristic of the HIR of etiolated seedlings. Therefore, it has been suggested that light-labile phytochrome A may be functioning in the perception of these day extensions in light-grown plants (Carr-Smith et al., 1989; Thomas, 1991).

Here we have investigated several aspects of the photophysiology of *phyA* mutants of *Arabidopsis*. Under a range of growth conditions, light-grown *phyA* mutants are elongated compared with wild-type seedlings, and seeds display a reduced induction germination by FR. Most strikingly, whereas the flowering behavior of *phyA* mutants under 8-h SD conditions is indistinguishable from that of wild-type seedlings, the phytochrome A-deficient mutants display a defective response to an inductive, low-fluence-rate incandescent daylength extension. Therefore, under these LD conditions *phyA* mutants are markedly late flowering.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Wild-type *Arabidopsis thaliana* (L.) Heynh. seeds were of the Landsberg *erecta* ecotype, and the *phyA* (alleles *phyA-1* and *phyA-2*, formerly *fhy2-1* and *fhy2-2*) and *fhy1* mutants were those described previously (Whitelam et al., 1993). Except in the construction of *phyA phyB* double mutants, the *phyA-1* allele was used throughout. The *phyA phyB* double mutant was constructed as follows. Plants homozygous for *phyA-2* were crossed with plants homozygous for *phyB* (allele *hy3*-Bo64) and the heterozygous F_1 progeny (*PHYA/phyA;PHYB/phyB*) were allowed to self-pollinate. Since *PHYA* and *PHYB* are located on different chromosomes, these loci segregate independently of one another. F_2 plants displaying an elongated hypocotyl in W (*phyB/phyB* homozygote) were selected and allowed to self-pollinate. From the progeny of these plants, F_3 seedlings displaying an elongated hypocotyl in FR were identified and used to establish a new line that was doubly homozygous for the *phyA* and *phyB* mutations (*phyA phyB*).

For experiments involving the photoregulation of germination, seeds were sown on 0.6% (w/v) agarose in BG11

mineral salts (Stanier et al., 1971). Seeds were incubated in the dark for 2 d before light treatments.

For experiments involving measurement of hypocotyl lengths of light-grown seedlings, seeds were sown on moistened compost:sand (3:1) in 10 × 1 × 1.5 cm high plexiglass troughs and chilled for 3 d at 4°C in darkness. Seedlings were grown under continuous W for 2 d before treatment. For EOD FR treatments, de-etiolated seedlings were transferred to 8-h light/16-h dark cycles, with or without 15 min of FR irradiation at the end of the light period. Treatment continued for 4 d. For R/FR ratio experiments, seedlings were transferred to continuous high or low R/FR ratio growth conditions for 5 d. For experiments to investigate hypocotyl elongation of the *phyA phyB* double mutant under low R/FR ratio, seeds were sown on 0.6% (w/v) agarose in BG11 mineral salts, chilled for 3 d at 4°C in darkness, then germinated for 4 d under continuous W before being transferred to continuous low R/FR ratio growth conditions for 5 d.

For experiments involving daylength perception, seeds were sown on moistened compost:sand in Petri dishes and chilled for 3 d at 4°C in darkness. Seeds were germinated and grown for 7 d in SD conditions (8 h of W, 16 h of dark). Uniform seedlings were selected and transferred to 5 × 5 cm pots and grown for a further 7 d in SD conditions before treatment under SD, extended SD, or LD conditions.

Light Sources

Broad-band R at a photon fluence rate (600–700 nm) of 3 μmol m^{-2} s^{-1} was obtained by filtering the output from Thorn EMI (Birmingham, UK) Deluxe natural 40-W fluorescent tubes through a 1-cm-deep layer of copper sulfate solution (1.5%, w/v) and one layer of red (No. 14) cinemoid (Rank Strand, Isleworth Middlesex, UK). Broad-band FR at a photon fluence rate (700–800 nm) of 33 μmol m^{-2} s^{-1} was obtained by filtering the output from water-cooled 100-W incandescent bulbs through black plexiglass (type FRF 700, West Lake Plastics, Lemmi Mills, PA).

