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Arabidopsis Mutants Lacking Blue Light-Dependent Inhibition of Hypocotyl Elongation

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We have isolated a new class of photomorphogenic mutants in *Arabidopsis*. Hypocotyl elongation is not inhibited in the mutant seedlings by continuous blue light but is inhibited by far red light, indicating that these mutations are phenotypically different from the previously isolated long hypocotyl (*hy*) mutants. Complementation analysis indicated that recessive nuclear mutations at three genetic loci, designated *blu1*, *blu2*, and *blu3*, can result in the *blu* mutant phenotype and that these mutants are genetically distinct from other long hypocotyl mutants. The *BLU* genes appear to be important only during seedling development because the *blu* mutations have little effect on mature plants, whereas hypocotyl elongation and cotyledon expansion are altered in seedlings. The genetic separation of the blue and far red sensitivities of light-induced hypocotyl inhibition in the *blu* and *hy* mutants demonstrates that two photosensory systems function in this response.

INTRODUCTION

Light is particularly important to plant growth because it regulates many aspects of plant development and is the energy source for photosynthesis. Examples of light-dependent developmental processes include stem growth inhibition, leaf and root growth, tropic responses, germination, and flower induction. Red, blue, and UV light are especially effective in inducing photomorphogenic responses through the action of the red/far red photoreceptor phytochrome, blue/UV-A photoreceptor(s), and UV-B photoreceptor(s) (Mohr, 1986). However, phytochrome is the only plant photoreceptor pigment to be identified at the genetic and biochemical levels (Quail et al., 1987; Sharrock and Quail, 1989).

In general, blue light responses can be separated into two classes, low fluence responses and high irradiance responses, based on their fluence and exposure time requirements (Mancinelli and Rabino, 1978). Low fluence responses are typically inductive and require only a brief exposure to low fluence rate light for maximal response (Mancinelli and Rabino, 1978). Phototropism is an example of a low fluence blue response. In contrast, high irradiance responses require extended exposures to high fluence rate light for full expression of the response (Mancinelli and Rabino, 1978). The inhibition of hypocotyl elongation is considered a high irradiance response.

Many blue light responses have action spectra that most closely resemble the absorption spectra of riboflavin and some carotenoids (Schmidt, 1980). However, most studies suggest that the blue light receptor has a flavin-type chromophore. For example, inhibitors of carotenoid synthesis had no effect on phototropism in corn (Vierstra and Poff, 1981), and normal blue light responses have been reported in carotenoidless mutants of *Euglena* (Checcucci et al., 1966), *Phycomyces* (Presti et al., 1977), and *Neurospora* (Sargent et al., 1966). In addition, when riboflavin auxotrophs of *Phycomyces* were provided with the flavin analog roseoflavin, the action spectra of phototropism had spectral shifts corresponding to the absorption properties of roseoflavin compared with riboflavin (Otto et al., 1981).

Several recent studies have identified possible components of blue light signal transduction pathways. Gallagher et al. (1988) described an in vivo blue light treatment of pea stem segments that markedly decreased the in vivo phosphorylation of a plasma membrane-associated protein. Kinetic properties and fluence response relationships for the phosphorylation activity were found to be comparable with the kinetic and fluence response properties for phototropism in pea (Short and Briggs, 1990). A blue light-activated GTP-binding protein, which may be a component in the signal transduction pathway of low fluence blue responses, has been identified in plasma membrane preparations in pea (Warpeha et al., 1990). In addition, a blue light-induced membrane depolarization, which is mediated by a plasma membrane H^+-ATPase, has been implicated in the high irradiance reaction leading to inhibition of stem elongation in cucumber (Spalding and Cosgrove, 1989, 1990).

Mutants of *Arabidopsis* are proving to be particularly useful for investigating signal perception and transduction

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systems in plants (Olsen et al., 1984; Bleecker et al., 1988; Chory et al., 1989b; Wilson et al., 1990). Photomorphogenic mutants are no exception. A class of photomorphogenic mutants, designated *hy*, that have long hypocotyls in high irradiance white light have been isolated in *Arabidopsis* (Koornneef et al., 1980; Chory et al., 1989a). Six complementation groups have been described and five of these have been, or are likely to be, identified as components of phytochrome-regulated perception and signal transduction pathways. Three of them (*hy1*, *hy2*, and *hy6*) lack spectrophotometrically detectable phytochrome but have normal levels of the phytochrome polypeptide as detected by protein gel blotting (Koornneef et al., 1980; Chory et al., 1989a; Parks et al., 1989). The *hy3* and *hy5* mutants are probably response mutants because they have altered red and far red light sensitivity but have wild-type levels of phytochrome (Koornneef et al., 1980; Chory et al., 1989a; Parks et al., 1989).

Arabidopsis is the only higher plant for which blue light-response mutants have been identified. One of these, the *hy4* mutant line, shows reduced hypocotyl inhibition in blue light while maintaining normal phytochrome levels and red/far red responses (Koornneef et al., 1980). Unfortunately, no further characterization of this mutant has been made. The other blue light-response mutants that have been isolated are phototropism mutants and include reduced response mutants (Khurana et al., 1989), "null" phototropism mutants, and presumptive photoreceptor mutants (Khurana and Poff, 1989).

