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# Phytochrome A Regulates Red-Light Induction of Phototropic Enhancement in Arabidopsis[1]

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**Phytochrome A (phyA) and phytochrome B photoreceptors have distinct roles in the regulation of plant growth and development. Studies using specific photomorphogenic mutants and transgenic plants overexpressing phytochrome have supported an evolving picture in which phyA and phytochrome B are responsive to continuous far-red and red light, respectively. Photomorphogenic mutants of *Arabidopsis thaliana* that had been selected for their inability to respond to continuous irradiance conditions were tested for their ability to carry out red-light-induced enhancement of phototropism, which is an inductive phytochrome response. We conclude that phyA is the primary photoreceptor regulating this response and provide evidence suggesting that a common regulatory domain in the phyA polypeptide functions for both high-irradiance and inductive phytochrome responses.**

The phytochromes are a related family of photoreceptors that have evolved to control several diverse aspects of plant growth and development during all phases of the life cycle. The basis for this control rests on their capacity to switch photoreversibly between inactive (Pr) and active (Pfr) forms of the molecule following the absorption of red or far-red light, respectively. This property underlies the photoreceptor's ability to monitor the intensity, quality, and duration of light in a given environment. Although the initial steps of biochemical action and signal transduction are still not known for any of the phytochromes, collected evidence from physiological, biophysical, biochemical, and genetic disciplines has resulted in the recognition of several basic characteristics of phytochromes and their function in plants.

An intriguing picture of the phytochromes has emerged supporting the contention that individual members of the phytochrome family monitor discrete elements of the light environment during plant development. Molecular evidence for a divergent family of expressed phytochrome genes (*PHYA, PHYB, PHYC, PHYD,* and *PHYE*) in Arabidopsis (Sharrock and Quail, 1989; Clack et al., 1994) and other higher plants (Pratt, 1995) provided verification of earlier biochemical and physiological results that had questioned the existence of a single phytochrome (Smith and Whitelam, 1990; Kendrick and Kronenberg, 1994). This discovery also provided support for the proposition that different phytochrome types within a single species may have unique roles. This appears to be the case for at least phyA and phyB. Physiological studies of defined photomorphogenic mutants and phytochrome-overexpressing transgenic plants have demonstrated that phyA is the primary or only photoreceptor responsible for controlling the FR-HIR, elicited by long exposure to far-red-enriched light (Dehesh et al., 1993; McCormac et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993; Reed et al., 1994; Whitelam and Harberd, 1994). In a similar fashion, it has been shown that phyB regulates the red-HIR, activated by long exposure to red-enriched light, as well as the shade-avoidance and end-of-day far-red responses (Somers et al., 1991; McCormac et al., 1992, 1993; Reed et al., 1993).

Recent studies of chimeric *A/B* phytochromes overexpressed in transgenic Arabidopsis have indicated that the photosensory specificities exhibited by phyA and phyB are governed by sequences in the $NH_2$-terminal domain of their respective polypeptides, whereas the COOH-terminal domains are functionally indistinguishable between phyA and phyB and contain sequences necessary for the regulatory capacity of the photoreceptors (D. Wagner, R. Kuhn, and P. Quail, unpublished results). These latter sequences have recently been explored in greater depth for phyA (Quail et al., 1995; Xu et al., 1995) and phyB (Wagner and Quail, 1995). Direct sequencing of mutant *phyA* and *phyB* sequences in photomorphogenic mutants that lack responsivity to continuous far-red or red light, respectively, have shown that the region between residues 624 and 777 (phyA coordinates) is critical for the regulatory function of these photoreceptors (Quail et al., 1995). This region is highly conserved between phyA and phyB, consistent with its being a regulatory domain in common between phyA and phyB.

