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Suppressors of an *Arabidopsis thaliana phyB* Mutation Identify Genes That Control Light Signaling and Hypocotyl Elongation

Jason W. Reed,* Rangasamy P. Elumalai* and Joanne Chory†

**Biology Department, University of North Carolina, Chapel Hill, North Carolina 27599-3280, and †Howard Hughes Medical Institute, Plant Biology Laboratory, The Salk Institute, San Diego, California 92186-5800*

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ABSTRACT

Ambient light controls the development and physiology of plants. The *Arabidopsis thaliana* photoreceptor phytochrome B (PHYB) regulates developmental light responses at both seedling and adult stages. To identify genes that mediate control of development by light, we screened for suppressors of the long hypocotyl phenotype caused by a *phyB* mutation. Genetic analyses show that the *shy* (*s*hort *hy*pocotyl) mutations we have isolated fall in several loci. Phenotypes of the mutants suggest that some of the genes identified have functions in control of light responses. Other loci specifically affect cell elongation or expansion.

PLANTS adjust their development in response to ambient wind, temperature, water, and light. Such adjustments allow plants to grow in a variety of sites and to adapt to seasonal changes in external conditions. Light is among the most relevant environmental signals because plants use light for photosynthesis and because light conditions reflect both the local growth environment and diurnal and seasonal time (Smith 1994). Plants sense the light environment using a battery of photoreceptors that specifically control development. These include red/far-red light photoreceptors called phytochromes, blue light photoreceptors called cryptochromes, and unnamed photoreceptors that mediate phototropism and UV light responses (von Arnim and Deng 1996; Fankhauser and Chory 1997).

The phytochromes are the most extensively characterized developmental photoreceptors in plants (Quail 1991; Furuya 1993). They are soluble dimeric proteins, and each ~120-kD monomer has a covalently attached linear tetrapyrrole chromophore. Phytochromes are synthesized in the dark in a red light–absorbing form called Pr. Upon absorption of red light, they are converted to a far-red light absorbing form called Pfr. On the basis of physiological, genetic, and biochemical studies, Pfr is thought to be the active form. Studies of seed germination have suggested that Pr may also have an activity that counteracts the activity of the Pfr form (Reed *et al.* 1994; Shinomura *et al.* 1994).

Although the mechanisms of phytochrome signal transduction are uncertain, several models have been proposed. A cyanobacterial phytochrome homolog signals by a phosphorelay mechanism (Yeh *et al.* 1997), suggesting that higher plant phytochromes might also signal in this fashion. Pharmacological studies have suggested that phytochromes may act through branched signaling pathways involving G proteins, cyclic GMP, and calcium (Shacklock *et al.* 1992; Neuhaus *et al.* 1993; Bowler *et al.* 1994). More recently, it has been reported that phytochrome B migrates to the nucleus in the light, suggesting that phytochromes may signal in the nucleus as well as in the cytoplasm (Sakamoto and Nagatani 1996).

The genetics of plant light responses have been studied most extensively in *Arabidopsis thaliana.* Arabidopsis has five genes that encode phytochrome apoproteins (Sharrock and Quail 1989; Clack *et al.* 1994), and mutations are known in three of these, *PHYA* (Dehesh *et al.* 1993; Whitelam *et al.* 1993; Reed *et al.* 1994), *PHYB* (Koornneef *et al.* 1980; Reed *et al.* 1993), and *PHYD* (Aukerman *et al.* 1997). Analyses of the phenotypes of these mutants have shown that these three phytochromes each mediate overlapping subsets of light responses, but often do so under distinct light conditions. For example, *phyA* mutant seeds germinate poorly in response to very low fluence light over a wide spectral range (Botto *et al.* 1996; Shinomura *et al.* 1996), whereas *phyB* mutant seeds germinate poorly in response to red light (Reed *et al.* 1994; Shinomura *et al.* 1994, 1996). *phyA* mutants fail to inhibit hypocotyl elongation in response to far-red light (Nagatani *et al.* 1993; Parks and Quail 1993; Whitelam *et al.* 1993), whereas *phyB* mutants fail to inhibit hypocotyl elongation in response to red light (Koornneef *et al.* 1980). Finally, *phyA* mutants flower later than wild-type plants in response to night breaks or day length extensions (Johnson *et al.* 1994; Reed *et al.* 1994), whereas *phyB* mutants flower earlier than wild-type plants under a variety of conditions (Goto *et al.* 1991; Reed *et al.* 1993;

Corresponding author: Jason W. Reed, Department of Biology, University of North Carolina, CB #3280, Coker Hall, Chapel Hill, NC 27599-3280. E-mail: jreed@email.unc.edu

Bagnall *et al.* 1995; Koornneef *et al.* 1995). Effects of *phyA* and *phyD* mutations on the inhibition of hypocotyl elongation by red light can be seen in a *phyB* mutant background (Reed *et al.* 1994; Aukerman *et al.* 1997). These results reveal that the multiplicity of phytochromes serves in part to increase the versatility of the plant in responding to different light environments. These phenotypic analyses also suggest that the signaling pathways initiated by the different phytochromes may overlap. *hy4* mutants deficient in the blue light photoreceptor cryptochrome 1 have some phenotypes in common with the phytochrome-deficient mutants (Koornneef *et al.* 1980; Young *et al.* 1992; Ahmad and Cashmore 1993), suggesting that blue light signaling pathways also converge with phytochrome signaling pathways. Physiological analyses of photoreceptor mutants have also suggested that blue and red light systems interact functionally (Casal and Boccalandro 1995; Ahmad and Cashmore 1997).

Mutations that identify possible downstream components of phytochrome signaling have been isolated in long hypocotyl screens, in screens for early flowering mutants, and in screens for seedlings with characteristics of light-grown plants in the dark. The *fhy1* and *fhy3* mutants have long hypocotyls in far-red light, suggesting that they may have lesions in a PHYA-specific signaling pathway (Whitelam *et al.* 1993; Barnes *et al.* 1996). *hy5* mutants have long hypocotyls under all light conditions, suggesting that HY5 may act downstream of the convergence of different photoreceptor pathways (Koornneef *et al.* 1980). Consistent with this idea, *HY5* encodes a basic leucine zipper transcription factor (Oyama *et al.* 1997). *elf3* mutants have an elongated hypocotyl under all light conditions, and they flower early (Zagotta *et al.* 1996). The *cr88*, *pef1*, *pef2*, *pef3*, and *red1* mutants have long hypocotyls in red light (*cr88*, *pef2*, *pef3*, and *red1*) or in both red and far-red light (*pef1*; Ahmad and Cashmore 1996; Lin and Cheng 1997; Wagner *et al.* 1997). These genes may encode positive regulators of light signaling.

Screens for mutants with short hypocotyls and leaf development in the dark have yielded candidate negative regulators. A series of *cop*, *det*, and *fus* mutants have short hypocotyls, develop leaves, and express light-induced genes in the dark (reviewed in Wei and Deng 1996; Fankhauser and Chory 1997). The biochemical functions of the products of these genes remain unclear. However, the DET1, COP1, COP9, and FUS6 proteins localize to the nucleus, suggesting that they may repress gene expression in the dark (Pepper *et al.* 1994; von Arnim and Deng 1994; Chamovitz *et al.* 1996; Staub *et al.* 1996). Double-mutant plants that carry photoreceptor mutations and *det/cop/fus* mutations have phenotypes consistent with the DET/COP/FUS gene products functioning downstream of photoreceptor pathways (Chory 1992; Ang and Deng 1994; Miséra *et al.* 1994; Wei *et al.* 1994a,b). More detailed analysis of *cop/det/fus* mutant phenotypes suggests that these genes may play a more general role in regulating gene expression in response to a variety of stimuli in addition to light. For example, *det1* seedlings express *LHCB* (encoding light harvesting chlorophyll a/b–binding protein) and other genes in roots (Chory and Peto 1990); and *cop1*, *det1*, and *cop9* mutants overexpress genes that are normally activated by pathogen infection, hypoxia, or developmental signals, as well as genes normally activated by light (Mayer *et al.* 1996). Finally, several other mutants with subsets of the phenotypes described above have been described, including *det2*, *det3*, *cop2*, *cop3*, *cop4*, and *doc1* (Chory *et al.* 1991; Cabrera y Poch *et al.* 1993; Hou *et al.* 1993; Li *et al.* 1994).

Although many genes involved in light signaling have been identified in these screens, it is likely that numerous other relevant loci remain to be discovered. In other systems, screens for suppressors and enhancers of mutations in a pathway have identified important new genes (for example, Karim *et al.* 1996). These genetic methodologies have been used less frequently in plants (Koornneef *et al.* 1982; Niyogi *et al.* 1993; Carol *et al.* 1995; Cernac *et al.* 1997; Silverstone *et al.* 1997), but promise to become very useful for dissecting light responses. A screen for suppressors of a *hy2* phytochrome chromophore–deficient mutation (Kim *et al.* 1996) and a screen for suppressors of a *det1* mutation (Pepper and Chory 1997) have recently revealed more candidate light-signaling mutations. To identify other genes involved in light signaling, we have conducted a screen for mutations that suppress the long hypocotyl phenotype caused by a *phyB* mutation. In this report, we describe the results of our initial screen. Following the precedent established by Kim *et al.* (1996), we have called these new mutations *shy* (for *s*hort *hy*pocotyl and *s*uppressor of *hy*). We have characterized various phenotypes of the mutants, allowing us to identify those that affect light signaling.