W for seedling de-etiolation and for use in the EOD FR experiments was provided by a bank of cool-white fluorescent tubes at a photon fluence rate (400–700 nm) of 127 μmol m^{-2} s^{-1}. The R/FR ratio treatment cabinets were the same as those described in detail by Keiller and Smith (1989). The high R/FR ratio cabinet (cool-white fluorescent light) provided a photon fluence rate (400–700 nm) of 87 μmol m^{-2} s^{-1} and a R/FR ratio of 5.82. The low R/FR cabinet (cool-white fluorescent light supplemented with FR) provided the same photon fluence rate (400–700 nm) but a R/FR ratio of 0.12.

SD, extended SD, and LD growth conditions were provided by Fitotron 600 growth cabinets (Fisons Scientific Apparatus, Loughborough, UK). SD conditions comprised 8 h of cool-white fluorescent light (photon fluence rate 400–700 nm, 181 μmol m^{-2} s^{-1}) followed by 16 h of darkness. Extended SD conditions comprised 8 h of cool-white fluorescent light (photon fluence rate 400–700 nm, 200 μmol m^{-2} s^{-1}) followed by 8 h of low-fluence rate incandescent light (photon fluence rate 400–700 nm, 2 μmol m^{-2} s^{-1}, provided by Thorn EMI 15-W tungsten bulbs) followed by 8 h of darkness. LD conditions comprised 16 h of cool-white fluorescent light (photon fluence rate 400–700 nm, 90 μmol m^{-2} s^{-1}) followed by 8 h of darkness. For all light treatments the temperature was maintained at 18 to 20°C throughout. All light measurements were made using a LI 1800/12 spectroradiometer (Li-Cor, Lincoln, NE).

Measurements of Germination, Hypocotyl Lengths, and Flowering

Germination was assessed as radicle emergence and was scored 4 d after the onset of light treatments. Data were derived from six independent replicates, each having at least 25 seeds, for each treatment.

Hypocotyl lengths of seedlings from EOD FR and R/FR ratio experiments were measured from projections of calibrated photographic transparencies. Mean values were calculated from at least 25 seedlings. Error bars in the figures represent the SE.

Flowering time was determined as the number of days from sowing to bolting. Seedlings were considered to have bolted following appearance of the first leaf on the cauline stem. Mean number of days to bolting, and leaf numbers at bolting, were calculated from at least 10 plants in each treatment.

RESULTS AND DISCUSSION

Photocontrol of Seed Germination

Batches of freshly harvested *Arabidopsis* seeds incubated in the dark displayed a wide variation in germination levels, ranging from a few percent to over 70% (data not shown). By selecting batches of wild-type, *phyA*, and *fhy1* mutant seeds that show very low dark germination, we studied the effects of R and FR treatments on germination. In these experiments seeds were allowed to imbibe for 2 d in the dark at 20°C prior to light treatments, since this enhances the sensitivity of *Arabidopsis* seeds to light (Cone, 1985). The germination of wild-type, *phyA*, and *fhy1* seeds was increased to nearly 100% by a 15-min pulse of low-fluence-rate R (Fig. 1). In the case of wild-type and *fhy1* seeds, a 15-min pulse of FR led to a significant increase in germination compared with seeds maintained in the dark (Fig. 1). Increasing the duration of FR exposure to 1 h led to increased germination in wild-type and *fhy1* seeds, whereas continuous FR exposure led to 100% germination in both seed types (Fig. 1). In contrast, seeds of the *phyA* mutant displayed very poor germination in response to both brief and prolonged FR exposure (Fig. 1). The germination of *phyA* seeds exposed to FR was similar to that of seeds maintained in the dark. The very high germination of *phyA* seeds in response to a R pulse indicated that the low germination observed in response to FR was not a consequence of low viability of this seed batch.

