Multiple Phytochromes Are Involved in Red-Light-Induced Enhancement of First-Positive Phototropism in Arabidopsis thaliana

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The amplitude of phototropic curvature to blue light is enhanced by a prior exposure of seedlings to red light. This enhancement is mediated by phytochrome. Fluence-response relationships have been constructed for red-light-induced enhancement in the phytochrome A (phyA) null mutant, the phytochrome B (phyB) deficient mutant, and in two transgenic lines of Arabidopsis thaliana that overexpress either phyA or phyB. These fluence-response relationships demonstrate the existence of two responses in enhancement, a response in the very-low-to-low-fluence range, and a response in the high-fluence range. Only the response in the high-fluence range is present in the phyA null mutant. In contrast, the phyB-deficient mutant is indistinguishable from the wild-type parent in red-light responsiveness. These data indicate that phyA is necessary for the very-low-to-low but not the high-fluence response, and that phyB is not necessary for either response range. Based on these results, the high-fluence response, if controlled by a single phytochrome, must be controlled by a phytochrome other than phyA or phyB. Overexpression of phyA has a negative effect and overexpression of phyB has an enhancing effect in the high-fluence range. These results suggest that overexpression of either phytochrome perturbs the function of the endogenous photoreceptor system in an unpredictable fashion.

Visible light induces adaptation in phototropism (Blaauw and Blaauw-Jansen, 1970; Iino, 1987, 1988; Golland, 1990; Janoudi and Poff, 1991). Adaptation consists of desensitization, a refractory period, recovery, and curvature enhancement (Janoudi and Poff, 1991). Action spectra indicate that one component of adaptation, enhancement of the first-positive phototropic curvature, is regulated by phytochrome (Chon and Briggs, 1966; Janoudi and Poff, 1992). Enhancement of phototropism is most easily illustrated by the effect of an exposure to red light on the subsequent phototropic curvature to blue light (Fig. 1). Although red light does not induce phototropism in Arabidopsis thaliana, maximum first-positive curvature to blue light is increased when the seedlings are exposed to red light up to 2 h prior to irradiation with unilateral blue light. Moreover, the increase in phototropic curvature, i.e. enhancement, increases as the fluence of red light is increased, eventually reaching saturation at fluences above 10 μmol m⁻², at least for the Estland ecotype (Janoudi and Poff, 1992).

Five phytochrome genes have been identified in A. thaliana (Sharrock and Quail, 1989; Clack et al., 1994) and a number of phytochrome mutants have been isolated (Koornneef et al., 1980; Parks and Quail, 1993; Dehesh et al., 1993; Nagatani et al., 1993; Whitelam et al., 1993; Reed et al., 1994). These mutants are deficient in one or more of the phytochromes. One of these mutants, phyA-101 (formerly hy8-1), is known to be null for the light-labile phytochrome, phyA (Dehesh et al., 1993; Parks and Quail, 1993), whereas a second mutant, phyB-1 (formerly hy3), is deficient for the light-stable phytochrome, phyB (Somers et al., 1991; Reed et al., 1993). Transgenic plants that overexpress either phyA or phyB (Boylan and Quail, 1991; Wagner et al., 1991; Reed et al., 1993) have been engineered in part to investigate the role of individual phytochromes in plant development. Transgenic plants overexpressing phyB have an increased sensitivity to red light (McCormac et al., 1993; Wagner et al., 1991), whereas seedlings overexpressing phyA exhibit an increased sensitivity to far red light as well as to red light (McCormac et al., 1992; Dehesh et al., 1993; Whitelam and Harberd, 1994).

Recently, Parks et al. (1996) reported that phyA is the primary photoreceptor pigment regulating the enhancement of phototropism by low-fluence red light. We also have studied the enhancement of phototropism, but over a broad range of fluences in an attempt to identify which phytochromes mediate enhancement at low fluences and at high fluences of red light. We have measured the fluence-

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Abbreviations: phyA, phyB, phytochrome A and B, respectively; phyA-101, phyB-1, mutations in the PHYA and PHYB genes.
response relationships for enhancement in the phyA-deficient mutant phyA-101, in the phyB-deficient mutant phyB-1, and in two transgenic lines of A. thaliana, one of which overexpresses phyA and the other of which overexpresses phyB. Our data for red-light-induced enhancement indicate the existence of one response in the very-low-to-low-fluence range (phase I) and a second response in the high-fluence range (phase II). Phase I requires phyA. However, if phase II is controlled by a single phytochrome, it must be a phytochrome other than phyA or phyB.