It is well established that phytochrome is not limited to monitoring the light environment under the high-irradi-

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Abbreviations: FR-HIR, far-red high-irradiance response; HIR, high-irradiance response; phyA, phyB, phytochrome A and B, respectively; *phyA, phyB*, mutations in the *PHYA* and *PHYB* genes.

ance conditions described above. One or more of these photoreceptors also regulates inductive, photoreversible responses to brief exposures of low-intensity illumination, where a response can be triggered by a red-light pulse and abrogated by a subsequent far-red pulse. Examples of inductive responses include seed germination, leaf movements, enhancement of phototropism, potentiation of gravitropic sensitivity, and growth inhibition (Kendrick and Kronenberg, 1994). Although both the HIR and inductive responses are regulated by phytochrome, it has been difficult to reconcile differences between these two classes of responses with a single molecular function of the photoreceptor. In an effort to understand the roles of phyA and phyB in a response effected under inductive conditions, we have examined phototropic enhancement in previously described phytochrome mutants of Arabidopsis. Specific phyA- and phyB-null mutants were used to determine which, if either, of these photoreceptors regulates this inductive response. Missense mutants possessing normal photosensory capacity but aberrant regulatory activity for the control of the FR-HIR were also used to test whether the domains necessary for HIR activity are also important for transduction of inductive stimuli.

## MATERIALS AND METHODS

### Plant Material

PhyA mutants were selected from an $M_2$ population of *Arabidopsis thaliana* L. as described previously (Parks and Quail, 1993). PhyA mutant alleles were defined by complementation (Parks and Quail, 1993), and later by direct sequencing of the *phyA* structural gene (Dehesh et al., 1993; Xu et al., 1995). All phyA mutant alleles used for these experiments were back-crossed once to the parent wild type (ecotype RLD). The *phyB-9* mutant Columbia ecotype (Reed et al., 1993) was obtained from the Arabidopsis Biological Resource Center (Columbus, OH). Mutant names follow nomenclature for phytochrome as described previously (Quail et al., 1994). Seeds used for all experiments were routinely sterilized for 30 min in a solution containing 1% hypochlorite and 0.2% SDS, rinsed several times with sterile distilled water, and dried on sterile filter paper. For phototropism experiments, seeds were embedded in 1 mM $KNO_3$ solidified with 0.8% agar (w/v) in eight-well microtiter strips (two seeds per well). These microtiter strips were then placed in humid, light-tight plastic boxes and stored at 4°C for 2 d. Germination was then induced by treating the seeds with saturating red light for 30 min at 25°C with the exception of *phyB-9*, which required 12 h of white fluorescent light to induce germination (Shinomura et al., 1994). PhyB mutant seeds were given this alternative light treatment to increase the percentage of germination in this particular line. No seedling emergence was detected after this 12-h light treatment, and the phototropic characteristics of seedlings germinated by this procedure were not different from populations of *phyB-9* that had germinated less efficiently with the standard red-light treatment (data not shown). Germination and growth were then allowed to proceed in darkness at 23°C in high humidity for 72 h, at which time seedlings were used for phototropism experiments. For protein extractions, seeds were sown in Petri dishes on half-strength Murashige-Skoog salts (GIBCO-BRL) solidified with 0.8% agar at pH 5.9. The seeds were cold-treated and induced to germinate as described above. Seedlings were harvested after 4 d of growth in darkness at 23°C, blotted dry, weighed, treated with saturating red light for 1 min to convert the endogenous phytochrome pool to Pfr, and frozen in liquid nitrogen.

### Phototropism Experiments

All manipulations for phototropism experiments were conducted in darkness at 25°C. Three-day-old, etiolated mutant and parental wild-type seedlings that were grown in microtiter wells were transferred into a humid, clear plastic box contained within a dark box with a blue window (No. 2045 acrylic, Rohm and Haas [Philadelphia, PA]) on one side. A unilateral phototropic stimulus was delivered as a 5-s pulse by opening a shutter in front of a cool-white fluorescent bulb (F15T8/CW [General Electric]).This filter/light source combination yielded broadband blue light (420–525 nm) with maxima at 436 and 468 nm. After 1 h, seedlings were photographed at a right angle to the direction of the phototropic stimulus. The angle of curvature was recorded directly from projected images of the photographic negatives. The intensity of blue light was varied by changing the distance between the light source and the seedlings or by introduction of metal neutral density screens. Enhancement of phototropism was induced by a 12-s red-light pulse (No. 2423 acrylic, Rohm and Haas; cool-white fluorescent bulb No. F40/CW [General Electric]; 600–700 nm with maximum intensity at 658 nm) given from above 2 h prior to the phototropic stimulus (Janoudi and Poff, 1991). For most experiments, the red-light intensity was saturating for the enhancement response (Janoudi and Poff, 1992). When necessary, the intensity of the red-light treatment was varied by the addition of metal neutral density screens. Enhancement experiments always included a nonenhanced control population. There were approximately 60 seedlings in each population of enhanced or nonenhanced seedlings for every treatment.