These observations suggest the operation of two separate light responses controlling *Arabidopsis* seed germination. The first is the R-mediated induction of germination, which, because it is unaffected by the *phyA* mutation, is mediated by a phytochrome species other than phytochrome A. Since seeds of the *phyB* mutant are poorly responsive to R pulses (Cone, 1985), it seems likely that phytochrome B is, at least partly, involved in this first response. The second light re-

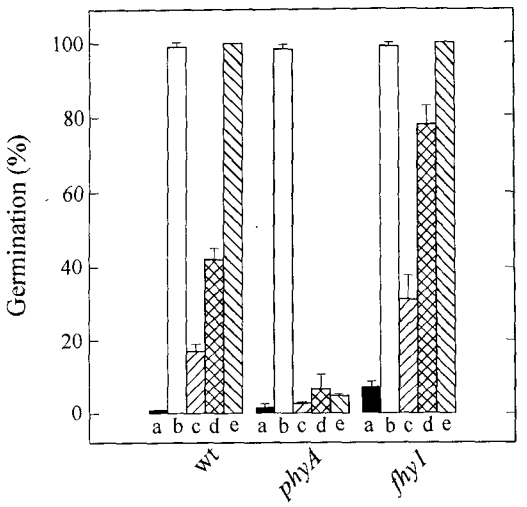

Figure 1. Light regulation of germination of wild-type (wt), *phyA*, and *fhy1* seeds. Seeds were allowed to imbibe for 2 d in the dark prior to exposure to darkness (a); 15 min of R (b); 15 min of FR (c); 60 min of FR (d); or continuous FR (e). Germination was assessed as radicle emergence 4 d after the onset of light treatments.

sponse, observed as a promotion of germination by increasing exposure to FR, is absent in *phyA* seeds, indicating that phytochrome A is the responsible photoreceptor.

Seeds of the *fhy1* mutant displayed somewhat higher germination than wild-type seeds in response to FR pulses, as well as in the dark. Thus, for this particular photoresponse the *FHY1* gene product appeared to be unnecessary for full display of phytochrome A action.

Elongation Growth Responses of Light-Grown Seedlings to EOD FR

Light-grown seedlings of wild-type plants showed a significant promotion of hypocotyl elongation in response to EOD FR treatments (Fig. 2). After 5 d of treatment, wild-type seedlings that had been irradiated with 15 min of FR at the end of 8-h photoperiods were significantly longer than those of control seedlings that did not receive the EOD FR. Light-grown *phyA* mutant seedlings, grown for 5 d in 8-h photoperiods, had longer hypocotyls than comparable wild-type seedlings, irrespective of whether EOD FR treatments were given (Fig. 2). Under control conditions (no EOD FR) the hypocotyls of *phyA* seedlings were about 30% longer than those of comparable wild-type seedlings, and under EOD FR conditions they were about 25% longer. Thus, *phyA* seedlings, although elongated compared with wild-type seedlings, displayed a more or less normal elongation response to EOD FR treatments. The retention of the EOD FR response by *phyA* mutant seedlings has been reported previously (Nagatani et al., 1993; Parks and Quail, 1993) and is fully consistent with the attribution of this response to the action of light-stable phytochrome B (Nagatani et al., 1991; Somers et al., 1991; Reed et al., 1992, 1993).

The elongated hypocotyl of light-grown *phyA* seedlings, compared with wild-type seedlings, under both control and EOD FR conditions has not been noted previously, although the data of Parks and Quail (1993) and Nagatani et al. (1993) do appear to show a similar effect. The longer hypocotyls of the phytochrome A-deficient mutants suggest that in light-grown, wild-type seedlings phytochrome A action leads to an inhibition of elongation growth. This proposed growth-inhibitory action of phytochrome A in wild-type seedlings is apparent only for seedlings that have been grown in light/dark cycles; the hypocotyls of wild-type and *phyA* seedlings grown under continuous W are indistinguishable (Whitelam et al., 1993; Fig. 3, a and b). The apparent requirement for periods of darkness to detect an inhibitory action of phytochrome A in wild-type seedlings may point to there being a reaccumulation of light-labile phytochrome A in the dark that is active during the next photoperiod. The levels of immunochemically detectable phytochrome A in light-grown *Arabidopsis* seedlings are at the limits of detection, so we did not detect any such oscillation in phytochrome A levels. Oscillations in the levels of an immunochemically detectable, low-abundance, type II phytochrome have been reported for oat seedlings grown in light/dark cycles, with the magnitude of phytochrome reaccumulation in the dark being increased by an EOD FR treatment (Stewart et al., 1992).