MATERIALS AND METHODS

Mutagenesis and genetic methods: Mutant *phyB-1* (previously called *hy3-Bo64*) is in the Landsberg *erecta* background and has a stop codon in the *PHYB* coding sequence (Koornneef *et al.* 1980; Reed *et al.* 1993; Quail *et al.* 1994). We incubated 5000 *phyB-1* M1 seeds overnight in 0.3% ethyl methane sulfonate and then rinsed several times with water. We collected M2 progeny in eight batches from ~600 M1 plants per batch. We screened ~14,000 M2 seedlings on MS/sucrose/agar plates (see below) under white light for short hypocotyl variants. To detect revertants of the starting *phyB-1* mutation and to follow the *phyB-1* mutation in subsequent genetic manipulations, we assayed for the presence (wild-type allele) or absence (*phyB-1* mutant allele) of an *AlwN*I restriction site in a *PHYB*-specific PCR product amplified from chromosomal DNA of the plant being tested (Reed *et al.* 1993). Mutants were judged to be independent if they came from different batches or if they had clearly distinguishable phenotypes.

To separate the *shy* mutations from the starting *phyB-1* mutation, we crossed the *phyB-1 shy* strains to wild-type Landsberg

erecta, allowed these F_1 plants to self-fertilize, and identified *PHYB/PHYB shy/shy* progeny in the F_2 or F_3 generation. For *shy* mutations that confer an obvious phenotype (such as dwarfism) in a wild-type background we identified *PHYB/PHYB shy/shy* plants in the F_2 generation. For mutations that confer a phenotype similar to the wild-type phenotype in a *phyB-1* mutant background and that confer no dramatic phenotype in the wild-type *PHYB* background, we identified *PHYB/phyB-1* heterozygous F_2 plants from the outcross to wild type that did not segregate tall (*phyB-1/phyB-1 SHY/SHY* or *phyB-1/phyB-1 SHY/shy*) F_3 progeny. The failure to segregate tall F_3 plants indicated that these F_2 plants were homozygous for the *shy* mutation (or that the *shy* mutation was linked to the *phyB-1* mutation, a possibility tested in the mapping experiments described below). From the F_3 progeny populations, we identified *PHYB/PHYB* individuals of genotype *PHYB/PHYB shy/shy.* In these genetic manipulations, we distinguished the *PHYB* and *phyB-1* alleles by the PCR-based assay described above.

We mapped the *shy* mutations using PCR-based SSLP and CAPS markers polymorphic between Landsberg and Columbia ecotypic backgrounds (Konieczny and Ausubel 1993; Bell and Ecker 1994). We crossed *phyB-1/phyB-1 shy/shy* plants (Landsberg *erecta* background) with *phyB-9/phyB-9* plants (Columbia background), and assayed DNA from individual F_2 progeny for Landsberg- or Columbia-specific polymorphisms. Both the *phyB-1* and *phyB-9* mutations create stop codons in the *PHYB*-coding sequence (Reed *et al.* 1993). Since suppressor mutations isolated in this screen should bypass the requirement for PHYB, we expected the *phyB-1* and *phyB-9* alleles to behave equivalently in the mapping populations. In cases where we suspected that a mutation was allelic to a previously known mutation, we performed complementation tests between the *shy* mutation and the previously described mutation, and we looked for lack of segregation of F_2 plants with a wild-type phenotype, indicating that the two mutations mapped to the same location. In cases where we found allelism to a previous locus, we have given the new mutation an allele designation that incorporates the established gene name.

Phenotypic tests: Seeds were surface sterilized and plated on Murashige and Skoog (MS)/agar plates [1× MS salts (GIBCO, Grand Island, NY), 0.8% phytagar (GIBCO), 1× Gamborg's B5 vitamin mix (Sigma, St. Louis, MO)] with or without 2% sucrose, stored overnight at 4°, and moved to the appropriate light condition. For hypocotyl length tests in red light, we used LED red light sources emitting light with a peak at 670 nm and a half bandwidth of 25 nm (Quantum Devices, Inc., Barneveld, WI). For far-red light, we used LED sources emitting light with a peak at 730 nm and a half bandwidth of 25 nm (Quantum Devices). For blue light, we used cool white fluorescent bulbs filtered through Schott blue glass filter No. 5-57 (Newport Industrial Glass, Costa Mesa, CA). For fluence rate/response experiments, light was filtered through various thicknesses of bronze plexiglass No. 2412 (Golden Rule Plastics, Haw River, NC). This filter causes minimal distortion of the light spectrum (data not shown). Light levels were measured with an LI-189 quantum radiometer (Li-Cor, Lincoln, NE), or extrapolated based on numbers of layers of plexiglass. For red and far-red fluence rate–response experiments, we grew seedlings on MS/agar plates (without sucrose) that were placed vertically behind various thicknesses of plexiglass, with the light source placed so as to project horizontally. After 5 days, we took digital images of the plates with a CCD camera and measured the hypocotyl lengths using image analysis software (NIH Image, Bethesda, MD). For root length experiments, we grew seedlings in red light (30–50 μmol · m^{-2} · sec^{-1}) on vertical MS/sucrose/agar plates for 5 days and measured roots against a ruler. In other experiments and for all blue light experiments, we grew plants on plates placed horizontally and measured hypocotyl lengths against a ruler.

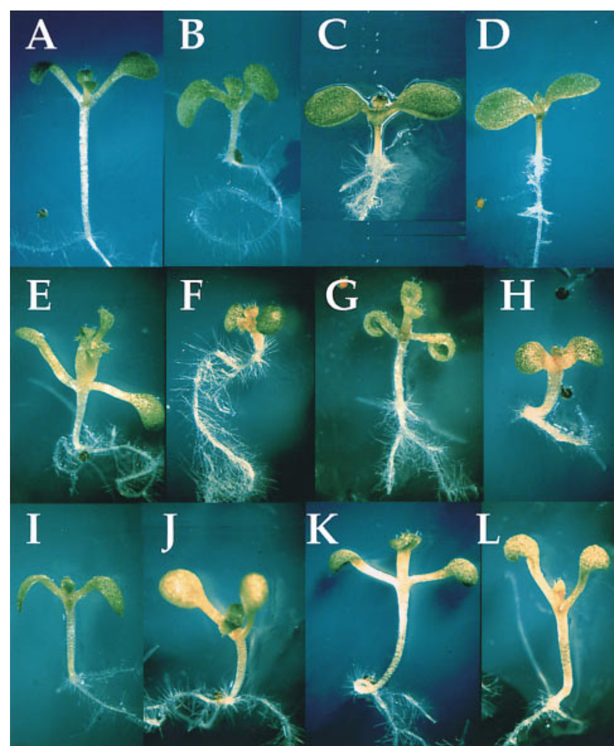

Figure 1.—*phyB-1 shy* seedlings grown in white light. Seedlings were grown for 8 days on MS/sucrose plates. Genotypes shown are (A) *phyB-1* (starting strain), (B) Landsberg *erecta* (wild-type parent), (C) *phyB-1 shy2-2*, (D) *phyB-1 shy2-3*, (E) *phyB-1 amp1-4*, (F) *phyB-1 shy-115*, (G) *phyB-1 pom1-15*, (H) *phyB-1 bot1-5*, (I) *phyB-1 shy3-1*, (J) *phyB-1 shy4-1*, (K) *phyB-1 shy5-1*, and (L) *phyB-1 shy6-1. shy4-1* and *shy4-2* seedlings appeared similar to each other, and *pom1-14* and *pom1-15* seedlings appeared similar to each other. Therefore, only one mutant at each locus is shown.

For flowering time determinations, we grew seedlings on MS/sucrose/agar plates for 10–14 days and then transplanted them to soil (Pro-Mix BX; Hummert, St. Louis, MO). Experiments were performed in a Conviron growth chamber at 21°. Light was provided on a 9 h:15 h day:night cycle from 12 fluorescent (F72T12/CW/VHO, 160 W) and six incandescent (60 W) bulbs, and had an intensity at plant height of 100–230 μmol · m^{-2} · sec^{-1}, depending on the experiment. We repeated the experiment six times, testing each genotype in between one and five different experiments (average = 2.5).

RESULTS

Isolation and genetic analysis of *shy* mutants: From our screen, we isolated 13 independent *shy* (*s*hort *hy*pocotyl, or *s*uppressor of *hy*) variants with mutations at sites distinct from the starting *phyB* mutation (see materials and methods). As described below, we have named those that turned out to be alleles of previously known loci according to the established gene names. Figure 1 shows *phyB-1 shy* mutant seedlings grown for 8 days in white light. They fall into several distinct phenotypic

TABLE 1

Classes of *shy* mutations

Mutation	Phenotype	Dom./rec.[a]	Allelism[b]
shy2-2	Leaves curl upward	Semi-D	*shy2-1*
shy2-3	Leaves curl upward	Semi-D	*shy2-2*
amp1-4	Extra leaf production	r	*amp/pt*
shy-115	Brassinosteroid-deficient dwarf	r	
shy-802	Brassinosteroid-deficient dwarf	r[c]	
pom1-14	Crooked hypocotyl in dark	Semi-D	*pom1-7*
pom1-15	Crooked hypocotyl in dark	r	*pom1-14*
bot1-5	Short, round tissues	r	*botero1-1*
shy3-1	Quantitative changes	Semi-D	
shy4-1	Quantitative changes	r	*shy4-2*
shy4-2	Quantitative changes	r	*shy4-1*
shy5-1	Quantitative changes	r	
shy6-1	Quantitative changes	r	

[a] Dominance, semidominance, or recessiveness for hypocotyl length in red light (Table 2).
[b] Indicated are mutations found to be allelic to *shy* mutations on the basis of complementation and mapping data (see text). In addition, we have recently obtained DNA sequence evidence that the partially dominant mutations *shy2-1*, *shy2-2*, and *shy2-3* each have a mutation in the *SHY2* gene (Q. Tian and J. W. Reed, unpublished result).
[c] Although we did not analyze the dominance or recessiveness of *shy-802* quantitatively (Table 2), observation of F_1 seed of a backcross indicated that the dwarf phenotype was substantially if not completely recessive (data not shown).

classes, as summarized in Table 1 and described in more detail below.