MATERIALS AND METHODS

Seedlings of Arabidopsis thaliana were grown as previously described (Steinitz and Poff, 1986) in strips of microassay wells containing 0.7% (w/v) agar/water. Seed germination was potentiated by chilling the planted seeds at 4°C for 3 d and exposing the seeds to white light for 18 h at 23°C. The strips were transferred to darkness for 42 h at a temperature of 25 ± 1°C. At the end of the dark period, the seedlings were given the appropriate irradiation treatment. Seedlings were maintained throughout a RH >90%. The wild-type ecotypes used in this study were NO-O and RLD. The phyA-deficient mutant used here was phyA-101 (Parks et al., 1996), whereas the phyB-deficient mutant was phyB-1, both in an RLD background. The phyA transgenic overexpressor line used in this study exhibits a 4-fold increase in the phyA level (Boylan and Quail, 1991). The phyB overexpressor line was that designated ABO in Wagner et al. (1991). The ABO line expresses phyB at levels that are about 18- to 30-fold higher than those of the wild-type parent RLD. By contrast, the threshold fluence that is required to induce enhancement in phyA-101 is about four orders of magnitude higher than that required by RLD. At the highest fluences tested, 100 μmol m⁻² or greater, the magnitude of curvature enhancement in the phyA-101 mutant is similar to that observed in the wild-type parent, RLD. By contrast, the mean response relationship for phyB-1 is indistinguishable from that of its RLD wild-type parent throughout the range tested (Fig. 2).

To further characterize the role of the different phytochromes in enhancement, the fluence-response relationships for enhancement were determined for transgenic plants overexpressing either phyA or phyB. For each of the phytochrome-overexpressing transgenic lines, enhancement exhibited an unexpectedly complex dependence on the fluence of red-light irradiation.

In the transgenic line that overexpresses phyA, curvature enhancement increased with increasing fluences of red-

Light Sources

The white light used to potentiate seed germination was provided by two Delux cool-white fluorescent tubes (General Electric). A slide projector equipped with a 900-W BVA tungsten-halogen lamp (Sylvania), in combination with the appropriate Corion (Holliston, MA) interference filter (10-nm one-half-band width; stray light blocked to >2000 nm), was used as the light source for the red (669 nm) light and blue (450 nm) light irradiation. Fluence rates were measured using an LI-190SA quantum sensor (Li-Cor, Lincoln, NE) in combination with a LI-1000 Datalogger (Li-Cor, Lincoln, NE). A Uniblitz shutter (Vincent Associates, Rochester, NY) was used to control the duration of irradiation.

Seedlings were bilaterally irradiated with red light as previously described (Janoudi and Poff, 1991) by sequentially irradiating the opposite sides of the seedlings with 669-nm light at equal fluences. A number of fluences of red light were tested over a range of about seven orders of magnitude from 0.001 to 2000 μmol m⁻². Two hours after the red-light irradiation, seedlings were unilaterally irradiated with blue light at a fluence of 0.5 μmol m⁻², which induces maximum first-positive phototropism.

Curvature Measurement

Seedling curvature was measured 70 min after the end of the blue-light irradiation, as previously described (Steinitz and Poff, 1986). The seedlings were mounted on transparent adhesive tape with the curvature in the plane of the tape surface. The tape was inserted into a photographic enlarger and the hypocotyl curvature was traced and then measured.

RESULTS

Phytochrome mutants of A. thaliana that are deficient in either phytochrome A (phyA-101) or phytochrome B (phyB-1), in addition to two transgenic lines that overexpress either phyA or phyB, were used to determine the involvement of phyA and phyB in enhancement. Based on the fluence-response relationship for enhancement in phyA-101 and its wild-type parent RLD (Fig. 2), the threshold fluence that is required to induce enhancement in phyA-101 is about four orders of magnitude higher than that required by RLD. At the highest fluences tested, 100 μmol m⁻² or greater, the magnitude of curvature enhancement in the phyA-101 mutant is similar to that observed in the wild-type parent, RLD. By contrast, the mean response relationship for phyB-1 is indistinguishable from that of its RLD wild-type parent throughout the range tested (Fig. 2).