The hypocotyls of the *fhy1* mutant were also more elongated than those of wild-type seedlings following growth in light/dark cycles, with or without an EOD FR treatment (Fig. 2). As is the case for *phyA* seedlings, the long-hypocotyl phenotype of light-grown *fhy1* seedlings was dependent on the dark periods because under continuous fluorescent W, *fhy1* hypocotyls are the same length as those of wild-type seedlings (Whitelam et al., 1993; Fig. 3). This similarity between *phyA* and *fhy1* mutant seedlings supports the view that the *FHY1* gene product is responsible for the transduction of W signals from phytochrome A that lead to alterations in elongation growth.

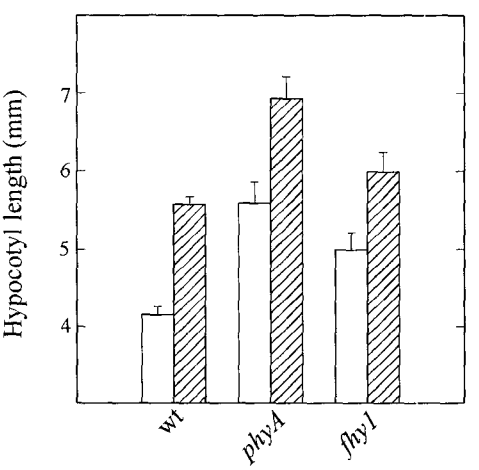

Figure 2. Effect of EOD FR on hypocotyl elongation in light-grown wild-type, *phyA*, and *fhy1* seedlings. Seedlings were grown in continuous W for 2 d prior to growth for 5 d under 8-h light/16-h dark cycles, without (open bars) or with (hatched bars) a 15-min EOD FR treatment.

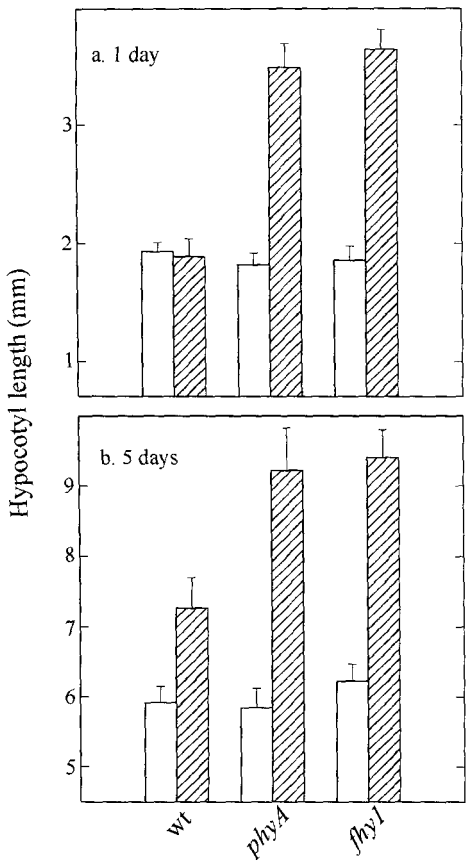

Figure 3. Hypocotyl lengths of light-grown wild-type, *phyA*, and *fhy1* seedlings exposed to high (open bars) or low (hatched bars) R/FR ratio conditions. Seedlings were grown in continuous W for 2 d prior to growth for 1 d (a) or 5 d (b) under W (R/FR ratio = 5.82) or W + FR (R/FR ratio = 0.12).