This paper presents the initial characterization of four mutant lines that fail to show blue light-dependent inhibition of hypocotyl elongation. Phytochrome-mediated inhibition of hypocotyl growth by far red light appears to be normal in these mutants. These mutants are genetically distinct from all of the long hypocotyl mutants previously isolated (Koornneef et al., 1980; Chory et al., 1989a) and represent three new loci involved in photomorphogenesis in seedlings of higher plants.

RESULTS

Isolation and Genetic Characterization of Blue Light-Response Mutants

To identify genes involved in blue light perception and signal transduction, we searched for mutants that exhibit altered blue light-regulated inhibition of hypocotyl elongation. Our approach was similar to one used in the isolation of the *hy* mutants of *Arabidopsis*, in which plants with long hypocotyls were selected from M_2 populations grown under high-intensity white light conditions that are inhibitory to hypocotyl elongation in wild-type plants (Redei and Hirono, 1964; Koornneef et al., 1980; Chory et al., 1989a). However, we used light only from the blue region of the electromagnetic spectrum to increase the probability of isolating blue light-specific response mutants. Under our blue light conditions, hypocotyl elongation in wild-type plants was 70% inhibited with respect to wild-type plants grown in the dark, as shown in Figure 1.

By using this selection scheme, we isolated 10 blue light-specific elongation mutants of *Arabidopsis* from 14 different populations of ethyl methanesulfonate-mutagenized M_2 seeds. Hypocotyl elongation in four of these mutants was not inhibited by blue light and resembled hypocotyl elongation in dark-grown seedlings (Figure 1). We designated this previously unidentified class of mutants as *blu*, for *b*lue *l*ight *u*ninhibited. Hypocotyl elongation in the remaining six mutants was intermediate between dark-grown and blue light-grown wild-type seedlings. We tentatively designated this class of mutants as *bli*, for *b*lue *l*ight *i*ntermediate. The histogram plots in Figure 2 of hypocotyl lengths of M_3 progeny homozygous for a *blu* and an intermediate mutant illustrate that these phenotypic classes are separable from wild-type plants when grown under blue light. However, we have not yet conducted further characterization of the intermediate class of mutants to determine whether any of them are leaky alleles of the *blu* mutants.

To determine the genetic basis for the *blu* phenotype, each mutant line was crossed to a wild-type plant and the hypocotyl phenotype was scored in F1 and F2 seedlings grown under continuous blue light. A summary of the genetic analysis is shown in Table 1. Each mutant line segregated three short hypocotyl plants to one long hypocotyl plant in the F2 generation. Reciprocal crosses indicated that each line represents a single recessive nuclear mutation. In addition, complementation analysis indicated that the four mutant lines represent three separate genetic loci: *blu1*, *blu2*, and *blu3*. Two of the mutations, *blu3-1* and *blu3-2*, are allelic.

Each of the *blu* mutant lines was crossed to each of the six *hy* mutant lines (Koornneef et al., 1980; Chory et al., 1989a) to test the genetic relationship between the *blu* and *hy* mutants. A summary of the complementation analysis between *blu* and *hy* mutants is shown in Table 2. All of the F1 progeny from these crosses had short hypocotyls when grown in continuous blue light, indicating that the *blu* mutant phenotype is genetically separable from that of the *hy* mutants and represents a new class of photomorphogenic mutants.

Characterization of Light Effects on *blu* Mutant Seedlings

Different regions of the electromagnetic spectrum vary greatly in the efficiency with which they inhibit hypocotyl elongation in wild-type plants, with blue and far red light being most effective (Kranz, 1977; Koornneef et al., 1980;

