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Genetic Separation of Phototropism and Blue Light Inhibition of Stem Elongation[1]

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ABSTRACT

Blue light-induced regulation of cell elongation is a component of the signal response pathway for both phototropic curvature and inhibition of stem elongation in higher plants. To determine if blue light regulates cell elongation in these responses through shared or discrete pathways, phototropism and hypocotyl elongation were investigated in several blue light response mutants in *Arabidopsis thaliana*. Specifically, the *blu* mutants that lack blue light-dependent inhibition of hypocotyl elongation were found to exhibit a normal phototropic response. In contrast, a phototropic null mutant (JK218) and a mutant that has a 20- to 30-fold shift in the fluence dependence for first positive phototropism (JK224) showed normal inhibition of hypocotyl elongation in blue light. F_1 progeny of crosses between the *blu* mutants and JK218 showed normal phototropism and inhibition of hypocotyl elongation, and approximately 1 in 16 F_2 progeny were double mutants lacking both responses. Thus, blue light-dependent inhibition of hypocotyl elongation and phototropism operate through at least some genetically distinct components.

Regulation of cell elongation is an intrinsic step in blue light-dependent inhibition of stem elongation and induction of phototropic curvature in higher plants (6, 8). It has been suggested that blue light inhibits longitudinal stem growth and induces phototropism through the same photoinhibition events at the cellular level (2), or that these responses share components in their signal transduction pathways (7, 9). However, other studies indicate that cell elongation is controlled by discrete signal transduction systems in these two responses (5, 15, 18). Most of the data regarding these hypotheses are based on physiological experiments that were designed to distinguish between what are normally superimposed responses.

Several blue light response mutants have been found in *Arabidopsis thaliana* that provide a means of genetically dissecting the relationships between phototropism and hypocotyl elongation. In particular, mutants have been isolated that lack blue light-dependent inhibition of hypocotyl growth, but curve in response to unilateral blue light (14). Here we report results from a detailed investigation of blue light-induced phototropic and longitudinal growth responses of these hypocotyl elongation mutants as well as two phototropism mutants. The results show that blue light induces phototropism and inhibition of hypocotyl elongation through genetically distinct pathways.

MATERIALS AND METHODS

Plant Material

Hypocotyl elongation mutants (*blu1*, *blu2*, and *blu3*) of *Arabidopsis thaliana* ecotype Columbia were described previously by Liscum and Hangarter (14). Phototropism mutants (JK218 and JK224) of *A. thaliana* ecotype Estland were described previously by Khurana and Poff (11). Specifically, the *blu* mutants lack blue light-dependent inhibition of hypocotyl elongation, JK218 seedlings lack phototropic curvature, and JK224 seedlings require 20- to 30-fold more actinic light to induce first positive phototropism than do wild-type seedlings.

Genetic Analysis

For genetic analysis, seeds were handled as described by Liscum and Hangarter (14). Surface-sterilized seeds were planted on agar medium and incubated at 4°C in the dark for 2 to 3 d. The seeds were then given 30 min of red light at 23°C, followed by 23.5 h in the dark, and then transferred to blue light (56 ± 2 μmol m^{-2} s^{-1}) given from directly above. After 4 d of growth in continuous blue light, the angle of the actinic light was changed to 55° from vertical for an additional 24 h to induce phototropic curvature. After this treatment, hypocotyls could be visually scored for both length and curvature for segregation analysis. When appropriate, seedlings were transferred to pots and grown to seed as described previously (14).

Allelism was tested by crossing the *blu* mutants (♂) to JK218 (♀), and patterns of inheritance were determined from the F_2 generation. Double mutants homozygous for a given *blu* mutation and JK218 were selected in blue light as F_2 seedlings that grew tall and straight under the conditions described above. Double mutant lines for each genetic combination were grown to maturity and F_3 seeds were collected. Samples from the F_3 populations were grown under the inductive conditions to confirm that the seedlings showed both mutant phenotypes.