Elongation Growth Responses to R/FR Ratio

When wild-type seedlings that were grown for 2 d in continuous fluorescent W (high R/FR ratio) were transferred to low R/FR ratio conditions for 5 d they showed a marked promotion of hypocotyl elongation (Fig. 3b). This increase in hypocotyl elongation was not detectable after 1 d of treatment (Fig. 3a), but by the 5th d of treatment the hypocotyls of low R/FR ratio-treated seedlings were about 25% longer than those of seedlings maintained in high R/FR ratio conditions. Whereas the hypocotyls of *phyA* seedlings maintained under continuous high R/FR ratio conditions were essentially the same length as those of comparable wild-type seedlings, the hypocotyls of low R/FR ratio-treated *phyA* seedlings showed an enhanced elongation response (Fig. 3, a and b). Thus, even after only 1 d of low R/FR ratio treatment the hypocotyls of *phyA* seedlings were significantly elongated compared with the hypocotyls of *phyA* seedlings maintained under high R/FR ratio conditions.

The more elongated hypocotyls of the *phyA* mutants under continuous, low R/FR ratio irradiation implies that under these conditions phytochrome A action in wild-type seedlings leads to an inhibition of hypocotyl elongation. This phytochrome A-mediated inhibition of hypocotyl elongation is not normally observed in de-etiolated wild-type seedlings because of a second phytochrome response that leads to increased hypocotyl elongation. This second response is the shade-avoidance response to low R/FR ratio, and is assumed to be largely mediated by phytochrome B (Whitelam and Smith, 1991; Robson et al., 1993). The suggestion that the actions of phytochrome A and phytochrome B, in relation to low R/FR ratio conditions, lead to opposing effects on elongation growth is supported by analysis of the effect of low R/FR ratio on hypocotyl elongation in *hy3* (=*phyB*) mutants and in *phyA phyB* double mutants (Table I). Thus, the hypocotyls of the *phyA phyB* double mutant are significantly longer than those of the already elongated monogenic *phyB* mutant. This indicates that the action of phytochrome A in the monogenic *phyB* mutant reduces the long hypocotyl phenotype that is caused by the phytochrome B deficiency.

That wild-type seedlings required more than 1 d of exposure to low R/FR ratio conditions before an increase in hypocotyl elongation was observed (Fig. 3, a and b) suggests that the magnitude of the phytochrome A-mediated growth inhibition decreases with time. It has been observed that, when exposed to low R/FR ratio conditions, etiolated seedlings of several plant species display a transient inhibition of elongation growth, and that subsequent to a period of light adaptation, elongation growth becomes promoted by low R/FR ratio (Whitelam and Johnson, 1980; McCormac et al., 1992a). It has been proposed that these different responses to low R/FR ratio reflect the predominance of the actions of phytochrome A and phytochrome B (McCormac et al., 1992a).

The data presented here indicate that the phytochrome A-mediated inhibition of hypocotyl elongation under low R/FR ratio conditions persists for several days in light-grown *Arabidopsis* seedlings despite the light lability of this phytochrome species. However, this inhibition of elongation growth is not usually observed in wild-type seedlings after several days of light treatment because of the phytochrome B-mediated promotion of elongation. This idea is supported by the behavior of etiolated phytochrome B-deficient mutant seedlings in response to low R/FR ratio conditions. Thus, in the absence of the phytochrome B-mediated promotion of elongation growth, the phytochrome A-mediated inhibition of elongation would be expected to predominate and seedlings should therefore continue to display a net growth inhibition as they de-etiolate under low R/FR ratio conditions.

Table I. *Hypocotyl lengths of light-grown wild-type, phyA, phyB (=hy3), and phyA phyB seedlings exposed to low R/FR ratio conditions*

Seedlings were grown for 4 d under continuous W prior to exposure to W + FR for 5 d.