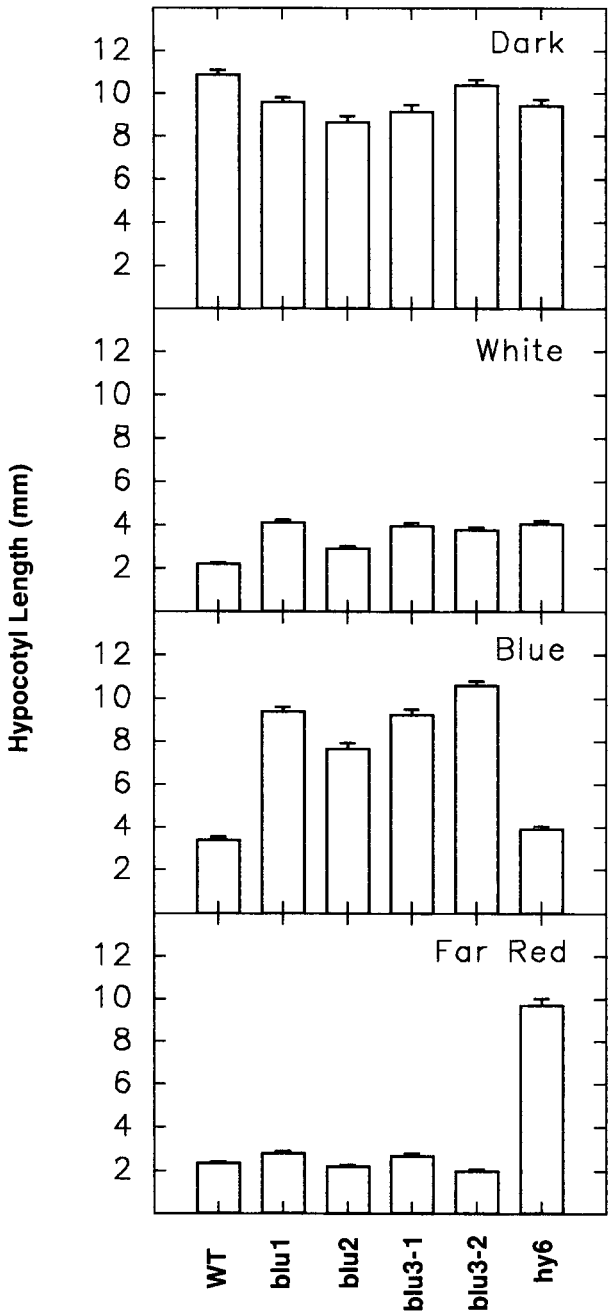

Figure 1. Effect of Light Quality on the Inhibition of Hypocotyl Elongation in Wild Type and *blu* and *hy6* Mutants of *Arabidopsis*.

Light was provided from above. Hypocotyl lengths (in millimeters) were measured after 5 days of growth. The *hy6* mutant is phytochrome deficient (Chory et al., 1989a). Each bar represents the mean measurement for 50 to 75 seedlings. Error bars indicate the standard error. Fluence rates were as follows: white, 45 ± 3 μmol m^{-2} sec^{-1}; blue, 56 ± 2 μmol m^{-2} sec^{-1}; far red, 30 ± 2 μmol m^{-2} sec^{-1}. WT, wild type.

J.C. Young and R.P. Hangarter, unpublished results). However, white light, which consists of all the visible wavelengths and represents more natural conditions, is more effective than blue or far red alone. Therefore, we investigated the response of *blu* seedlings to blue light, far red light, and white light.

As shown in Figure 1, hypocotyl elongation in seedlings of all four *blu* mutants grown in the dark or in continuous far red light was similar to wild-type seedlings grown under the same conditions. However, in continuous white light, *blu1*, *blu3-1*, and *blu3-2* seedlings were approximately twice as long as wild type, whereas *blu2* seedlings were only slightly longer than wild type. The most striking differences between the *blu* and wild-type seedlings are seen when seedlings are grown in blue light. In continuous blue light, wild-type hypocotyls were 70% shorter than their dark controls, whereas the *blu* mutants were uninhibited. In contrast, hypocotyls in the phytochrome-deficient mutant *hy6* were inhibited by blue light but in far red light grew as long as etiolated controls. Like the *blu* mutants, *hy6* was longer than wild type in white light.

Figure 3 is a representative photograph of wild type, *blu1*, and *hy6* under the different light conditions. Figure 3 also shows the phenotype of double mutants homozygous for *blu1* and *hy6*. The double mutants were essentially blind to blue and far red light, and even after 5 days of growth in continuous white light they had a typical etiolated phenotype. Although the double mutants eventually developed and reproduced, the plants were weak and their seed production was low. When enough seed of the double mutants become available, their phenotype will be characterized in detail.

After 5 days of growth, hypocotyl elongation varied only slightly among the different *blu* mutant lines in a given light condition (Figure 1). For example, *blu1* and *blu3-1* elongated the same amount for a given light condition. The mean hypocotyl length of *blu2* was 1 to 2 mm shorter than *blu1* and *blu3-1* in all light conditions. However, the germination experiment shown in Figure 4 indicates that the small differences in hypocotyl length may reflect the fact that radicle emergence occurred 6 to 12 hr later in *blu2* than in the other *blu* mutants. Seeds germinated without the red light pretreatment showed the same time course, but the germination percentages were lower for all the genotypes, indicating that the slower germination in the *blu* mutants is not light dependent (data not shown). Moreover, the *blu* phenotype was similar whether or not the seeds received the red light treatment, although germination was more uniform in the red light-treated seed.

Light-induced cotyledon expansion in the *blu* mutants was different from wild type, as illustrated in Table 3. In blue light, cotyledon expansion was reduced by about 60% in all of the *blu* mutants. In white light, cotyledon expansion was reduced by about 30% in *blu1* and *blu2* seedlings, whereas *blu3-1* and *blu3-2* cotyledons expanded similarly to wild-type cotyledons. Under far red