To assess the degree of dominance or recessiveness of the *shy* mutations in the *phyB-1* mutant background, we measured hypocotyl lengths after growth in constant red light, as this parameter is sensitive and easy to score. We compared hypocotyl lengths of *phyB-1/phyB-1 SHY/shy* F_1 plants with those of the *phyB-1/phyB-1 SHY/SHY* and *phyB-1/phyB-1 shy/shy* parents. As shown in Table 2, the *amp1-4*, *shy-115*, *shy-802*, *pom1-15*, *bot1-5*, *shy4-1*, *shy4-2*, *shy5-1*, and *shy6-1* heterozygous seedlings were the same height as *SHY/SHY* seedlings, indicating that these mutations are each recessive for hypocotyl length. In contrast, the *shy2-2*, *shy2-3*, *pom1-14*, and *shy3-1* heterozygous seedlings were significantly shorter than the *SHY/SHY* seedlings and significantly taller than the corresponding *shy/shy* seedlings, indicating that these mutations are each partially dominant.

To determine whether the short hypocotyl phenotype of these mutants was caused by mutation at a single locus, we checked the segregation of the short hypocotyl phenotype in the F_2 generation of these backcrosses. For each mutant, the phenotype segregated in a manner consistent with a mutation at a single locus (Table 3).

We found three allelic pairs in our screen, at the *SHY2*, *POM1*, and *SHY4* loci. As the *shy2* mutations are partially dominant, our assessment that they are alleles of *SHY2* (and of each other) is based on their conferring similar phenotypes as *shy2-1* does (Kim *et al.* 1996), as well as mapping to the same location (Table 4). In addition, we have recently obtained independent molecular evidence that *shy2-1*, *shy2-2*, and *shy2-3* are allelic (Q. Tian and J. W. Reed, unpublished data). As we identified single mutations at the remaining seven loci described here, the screen has not been saturated, and there are probably several other loci that can be mutated to give a shy phenotype.

We mapped the *shy* mutations by outcrossing them to a different ecotype and assaying polymorphic markers or by establishing allelism with previously mapped mutations (see materials and methods). Mapping results are summarized in Table 4. We obtained linkage to a polymorphic marker for all the loci except for the *shy5-1* mutation, for which different mapping populations failed to show consistent linkage (data not shown). The 13 mutations fall in three previously known genes, *AMP*, *SHY2*, and *POM1* (Chaudhury *et al.* 1993; Hauser *et al.* 1995; Kim *et al.* 1996); two probable *DWF* genes (mutated in *shy-115* and *shy-802*, but not assigned by complementation—see below; Feldmann *et al.* 1989); and five new genes, *BOT1* (*BOTERO1*; H. Höfte, personal communication), *SHY3*, *SHY4*, *SHY5*, and *SHY6*.

To determine whether any of the *shy* mutations completely quench PHYB signaling, we tested whether the *shy* mutations were epistatic to the *phyB-1* mutation. We compared the hypocotyl lengths of *phyB-1 shy* double mutants with that of the *phyB-1* single mutant and with those of the corresponding *shy* single mutants. For each mutant, we identified *PHYB shy* plants among progeny of an outcross to a wild-type Landsberg *erecta* plant (see

TABLE 2

Hypocotyl lengths of *phyB-1/phyB-1 shy/shy* and *phyB-1/phyB-1 SHY/shy* seedlings in red light[a]

	shy/shy seedlings					*SHY/shy* seedlings				
shy mutation	*x*	σ	*n*	t^b	P^b	*x*	σ	*n*	t^c	P^c
shy2-2	2.5	0.4	20	13.9	<0.001	4.1	0.3	20	17.1	<0.001
shy2-3	4.2	1.0	20	10.12	<0.001	7.4	0.8	16	6.50	<0.001
amp1-4	4.4	1.1	20	10.4	<0.001	9.7	2.2	20	0.51	>0.5
shy-115	4.0	1.0	20	9.89	<0.001	8.9	1.3	7	1.71	<0.1
pom1-14	2.2	0.4	40	12.0	<0.001	7.5	2.7	22	6.07	<0.001
pom1-15	2.6	0.7	20	16.47	<0.001	10.1	1.7	10	0.40	>0.5
bot1-5	2.6	0.6	40	13.3	<0.001	9.8	3.3	26	0.25	>0.5
shy3-1	6.5	1.0	20	3.98	<0.001	8.2	1.6	22	4.80	<0.001
shy4-1	5.8	1.7	20	5.28	<0.001	9.3	2.3	19	1.48	<0.2
shy4-2	7.2	1.4	19	3.77	<0.001	10.7	3.4	10	1.40	<0.2
shy5-1	6.8	1.7	40	2.46	<0.025	9.6	6.9	40	0.47	>0.5
shy6-1	8.2	1.3	20	4.04	<0.001	10.4	1.9	17	1.23	<0.4

[a] Hypocotyl lengths in millimeters.

[b] *t*-test and *P* value from comparing the *phyB-1/phyB-1 shy/shy* and *phyB-1/phyB-1 SHY/shy* seedling hypocotyl lengths. The low *P* values indicate that for all of the mutations, the hypocotyl length of the *shy/shy* homozygote is significantly shorter than that of the *SHY/shy* heterozygote, and that therefore none of the *shy* mutations is completely dominant.

[c] *t*-test and *P* value from comparing the *phyB-1/phyB-1 SHY/SHY* and *phyB-1/phyB-1 SHY/shy* seedling hypocotyl lengths. For the *phyB-1/phyB-1 SHY/SHY* single mutant, the hypocotyl measurements were $x = 9.9$, $\sigma = 1.5$, and $n = 140$. The results indicate that only *shy3-1, pom1-14*, *shy2-2*, and *shy2-3* heterozygotes are significantly shorter than the *phyB-1/phyB-1* single mutant and, therefore, are not recessive.

materials and methods). Table 5 shows hypocotyl lengths in red light of each of the *PHYB shy* and *phyB-1 shy* seedlings. In most cases, the *phyB-1 shy* double mutant is significantly taller than the *PHYB shy* single mutant, indicating lack of epistasis (Table 5). For *pom1-14*, the difference between *PHYB* and *phyB-1* genotypes was not significant, indicating that *pom1-14* is epistatic to *phyB-1* for this phenotype.

Phenotypic analyses of *shy* mutant plants: To assess whether the *shy* mutations affect genes involved in PHYB signal transduction or, more generally, in light signaling, we checked several phenotypes. Because a number of photomorphogenic mutations affect dark growth, we examined the morphology of *shy* seedlings in the dark. We also examined whether the *shy* mutations affect light-dependent phenotypes. As described above, *phyB* mutants have long hypocotyls in red light, have short roots, and flower early. We expected that mutations specifically limiting hypocotyl cell enlargement or elongation would suppress only the long hypocotyl phenotype (and perhaps other elongation phenotypes such as elongated root hairs or bolting stems), but not the flowering time or short root phenotypes. In contrast, mutations that affect a general control function might suppress multiple phenotypes caused by the *phyB-1* mutation. These phenotypic criteria have indeed allowed us to distinguish mutations that affect elongation from those that affect putative control functions. Within this broad classification, many of the mutants have unique characteristics that define distinct roles in development (Table 1).

TABLE 3

Segregation of *shy* mutant phenotypes among F_2 progeny of backcrosses

shy mutation	Tall progeny[a]	Short progeny[a]	χ^{2b}	*P*
shy2-2	34	89	0.46	>0.25
shy2-3	52	163	0.08	>0.5
amp1-4	146	52	0.17	>0.5
shy-115	133	48	0.22	>0.5
shy-802	50	16	0.02	>0.5
pom1-14	47	22	1.74	>0.1
pom1-15	34	11	0.01	>0.5
bot1-5	365	104	2.00	>0.1
shy3-1	45	100	2.82	>0.05
shy4-1	78	23	0.27	>0.5
shy4-2	381	136	0.47	>0.25
shy5-1	96	39	1.09	>0.25
shy6-1	104	35	0.01	>0.5

[a] Tall and short F_2 progeny were distinguished by eye in populations grown in dim white light. For those mutations with quantitative effects (*shy3-1*, *shy4-1*, *shy4-2*, *shy5-1*, and *shy6-1*), we measured hypocotyl lengths of representative F_2 seedlings and found that the two visible groups (tall and short) formed a frequency distribution with two peaks, with no seedlins of intermediate hypocotyl length.

