shl, a New Set of Arabidopsis Mutants with Exaggerated Developmental Responses to Available Red, Far-Red, and Blue Light[1]

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The interaction of light perception with development is the subject of intensive genetic analysis in the model plant Arabidopsis. We performed genetic screens in low white light—a threshold condition in which photomorphogenetic signaling pathways are only partially active—for ethyl methane sulfonate-generated mutants with altered developmental phenotypes. Recessive mutants with exaggerated developmental responses were obtained in eight complementation groups designated *shl* for seedlings hyperresponsive to light. *shl1, shl2, shl5,* and *shl3 shl4* (double mutant) seedlings showed limited or no phenotypic effects in darkness, but showed significantly enhanced inhibition of hypocotyl elongation in low-white, red, far-red, blue, and green light across a range of fluences. These results reflect developmental hyper-responsiveness to signals generated by both phytochrome and cryptochrome photoreceptors. The *shl11* mutant retained significant phenotypic effects on hypocotyl length in both the *phyA* mutant and *phyB* mutant backgrounds but may be dependent on *CRY1* for phenotypic expression in blue light. The *shl2* phenotype was partially dependent on *PHYB*, *PHYA*, and *CRY1* in red, far-red, and blue light, respectively. *shl2* and, in particular, *shl1* were partially dependent on *HY5* activity for their light-hyperresponsive phenotypes. The *SHL* genes act (genetically) as light-dependent negative regulators of photomorphogenesis, possibly in a downstream signaling or developmental pathway that is shared by *CRY1*, *PHYA,* and *PHYB* and other photoreceptors (*CRY2*, *PHYC*, *PHYD*, and *PHYE*).

Light is a critical environmental signal that effects nearly every aspect of plant development, including seed germination, seedling morphogenesis, and floral initiation. A complex network of photoreceptors and signaling pathways have evolved to regulate developmental responses to light quantity, quality, and duration. The photoreceptors include the red (R)- and far-red (FR)-responsive phytochromes and several blue (B) and UV receptors, including the cryptocromes. Light perception has been the subject of intensive genetic analysis, primarily in Arabidopsis (Deng and Quail, 1999; Neff et al., 2000), and has become a model for interactions of environment with development (Smith, 2000, and references therein). Phytochrome apoproteins are encoded by five genes in Arabidopsis (*PHYA–PHYE*), and cryptochromes are encoded by two genes, *CRY1* and *CRY2*. Pioneering genetic screens identified the long *hypocotyl* (*hy*) mutants in white light (Koorneef et al., 1980), which were defective in *PHYB*, *CRY1*, and *HY5*, a transcriptional regulator, as well as several gene products involved in the biosynthesis of the phytochrome chromophore (Ahmad and Cashmore, 1993; Reed et al., 1993; Somers et al., 1993; Oyama et al., 1997). Screens for mutants with a light-grown or "de-etiolated" phenotype in darkness (Chory et al., 1989; Deng et al., 1991) have identified several nuclear genes that act as negative regulators of photomorphogenesis (Deng et al., 1992; Pepper et al., 1994; Wei et al., 1994a). "Second generation" genetic screens included specific, physiology-based strategies, such as the search for *phyA* mutants in FR light (Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993), as well as screens for extragenic suppressors of "first generation" mutants such as *hy2* (Kim et al., 1996), *det1* (Pepper and Chory, 1997), *phyB* (Reed et al., 1998), and *phyA* (Hoecker et al., 1998). Other screens made use of floral initiation rather than seedling morphology in primary mutant screens (Ahmad and Cashmore, 1996). Some recent screens have exploited a light-inducible *CAB2-LUC* promoter-reporter transgene (Genoud et al., 1998) or have identified extragenic suppressors of a *PHYB* overexpressing transgene (Huq et al., 2000). Finally, important photoregulatory genes have also recently been identified by protein-protein interactions (Ni et al., 1998; Choi et al., 1999; Fankhauser et al., 1999).