### Protein Extraction and Immunochemical Analysis

Crude protein extracts were obtained after the first centrifugal separation in the method described by Somers et al. (1991). For these extracts, 4 mM PMSF was substituted for all other cited protease inhibitors (Somers et al., 1991). Extracted proteins were separated in 6% polyacrylamide gels using SDS-PAGE, followed by electroblotting and subsequent immunochemical detection as described previously (Parks et al., 1987, 1989). Hybridoma supernatants and their working concentrations for selectively detecting *PHYA*-encoded peptides are described by Parks et al. (1989).

Crude extracts used for proteolysis were prepared in the absence of protease inhibitor (PMSF) and then frozen in liquid nitrogen. Aliquots of these extracts were assayed for

total protein (Bradford, 1976), and the remainder of the frozen extracts were adjusted to a final protein concentration of 1.2 mg $mL^{-1}$ with half-strength extraction buffer. Aliquots of these balanced extracts were divided in half and irradiated for 1.5 min at 0°C with either saturating red (660 nm) or far-red (738 nm) light. Subtilisin (25 $\mu$g $mL^{-1}$ in extraction buffer) was added to the irradiated extracts at a dilution of 1:30 (protease:extract). Proteolysis at 24°C was terminated after 20 min by the addition of an equal volume of sample buffer (125 mM Tris, pH 6.8, 1.4 M 2-mercaptoethanol, 4% SDS, 7.5% glycerol, 0.02% bromphenol blue, supplemented with 8 mM PMSF) followed by rapid freezing in liquid nitrogen. All samples were boiled for 3 min prior to loading on the gel.

## RESULTS

### Phototropic Enhancement Is Regulated Primarily by PhyA

Previously isolated photomorphogenic mutants of Arabidopsis that are selectively deficient in phyA were used to test whether this photoreceptor regulates red-light-induced enhancement of phototropism. Figure 1 shows fluence-response measurements of phototropism in red-pretreated and nonpretreated controls of the phyA-null mutants, *phyA-101* and *phyA-102*, and their parental wild type. The wild-type response was comparable to previously described results, where first-positive phototropic curvature in nonpretreated wild-type seedlings increased over the tested fluence range to a maximum response at 0.15 $\mu$mol $m^{-2}$ $s^{-1}$ before declining at higher fluence rates. The decrease in the phototropic response seen at higher fluences is characteristic of first-positive curvature and has been described elsewhere (Steinitz and Poff, 1986).

Wild-type seedlings that had been pretreated with a red-light pulse prior to the blue-light phototropic stimulus displayed a 2-fold increase in seedling curvature over the most effective blue-light fluence range, similar to that shown in previous analyses of this species (Janoudi and Poff, 1991). When the two phyA-null mutants were tested for enhancement of phototropism, curvature in red-light-pretreated seedlings was similar to that in nonpretreated seedlings over the entire fluence range tested. Although phototropic curvature in nonpretreated controls of the wild type and the *phyA-102* mutant were similar, the amplitude of curvature seen for the *phyA-101* mutant was lower. The reason for the weak phototropic response of the *phyA-101* mutant is unclear, but may be the result of an unknown mutation in this line. However, the observation that *phyA-102* and *phyA-101* are severely affected in red-light-induced phototropic enhancement indicates that phyA is necessary for this inductive phytochrome response in Arabidopsis and that none of the other phytochromes (phyB, phyC, phyD, or phyE) can substitute for phyA in inducing this response.

The suggestion that phyA is the primary regulator of phototropic enhancement was further supported by a similar analysis of a phyB-deficient mutant. The previously identified *HY3* locus in Arabidopsis is now known to be the *PHYB* structural gene (Reed et al., 1993). The majority of phyB