[b] χ^2 values were calculated for the null hypothesis of 3 tall:1 short segregation, except for the semidominant or dominant mutations *shy3-1*, *shy2-2*, and *shy2-3*, for which the null hypothesis was 1 tall:3 short.

TABLE 4

Mapping of *shy* loci

shy mutation	Marker	Recombinant chromosomes	Deduced map position
shy2-2	*PVV4*	15/320	Chr. 1, 5 cM below *PVV4*,
	nga63	8/320	3 cM above *nga63*, close
	shy2-1	0/102[a]	to *shy2-1*
shy2-3	*shy2-2*	0/109[a]	Chr. 1, close to *shy2-2*
amp1-4	*pt*[b]	[b]	Chr. 3, position 81
shy-115	*GL1*	19/92	Chr. 3, 21 cM from *GL1*
shy-802	*BGL1*	3/56	Chr. 3, 5 cM from *BGL1*
pom1-14	*PVV4*	4/60	Chr. 1, 8 cM below *PVV4*,
	NCC1	5/60	8 cM above *NCC1*
pom1-15	*PVV4*	6/60	
	NCC1	5/60	
bot1-5	*ADH*	18/300	Chr. 1, 6 cM below *ADH*
shy3-1	*m323*	6/76	Chr. 2, 8 cM below *m323*,
	m429	56/840	5 cM below *m429*
shy4-2	*nga8*	14/58	Chr. 4, 24 cM below *nga8*,
	GA1	8/58	14 cM above *GA1*
shy6-1	*PVV4*	11/60	Chr. 1, close to *NCC1*
	NCC1	0/52	

[a] For mapping semidominant *shy2* alleles relative to each other, we crossed two *shy2* mutants, crossed the resulting F_1 double heterozygote with wild type, and looked for phenotypically wild-type segregants among the F_1 progeny of the outcross to wild type. Also included in the *shy2-2* × *shy2-3* data are 1065 F_2 progeny, among which we observed no wild-type plants. This is equivalent to observing $\sqrt{1065} = 33$ chromosomes in this cross of dominant mutations.

[b] *pt* is an allele of *amp* in the Landsberg *erecta* background. *amp1-4* failed to complement *pt*, and no wild-type plants were observed among several hundred F_2 progeny. Chr., chromosome.

We describe the mutants briefly here to help clarify the presentation below.

The *shy2-2* and *shy2-3* mutants have leaves that curl up at the edges. The *shy2-2* and *shy2-3* mutations were each semidominant (Table 2). In these respects, these mutants resemble the *shy1* and *shy2-1* mutants isolated as suppressors of a *hy2* mutation (Kim *et al.* 1996) and the *axr3* mutants isolated as having auxin-resistant root

TABLE 5

Hypocotyl lengths of *phyB-1* shy and *PHYB shy* seedlings in red light[a]

	PHYB shy seedlings			*phyB-1 shy* seedlings				
shy mutation	x	σ	n	x	σ	n	t[b]	P[b]
shy2-2	2.0	0.2	40	2.6	0.2	8	7.58	<0.001
shy2-3	2.1	0.5	30	4.8	1.8	23	7.68	<0.001
amp1-4	2.4	0.7	8	4.6	0.9	14	5.68	<0.001
shy-115	1.2	0.3	10	2.0	0.6	6	3.31	<0.005
pom1-14	2.3	0.8	27	2.4	0.5	29	0.55	>0.5
pom1-15	2.5	0.7	27	3.2	0.7	26	3.57	<0.001
bot1-5	1.7	0.5	37	2.5	0.8	37	4.88	<0.001
shy3-1	2.5	0.5	18	4.7	1.0	21	8.25	<0.001
shy4-1	2.4	0.7	15	5.6	1.4	15	7.65	<0.001
shy4-2	1.8	0.6	18	4.2	1.2	17	7.33	<0.001
shy5-1	3.1	1.3	16	5.1	1.3	9	3.54	<0.005
shy6-1	2.1	0.9	11	3.7	1.1	16	3.84	<0.001

[a] Hypocotyl lengths in millimeters. The red light level was 31–37 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. Data for *bot1-5*, *pom1-14*, *shy2-2*, *shy2-3*, and *pom1-15* were combined from two experiments. All *phyB-1 shy* seedlings were significantly shorter than *phyB-1* seedlings (data not shown).

[b] *t*-test and *P* values calculated for comparison of *phyB-1 shy* hypocotyl lengths with corresponding *PHYB shy* hypocotyl lengths.

growth (Leyser *et al.* 1996). The *shy2-2* mutation conferred more extreme phenotypes than the *shy2-3* mutation (see below), suggesting that it is the stronger allele. As described below, these mutants may identify an important control function in light-regulated development.

Mutations in the *AMP* (*a*ltered *m*eristem *p*rogram) gene confer altered phyllotaxy and partially de-etiolated growth in the dark (Chaudhury *et al.* 1993; Chin-Atkins *et al.* 1996). Like the previously described *amp* mutants, the *amp1-4* mutant we isolated appears dwarfed and has pale leaves and decreased apical dominance. In the light, *amp1-4* seedlings formed more leaves than wild-type plants (Figure 1). In the dark, they showed partial leaf development (see below).

The *shy-115* and *shy-802* mutants are brassinosteroid-deficient dwarfs. These resemble the previously characterized *bri1*, *cbb*, *det2*, *dim*, and *dwf* mutants in having small dark green leaves (Feldmann *et al.* 1989; Chory *et al.* 1991; Takahashi *et al.* 1995; Clouse *et al.* 1996; Li *et al.* 1996; Szekeres *et al.* 1996). Such mutants have been shown to have deficiencies in brassinosteroid synthesis or response. Both *shy-115* and *shy-802* mutants responded to exogenous brassinolide, suggesting that they are deficient in brassinosteroid synthesis (data not shown). *shy-115* plants were fully fertile, whereas *shy-802* plants were almost sterile and produced few seed. Both *shy-115* and *shy-802* complemented *det2-1*, and they also complemented each other. These equations therefore represent distinct loci. On the basis of their map positions, *shy-115* may be allelic with *DWF1* (also called *DIM*), and *shy-802* may be allelic with *DWF4.* Because we obtained few *shy-802* mutant seeds, it was difficult to subject the *shy-802* mutant to extensive phenotypic analyses. Moreover, light-related phenotypes of brassinosteroid-deficient mutants have been described extensively. Therefore, we omitted the *shy-802* mutant from many of the experiments described below.

pompom1 (*pom1*) mutants were first isolated as having abnormal root elongation (Hauser *et al.* 1995). The *pom1-14* and *pom1-15* mutants have similarly deficient root growth (see below). These mutants also have unusual hypocotyl morphology in the dark, and may be deficient in some aspect of cell elongation (see below).

The *bot1-5* mutant has morphological characteristics suggestive of a general deficiency in cell enlargement. Cotyledons, leaves, and flower parts were all foreshortened, and the mutant produced very few seed (Figure 1; data not shown). By complementation analyses, we determined that *bot1-5* is allelic to the *botero1-1* mutation (data not shown). This mutation confers similar phenotypes and maps to the same vicinity as *bot1-5* (H. Höfte, personal communication).

Finally, in contrast to the other mutants, the *shy3*, *shy4*, *shy5*, and *shy6* mutants have no unusual morphological characteristics; instead, they exhibit phenotypes within the normal range of wild-type growth patterns. Mutations at three loci, *shy4*, *shy5*, and *shy6*, are recessive, and one mutation, *shy3-1*, is semidominant (Table 2). As described below, *shy3-1* and *shy5-1* mutants have quantitative phenotypes that suggest that they may affect PHYB signaling.

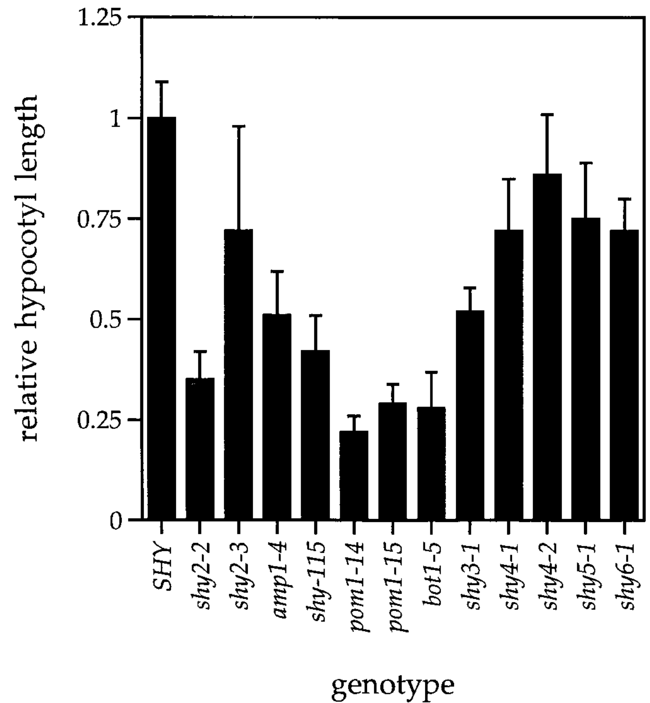

Figure 2.—Mean hypocotyl lengths of *PHYB shy* seedlings after 6 days of dark growth. Seedlings were grown on MS plates containing 2% sucrose, and hypocotyl lengths were normalized to the hypocotyl length of wild-type seedlings. Data from three experiments were pooled. Error bars indicate standard deviations. In the experiment in which *shy2-3*, *bot1-5*, and *shy4-2* data were gathered, the wild-type measurement had a relative standard deviation of 2.5 times that shown. In each case, the *shy* mutant hypocotyl lengths were statistically significantly shorter than the wild-type hypocotyl length ($P < 0.05$ for *shy4-2*, $P < 0.001$ for the rest).