In the present study, we screened for mutants with phenotypic effects in low light—a threshold condition in which the normal photoperception pathways are only partially active, leading to limited de-etiolation responses in wild-type (WT) seedlings. Using screens performed in low light, we obtained two classes of mutants: 1) those which had completely etiolated phenotypes, and 2) those which had com-

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pletely de-etiolated phenotypes. Whereas some of the mutations in the former class mapped to known genetic loci (*PHYB, CRY1*), others appeared to be novel genetic loci (characterization of these will be presented elsewhere). Here, we present our initial analysis of several mutants with exaggerated developmental responses to available light.

RESULTS

Identification of *shl* Mutants

To identify novel regulatory components at the interface of light signaling and development, we screened M_2 seed pools from ±28,000 individual ethyl methane sulfonate mutagenized M_1 plants. Aliquots from 16,420 seed pools were divided and screened simultaneously in low-intensity white light (4 μmol m^{-2} s^{-1}) and in darkness. An additional 9,540 seed pools were screened in darkness and under a yellow-green filter (24 μmol m^{-2} s^{-1}) that depleted much of the photomorphogenetically active B, R, and FR regions of the spectrum (the yellow-green filter was technically advantageous in that WT seedlings showed less phenotypic variance than that observed in low white light). Under each of these conditions, WT seedlings displayed a long hypocotyl and unfolded but poorly developed cotyledons. We identified 380 M_2 families that segregated multiple individuals with short hypocotyls and expanded cotyledons in low light. In darkness, 202 of the 380 M_2 families segregated individuals with de-etiolated phenotypes, and an additional 99 families segregated individuals with severe developmental abnormalities (e.g. no root, fused cotyledons, and fasciated). The remaining 79 M_2 families had normal etiolated phenotypes in darkness. In the M_3 generation, 15 of these families (±19%) exhibited heritable light-hyperresponsive phenotypes. The candidate mutants obtained from these families were designated *shl* for seedlings hyperresponsive to light.

Genetic Characterization of *shl* Mutants

All 15 *shl* mutants were recessive in back-crosses to WT Columbia ecotype (Col-0). Mutant lines were assigned to complementation groups by F_1 complementation analysis. Three complementation groups, designated *shl1*, *shl2*, and *shl5* contained multiple alleles (with five, four, and two alleles, respectively). Various alleles of *shl1* and *shl2* were obtained from both the yellow-green light and the low-intensity white light conditions, indicating that the two light regimes were effectively similar. The remaining four mutant lines fell into mono-allelic complementation groups, indicating that our screens were far from exhaustive or "saturating."

Phenotypic analysis of the F_2 progeny from back-crosses to Columbia (Col-0 or Col-0 seeds carrying the *glabrous* mutation [Col-*gl1*]) indicated that in 14 of 15 mutant lines, the light-hypersensitive trait was conditioned by a single gene (a subset of these data is presented in Table I). In the remaining line, mutant progeny were observed segregating in a ratio near 1:15 ($P > 0.70$), suggesting that the mutant phenotype in this line was due to recessive alleles at two unlinked loci. F_3 seeds were obtained by selfing of 20 of these F_2 progeny. Ten of the F_3 families segregated *shl* mutant individuals. This result closely fits ($P > 0.4$) the expectation for an F_2 population segregating two unlinked recessive loci, in which 7/16 of the individuals with WT phenotypes would be expected to carry at least one mutant allele at both loci. Furthermore, mutant to WT ratios near 1:15 were consistently obtained in subsequent back-crosses to Col-0 and in out-crosses to Landsberg *erecta*. The putative double-mutant line complemented all other lines, and the loci were tentatively designated *shl3* and *shl4*. Neither *shl3* nor *shl4* had an obvious morphological phenotype in the single-mutant homozygous state, although one of these loci had a subtle quantitative effect on hypocotyl length in high-irradiance FR light.

After two back-crosses to Col-0, representative alleles of the *shl1*, *shl2*, *shl5* complementation groups, as well as the putative *shl3 shl4* double mutant, were out-crossed to Landsberg *erecta* to create F_2 mapping populations. Molecular genotyping of 94 mutant F_2 individuals using PCR-based markers localized *shl1* to the top of chromosome 1, showing complete co-segregation with single sequence length polymorphism (SSLP) marker nga59. A mapping population of 94 mutant F_2 individuals was used to map *shl2* to a location on chromosome 2, ±7.0 cM telomeric to *PHYB*. Genetic mapping of *shl3* and *shl4* were limited by the relatively small number of mutant individuals in the F_2 generation. However, we found convincing

Table I. *Segregation analysis of shl mutants*

Mutants were back-crossed to WT Col-0 ecotype, and F_2 progeny were scored in low light for WT or light hyperresponsive (shl^-) phenotypes. Chi-squared (χ^2) analysis was applied using the null hypotheses (n.h.). Hypotheses indicated by (r) were rejected.