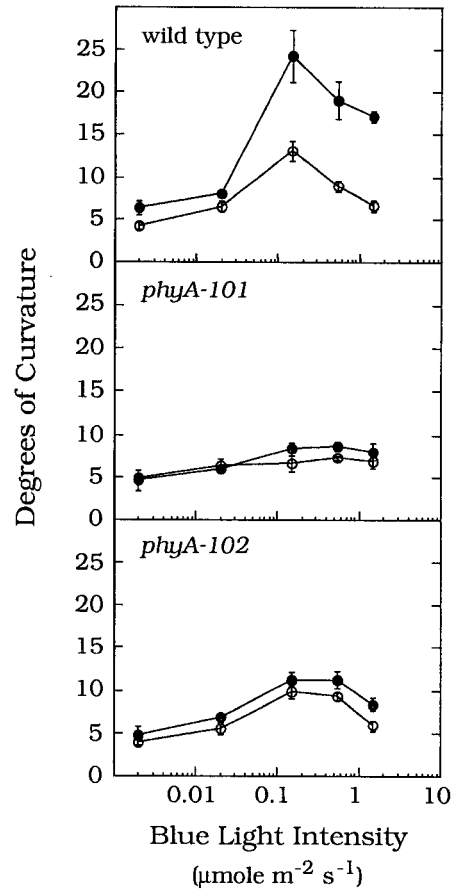


**Figure 1.** Phototropism fluence-response measurements in red-light-pretreated (●) and nonpretreated (○) phyA mutant and wild-type seedlings. Three-day-old etiolated seedlings were tested for phototropic curvature as described in "Materials and Methods." The intensity of the 12-s red-light pretreatment was 4.0 $\mu$mol $m^{-2}$ $s^{-1}$. Each point represents the mean ± SE of four separate experiments.

mutants have been isolated in the Landsberg background, which was found to respond poorly to a unilateral blue-light stimulus in preliminary phototropic measurements (data not shown). Therefore, we examined a phyB mutant in the Columbia ecotype (*phyB-9*). Sequence analysis of the *phyB-9* mutant suggested that this mutant line would be deficient in phyB because it carries a nonsense mutation at amino acid 397 (Reed et al., 1993). Western analysis of enriched-protein extracts of the phyB-9 mutant using monoclonal antisera selective for phyB confirmed the prediction that this allele contains no detectable full-length or truncated phyB polypeptides (data not shown). Figure 2 shows the analysis of phototropic enhancement in the *phyB-9* mutant and its wild-type parent. The Columbia wild type displayed phototropism and enhancement properties comparable to those seen for the RLD ecotype (Figs. 1 and 2). However, red-treated seedlings of the *phyB-9* mutant showed significant phototropic enhancement, but at slightly reduced levels compared to the wild type (Fig. 2). Although these results support the previous contention that phototropic enhancement is regulated primarily by

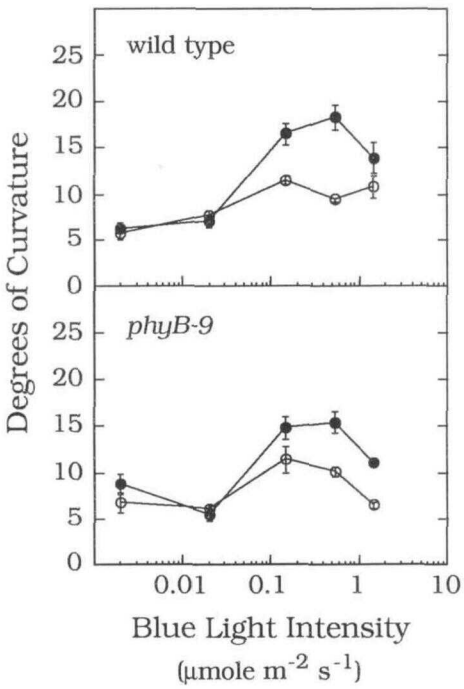


**Figure 2.** Phototropism fluence-response measurements in red-light-pretreated (●) and nonpretreated (○) phyB mutant and wild-type seedlings. Growth conditions and experimental parameters are identical to those described in Figure 1.

phyA, the reduced enhancement response seen for the *phyB-9* mutant may indicate a minor role for phyB.

### FR-HIR Regulatory Mutants of PhyA Show an Impaired Response to an Inductive Light Stimulus

The role of phyA in phototropic enhancement was investigated further using other mutant alleles of phyA that synthesize the full-length polypeptide but contain missense lesions in a proposed regulatory region for this photoreceptor (Dehesh et al., 1993; Xu et al., 1995). Several molecular characteristics of these mutant phytochromes have been described recently and include measurements of spectral activity, light-induced lability of the chromoprotein in vivo, and dimerization properties of the holoprotein (Xu et al., 1995). Figure 3 shows the relative protein levels and in vitro proteolysis characteristics for phyA in the wild-type and 12 independently isolated phyA mutant lines. The absolute protein levels in crude extracts of these etiolated mutant seedlings are consistent with other recent results (Xu et al., 1995). Results for one previously uncharacterized line (*phyA-111*) are also included (Fig. 3A).