Dark phenotypes: Mutations that activate light-response pathways constitutively might be expected to cause phenotypes in the dark. In fact, a number of genes thought to play important roles in photomorphogenesis were identified in screens for mutants that make leaves in the dark (see above). After 6 days in the dark, wild-type seedlings had a long hypocotyl, an unopened apical hook, small unexpanded cotyledons, and no leaf primordia (Figures 2 and 3). After 23 days, the apical hooks had opened, but the cotyledons were unexpanded and very few seedlings had visible leaf primordia (Figure 4, Table 6). We grew *shy* seedlings in the dark, and found that after 6 days all of them had significantly shorter hypocotyls than the wild-type seedlings (Figure 2). Some of the *shy* mutants also had open cotyledons or leaf development, morphological characteristics that are normally limited to light-grown plants (Table 6, Figure 4).

The *shy2-2* and brassinosteroid-deficient *shy-802* seed-

Figure 3.—*PHYB shy* seedlings after 6 days of dark growth. Seedlings were grown on MS/sucrose plates. Genotypes shown are (A) Landsberg *erecta*, (B) *shy2-2*, (C) *shy2-3*, (D) *amp1-4*, (E) *shy-115*, (F) *pom1-15*, (G) *bot1-5*, (H) *shy3-1*, (I) *shy4-1*, (J) *shy5-1*, and (K) *shy6-1*. *shy4-1* and *shy4-2* seedlings appeared similar to each other, and *pom1-14* and *pom1-15* seedlings appeared similar to each other. Therefore, only one mutant at each locus is shown.

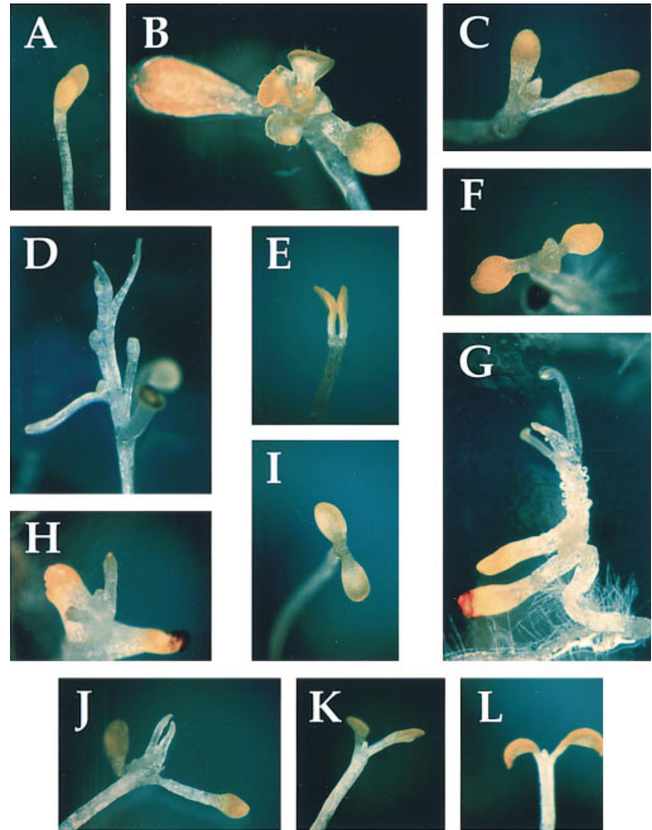

Figure 4.—*PHYB shy* seedlings after 23 days of dark growth. Seedlings were grown on MS/sucrose plates. Genotypes shown are (A) Landsberg *erecta*, (B) *shy2-2*, (C) *shy2-3*, (D) *amp1-4*, (E) *shy-115*, (F) *shy-802*, (G) *pom1-15*, (H) *bot1-5*, (I) *shy3-1*, (J) *shy4-1*, (K) *shy5-1*, and (L) *shy6-1*. *shy4-1* and *shy4-2* seedlings appeared similar to each other, and *pom1-14* and *pom1-15* seedlings appeared similar to each other. Therefore, only one mutant at each locus is shown.

lings resembled light-grown seedlings most closely, in that after 23 days they had structures resembling true leaves. Petioles were very short, and trichomes were visible (Figure 4). In this respect, these two mutants resembled the de-etiolated mutants, *det1*, *det2*, and *cop1*, described previously (Chory *et al.* 1989; Deng *et al.* 1991). Mutants carrying the weaker allele of *SHY2*, *shy2-3*, usually had expanded cotyledons after 23 days, but no visible leaf primordia (Figure 4, Table 6). The weaker brassinosteroid-deficient mutant *shy-115* appeared similar to the wild type in the dark.

Several mutants had phenotypes that superficially resembled a de-etiolated phenotype, but diverged from a normal light growth pattern. *amp1-4* seedlings had the most extreme phenotype after six days, having an open apical hook, expanded cotyledons, and appearance of leaf primordia. However, after 23 days, the organs in the positions where leaves would normally arise resembled elongated petioles or stems more than leaves, and yellow leaf blade material was absent or extremely abbreviated (Figure 4, Table 6). Similarly, the *shy4-1*, *pom1-14*, *pom1-15*, and *bot1-5* mutants frequently developed branches resembling petioles (Figure 4, Table 6). This curious phenotype may represent a partial commitment to leaf development in these mutants. Alternatively, enhanced petiole or stem development may arise in conditions where the hypocotyl would otherwise elongate but is prevented from doing so by some physiological limitation caused by a *shy* mutation.

As well as making numerous petiole-like structures, the *pom1-14* and *pom1-15* mutants had a unique dark hypocotyl morphology. The hypocotyls were quite crooked (Figures 3 and 4), and upon closer inspection, they appeared somewhat disorganized, with a rough surface (data not shown).

The remaining mutants (*shy3-1*, *shy4-2*, *shy5-1*, and *shy6-1*) had short hypocotyls, but otherwise looked similar to wild-type in the dark. *shy3-1* seedlings had a slightly open apical hook after 6 days. All had open apical hooks after 23 days, but the cotyledons were unexpanded and few seedlings had leaves (Figure 4, Table 6). Each of the mutants had a slightly higher incidence of leaf primordium formation than the wild type (Table 6). We do not know the reason for this quantitative difference, but it may conceivably be an indirect consequence of having shorter hypocotyls.

Hypocotyl elongation responses to red, blue, and far-red light: If a SHY protein activity is normally modulated by a light-signaling pathway, then mutation of the gene encoding that protein might cause the pathway to be more constitutive and less regulatable. In such a case, *PHYB shy* mutant seedlings would have a decreased response to light because of their "partially responding" baseline state, and they should be less sensitive to additional red light than wild-type seedlings. To examine this possibility, we tested the fluence rate–response behavior of the *PHYB shy* plants for hypocotyl elongation in constant red light. Figure 5, A and B, show the hypocotyl lengths of 6-day-old *shy* mutant seedlings grown under

TABLE 6

Leaf formation by *shy* mutants after 23 days in the dark

shy genotype	Zero leaves[a]	Two or more leaves[a]	Fraction with leaves[a]
SHY	68	9	0.12
shy2-2	3	58[c]	0.95
shy2-3	39	19	0.33
amp1-4	0	55[b]	1.00
shy-115	16	13	0.45
shy-802	0	22[c]	1.00
pom1-14	0	44[b]	1.00
pom1-15	0	25[b]	1.00
bot1-5	0	25[b]	1.00
shy3-1	18	9	0.33
shy4-1	2	17[b]	0.89
shy4-2	18	0	0
shy5-1	28	8	0.22
shy6-1	17	5	0.23

[a] Number of seedlings with leaves or other lateral organs. Branches, leaves, petioles, and undifferentiated primordia arising from the apex were all counted.

[b] Leaves resembled elongated stems or petioles with small or absent leaf blades (Figure 4).

[c] Leaves resembled true leaves (Figure 4).

different fluence rates, normalized in each case to the hypocotyl length of the same strain in the dark. Most of the *shy* mutants responded to the same range of fluence rates as the wild type did, having hypocotyl lengths of ~30% of their hypocotyl lengths in the dark at the highest fluence rate tested (Figure 5, A and B). As expected, the *phyB-1* mutant was less sensitive to red light, showing a minimum hypocotyl length of ~80% of its dark hypocotyl length.

The *shy2-2*, *pom1-14*, and *pom1-15* mutants showed altered hypocotyl responses to red light. *shy2-2* had a decreased response at the highest fluence rate tested (40–45 μmol · m^{-2} · sec^{-1}), with a minimum hypocotyl length of ~60% of its dark hypocotyl length (Figure 5A). In contrast, the *pom1-14* and *pom1-15* mutants had longer hypocotyls at very low fluence rates (<0.1 μmol · m^{-2} · sec^{-1}) than in the dark (Figure 5A). They thus showed the opposite response from the wild type in this fluence range. At higher fluence rates (>1 μmol · m^{-2} · sec^{-1}), they had a normal response to red light, reaching a minimum hypocotyl length of ~70% of their dark hypocotyl length (Figure 5A). Because the *shy2-2*, *pom1-14*, and *pom1-15* mutants are each extremely short in the dark (Figures 2 and 3, data not shown), these observations may reflect an inherent limitation in the degree to which red light can inhibit hypocotyl elongation. However, the *bot1-5* mutant, responded proportionately similarly to the wild type despite having a very short hypocotyl in the dark (Figures 2 and 5B). This observation suggests that the *shy2-2*, *pom1-14*, and *pom1-15* mutants do not reach a minimum attainable hypocotyl length in these experiments, and that the mutations may thus affect red light signaling or response pathways.