Genotype	Hypocotyl Length
	mm
Wild type	5.32 ± 0.16
phyA	6.71 ± 0.22
phyB	7.39 ± 0.39
phyA phyB	12.83 ± 0.38

This has been shown to be the case for the *phyB Arabidopsis* mutant (McCormac et al., 1992a), the *Brassica ein* mutant (P.F. Devlin and G.C. Whitelam, unpublished data), and the pea *lv* mutant, a putative phytochrome B signal transduction mutant (Weller and Reid, 1993). Transgenic seedlings that constitutively express high levels of *PHYA* cDNAs also display a net inhibition of elongation growth by low R/FR ratio that persists throughout their development (McCormac et al., 1992b; Whitelam et al., 1992).

As may be expected, de-etiolated *fhy1* seedlings respond to low R/FR ratio conditions in the same manner as *phyA* seedlings; they too show an exaggerated shade-avoidance response (Fig. 3, a and b).

Flowering Responses to R/FR Ratio

In common with other LDPs *Arabidopsis* seedlings show an acceleration of flowering in response to low R/FR ratio (e.g. Whitelam and Smith, 1991; Bagnall, 1992). The data in Table II show that wild-type seedlings exposed to continuous, low R/FR ratio irradiation flowered approximately 7 d earlier and with approximately three fewer leaves than comparable seedlings exposed to a high R/FR ratio. A virtually identical flowering response to low R/FR ratio was observed for the *phyA* seedlings (Table II). This indicates that this particular response to supplementary FR was not dependent on the action of phytochrome A. Furthermore, this response was not significantly modified by the absence of phytochrome A.

Photoperiod and Flowering

In addition to the perception of light quality, the other major function of phytochrome in light-grown plants is the perception of daylength. To study the role of phytochrome A in the LD flowering responses of *Arabidopsis*, wild-type, *phyA*, and *fhy1* seedlings were maintained under three photoperiods: (a) short days, 8 h of high-fluence-rate fluorescent W followed by 16 h of darkness; (b) extended short days, 8 h of high-fluence-rate fluorescent W followed by 8 h of low-fluence-rate incandescent light followed by 8 h of darkness; (c) long days, 16 h of high-fluence-rate fluorescent W followed by 8 h of darkness. The extended SD treatment achieved a LD exposure that did not alter the photosynthetic input relative to the SD treatment. The low-fluence-rate incandescent extension did not contribute photosynthetically (King and Evans, 1991) and was optimal with regard to phytochrome action (e.g. Downs and Thomas, 1982).

Under SD conditions, flowering time, measured either as the number of days to bolting or as the number of rosette leaves at bolting (see Koornneef et al., 1991), was very similar for wild-type, *phyA*, and *fhy1* seedlings (Fig. 4). Thus, for all genotypes flowering occurred at between 68 and 72 d from sowing, when the plants had between 23 and 29 leaves. For wild-type seedlings, flowering time was reduced by about 30 d under conditions where the short day was extended with low-fluence-rate incandescent light (Fig. 4). Under these conditions wild-type plants flowered with only about 10 leaves. In marked contrast, *phyA* mutant seedlings were only poorly responsive to incandescent daylength extensions (Fig. 4). Consequently, under these photoperiodic conditions, *phyA* seedlings flowered about 20 d later, and with nine leaves more, than wild-type seedlings. This late-flowering behavior was similar to that displayed by any of the previously characterized late-flowering mutants of the Landsberg *erecta* ecotype of *Arabidopsis* (Koornneef et al., 1991). For light-dominant plants such as *Arabidopsis*, common responses to light quality include action maxima near 710 to 720 nm for daylength extensions as well as a greater effectiveness of incan-