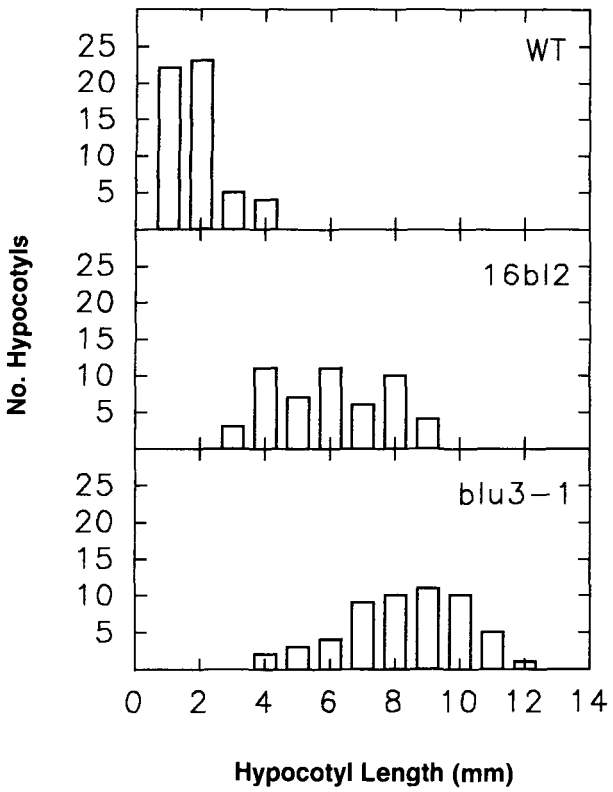

Figure 2. Histograms of Hypocotyl Lengths for Wild Type and Blue Light-Response Mutants of *Arabidopsis*.

Blue light was provided from above at a fluence rate of 56 ± 2 μmol m^{-2} sec^{-1}. Hypocotyl lengths (in millimeters) were measured after 5 days of growth. Line 16bl2 represents a typical *bli* type mutant, and line *blu3-1* represents a typical *blu* mutant. WT, wild type.

light, cotyledon expansion was reduced slightly in *blu2* seedlings, but expansion was enhanced in *blu3-1* and *blu3-2* when compared with wild-type cotyledons.

To determine whether the *blu* mutant phenotype was related to differences in photosynthetic capacity of the cotyledons, chlorophyll contents were measured. Table 4 shows that within each light condition, chlorophyll content and the chlorophyll *a*/*b* ratios were the same in *blu1* and wild-type cotyledons, indicating that chloroplast development was unaffected by the *blu* mutations.

Growth Features of Mature *blu* Mutant Plants

To determine whether the *blu* mutations exhibit pleiotropic effects in mature plants, a number of growth characteristics were examined in plants grown under white light. The data in Table 5 show that the morphology of 21- and 35-day-old *blu* mutants and wild-type plants was similar. Although chloroplast development is controlled partly by blue light (Richter and Ottersbach, 1990), total leaf chlorophyll content was similar in the *blu* mutants and wild-type plants grown in white light. The chlorophyll *a*/*b* ratio was between 2.6 and 2.8 in all of the plants. However, the accumulation of anthocyanin pigments, which is regulated by high-irradiance blue and UV light (Mancinelli and Rabino, 1978; Ohl et al., 1989), was enhanced threefold in *blu1* plants and reduced threefold in *blu2* plants exposed to high-irradiance white light. Anthocyanin induction was normal in *blu3-1* and *blu3-2*. Although anthocyanin induction in *blu1* and *blu2* was altered, it was the only consistently large difference observed among the various parameters measured between adult wild-type and *blu* plants. These results, when taken together, indicate that the *blu* mutations, which were identified at the seedling level, exert relatively benign effects during adult growth stages.

DISCUSSION

The common phenotype of the *blu* class of photomorphogenic mutants is the lack of blue light-dependent inhibition

Table 1. Genetic Analysis of *blu* Mutants[a]

Crosses		Short[a]	Long[a]	χ^2 [b]
blu1/blu1 × *BLU1/BLU1*[c]	F1	34	0	–
BLU1/blu1 × *BLU1/blu1*	F2	374	118	0.271[d]
blu2/blu2 × *BLU2/BLU2*[c]	F1	37	0	–
BLU2/blu2 × *BLU2/blu2*	F2	238	83	0.126[d]
blu3-1/blu3-1 × *BLU3-1/BLU3-1*[c,e]	F1	46	0	–
BLU3-1/blu3-1 × *BLU3-1/blu3-1*	F2	450	161	0.594[d]
blu1/blu1 × *blu2/blu2*	F1	18	0	–
blu1/blu1 × *blu3-1/blu3-1*	F1	27	0	–
blu1/blu1 × *blu3-2/blu3-2*	F1	16	0	–
blu2/blu2 × *blu3-1/blu3-1*	F1	39	0	–
blu2/blu2 × *blu3-2/blu3-2*	F1	16	0	–
blu3-1/blu3-1 × *blu3-2/blu3-2*	F1	0	14	–

[a] Wild type have short hypocotyls (≤6 mm); mutants have long hypocotyls (>6 mm).
[b] $P > 0.05$.
[c] Data represent mutant (♂):wild-type (♀) crosses; similar results were obtained with wild-type (♂):mutant (♀) crosses.
[d] χ^2 expected ratio, 3 wild type:1 mutant.
[e] Similar results were obtained with *blu3-2*.