All lines except *phyA-101*, *phyA-102*, *phyA-110*, and *phyA-111* showed levels of phyA equivalent to that of the parental wild type. The slight apparent variation in molecular mass for these proteins was not consistently observed on other immunoblots (data not shown) and probably reflects slight electrophoretic variability within the gel matrix during separation. The *phyA-111* mutant, like the previously described *phyA-101* and *phyA-102* (Parks and Quail, 1993), was also deficient in detectable phyA, whereas *phyA-110* possessed severely reduced levels of immunochemically detectable photoreceptor (approximately 10% of wild-type levels). In the present analysis, *phyA-107* appeared to have slightly reduced levels of phyA (approximately 95% of wild-type levels).

Analysis of the pattern of in vitro proteolysis of the mutant phyA proteins in their Pr and Pfr forms (Fig. 3B) showed the characteristic differences that are typical for wild-type phyA (Parks et al., 1989). This test not only offers an assay for the photochemical capacity of phyA, but also permits a reliable assessment of the conformational properties of the photoreceptor in its two forms. Although no gross conformational differences between the wild type and phyA-positive mutants were detected, slight variations in the relative quantities of various peptide fragments were detected among the different mutant lines, which may reflect small differences in the quality of the individual crude protein extracts. *PhyA-107* exhibited the only major difference from wild type. Although the pattern of proteolysis for this mutant was similar to that of the wild type, the overall rate of proteolysis in vitro appeared to be greater, thereby yielding a marked reduction in the levels of immunochemically detectable fragments. This observation was observed repeatedly in separate extracts from different lots of seedlings (data not shown). The reason for this difference is unclear at present, but this result implies that the mutation in this photoreceptor may cause greater

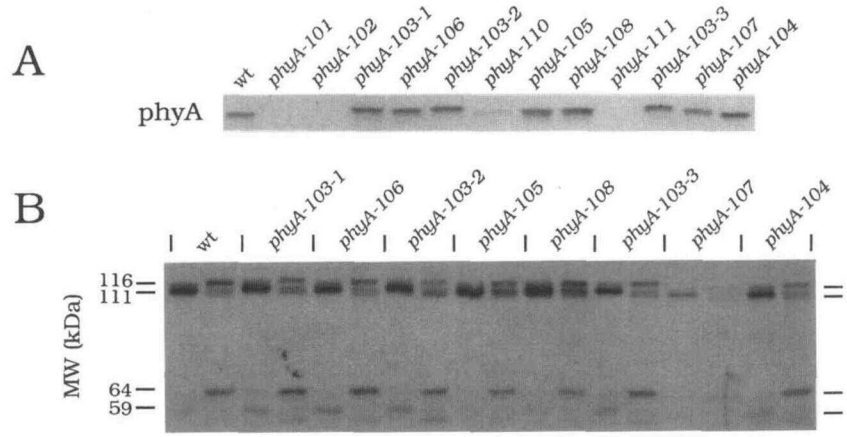


***Figure 3.*** *Protein levels and in vitro proteolysis of phyA in crude extracts of wild type (wt) and various phyA mutants. Crude extracts were generated for both panels as described in "Materials and Methods." A, The lanes were loaded with 40 μg of total protein with the exception of phyA-110 (120 μg), and the immunoblot was probed with a type-selective monoclonal antibody against phyA. B, The lanes contained 30 μg of total protein with the exception of phyA-107 (60 μg) after proteolysis as described in "Materials and Methods" and were probed with the same monoclonal antibody used in A. The left and right lanes of each crude extract pair were digested as Pr and Pfr, respectively. MW, Molecular mass.*