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Figure 1. Diagrammatic representation of the enhancement effects of various fluences of red light on the magnitude of first positive curvature in the Estland ecotype. Fluence-response relationships represent curvatures in response to blue (450 nm) light in seedlings that received no red light preirradiation (a), a red light preirradiation at fluences of 0.01 (b), 0.1 (c), 10 (d), or 100 (e) μmol m⁻². This diagrammatic representation was drawn as symmetrical curves based on the actual data for maximum first positive curvature following a preirradiation with 669-nm light, as previously reported by Janoudi and Poff (1992).
Figure 2. Fluence-response relationships for the effect of a red-light preirradiation on phototropic curvature in response to a subsequent unilateral irradiation with blue light. Etiolated seedlings of RLD (■), phyB-1 (○), and phyA-101 (□) were first irradiated bilaterally with red light at the indicated fluence, then, 2 h later, unilaterally with blue light at a fluence of 0.5 μmol m\(^{-2}\) s\(^{-1}\), sufficient to induce maximal first-positive phototropism.

Light irradiation, reaching a maximum at about 10 μmol m\(^{-2}\) (Fig. 3a). Further increases in the fluence of red light led to a decrease in enhancement. At the highest fluence of red light tested, there was no apparent enhancement in curvature as a result of the red-light treatment. The threshold fluences for induction of enhancement and the magnitude of enhancement were similar in the phyA transgenic line (Fig. 3a) and its NO-O wild-type parent (Fig. 3c).

In the phyB-overexpressing line, enhancement exhibited a biphasic dependence on the fluence of red-light irradiation (Fig. 3b). In the very-low-to-low-fluence phase of the response (phase I), the fluence threshold and the magnitude of enhancement appeared to be similar to those observed in the NO-O wild type (Fig. 3c). The high-fluence phase of the response (phase II) had an apparent threshold of about 10 μmol m\(^{-2}\). At fluences above about 100 μmol m\(^{-2}\), the response in the transgenic line was significantly higher than in the wild type.

DISCUSSION

Red-light irradiation of etiolated Arabidopsis seedlings results in an enhancement of phototropic responsiveness to subsequent irradiation, with blue light in the range of first-positive phototropism (Janoudi and Poff, 1992) and second-positive phototropism (A.-K. Janoudi and K. Poff, unpublished data). This enhancement process is mediated by one or more of the phytochromes and is dependent on the fluence of red light (Chon and Briggs, 1966; Janoudi and Poff, 1992). We have reasoned that an alteration in the level of phytochrome expression may lead to a change in the sensitivity and/or the responsiveness of etiolated seedlings to red light. Thus, in phytochrome-deficient mutants, we might expect a decrease in sensitivity that would be reflected by a shift in the fluence threshold for enhancement to higher fluences. Conversely, in mutants overexpressing phytochrome, we might expect an increase in sensitivity accompanied by a shift in the fluence threshold to lower fluences.

Using mutants and transgenic lines that exhibit altered levels of either phyA or phyB, we have been able to assess the roles and interactions of these phytochromes in enhancement of first-positive phototropism. It is apparent that a complex relationship exists between the level of expression of phytochromes and the plant's responsiveness to red light.

The difference in the fluence-response relationships of the RLD wild-type parent and phyA-101 cannot be attributed to a decreased concentration of a single photoreceptor pigment that mediates enhancement over the entire range from very low fluence through high fluence. If this were...
the case, the response curve for phyA-101 would simply be shifted to a higher fluence. Instead, we found that the slope of the phyA-101 fluence-response relationship is radically different from that of the RLD wild-type parent. Moreover, phyA-101 is a null mutant; that is, the amount of phyA is reduced to zero, and the threshold for the phyA-101 relationship is not infinitely greater than that of the RLD wild-type parent. Thus, these data suggest the involvement of (an)other photoreceptor pigment(s) in addition to phyA in enhancement.

The results presented here demonstrate the existence of two responses in enhancement, one that occurs at very low to low fluences (phase I) and another that is exhibited at high fluences (phase II). We observed a shift in the fluence threshold for enhancement, rather than a total loss of response to red light in the phyA-deficient mutant phyA-101. The shift in the fluence threshold for enhancement in phyA-101 toward higher fluences is expected if phyA mediates enhancement. This directly implicates phyA in enhancement by low fluences of red light, but does not explain the normal level of enhancement that we observed in the mutant at high fluences of red light. In phase I these results are in agreement with the recently published findings of Parks et al. (1996), who tested the phyA-deficient mutants phyA-101 and phyA-102 over the limited fluence range from 0.01 to 48 μmol m⁻² and consequently observed only the low-fluence-response component of enhancement. Based on these limited fluence-response relationships, they concluded that phyA is the primary phytochrome in enhancement.