Table 2. Complementation of *blu* Mutants with *hy* Mutants[a]

Crosses[b]		Short[a]	Long[a]
blu1/blu1 × *hy1/hy1*	F1	13	0
blu1/blu1 × *hy2/hy2*	F1	41	0
blu1/blu1 × *hy3/hy3*	F1	24	0
blu1/blu1 × *hy4/hy4*	F1	49	0
blu1/blu1 × *hy5/hy5*	F1	74	0
blu1/blu1 × *hy6/hy6*	F1	11	0
blu2/blu2 × *hy1/hy1*	F1	25	0
blu2/blu2 × *hy2/hy2*	F1	17	0
blu2/blu2 × *hy3/hy3*	F1	14	0
blu2/blu2 × *hy4/hy4*	F1	21	0
blu2/blu2 × *hy5/hy5*	F1	19	0
blu2/blu2 × *hy6/hy6*	F1	81	0
blu3-1/blu3-1 × *hy1/hy1*[c]	F1	47	0
blu3-1/blu3-1 × *hy2/hy2*[c]	F1	30	0
blu3-1/blu3-1 × *hy3/hy3*[c]	F1	56	0
blu3-1/blu3-1 × *hy4/hy4*[c]	F1	18	0
blu3-1/blu3-1 × *hy5/hy5*[c]	F1	70	0
blu3-1/blu3-1 × *hy6/hy6*[c]	F1	33	0

[a] Wild type have short hypocotyls (≤6 mm); mutants have long hypocotyls (>6 mm).
[b] Data represent *hy* (♂):*blu* (♀) crosses.
[c] Similar results were obtained with *blu3-2*.

of hypocotyl elongation. It appears that the *BLU* genes that control the blue light-induced inhibition of hypocotyl growth have little to do with growth of adult plants, suggesting that these genes are primarily involved in seedling development in *Arabidopsis*. This is different from the phytochrome-related *hy* mutants that show pleiotropic effects in adult plants (Koornneef et al., 1980; Chory et al., 1989a). The *blu* mutants, which are not inhibited in hypocotyl elongation by blue light, are, therefore, different from the previously isolated blue light-response mutant *hy4*, which is inhibited in hypocotyl elongation 35% to 40% by blue light (Koornneef et al., 1980). Furthermore, heterozygotes obtained from crosses between *blu* and *hy4* mutants have short hypocotyls, demonstrating genetic separation of the mutant phenotypes.

Because phytochrome can absorb blue and near-UV light, it has been difficult to exclude the possibility that phytochrome itself is involved in the blue light absorption responsible for high irradiance-dependent responses (Hartmann, 1966; Wildermann et al., 1978). However, until now, experiments testing this possibility relied on simultaneous exposures to different levels of blue and red or far red light to modulate the phytochrome photoequilibrium. The availability of mutants altered in specific blue light responses allows more direct approaches to testing the role of phytochrome in the high irradiance responses. Under the conditions we used to isolate them, the *blu* mutants lack blue light-mediated inhibition of hypocotyl elongation but exhibit normal far red inhibition, which is consistent with the involvement of two distinct photoreceptor systems. Similarly, the *hy4* mutant line has reduced sensitivity to blue light but retains red/far red sensitivity (Koornneef et al., 1980). Furthermore, the phytochrome-deficient *hy* mutants lack red/far red-dependent inhibition but show no change in blue light responsiveness (Koornneef et al., 1980; Chory et al., 1989a). As shown here, double mutants homozygous for *blu1* and the phytochrome-deficient *hy6* were insensitive to blue, far red, and white light. Together, these studies with the *blu* and *hy* mutants demonstrate unequivocally that two genetically distinct photosensory systems function in the high irradiance-induced inhibition of hypocotyl elongation and confirm physiological observations that suggest the existence of a separate blue light perception system (Holmes and Schafer, 1981; Cosgrove, 1982; Gaba et al., 1984; Laskowski and Briggs, 1989;

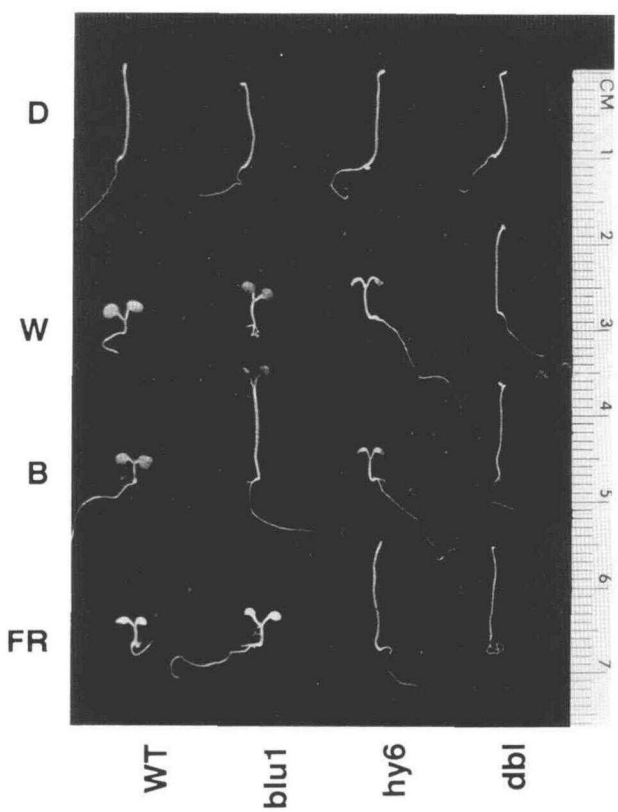

Figure 3. *Comparison of Wild-Type and Mutant Seedlings Grown* under Various Light Conditions.

Seedlings were photographed when 5 days old. Light conditions were as described in Figure 1. D, dark; W, white light; B, blue light; FR, far red light; dbl, *blu1/blu1 hy6/hy6* double mutant; WT, wild type.