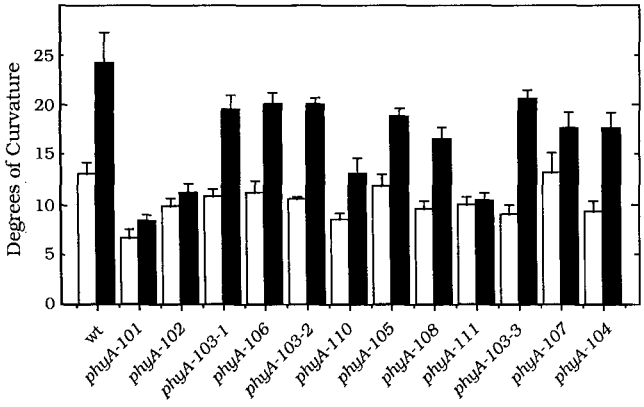


**Figure 4.** Measurement of phototropic enhancement in wild type (wt) and various phyA mutants. Three-day-old etiolated seedlings were germinated and tested for phototropic curvature as described in "Materials and Methods." The intensity of the 12-s red-light pulse used to induce enhancement of phototropic curvature was 4.0 μmol $m^{-2}$ $s^{-1}$. The intensity of the 5-s blue-light pulse used to induce phototropic curvature was 0.15 μmol $m^{-2}$ $s^{-1}$. Open and solid bars represent nonpretreated and red-pretreated seedlings, respectively. Each bar is the average ± SE of four separate experiments.

instability in vitro and may account for the lower levels of this protein in crude extracts (Fig. 3A). In general, however, these results suggest that the major conformational properties of PrA and PfrA in the majority of the phyA-positive mutants are similar to the conformational changes observed in the wild type and provide further support for the proposal that these mutant photoreceptors are not impaired in their basic physical properties (Xu et al., 1995).

Figure 4 shows the phototropic enhancement properties of the different phyA mutants. The new phyA-null mutant, *phyA-111*, showed a deficiency in phototropic enhancement similar to that seen for *phyA-101* and *phyA-102*, consistent with the proposal that phyA is the primary regulator of this response. Mutant lines that possess reduced levels of phyA (*phyA-110* and possibly *phyA-107*) showed reduced phototropic enhancement relative to dark controls. This result suggests that the magnitude of this inductive response is proportional to the amount of photochemically functional photoreceptor, although this has not been quantified for this inductive response. The protein-positive phyA mutants that appear to be altered only in their capacity to transduce a perceived far-red high-irradiance light signal all displayed phototropic enhancement induced by red-light pulses that saturate the wild-type response. However, for some mutants, the degree of enhancement was slightly lower than for the wild type, suggesting that the domain that regulates this inductive response may be similar to the region important for the transduction of the FR-HIR.

We focused on the enhancement properties of one regulatory mutant (*phyA-103–1*) that showed strong enhancement to address the possibility that a common phyA regulatory domain is important for both inductive and HIRs. Figure 5 shows fluence-response measurements for phototropism and phototropic enhancement in the *phyA-103–1*