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Measurement of Phototropism and Inhibition of Hypocotyl Elongation

For phototropism experiments, seeds were handled as described for the genetic analysis except that they were sown on 1.0 mM KNO_3 containing 1.0% (w/v) agar in microtiter wells (0.3 mL). Phototropic curvature was measured as degrees from vertical, as described by Janoudi and Poff (10).

For hypocotyl elongation experiments, seeds were handled as described by Liscum and Hangarter (14), except that unsupplemented, half-strength Murashige and Skoog nutrients (16) were used. After the cold and red light treatments to induce germination, seeds were transferred to darkness at 23°C for 23.5 h, followed immediately by 2 d of continuous blue light given from directly above at the indicated fluence rates. At the end of the blue light treatment, the seedlings were placed onto transparent tape. Using a projector, lengths of enlarged images (×10) of the taped seedlings were measured with a resolution of ± 0.05 mm using SigmaScan (Jandel Scientific, Sausalito, CA).

Light Sources

Red light (15 μmol m^{-2} s^{-1}) for induction of germination was obtained by filtering light from cool-white fluorescent lamps (F48T12-CW-1500) and four incandescent bulbs (GE 100 W) through one layer of Rohm and Haas red Plexiglas number 2444 (3.18 mm thick), one layer of yellow Roscolux number 10, and 1 cm of a 1.5% (w/v) $CuSO_4 \cdot 7H_2O$ solution. This filter combination cut off wavelengths below 580 nm and had a peak intensity at 658 nm. The 660:730 nm light ratio was 1.4. Light for potted plants was provided continuously at 65 ± 5 μmol m^{-2} s^{-1} from cool-white fluorescent lamps (F96T12-CW).

For genetic analysis, blue light was obtained by filtering light from four halogen flood lamps (GE 150 W Quartzline) through 5 cm of 1.5% $CuSO_4 \cdot 7H_2O$ and a layer of Rohm and Haas blue Plexiglas number 2045 (3.18 mm thick). The resulting spectral output had a peak intensity at 480 nm and a 100-nm half-bandwidth. The lights were mounted in a row on a frame that could be rotated to change the angle of irradiation. The $CuSO_4 \cdot 7H_2O$ solution was cooled by running cold tap water through copper tubing submerged in the solution.

Unilateral blue light for phototropism experiments was obtained by filtering light from a 750-W (GE) projector lamp through 3 cm of 1.5% (w/v) $CuSO_4 \cdot 7H_2O$ and a 450-nm interference filter with a half-bandwidth of 10 nm (Ealing Electro-Optics, Inc., Holliston, MA). The fluence rate was changed with neutral density filters, and fluence was varied by changing exposure time.

Blue light for hypocotyl elongation experiments was obtained by filtering light from a halogen flood lamp (GE 150 W Quartzline) through 6.5 cm of water-cooled 1.5% $CuSO_4 \cdot 7H_2O$ and a 450-nm interference filter. The fluence rate was varied by using a voltage rheostat and by changing the distance between the plant material and light source. Fluence rates for all experiments were measured with a LI-189 quantum photometer or a LI-1800 portable spectroradiometer (LiCor, Inc.).

RESULTS

The phenotypes of wild-type and mutant *Arabidopsis* seedlings after 4 d of continuous blue light from above followed by 24 h of unilateral blue light are shown in Figure 1. Under these conditions, hypocotyl elongation was inhibited and phototropic curvature occurred in wild-type seedlings. Hypocotyl elongation in the *blu1* mutant was not inhibited, but phototropic curvature was similar to that in wild-type seedlings. In contrast to wild type and *blu1*, JK218 seedlings showed no measurable curvature in the direction of the phototropic stimulus. However, inhibition of hypocotyl elongation in JK218 was similar to that in wild-type seedlings. Although not shown, the responses of *blu2*, *blu3–1*, and *blu3–2* were similar to those shown for *blu1* in Figure 1. JK218-*blu1* double mutants showed both parental phenotypes. Thus, in the double mutants, hypocotyl elongation was not inhibited and the seedlings did not bend toward blue light.