Cross	WT	shl^-	Ratio	n.h.	χ^2	*P*
shl1-1 × WT Col-0	624	200	3.12:1	3:1	0.235	>0.70
shl2-2 × WT Col-0	471	163	2.89:1	3:1	0.161	>0.70
shl3 shl4 × WT Col-0	521	32	16.28:1	3:1	108.9	<0.01 (r)
				15:1	0.211	>0.70
shl5-1 × WT Col-0	425	123	3.46:1	3:1	1.9	>0.15

linkage of one of these loci to chromosome 1, between SSLP marker nga63 (11.48 cM) and cleaved amplified polymorphic sequence (CAPS) marker CAT3 (29.91 cM). A smaller mapping population (38 mutant individuals) was used to locate *shl5* to chromosome 5, in close proximity to SSLP marker nga225 (±1.3 cM).

shl Mutant Phenotypes

After 7 d in low white light, *shl1*, *shl2*, *shl5*, and the *shl3 shl4* double mutant had comparatively short hypocotyls and expanded cotyledons relative to WT (Fig. 1). Precocious development of the first set of true leaves was readily apparent in *shl2* and *shl3 shl4* and was also evident in *shl1* and *shl5*—particularly after 8 to 9 d in low light. All four mutant lines had a normal etiolated morphology in darkness (Fig. 1). A minority of *shl5-1* seedlings had partially open, but not expanded, cotyledons (as shown). The frequency of such seedlings was not reproducible from experiment to experiment.

Hypocotyl length was used as simple quantitative measure of seedling developmental sensitivity to light (Fig. 2). In darkness, the strongest allele of *shl1* had a slightly shorter hypocotyl than WT. The *shl3 shl4* double mutant, and the strongest alleles of the *shl2* and *shl5* complementation groups had dark-grown hypocotyl lengths that were indistinguishable from WT. However, each of the mutants showed enhanced sensitivity to white light over a wide range of white light fluence conditions. For example, *shl1-1* showed 26% inhibition of hypocotyl growth at 0.37 μmol m^{-2} s^{-1}—a condition that had no effect on WT hypocotyl length. All of the *shl* mutants showed significantly enhanced inhibition of hypocotyl growth in the range of 1 to 30 μmol m^{-2} s^{-1}. At an intensity of 110 μmol m^{-2} s^{-1}, growth of WT and *shl* mutant hypocotyls was similarly inhibited.

To determine the spectral dependence of expression of the *shl* phenotypes, *shl1-1*, *shl2-1*, *shl3 shl4*, and *shl5-1* were examined in narrow-spectrum R, FR, B, and green (G) light (Fig. 3). Each mutant displayed enhanced responsiveness to light of each of these spectral conditions. The *shl5* mutant showed comparatively less responsiveness to FR and (to a lesser extent) B than the other *shl* mutants, which showed similar patterns of responsiveness in the light conditions tested. Additional alleles of *shl1* (*shl1-2*) and *shl2 (shl2-2, shl2-3, shl2-4)* showed qualitatively similar responses to those of the reference alleles shown in Figure 3. In all cases, the shorter hypocotyl length of the *shl* mutant was accompanied by increased expansion of the cotyledons relative to the WT controls.

Phenotypes of *shl* mutants were also examined in mature plants. All of the *shl* mutants displayed shorter petioles and a more compact rosette than WT. Plants carrying the most severe mutant allele of *shl1* showed a dramatic reduction in fertility and a moderate decrease in apical dominance (Table II). Whereas the *shl2-1* mutation and the *shl3 shl4* double mutation resulted in modest increases (±2-fold) in the accumulation of anthocyanin, the *shl5* mutation resulted in more dramatic increases (±10-fold). Finally, severe *shl2* alleles showed a moderate late-flowering phenotype.