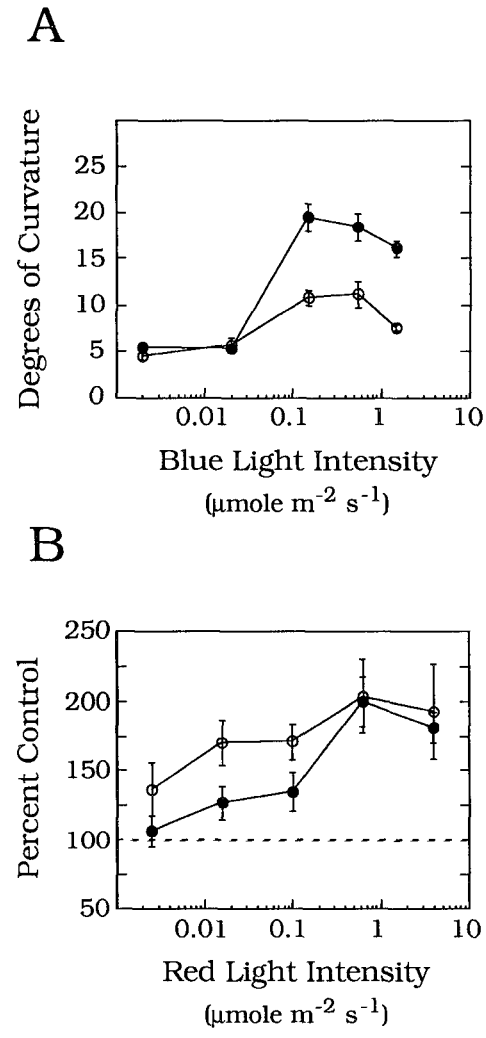


**Figure 5.** Fluence response for phototropism (A) and phototropic enhancement (B) in *phyA-103–1*. Three-day-old etiolated seedlings were tested for phototropic curvature as described in "Materials and Methods." A, The intensity of the 12-s red-light pulse used to induce enhancement of phototropic curvature was 4.0 μmol $m^{-2}$ $s^{-1}$. ●, Red-light-pretreated seedlings; ○, nonpretreated seedlings. B, The intensity of the 5-s blue-light pulse used to induce phototropic curvature was 0.15 μmol $m^{-2}$ $s^{-1}$, and data are displayed as a percent of the nonpretreated control population that was tested in tandem with the red-light-pretreated experimental population. ●, *phyA-103–1* mutant; ○, wild type. For both panels, each point is the mean ± SE of four separate experiments.

mutant. Fluence-response measurements of phototropism in red-light-pretreated and nonpretreated control seedlings of *phyA-103–1* showed that the phototropic and enhancement properties of this mutant were similar to those of the parental wild type, following saturating red-light pretreatment with a maximum response at 0.15 μmol $m^{-2}$ $s^{-1}$ (Figs. 1 and 5A). To test whether *phyA-103–1* possessed impaired sensitivity to red light, it was necessary to conduct a fluence-response test in which the intensity of red light was varied prior to an optimal blue-light treatment. The enhancement response of *phyA-103–1* became

distinct from that of the wild type as the fluence rate of the red-light pretreatment was lowered (Fig. 5B). At lower red-light fluence rates, *phyA-103–1* was less sensitive to red light compared to the wild type, with a response threshold shifted toward higher fluence. This shift in the fluence-response curve is expected for a photoreceptor with impaired sensitivity and implies that a domain critical for the response to a red-light pulse is similar to that required for the proper function of the FR-HIR.

## DISCUSSION

Because inductive phytochrome responses are commonly witnessed in etiolated seedlings where phyA is the predominant species, it was assumed that phyA was the regulator of these events. However, reports that implicated a role for phyB in these types of responses put this assumption in question (e.g. Liscum and Hangarter, 1993; Reed et al., 1993). We have utilized a series of photomorphogenic mutants containing lesions within the *PHYA* and *PHYB* structural genes to address their roles in an inductive phytochrome response. The results reported in this paper for null mutants of phyA and phyB show that the enhancement of phototropism by phytochrome is controlled primarily by phyA. Although it has been established that phyA is the dominant, or sole, regulator of de-etiolation under FR-HIR conditions (Quail et al., 1995), this present study indicates that phyA is not limited to the FR-HIR and that a comprehensive model of light perception by this photoreceptor must also incorporate an explanation for sensitivity to inductive red-light treatments.

The apparent dual photosensory roles of phyA for the detection of inductive red-light pulses and continuous far-red light can be understood in terms of the unique properties associated with this photoreceptor. In addition to being the predominant phytochrome type in etiolated seedlings, phyA differs from the other phytochromes in its capacity as PfrA to auto-regulate its expression and simultaneously trigger its rapid turnover (Quail, 1991). These properties can be used to explain how phyA can respond positively to both red and far-red light. The high sensitivity of inductive responses to low levels of red light coupled with short escape times for photoreversibility by far-red light implies that only small quantities of PfrA are required for brief periods to affect these types of responses. High levels of phyA in etiolated tissue would permit significant PfrA production in low-intensity red light in comparison to the other less-abundant phytochromes. The reduction in the phyA pool caused by continuous red illumination would not interfere with the capacity of phyA to affect inductive responses, since the initial moments of red irradiation would be sufficient to trigger the transduction sequence. Moreover, since continuous far-red light sustains the level of phyA at reduced but physiologically sufficient levels (Boylan et al., 1994), a scenario emerges in which phyA would shift from inductive responses to the FR-HIR during the early phases of de-etiolation.