To test whether the *shy* mutations may affect response pathways downstream of other photoreceptors, we also performed fluence rate response experiments in blue and far-red light. We found that all the mutants responded to both types of light with the same threshold and saturation characteristics as the wild type (Figure 5, C and D; data not shown). In particular, the *shy2-2* and *pom1-15* mutants had normal responses to both blue light (Figure 5C) and far-red light (Figure 5D). Therefore, the mutations have not affected a blue or far-red light signaling pathway.

Root elongation: Rather than repressing cell expansion in all seedling tissues, PHYB causes a redistribution of growth away from the hypocotyl and toward the roots and cotyledons. Thus, light-grown *phyB* mutant seedlings have a longer hypocotyl but a shorter root and smaller cotyledons than wild-type seedlings (Reed *et al.* 1993; Neff and Van Volkenburgh 1994). To determine whether the *shy* mutations affect elongation in multiple tissues, we measured the root lengths of *phyB-1 shy* seedlings grown in red light. This assay was particularly useful for distinguishing between mutants affected in regulatory functions, which should have roots longer than the *phyB-1* seedlings, and mutants affected specifically in elongation functions, which would be expected to have shorter roots than *phyB-1* seedlings. Mutants with roots the same length as *phyB-1* seedlings could belong to either group. As shown in Figure 6, wild-type (*PHYB SHY*), *amp1-4*, *shy2-2*, and *shy5-1* seedlings had significantly longer roots than *phyB-1* seedlings, suggesting that these mutations may affect the redistribution of growth controlled by light. *shy2-3*, *shy-802*, *pom1-14*, *pom1-15*, *bot1-5*, *shy4-2*, and *shy6-1* seedlings had significantly shorter roots than *SHY* seedlings, suggesting that these mutations affect functions specific to cell elongation. The remaining mutants (*shy-115*, *shy3-1*, and *shy4-1*) had roots of similar length as the *phyB* parent strain.

Flowering time: In addition to having elongation phenotypes, *phyB* mutants flower early (Goto *et al.* 1991; Reed *et al.* 1993; Bagnall *et al.* 1995). Plants that overexpress *PHYB* also flower early (Bagnall *et al.* 1995). We assessed flowering times in short days, under which conditions the difference in flowering time between wild-type and *phyB* plants is greatest. We measured both the time the plants took to flower (DF, days to flowering) and the number of leaves at the time of flowering (LN, leaf number) in both *PHYB* and *phyB-1* backgrounds. The results differed slightly from experiment to experiment, possibly because light conditions in our growth chamber varied (see materials and methods). Therefore, we consider as meaningful only results where we observed a statistically significant difference between *SHY* and *shy* plants in the majority of experiments. We present data for a subset of the mutants in Figure 7 and

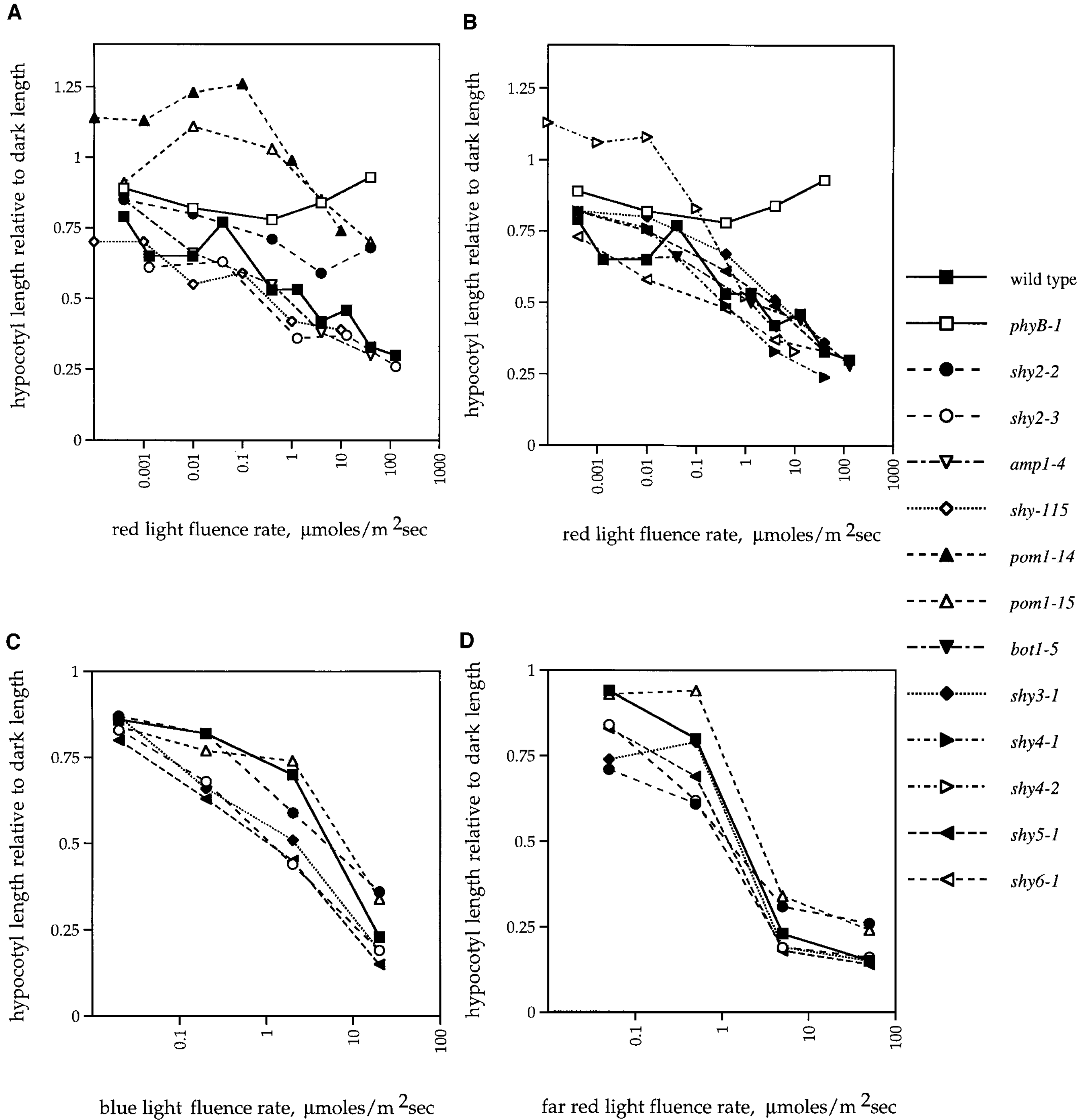

Figure 5.—Hypocotyl length response of *shy* seedlings to different fluence rates of red, blue, or far-red light. For each curve, data are normalized to the hypocotyl length of the same genotype in the dark. (A and B) Response to red light. Curves are split into two graphs for clarity, and data for wild type and *phyB-1* are shown in both graphs. Each data point is the mean hypocotyl length of 20–40 seedlings, and standard deviations were generally 10–20% of the mean. Repetitions of this experiment gave similar results (data not shown). (C and D) Response to blue light (C) and far-red light (D). Shown are *shy2-2*, *shy2-3*, *pom1-15*, *shy3-1*, and *shy5-1* mutants. *amp1-4*, *shy-115*, *bot1-5*, *shy4-1*, *shy4-2*, and *shy6-1* mutants all responded similarly to wild type in both blue and far-red light (data not shown).

summarize our consensus results from six experiments in Table 7. Four of the mutations affected flowering time significantly.

One *shy* mutation, *shy5-1*, delayed flowering in both *PHYB* and *phyB-1* backgrounds (Figure 7, Table 7). In the *PHYB* background, *shy5-1* also caused more leaves to form before flowering, whereas in the *phyB-1* background, the leaf number was normal. These data suggest that the *SHY5* gene product may normally antagonize the activity of PHYB.