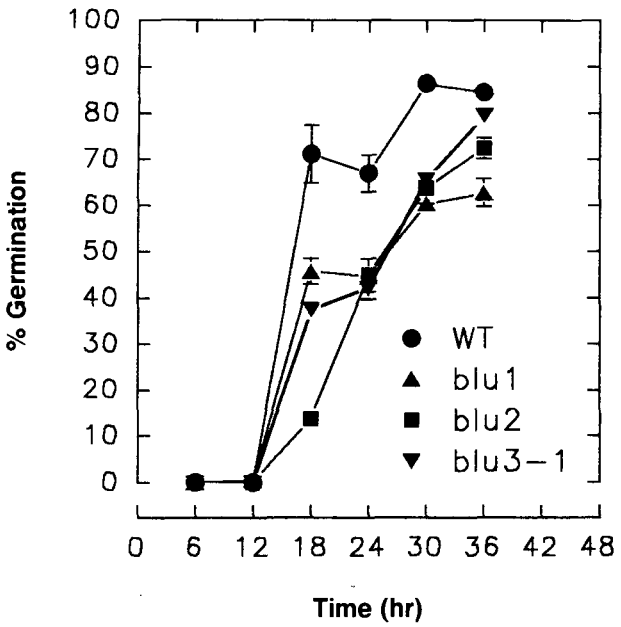

Figure 4. Time Course of Germination of Wild-Type and *blu* Mutant Seeds.

Seeds were given a standard cold and red light treatment (see Methods) and then incubated in the dark. A seed was considered to have germinated when the radicle emerged from the seed coat. The data are presented as the percentage of the seeds that had germinated at each time point. WT, wild type.

Warpeha and Kaufman, 1989). Moreover, the results with the *blu* mutants indicate that blue light-induced phytochrome photoconversion plays a relatively minor role in suppression of hypocotyl elongation in *Arabidopsis*.

Sarkar and Song (1982) have suggested that blue light may act through phytochrome transduction pathways by direct energy transfer from a blue light-absorbing flavin molecule to phytochrome. If blue and red/far red responses do share transduction pathways, the isolation of four mutant lines (the three *blu* mutants and *hy4*) altered in the blue light component of the inhibition of hypocotyl elongation but retaining phytochrome control suggests that at least four steps are unique to the blue light response system. Furthermore, no single gene mutants have yet been isolated that lack both blue- and red/far red-dependent inhibition of hypocotyl elongation. Thus, it is unlikely that high-irradiance blue and red/far red response systems have many signal transduction steps in common.

In addition to the inhibition of stem growth, blue and red/far red light regulate many other processes in the normal development of dicotyledonous plants, including chlorophyll and anthocyanin biosynthesis, chloroplast development, apical hook opening, and cotyledon expansion (Attridge, 1990). The results from our *blu* mutant lines suggest that cotyledon expansion and inhibition of stem elongation may be genetically coupled. The lack of blue light-dependent hypocotyl growth inhibition exhibited by the *blu* mutants was accompanied by a 60% reduction of cotyledon expansion. Furthermore, cotyledon expansion in *blu1* and *blu2* was greater in white light, which contains some red and far red light, than in blue light, suggesting that phytochrome is involved in the expression of the full developmental response. The induction of anthocyanin biosynthesis by high-irradiance light seems to be affected in *blu1* and *blu2*. In parsley, the UV light-induced activation of chalcone synthase was found to be modulated by a blue light-derived signal (Ohl et al., 1989). It is possible that the *blu1* and *blu2* mutations may be related to a similar modulation system in *Arabidopsis*. However, linkage analysis between the long hypocotyl phenotype and anthocyanin induction has to be completed before this possibility can be tested.

Several other blue light-sensitive photomorphogenic responses appear to be normal in the *blu* mutants. For example, opening of the apical hook appears qualitatively normal, but we need to examine fluence response relationships to determine whether the light conditions used in this study were saturating a low fluence response that might be altered in the mutants. Chlorophyll accumulation is apparently not affected by the *blu* mutations in seedlings or mature plants, indicating that the blue light response system controlling chloroplast development is different from the system controlling hypocotyl elongation. Interestingly, during the characterization of the *blu* mutants, we noticed that all of the *blu* mutant lines responded to phototropic stimuli (data not shown). As already mentioned, phototropism is a low fluence response, and the inhibition of hypocotyl elongation is a high irradiance response. However, both responses are dependent on changes in cell elongation and have similar blue light action spectra (Senger, 1980). Because hypocotyl elongation was not inhibited in the *blu* mutants when blue light was given from above but differential growth occurred when they

Table 3. Light-Dependent Cotyledon Expansion in *blu* Mutants

	Cotyledon Area (mm^2)[b]			
Light[a]	Wild Type	*blu1*	*blu2*	*blu3-1*[c]
White	41 ± 3	31 ± 4	30 ± 2	42 ± 3
Blue	46 ± 3	18 ± 1	18 ± 2	20 ± 3
Far red	24 ± 1	25 ± 3	19 ± 2	33 ± 1

[a] Actinic light conditions were as described in Figure 1.
[b] Data represent mean ± SE of 10 cotyledon pairs. Cotyledon area of dark-grown seedlings averaged 0.34 ± 0.01 mm^2 for all genotypes.
[c] Similar results were obtained with *blu3-2*.