The impairment of phototropic enhancement described here for *phyA* missense mutants suggests that at least one domain that controls the FR-HIR and red-low-fluence phytochrome responses is shared. $NH_2$-terminal missense *phyA* mutations have generally resulted in a decrease in the photochemically and immunochemically detectable levels of the photoreceptor (Quail et al., 1995; Xu et al., 1995), implying a proportional relationship between photoreceptor abundance and/or activity and the measured response. Our results with two $NH_2$-terminal mutants (*phyA-110* and *phyA-107*) suggests that a similar relationship is also valid for an inductive photoresponse. Although previous analyses of *phyA-107* indicated that phyA levels in this $NH_2$-terminal mutant were normal (Xu et al., 1995), in vitro analysis of the molecular properties of the mutant phyA suggest that it is less stable than the wild-type photoreceptor in vitro (Fig. 3B).

For the COOH-terminal portion of the molecule, the aberrant enhancement response of the *phyA-103–1* missense mutant, revealed at a low red-light fluence rate (Fig. 5B), shows the importance of the mutated residue (727, Gly → Glu) (Dehesh et al., 1993) in the regulation of this response. This critical residue is highly conserved among all phytochrome types and is part of a proposed regulatory domain of about 160 amino acids in the COOH terminus of phyA (Xu et al., 1995). Although comparable red-light fluence rate analyses of enhancement have not been conducted for the other regulatory mutants studied here (*phyA-103–2, -103–3, -104, -105, -106,* and *-108*), two of these mutants (*phyA-103–2* and *-103–3*) are independent isolates of the *phyA-103* allele, and the others all lie within the same putative regulatory domain for function of the FR-HIR (Quail et al., 1995). It is therefore anticipated that deficiencies in their response to inductive red-light stimuli (Fig. 4) would also be more evident at a lower fluence rate.

The mechanism by which phyA can increase the phototropic response to a blue-light stimulus is not known. Although phytochrome is capable of absorbing and responding to blue light (Kendrick and Kronenberg, 1994), it is known that blue-light-induced phototropism is controlled by a separate, unrelated sensing system (Liscum and Briggs, 1995). Therefore, it is evident that a form of interaction occurs between these two distinct sensory-response systems. Previous studies have shown that enhancement is maximized when the red-light stimulus precedes the blue-light phototropic stimulus by 2 h (Janoudi and Poff, 1991), and it was suggested that phytochrome affects the phototropic response by modulating a component of the blue-light signal transduction sequence (Janoudi and Poff, 1992). It is also possible that the effect of phytochrome on phototropism is indirect. For example, because vertically oriented shoots that are induced to curve by a phototropic stimulus will experience lateral gravitropism as soon as their orientation departs from vertical (Shen-Miller and Gordon, 1967), the resulting curvature response represents a compromise between positive phototropism and negative gravitropism.

Indeed, plants that received a phototropic stimulus and were placed on a clinostat during development of curvature exhibited greater bending than nonclinostated controls (Nick and Schäfer, 1988). Moreover, phytochrome is known to modulate gravitropism in different species (McArther and Briggs, 1979; Feldman and Briggs, 1987) and, in Arabidopsis, red light greatly attenuates gravitropism (Liscum and Hangarter,

1993). The red-light effect on gravitropism is primarily due to phyB, but phyA also plays a role (Liscum and Hangarter, 1993; J. Kim, B. Tamot and R.P. Hangarter, unpublished results). Even though the response time for visible gravitropic changes is generally greater than the 2-h interval used for the enhancement studies, the presentation time required to induce gravitropic curvature in Arabidopsis roots is close to 1 min (Kiss et al., 1989). Thus, it is reasonable to suggest that red light enhances the phototropic response by attenuating the gravitropic response. However, a recent experiment on phototropism in wheat coleoptiles conducted in space indicated that the promotion of phototropic curvature in microgravity was rather weak (Heathcote et al., 1995). Similar experiments designed to test phototropic enhancement of Arabidopsis in microgravity would help determine whether phytochrome affects phototropism through changes in the gravitational response system.

This work has shown that phyA is the primary photoreceptor regulating the induction of first positive phototropic enhancement in Arabidopsis. In addition, phyA may share a regulatory domain that has been implicated in the control of the FR-HIR. However, unlike the FR-HIR, inductive photoresponses are characterized by nonsaturating fluence requirements, reciprocity, and photoreversibility. These properties are beneficial to photobiological analyses and may facilitate the elucidation of the primary transduction events for responses regulated by phyA and lead to a better understanding of the mechanism of phytochrome action.

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