Genetic Interactions with Photoreceptors *PHYA*, *PHYB*, and *CRY1*

The photoreceptors PHYA, PHYB, and CRY1 play predominant—but not exclusive—roles in seedling photomorphogenetic responses to FR, R, and B, respectively (Whitelam et al., 1993; Reed et al., 1994; Ahmad and Cashmore, 1997; Neff and Chory, 1998; Casal and Mazzella, 1998). To test for functional dependence of the *shl* phenotypes on each of these photoreceptors, *shl1-1* and *shl2-2* were placed in double-mutant combinations with *phyA-211*, *phyB-9*, and *cry1-B36* (in the Col-0 genetic background). Hypocotyl phenotypes of *shl phyA*, *shl phyB*, and *shl cry1* double mutants were determined in FR, R, and B, respectively.

As shown in Figure 4, the *shl1* mutant retained significant phenotypic effects on hypocotyl length in both the *phyA* mutant and *phyB* mutant backgrounds. In the cross of *shl1* to *cry1*, five homozygous *cry1* mutant individuals were identified in the F_2 generation by PCR; all had a long hypocotyl phenotype in B,

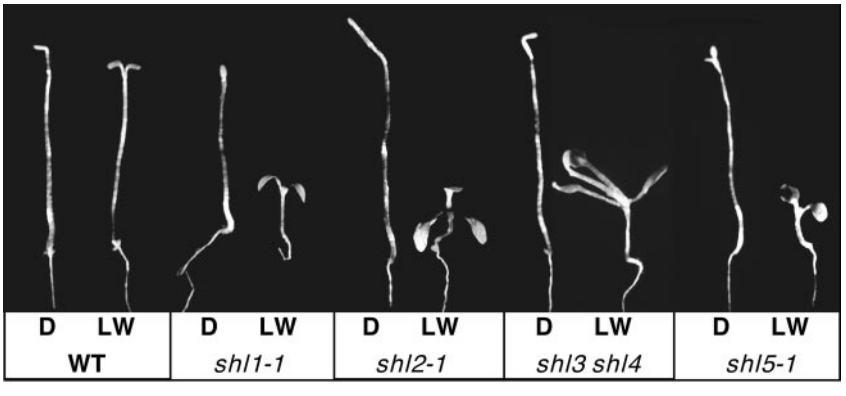

Figure 1. Morphologies of WT and *shl* mutant seedlings. Seedlings were grown for 7 d on Murashige and Skoog/phytagar/2% (w/v) Suc media in darkness (D) or in low white light (LW) at a fluence of 4 μmol m^{-2} s^{-1}.

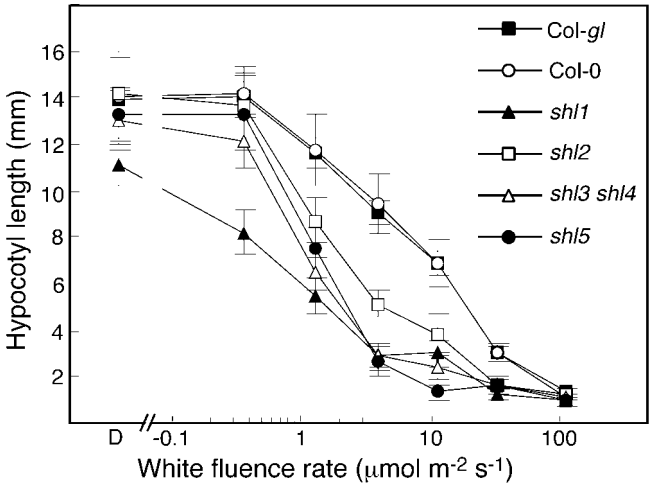

Figure 2. Hypocotyl length responses to white light of varying intensity in WT and *shl* mutant seedlings. Hypocotyls were measured in seedlings grown for 6 d. Error bars = SD.

similar to the *cry1* control. However, in the F_3 progeny from these five F_2 individuals, no novel phenotypes were observed. Thus, we could not definitively identify a phenotype for the *shl1 cry1* double mutant. Given that *shl1* is not linked to *cry1*, we would have expected that two-thirds of the five F_2 individuals would have been heterozygous for *shl1*. The probability that at least one of the five F_2 individuals was heterozygous for *shl1* is approximately 99.6%. Thus, there is a strong possibility that the phenotype of *shl1* in B light is strictly dependent on *CRY1* activity. This hypothesis is supported by the fact that there were no homozygous *cry1* individuals with a hypocotyl phenotype that was shorter that the *cry1* mutant control. However, it remains remotely possible that *shl1* does indeed exert an effect in the *cry1* mutant background and that none of the homozygous *cry1* F_2 individuals were heterozygous or homozygous for *shl1*.