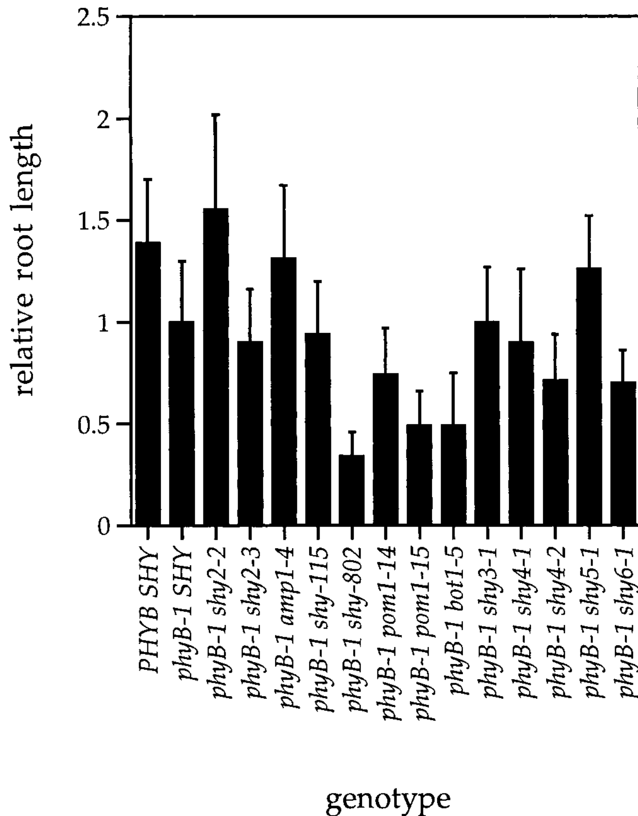

Figure 6.—Root lengths of *phyB-1 shy* seedlings in red light. *shy* seedling root lengths were normalized to the root length of *phyB-1* seedlings. Data from four experiments were combined. Error bars indicate standard deviations of measurements. Normalized root lengths were tested for significant difference from *phyB-1* root length by *t*-test. All genotypes except *phyB-1 shy-115*, *phyB-1 shy3-1*, and *phyB-1 shy4-1* showed a difference from *phyB-1* root lengths at 95% confidence or more. *P* values from *t*-tests for comparison with *phyB-1* root lengths were wild type, $P < 0.001$; *phyB-1 shy2-2*, $P < 0.001$; *phyB-1 shy2-3*, $P < 0.05$; *phyB-1 amp1-4*, $P < 0.001$; *phyB-1 shy-115*, $P < 0.5$; *phyB-1 shy-802*, $P < 0.001$; *phyB-1 pom1-14*, $P < 0.025$; *phyB-1 pom1-15*, $P < 0.001$; *phyB-1 bot1-5*, $P < 0.001$; *phyB-1 shy3-1*, $P = 1$; *phyB-1 shy4-1*, $P < 0.1$; *phyB-1 shy4-2*, $P < 0.001$; *phyB-1 shy5-1*, $P < 0.001$; and *phyB-1 shy6-1*, $P < 0.001$.

The *shy2-2* and *shy3-1* mutations caused early flowering in the *PHYB* background, but had no effect on flowering time in the *phyB-1* background. Both *PHYB shy2-2* and *PHYB shy3-1* mutant plants also flowered with fewer leaves than wild-type plants. Although they flowered at the normal time, *phyB-1 shy3-1* plants had extra leaves. The early flowering of *shy2-2* and *shy3-1* mutants in the *PHYB* background suggests that SHY2 and SHY3 have significant regulatory functions.

As mentioned above, *amp1-4* plants made leaves more quickly than *AMP* plants. This accelerated leaf production was accompanied by an acceleration of flowering in both *PHYB* and *phyB-1* backgrounds. However, the effect of the *amp1-4* mutation on the number of leaves made before flowering correlated poorly with its effect on flowering time. Thus, in some experiments, the leaf number was significantly greater than for the corresponding *SHY* plants, and in other experiments, the leaf number was significantly smaller (data not shown). This observation suggests that the accelerated flowering of *amp1-4* plants may be a secondary consequence of its rapid leaf production.

The remaining *shy* mutations (*shy2-3*, *shy-115*, *pom1-14*, *pom1-15*, *bot1-5*, *shy4-1*, *shy4-2*, and *shy6-1*) had no effect on the number of days to flowering in either *PHYB* or *phyB-1* backgrounds (Table 7). Three of these, *shy2-3*, *bot1-5*, and *shy6-1*, affected leaf number in one genetic background (Table 7). (The *shy2-3* mutation caused extra leaf formation in the *phyB-1* background, the *bot1-5* mutation caused flowering with fewer leaves in the *PHYB* background, and the *shy6-1* mutation caused extra leaf formation in the *PHYB* background.)

DISCUSSION

The *phyB-1* mutation creates a stop codon and is probably a null allele (Reed *et al.* 1993). Therefore, the suppressor mutations described here most likely bypass the requirement for PHYB for inhibiting hypocotyl elongation. The *shy* mutations may identify downstream mediators of PHYB signaling, regulators of other environmental response pathways, or biochemical or metabolic functions needed for hypocotyl elongation. The occurrence of single mutations at several of the loci indicates that we have not saturated the screen. Additional known loci that can mutate to give a short hypocotyl phenotype include the *AXR* genes and the *DET*/*COP*/*FUS* genes (Fankhauser and Chory 1997).

Based on the mutant phenotypes, the best candidates for genes that regulate light responses are *SHY2*, *SHY3*, and *SHY5*. The recessive *shy5-1* mutation suppresses all of the *phyB-1* phenotypes we tested. *phyB-1 shy5-1* plants have a shorter hypocotyl than the starting *phyB-1* mutant, they have a longer root, and they flower late. For each phenotype, the suppression is partial in that the *phyB-1 shy5-1* plants still differ from wild-type plants. This may indicate that the mutation is a partial loss-of-function allele or that the mutated function is encoded by more than one gene. Taken together, the results suggest that the *SHY5* gene may encode a function that opposes the activity of PHYB, acting either downstream of PHYB in a light regulatory pathway or in a separate branch of the control network.

The *shy2* mutants have several striking phenotypes that suggest that SHY2 may be an important mediator of red light responses. *shy2-2* plants make leaves in the dark, respond only slightly to red light for control of hypocotyl elongation, have an elongated root in the *phyB-1* background, and flower early in the *PHYB* background. The weaker *shy2-3* mutation caused less profound effects on development than *shy2-2*, *e.g.*, expanded cotyledons without obvious leaf formation in the dark. Like the *shy2-2* and *shy2-3* mutations described

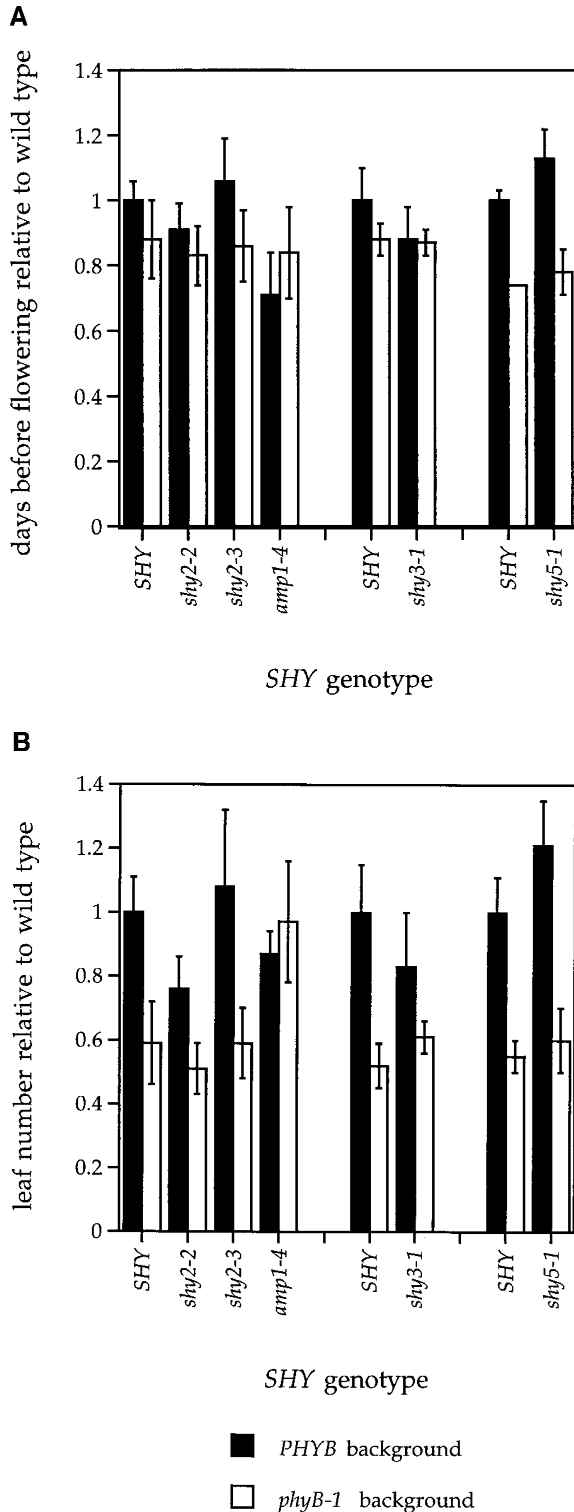

Figure 7.—Flowering of selected *PHY shy* and *phyB-1 shy* plants in short days. (A) days to flower; (B) leaf number at time of flowering. Error bars indicate standard deviations. Shown are data for selected genotypes from three separate experiments. To facilitate comparison between different experiments, data have been normalized to the wild-type flowering time and LN in each case. In the experiments shown, the flowering times (A) for *PHYB shy2-2, PHYB amp1-4, PHYB shy3-1, PHYB shy5-1*, and *phyB-1 shy5-1* were significantly different from the corresponding *SHY* plants ($P < 0.05$ or less);- and the leaf numbers, (B) for *PHYB shy2-2, phyB-1 shy2-2, PHYB amp1-4, phyB-1 amp1-4, PHYB shy3-1, phyB-1 shy3-1*, and *PHYB shy5-1* were significantly different from the corresponding *SHY* plants ($P < 0.05$ or less). Flowering data from several experiments are summarized in Table 7.

here, the previously described *shy2-1* mutation is semidominant, causes cotyledon expansion in the dark, and causes upcurled leaves in the light (Kim *et al.* 1996). The semidominance of all three *shy2* alleles is consistent with the mutations being hypomorphic, hypermorphic, or neomorphic. The frequency with which we have obtained *shy2* alleles might indicate that the mutations are hypomorphic (decreased function), in which case SHY2 may normally repress phytochrome-mediated development in the dark. If the mutations are hypermorphic, SHY2 may normally activate de-etiolation in response to light. If the mutations are neomorphic, they may reveal otherwise cryptic effects of some other regulatory pathway on seedling development.