Table 4. Chlorophyll Content in Cotyledons

	Wild Type		*blu1*[b]	
Light[a]	Total Chlorophyll[c]	Chlorophyll *a*/*b* Ratio	Total Chlorophyll[c]	Chlorophyll *a*/*b* Ratio
Dark	0.79	1.17	0.62	1.65
White	61.98	2.30	47.56	2.35
Blue	82.98	2.39	119.54	2.60
Far red	3.25	2.71	2.79	2.41

[a] Actinic light conditions were as described in Figure 1.
[b] Total chlorophyll is expressed as micromoles per microgram of total protein.
[c] Similar results were obtained with the other *blu* mutants.

were exposed to blue light from the side (data not shown), the two responses are probably independent, consistent with the demonstration that the onset of these responses can be kinetically separated in cucumber hypocotyls (Cosgrove, 1985). A detailed investigation of the phototropic response in the *blu* mutants is in progress.

The complexity of photomorphogenic responses in the dicot seedling is astounding (Attridge, 1990). Isolation of mutants that have attenuated or nullified photomorphogenic responses demonstrates the potential for identifying, at the molecular level, components of light perception/transduction pathways leading to those responses. The *blu* mutants represent a new class of genes that will aid in the dissection of the complex network of light-regulated responses.

METHODS

Plant Materials, Growth Conditions, and Light Sources

Arabidopsis thaliana ecotype Columbia homozygous for the recessive *gl1* mutation (Koornneef et al., 1982) was the parental strain used for the isolation of blue light-response mutants. The *hy6* mutant was also in the Columbia background (Chory et al., 1989a). The other *hy* mutants were in the Landsberg *erecta* background (Koornneef et al., 1980). Seeds were surface sterilized for 20 min in 30% (v/v) commercial bleach, rinsed several times with sterile H_2O, and planted in Petri dishes containing growth medium consisting of Murashige and Skoog salts (Murashige and Skoog, 1962), 2% (w/v) sucrose, and 0.8% (w/v) agar. Seeds were incubated on this medium in the dark for 2 to 3 days at 4 ± 1°C. The cold-conditioned seeds were exposed to saturating red light for 30 min to induce germination (Cone and Kendrick, 1985), then transferred to the dark for 23.5 hr at 23 ± 2°C. The dishes were then moved to the various light conditions. When appropriate, seedlings (5 to 7 days old) were transferred (four seedlings/10-cm pot) to a soil-less growth medium (Cornell-Mix:sand, 3:1) saturated with nutrient solution (Estelle and Somerville, 1987) and grown to maturity under constant illumination (65 ± 5 μmol m^{-2} sec^{-1}) at 23 ± 2°C. Potted plants were watered daily with distilled H_2O and once a week with nutrient solution (Estelle and Somerville, 1987).

Light for potted plants and white light treatments was provided from cool-white fluorescent lamps (F96T12-CW). Red light (10 μmol m^{-2} sec^{-1}) for induction of germination was obtained by filtering light from cool-white fluorescent lamps (F48T12-CW-

Table 5. Growth Features of *blu* Mutants

	Wild Type	*blu1*	*blu2*	*blu3-1*[a]
21 days				
Rosette diameter (cm)	3.2 ± 0.1	3.9 ± 0.1	2.7 ± 0.2	3.7 ± 0.1
Blade length/width ratio[b]	1.3 ± 0.1	2.0 ± 0.1	1.5 ± 0.1	1.7 ± 0.1
Petiole/blade ratio[b]	1.0 ± 0.1	0.7 ± 0.1	1.2 ± 0.2	1.0 ± 0.1
Chlorophyll content (mmol g^{-1}, fresh weight)[c]	426 ± 15	451 ± 12	493 ± 18	371 ± 20
Anthocyanin induction[d]	6.7 - 6.8	19.9 - 20.0	2.0 - 2.1	5.1 - 5.2
35 days				
Length of main stem (cm)	21.4 ± 1.0	22.8 ± 0.9	17.4 ± 1.5	19.8 ± 1.5
Apical dominance[e]	1.3 ± 0.2	2.1 ± 0.3	1.1 ± 0.2	2.0 ± 0.2

Seeds were grown on agar plates for 7 days in continuous white light (45 ± 3 μmol m^{-2} sec^{-1}) and then transferred to soil and grown in continuous white light at a fluence rate of 65 ± 5 μmol m^{-2} sec^{-1}. Wild-type data represent mean ± SE from six plants. *blu* mutant data represent mean ± SE from 10 plants.
[a] Similar results were observed with *blu3-2*.
[b] Determined from measurements on the longest rosette leaf of each plant.
[c] Chlorophyll *a*/*b* ratios were consistent (2.6 to 2.8) between genotypes.
[d] Data represent the ratio of anthocyanin content in induced versus uninduced plants (see Methods for details).
[e] Data represent the number of out-grown side shoots in the rosette.