Crosses between the *blu* mutants and JK218 resulted in complementation of the mutant phenotypes so that F_1 progeny had short, curved hypocotyls similar to wild-type seedlings after the blue light treatment (Table I). Segregation of F_2 progeny into wild-type, *blu*, JK218, and *blu*-JK218 double mutant phenotypes was as expected for two independently segregating, recessive nuclear genes.

The results in Figure 1 and Table I were obtained using saturating levels of light for both the longitudinal growth inhibition and phototropic response. To determine if the responses were normal under limiting light conditions, fluence-response relationships for phototropism (Fig. 2) and fluence rate-response relationships for inhibition of hypocotyl elongation (Fig. 3) were examined. The fluence and time thresholds for phototropism (Fig. 2) were the same in *blu1* and Columbia wild-type seedlings and are consistent with previous results reported for Estland wild-type seedlings (10).

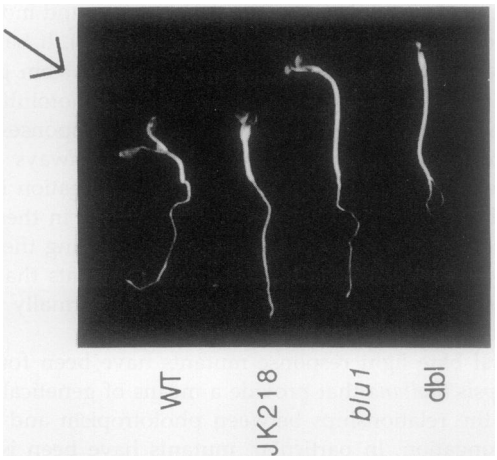

Figure 1. Comparison of blue light-induced phototropism and inhibition of hypocotyl elongation in wild-type and mutant *Arabidopsis* seedlings. Seedlings were grown under blue light (56 ± 2 μmol m^{-2} s^{-1}) given from above for 4 d, followed by 24 h of illumination at 55° from vertical, as indicated by the arrow. The photograph shows representative seedlings. WT, Columbia wild type; dbl, double mutant homozygous for *blu1* and JK218.

Table I. *Genetic Analysis of JK218 and blu Mutants*

Cross (♂ × ♀)		Hypocotyl Phenotype				
		Short/curved	Long/curved	Short/straight	Long/straight	χ^2
blu1 × JK218	F_1	10	0	0	0	
	F_2[a]	164	51	36	13	0.792[b]
blu2 × JK218	F_1	14	0	0	0	
	F_2[a]	182	49	41	13	1.300[b]
blu3-1 × JK218[c]	F_1	45	0	0	0	
	F_2[a]	146	44	46	15	0.032[b]