Like *shy2* mutants, mutants of the *det/cop/fus* class make leaves in the dark. However, the morphological phenotypes of *shy2* mutants are quite distinct from the phenotypes of mutants of the *det/cop/fus* class, such as *det1-1*. For example, *det1-1* mutant seedlings do not have curled leaves characteristic of *shy2* seedlings, and *shy2* mutant seedlings do not overproduce anthocyanin as *det1* seedlings do. Thus, SHY2 probably regulates development in a manner different from the DET/COP/FUS gene products.

The last of the mutations with a substantial effect on both flowering and hypocotyl elongation is *shy3-1*. Interestingly, *shy3-1* partially suppressed the flowering phenotype caused by a *phyB-1* mutation, but in a *PHYB* background, it caused early flowering, as *phyB-1* does. Thus, *PHYB shy3-1* plants flowered with reduced leaf number compared to wild-type plants, whereas *phyB-1 shy3-1* plants flowered with increased leaf number compared to *phyB-1* plants. This dampening of the effect of PHYB on leaf number may indicate that SHY3 interacts with PHYB to control flowering (but see below). The semidominance of the *shy3-1* mutation for hypocotyl length phenotypes is consistent with either a gain-of-function or loss-of-function type of allele, so the normal role of SHY3 in development could be either to transmit the PHYB signal or to antagonize it.

At first blush, the early flowering conferred by the *shy2-2* and *shy3-1* mutations would seem counter to the expectation (fulfilled by *shy5-1*) that a regulatory suppressor mutation should affect any phenotypes in the opposite sense as the starting *phyB-1* mutation. However, interpretation of the flowering phenotypes is complicated by the finding that when overexpressed, *PHYB* causes early flowering (Bagnall *et al.* 1995). This suggests either that overactivation of PHYB response pathways causes early (rather than late) flowering or that overexpressed PHYB can co-opt another pathway that

TABLE 7

Summary of developmental phenotypes of *shy* mutants[a]

shy genotype	Dark development	RL response	Root length	Flowering[c]			
				PHYB background		*phyB-1* background	
				DF	LN	DF	LN
SHY	Long hyp., small cotyledons	+	Medium	Normal	Normal	Normal	Normal
shy2-2	Leaves	+/−	Long	Early	Low	Normal	Normal
shy2-3	Expanded cotyledons	+	Short	Normal	Normal	Normal	High
amp1-4	Stem-like organs	+	Long	Early	Low	Normal	High
shy-115	Normal	+	Normal	Normal	Normal	Normal	Normal
shy-802	Leaves		Short				
pom1-14	Twisted hyp.; stem-like organs	+/−[b]	Short	Normal	Normal	Normal	Normal
pom1-15	Twised hyp.; stem-like organs	+/−[b]	Short	Normal	Normal	Normal	Normal
bot1-5	Stem-like organs	+	Short	Normal	Low	Normal	Normal
shy3-1	Normal	+	Normal	Early	Low	Normal	High
shy4-1	Stem-like organs	+	Normal	Normal	Normal	Normal	Normal
shy4-2	Normal	+	Short	Normal	Normal	Normal	Normal
shy5-1	Normal	+	Long	Late	High	Late	Normal
shy6-1	Normal	+	Short	Normal	High	Normal	Normal

[a] Phenotypes of *shy* mutants are described relative to the corresponding *SHY* strain.
[b] *pom1-14* and *pom1-15* seedlings have an altered response to low fluence rates (Figure 5).
[c] Shown are the consensus results for days to flower (DF) and leaf number (LN) at time of flowering. In each column, the phenotype of the *SHY* plants is taken as normal. Thus, although *phyB-1 SHY* plants flower earlier than *PHYB SHY* plants, both are considered ''normal'' for the purpose of comparing their phenotypes to those of the corresponding *shy* plants. hyp., hypocotyl.

normally promotes flowering. For example, phytochrome A normally inhibits elongation and also promotes flowering (Nagatani *et al.* 1993; Parks and Quail 1993; Whitelam *et al.* 1993; Johnson *et al.* 1994; Reed *et al.* 1994). In either case, a *shy* mutation might cause early flowering by acting similarly to overexpressed PHYB. Another possible explanation for why these *shy* mutations cause early flowering could be that they affect flowering time indirectly through effects on elongation. *phyB-1* plants make leaves at a slower rate than wild-type plants, perhaps as a result of extra elongation growth at the expense of new organ formation (Koornneef *et al.* 1995; data not shown). If flowering depends in part on formation of a threshold number of leaves, then the slower rate of leaf formation in *phyB-1* plants may actually delay flowering and partially compensate for the propensity of such plants to flower with fewer leaves. In that case, a mutation that suppressed the elongation phenotypes of a *phyB-1* mutant would cause earlier flowering, as the *shy2-2* and *shy3-1* mutations do.

Our finding of an allele of *AMP* (*amp1-4*) and presumed brassinosteroid auxotrophs (*shy-115* and *shy-802*) in this screen underscores the probable relevance of plant hormone signaling pathways to seedling de-etiolation. The *amp* mutant overproduces cytokinin (Chaudhury *et al.* 1993; Chin-Atkins *et al.* 1996), and applications of cytokinin to dark-grown seedlings can induce aspects of a de-etiolated phenotype (Chory *et al.* 1994; Chin-Atkins *et al.* 1996). However, dark-grown *amp* mutant seedlings make organs resembling stems or petioles rather than leaves, and the altered phyllotaxy of *amp* mutants cannot be mimicked by altering light conditions. These observations suggest that the connection of AMP to photomorphogenesis may be indirect. Brassinosteroids have been implicated in repressing seedling de-etiolation because mutants isolated as having partially de-etiolated phenotypes in the dark have turned out to be brassinosteroid auxotrophs (Li *et al.* 1996; Szekeres *et al.* 1996). Like *shy-802*, mutants such as *det2-1* make leaves in the dark, although the plastids do not differentiate as they do in other de-etiolated mutants such as *det1* (Chory *et al.* 1991). It remains to be determined whether these observations reflect direct regulation of brassinosteroid physiology by light. Other workers have reported evidence suggesting that auxin and light signaling are connected. For example, *Nicotiana plumbaginifolia* phytochrome mutants have been found to have increased auxin levels (Kraepiel *et al.* 1995), and some auxin-resistant mutants are short and have partially de-etiolated phenotypes in the dark (Lincoln *et al.* 1990; Timpte *et al.* 1992; Leyser *et al.* 1996; Cernac *et al.* 1997; A. Sonawala and J. W. Reed, unpublished observations). In this regard, it is interesting that *shy2* mutants share some phenotypes, such as curled leaves, with *axr3* mutants (Leyser *et al.* 1996).

The remaining *shy* mutations suppress only the long hypocotyl phenotype of the starting *phyB-1* mutant, but do not suppress the root elongation or flowering time phenotypes. The *pom1-14*, *pom1-15*, *bot1-5*, *shy4-2*, and *shy6-1* mutations actually cause the root to be shorter

than that of *phyB-1* plants. This observation suggests that either these mutations primarily affect a cell elongation or enlargement function, or they affect cell division rates in the root. The morphological variety among these mutants suggests that the different mutations may affect distinct aspects of growth. Those with quantitative effects but having otherwise normal shape (*e.g.*, *shy4-2* or *shy6-1*) may affect a control function. Those conferring aberrant morphology (*e.g.*, *pom1-14* or *bot1-5*) may affect part of the cellular machinery that elongates cells, synthesizes cell walls, or determines cell polarity.

The twisted hypocotyls of dark-grown *pom1* mutants resemble those of dark-grown *procuste1* mutants (Desnos *et al.* 1996), suggesting that *POM1* and *PROCUSTE1* have related functions. Apparently, these genes are required for proper hypocotyl elongation in the dark. Whereas the *procuste1* mutants show a biphasic hypocotyl elongation curve in blue light, however, our *pom1* mutants have a normal blue light response but an aberrant response in red light. POM1 is also required for elongation and control of cortical cell enlargement in roots (Hauser *et al.* 1995) and for proper epidermal cell differentiation (Schneider *et al.* 1997). POM1 therefore plays a role in growth and/or shape determination of cells of various organs.

None of the putative regulatory mutations we have identified (*shy2-2*, *shy2-3*, *shy3-1*, or *shy5-1*) is epistatic to the *phyB-1* mutation for the hypocotyl length phenotype. This indicates that these mutations either cause incomplete defects in the corresponding genes or affect redundant functions. The results reinforce our view of light signal transduction as a network of interacting components rather than as a collection of linear pathways (Reed and Chory 1994). As well, light signaling is surely intimately coupled to more general developmental control mechanisms. Further analysis of the *shy* mutants reported here may contribute to unraveling the complex regulatory pathways that mediate control of plant development by a variety of environmental factors.

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