Our observation that phyA-101 retains the capacity to undergo enhancement in response to high fluences of red light indicates that high-fluence enhancement, if controlled by a single phytochrome, must be controlled by a phytochrome other than phyA. Thus, phytochromes other than or in addition to phyA can also mediate the red-light-induced response of enhancement.

We have tested the possible involvement of phyB in enhancement by using the mutant phyB-1, which lacks phyB. We postulated that if phyB can mediate enhancement, then a decrease in phyB should lead to a shift in the fluence threshold or a decrease in enhancement. Parks et al. (1996) observed a slight reduction in enhancement in the phyB-deficient mutant phyB-9 and concluded that phyB has a minor role in enhancement. However, the fluences used in their study to test phyB-9 were in the low-fluence range, where phyA acts as the major photoreceptor pigment for enhancement. In our experiments the phyB-deficient mutant phyB-1 showed enhancement, which was indistinguishable from that of its wild-type parent throughout the very-low to high-fluence range. Based on these results, we conclude that phyB is neither necessary nor sufficient for low-fluence enhancement, and that phyB is also not required for high-fluence enhancement.

The use of transgenic lines with overexpression of either phyA or phyB gave less apparent information than did the use of the phyA and phyB mutants. We reasoned that an increase in the level of phytochrome should lead to an increased sensitivity or increased maximum response to red light. An increased sensitivity would be reflected by a lower fluence threshold and was not observed in the transgenic plants overexpressing either phyA or phyB. Based on these data, an element other than phyA is the rate-limiting factor in phase I enhancement at phyA concentrations above that of the wild-type parent. Moreover, neither phyA nor phyB alone is the rate-limiting factor in phase II enhancement.

Overexpression of phyA did not have a detectable effect on the extent of enhancement induced by low fluences of red light. This indicates that the quantity of phytochrome that is present in the wild type is sufficient to achieve the maximum level of enhancement that can be mediated by phyA. The decrease in enhancement at higher fluences that was observed in the phyA-overexpressing line is apparently due to a direct or indirect negative effect of phyA on some process in enhancement. We have no data with which we can identify this second process that is affected by phyA overexpression. In contrast, the transgenic line with overexpressed phyB exhibited a significant increase in enhancement at fluences above 100 μmol m⁻² (Fig. 3b) over that seen in the wild-type parent (Fig. 3c). Based on these data, phyB can mediate enhancement under these conditions.

Both overexpression effects, the negative effect of overexpressed phyA in the high-fluence range and the positive effect of overexpressed phyB in the high-fluence range, appear, at least superficially, to be at odds with the data obtained with the mutants lacking either phyA or phyB. This conflict could be the result of ectopic expression, or some interaction caused by overexpression such as competition for a limited pool of chromophore. We have no data with which we can resolve this apparent conflict.

The involvement of multiple phytochromes in enhancement is not surprising. PhyA and phyB are known to have overlapping functions in plant development (Reed et al., 1994). In the case of enhancement, multiple phytochromes may act to extend the plant’s sensitivity to red light, such that the plant is sensitive over the range from very low through high fluence. It is conceivable that phyA acts to optimize phototropism in germinating etiolated seedlings beneath the soil and that an additional phytochrome assumes a role as the seedlings are exposed to high fluences of light. Our finding that phyA mediates the very-low-fluence-enhancement response is consistent with other phyA-mediated responses in plants (Parks and Quail, 1993; Reed et al., 1994; Botto et al., 1996). The data presented in this study demonstrate the involvement of phyA in low-fluence enhancement and suggest the involvement of other photoreceptor pigments (phytochromes) in high-fluence enhancement. This possibility should be studied by measuring enhancement in double mutants lacking both phyA and phyB as well as mutants deficient in phyC, phyD, and phyE. Such a study would provide additional support for the existence of overlapping signal transduction pathways for the different phytochromes.

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Multiple Phytochromes in Phototropic Enhancement

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