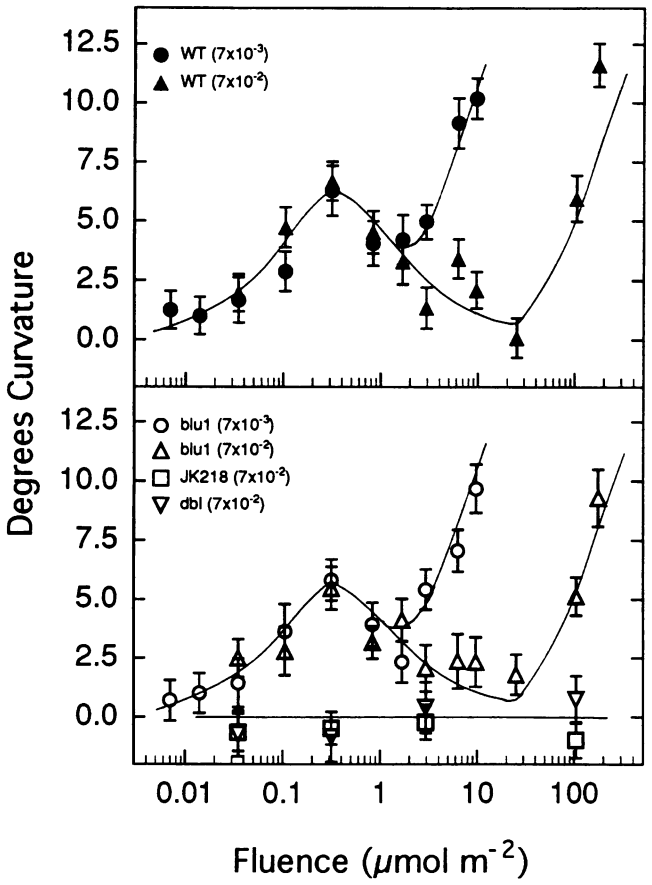

Figure 2. Fluence-response relationships for phototropism in wild-type and mutant *Arabidopsis* seedlings. The top panel shows the angle of phototropic curvature in wild-type seedlings after single pulses of 450-nm light at the indicated fluence rates (μmol m^{-2} s^{-1}). The data are from experiments with Columbia wild type (genetic background of the *blu* mutants), but similar results were obtained with Estland wild type (genetic background of JK218 and JK224). The bottom panel shows the angle of phototropic curvature in mutant seedlings after exposure to single pulses of 450-nm light. Data points represent the mean ± SE of 70 to 90 seedlings.

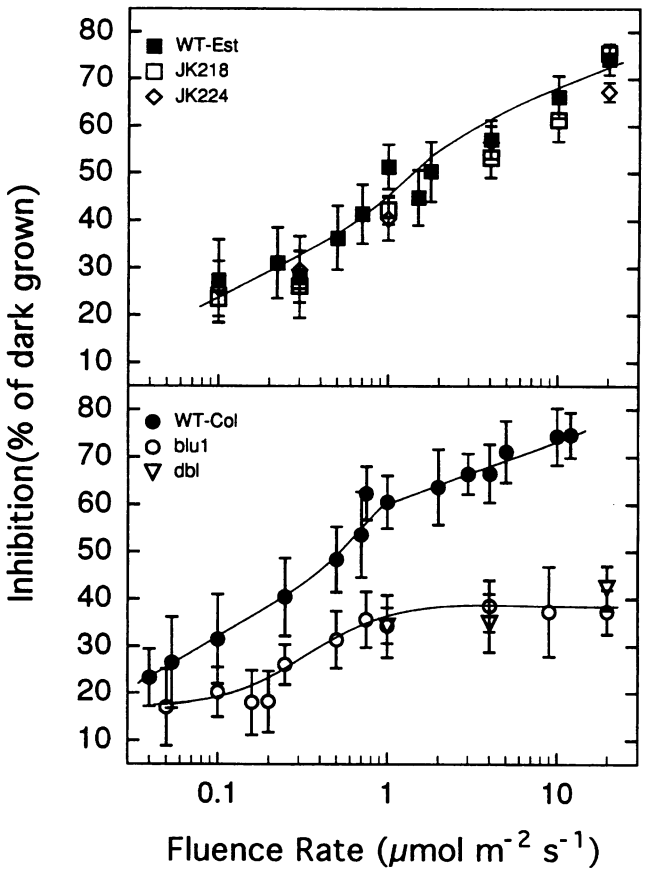

Figure 3. Fluence rate-response relationships for inhibition of hypocotyl elongation in wild-type and mutant *Arabidopsis* seedlings. Inhibition in Estland wild type, JK218, and JK224 seedlings is shown in the top panel. Inhibition in Columbia wild type, *blu1*, and *blu1*-JK218 double mutant seedlings is shown in the bottom panel. Each data point represents the mean of 25 to 50 seedlings exposed to continuous 450-nm light for 2 d. Vertical error bars represent the combined SE for the dark- and light-grown seedlings.