1500) through one layer of Rohm and Haas red plexiglass no. 2444 (3.18 mm thick, Dayton Plastics, Columbus, OH), one layer of yellow Roscolux no. 10, and 1 cm of a 1.5% (w/v) $CuSO_4 \cdot 7H_2O$ solution. Blue light used for isolation of mutants was obtained by filtering light from four halogen flood lamps (GE 150W Quartzline) through 5 cm of 1.5% $CuSO_4 \cdot 7H_2O$ and one layer of Rohm and Haas blue plexiglass no. 2045 (3.18 mm thick, Dayton Plastics). The $CuSO_4 \cdot 7H_2O$ solution was cooled by running cold tap water through copper tubing submerged in the solution. The resulting spectral output had a peak intensity at 480 nm and a 100 nm half-bandwidth. Blue light for characterization of mutants was obtained by filtering light from one halogen flood lamp through 3 cm of water-cooled 1.5% $CuSO_4 \cdot 7H_2O$ and a wide-band blue interference filter (5 cm^2). The filtered light had a peak intensity at 470 nm and a 100 nm half-bandwidth. Far red light was obtained by filtering light from one halogen flood lamp through 3 cm of H_2O and one layer of far red plexiglass no. FRF 700 (3.18 mm thick, Westlake Plastics Co., Lenni, PA). The fluence rate for far red light was measured for wavelengths between 710 and 750 nm because far red-absorbing phytochrome absorbs maximally at 730 nm. Wavelengths above 750 nm were assumed to be inactive in the responses we tested. Fluence rates were adjusted by changing the lamp voltage or by changing the distance between plant material and the light source. Fluence rates at the level of seedlings were measured with an LI-1800 portable spectroradiometer (LiCor, Inc., Lincoln, NE).

Isolation of Mutants and Genetic Analysis

Ethyl methanesulfonate-mutagenized M_2 seeds were obtained from Lehle Seeds (Tucson, AZ). Approximately 3000 to 3500 M_2 seeds were screened from each of 14 independent populations. After the cold and red light germination-induction treatment, M_2 seeds were transferred to 56 ± 2 μmol m^{-2} sec^{-1} of blue light given from directly above. Seedlings were scored for hypocotyl length after 4 days of growth in continuous blue light. Forty-seven putative long hypocotyl mutants were recovered using this selection. Thirty-one putative mutants survived to maturity after transfer to soil-less growth medium and were allowed to self-pollinate. The resulting M_3 seeds were rescreened for the long hypocotyl phenotype in blue light. Ten nonlethal, fertile, blue light-response mutants were recovered from these M_3 populations. The four mutants with the longest hypocotyls were analyzed further in the M_4 generation. Each of these four mutants was crossed to wild type to determine patterns of inheritance. Allelism was tested by crossing the blue light-response mutants to each other and to the six *hy* mutants (Koornneef et al., 1980; Chory et al., 1989a). Double mutants homozygous for *blu1* and *hy6* were obtained from F2 progeny of a cross between *blu1* and *hy6*. Selection was done under white light where the double mutants grew as long as dark controls. A number of the double mutants survived to maturity and set seed. Before using these F3 seeds for the experiment in Figure 3, several individual seeds were grown under blue and far red light to confirm that the seedlings showed both *blu1* and *hy6* phenotypes.

Hypocotyl lengths were measured to the nearest millimeter on 5-day-old seedlings with opened cotyledons and no seed coat attached so that late-germinating seeds were not included in the measurements. During the segregation analysis with the *blu* mutants, hypocotyls were considered long if they were longer than 6 mm (Figure 2).

Measurement of Cotyledon Expansion

Cotyledon pairs were excised from 5-day-old seedlings and placed onto transparent tape. Images of the cotyledons were projected from a photographic enlarger (magnification ×10) and traced on paper. The paper tracings were cut out and weighed, and the areas were calculated from the weight.

Extraction and Analysis of Chlorophyll and Anthocyanin Pigments

For chlorophyll determination in seedlings, cotyledon pairs were excised from 5-day-old seedlings grown under the indicated light conditions. For chlorophyll determinations in leaves, plants were grown in continuous white light at a fluence rate of 65 ± 5 μmol m^{-2} sec^{-1}. Anthocyanin pigments were induced to form by transferring 19-day-old plants to the dark for 24 hr, followed by 24 hr of high-intensity white light (300 μmol m^{-2} sec^{-1}). Control plants were left in the normal growth conditions for the entire 21 days.

Leaves (0.1 to 0.5 g, fresh weight) or cotyledons (10 pairs) were ground in 200 μL of ethanol to extract chlorophylls (McCourt et al., 1987) and anthocyanins. The sample was centrifuged in a microcentrifuge for 5 min at 10,000 rpm and the supernatant removed. The pellet was re-extracted in 50 μL of ethanol, the supernatants were combined, and acetone was added to give a final acetone concentration of 80% (v/v). Chlorophyll contents were determined in the final extract as described by Graan and Ort (1984).

To determine the anthocyanin content, the acetone and ethanol in the final extract were evaporated at reduced pressure at 65°C. The remaining aqueous phase was extracted with chloroform (2:1) to remove chlorophyll. The aqueous phase was acidified with 20 μL of concentrated HCl (Chory et al., 1989a) and brought to a final volume of 2 mL with H_2O. Quantification of anthocyanin pigments followed the methods of Feinbaum and Ausubel (1988) and were normalized to the fresh weight used in each sample.

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