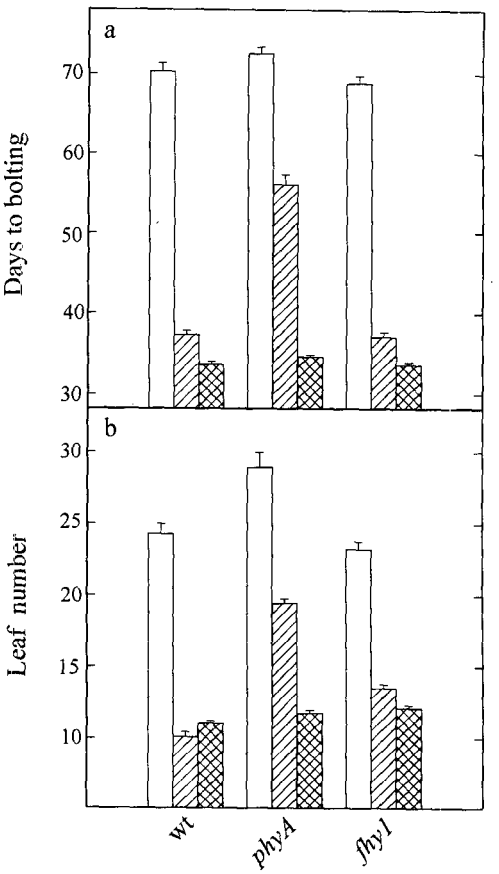

Figure 4. Effect of daylength on flowering time of wild-type, *phyA*, and *fhy1* seedlings. Seedlings were grown under SD conditions (8 h of fluorescent W/16 h of dark, open bars), extended SD conditions (8 h of fluorescent W/8 h of low-fluence-rate incandescent W/8 h of dark, hatched bars), or LD conditions (16 h of fluorescent W/8 h of dark, cross-hatched bars) until bolting. Flowering time was assessed as either days to bolting (a) or the number of rosette leaves at bolting (b).

Table II. *Effect of R/FR ratio on flowering time in wild-type and phyA seedlings*

Genotype	R/FR Ratio	Flowering Time	
		Days to bolting	Leaf number[a]
Wild type	5.82	27.3 ± 0.4	7.5 ± 0.2
	0.12	20.2 ± 0.2	4.6 ± 0.1
phyA-1	5.82	27.8 ± 0.4	8.3 ± 0.3
	0.12	20.1 ± 0.2	4.6 ± 0.1

[a] Number of rosette leaves at bolting.

descent light, rather than fluorescent light, for daylength extensions (Downs and Thomas, 1982; Vince-Prue, 1986; Thomas, 1991). The action maximum at about 710 to 720 nm, which resembles that of the HIR of etiolated seedlings, has been interpreted in terms of the action of light-labile phytochrome A (Thomas, 1991). The data presented here provide direct evidence that phytochrome A is necessary for the perception of low-fluence-rate incandescent daylength extensions. Clearly, light-labile phytochrome A is present and active in mature, light-grown plants.

The flowering of *phyA* seedlings showed a small promotion (about 15 d) in response to the incandescent daylength extension. It is believed that this is due, at least partially, to the reduction in R/FR ratio that occurs at the end of the extended day. Thus, seedlings grown under extended SD conditions displayed mild symptoms of increased elongation of petioles, characteristic of the EOD FR effect (Table III). It has previously been observed that EOD FR treatments promote flowering in wild-type *Arabidopsis* seedlings (Goto et al., 1991), and we observed the same effect for *phyA* seedlings (data not shown). The marked promotion of flowering by relatively short periods of broad-band FR given after a short day (see Goto et al., 1991) have precluded an investigation of the effects of prolonged broad-band FR daylength extensions.

Unlike *phyA* seedlings, *fhy1* seedlings were not particularly late flowering compared with wild-type seedlings under extended SD conditions (Fig. 4). Thus, *fhy1* seedlings bolted at the same time as wild-type seedlings, but had three more leaves at this time. This suggests that whereas the *FHY1* gene product appears to be required for the transduction of some light signals from phytochrome A, this does not appear to be the case for all phytochrome A-mediated responses.

For seedlings maintained under LD conditions (16 h of light, 8 h of dark), wild-type seedlings bolted at about 33 d, with 11 leaves (Fig. 4). This was very similar to their behavior under extended SD conditions. Under LD conditions *phyA* seedlings displayed only a very small late-flowering phenotype, as they did under continuous irradiation conditions (Table I). The almost wild-type photoperiodic responsiveness of *phyA* seedlings to 16-h, high-fluence-rate long days indicated that daylength perception per se was not dependent on the presence and action of phytochrome A. This implies that under low-fluence-rate incandescent daylength extension conditions, phytochrome A plays the major role in photoperiod perception, but that under high-fluence-rate, fluorescent LD conditions, phytochrome A plays only a very minor role.