Moreover, the magnitude of the response was the same in wild-type and *blu1* seedlings. Similar results were found with *blu2*, *blu3–1*, and *blu3–2* (data not shown). In contrast to *blu1* and wild-type seedlings, JK218 and JK218-*blu1* double mutant seedlings did not curve in response to unilateral blue light at any fluence tested. Similar results were found with JK218-*blu2*, JK218-*blu3–1*, and JK218-*blu3–2* double mutants (data not shown).

Wild-type Columbia and Estland seedlings had similar fluence rate-response relationships for inhibition of longitudinal hypocotyl elongation (Fig. 3). Elongation in the wild-type seedlings was inhibited by about 73% at 10 μmol m^{-2} s^{-1}, which is about the maximum level of inhibition caused by saturating amounts of white light (14). Hypocotyl inhibition in the phototropism null mutant JK218 was indistinguishable from that of wild-type seedlings. The degree of inhibition of hypocotyl elongation in JK224, which is a mutant that exhibits a shift in the fluence dependence for first positive phototropism (11), was similar to that of the wild type. In contrast to JK218 and JK224, hypocotyl inhibition in *blu1* and *blu1*-JK218 double mutant seedlings saturated at 1 μmol m^{-2} s^{-1} with a maximal response of only 37%. Similar results were obtained with double mutants between JK218 and the other *blu* mutants (data not shown).

DISCUSSION

The results presented here show that blue light-induced inhibition of hypocotyl elongation and phototropism are mediated by genetically separate signal transduction systems. These systems require at least five distinct gene products coded for by *BLU1*, *BLU2*, *BLU3*, and the wild-type genes for the mutations in strains JK218 and JK224. Unfortunately, these results are not sufficient to indicate where in the signal transduction chains these genes function. However, because hypocotyl elongation and phototropism are ultimately dependent on changes in basic processes that affect cell elongation patterns, these responses might be expected to share functions near the end of their signal transduction chains. However, the mutations examined here affect one of the responses, but not both. Thus, if the responses share functions, the gene products affected by these mutations probably function relatively early in signal transduction.

At this point, it is not possible to determine with certainty if the responses are controlled by a photoreceptor with diverging signal transduction pathways before influencing cell elongation, or if the responses are controlled by different photoreceptors. However, the JK224 mutant, in which the light threshold for first positive phototropism is shifted, has recently been suggested to be a photoreceptor mutant (11, 12). Therefore, the observation made here, that blue light-induced inhibition of hypocotyl elongation is unaffected in JK224, supports the involvement of different blue light photoreceptors in these different responses.

In addition to the genetic data presented here, several physiological observations suggest that blue light-dependent inhibition of stem elongation and phototropism are distinct processes. For example, pea epicotyls are far more sensitive to blue light for induction of phototropism than for inhibition of epicotyl elongation (1, 13). In addition, blue light-induced inhibition of stem elongation occurs within 15 to 150 s in etiolated seedlings of *Sinapis*, *Cucumis*, *Pisum*, *Cucurbita*, *Helianthus*, and *Phaseolus* species, whereas the onset of phototropism usually takes orders of magnitude longer (3, 4). In a detailed study with cucumber, longitudinal growth inhibition occurred within 30 s of blue light exposure, but phototropism did not occur until 4.5 h after the start of irradiation (5). Because inhibition of stem elongation occurs more rapidly than phototropism in several different species (3–5), the same probably holds true for *Arabidopsis*. Although the time course for inhibition of hypocotyl elongation has not been determined in *Arabidopsis*, the onset of phototropism was found to occur 10 to 20 min after blue light irradiation in *Arabidopsis* (17). Kinetic separation alone, however, is not sufficient evidence to show that the responses are independent. For example, the slower phototropic response may be initiated by earlier effects on elongation of the stem cells.

The results obtained here with the blue light response mutants are consistent with the conclusions of many physiological experiments: the responses occur through distinct signal transduction systems. Moreover, the altered responses in the mutant plants demonstrate that phototropism and inhibition of stem elongation are controlled by genetically distinct products and possibly different photoreceptor pigments.

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