Table III. *Effect of low-fluence-rate, incandescent day extensions on petiole lengths of wild-type, phyA, and fhy1 seedlings*

Genotype	Mean Leaf Length[a]	
	Short days	Extended short days
	cm	
Wild type	2.56 ± 0.11	5.23 ± 0.15
phyA-1	2.80 ± 0.10	6.08 ± 0.27
fhy1	2.96 ± 0.12	5.70 ± 0.20

[a] Lengths of the first pair of leaves (petiole + lamina) for each seedling were determined at 40 d from sowing.

Alternatively, it is possible that in wild-type plants phytochrome A is predominant in the perception of even high-fluence-rate, fluorescent W long days, but that in *phyA* seedlings other photoreceptors are able to compensate for the phytochrome A deficiency. Whichever is the case, it is clear that photoreceptors other than phytochrome A participate in photoperiod perception and that these other photoreceptors do not play a significant role in the perception of low-fluence-rate incandescent day extensions.

That more than one phytochrome species is involved in photoperiod perception has been proposed many times (e.g. Takimoto and Saji, 1984; Thomas, 1991). Similarly, it has been speculated that light may have both inhibitory and promotional actions in the flowering of LDPs (Thomas, 1991). The phytochrome B-deficient *hy3* mutant, which is early flowering, shows a substantial reduction in its flowering response to photoperiod (Goto et al., 1991). This has led to the suggestion that phytochrome B action has an inhibitory function in photoperiodic floral induction in LDPs (Goto et al., 1991; Weller and Reid, 1993). The data presented here indicate that, at least under some conditions, phytochrome A action normally promotes flowering in a LDP. This conclusion is supported by the observation that transgenic *Arabidopsis* seedlings that overexpress an oat *PHYA* cDNA display an early-flowering phenotype and a reduced response to photoperiod (D.J. Bagnall, G.C. Whitelam, R.W. King, M.T. Boylan, D. Wagner, P.H. Quail, unpublished data). Photoreceptors other than the phytochromes may also influence the photoperiodic flowering responses of *Arabidopsis* seedlings, and a role for a blue-light photoreceptor and for a photosynthetic component cannot be excluded (see King and Evans, 1991; Eskins, 1992).

CONCLUSIONS

Phytochrome A null mutants of *Arabidopsis* display numerous abnormalities in their photomorphogenic behavior. Many of these abnormalities are displayed in light-grown seedlings, indicating that although phytochrome A is light labile, the low levels of the photoreceptor that persist in light-grown plants play significant roles in the perception of light quality and photoperiod.

Phytochrome A in light-grown wild-type seedlings mediates responses to supplementary FR, such as the inhibition of hypocotyl elongation. This action of phytochrome A is "antagonistic" to the action of phytochrome B, which appears to mediate a promotion of elongation growth in response to supplementary FR irradiations. Phytochrome A is also active in mediating an inhibition of hypocotyl elongation under light/dark photoperiodic conditions.

Perhaps the most striking consequence of the absence of phytochrome A from *phyA* seedlings is their reduced ability to perceive inductive daylength extensions of low-fluence-rate incandescent light. This indicates that phytochrome A normally functions to promote flowering under this photoperiodic condition. However, *phyA* seedlings show a near wild-type flowering response to higher-fluence-rate long days. This indicates that other photoreceptors are capable of photoperiod perception.

The photoresponses of the *fhy1* mutant show that it is

affected in some but not all of the responses mediated by phytochrome A. Interestingly, the responses affected are those involved with hypocotyl growth regulation (Whitelam et al., 1993). Thus, phytochrome A may mediate its effects via a branched signal-transduction chain, such that one branch contains the *FHY1* gene product as a component and controls hypocotyl elongation. An additional branch (or branches), which does not contain the *FHY1* gene product component, would then mediate phytochrome A control of seed germination and photoperiod perception.

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