The light-hyperresponsive phenotype of the *shl2* mutant was only partially dependent on *PHYB*, *PHYA*, and *CRY1* in R, FR, and B, respectively (Fig. 4). For example, although the *shl2 phyB* had a slightly longer hypocotyl than WT, it was still significantly inhibited compared to the *phyB* single mutant.

Genetic Interactions with *HY5*

HY5 is a basic-Leu zipper transcription factor that positively regulates seedling de-etiolation and in the process actively promotes the inhibition of hypocotyl elongation (Koorneef et al., 1980; Oyama et al., 1997). The hypocotyl phenotypes of *shl1 hy5* and *shl2 hy5* double mutants were examined in moderate white light (Fig. 5). The phenotypes of the double mutants were additive, with both *shl* mutants showing a partial dependence on *HY5* activity for expression of their light-hyperresponsive phenotypes. Interestingly, *shl1*, which as a single mutant showed the greater inhibition of hypocotyl length in this light condition, also showed the greater degree of dependence on *HY5* activity.

DISCUSSION

To identify mutants in genes acting at the interface of light perception and developmental pathways—"downstream" from the photoreceptors and photoreceptor-specific signaling elements—we employed broad-spectrum white light to cast a "wide net" for mutants that were light hyposensitive or hypersensitive to a wide range of spectral conditions. At the onset, mutant seed pools were "counter-screened" in darkness to eliminate mutants in the *det/cop/fus* class and those with severe pleiotropic developmental defects. In pilot experiments, we found that under low-light conditions, even unmutagenized WT seed stocks gave rise to abnormal seedlings with a relatively short hypocotyl and well-developed cotyledons at a low, but potentially problematic, frequency. This frequency appeared to increase with the age of the seeds, and with the length of time that the seeds are stored in an imbibed state. We concluded that a typical en masse screen of M_2 seedlings for mutants with exaggerated de-etiolation responses would yield an overwhelming number of seedlings with phenotypes that were not due to heritable mutation. To avoid this source of false mutants, we screened M_2 families derived from single M_1 plants and identified pools that segregated multiple individuals with light-hyperresponsive phenotypes. By this strategy, we isolated recessive light-hyperresponsive mutants in eight genetic loci.

On the basis of their recessive nature, we expect that the *SHL* genes act as negative regulators of photomorphogenesis. However, they are functionally distinct from mutants in *det/cop/fus* class in that they give rise to phenotypes that are hyperresponsive to available light, rather than light independent.

There is a formal possibility that *shl* mutants are extremely weak alleles of mutants in *det/cop/fus* class that express overt phenotypes only in the light. However, the overwhelming majority of mutants in the *det/cop/fus* class have been mapped (Chory et al., 1989; Chory et al., 1991; Deng et al., 1991; Wei and Deng, 1992; Miserá et al., 1994; Wei et al., 1994b; Franzmann et al., 1995). *shl1* and *shl5* do not appear to be closely linked to any of these mapped loci. *shl2* mapped to within 10 cM of the published map position of *fus12* (also known as *cop12*) on chromosome 2 (Miserá et al., 1994), but a complementation test demonstrated that *shl2* is not an allele of *fus12*. Thus *shl1*, *shl2*, and *shl5* do not appear to be new alleles of mapped *det*, *cop*, or *fus* loci.

The *shl1*, *shl2*, *shl5* mutants and the postulated *shl3 shl4* double mutant are phenotypically distinct from other recently identified light-hypersensitive mutants. The *spa1* (Hoecker et al., 1998) and *eid1* (Buche et al., 2000) mutants appear to be FR-specific in their

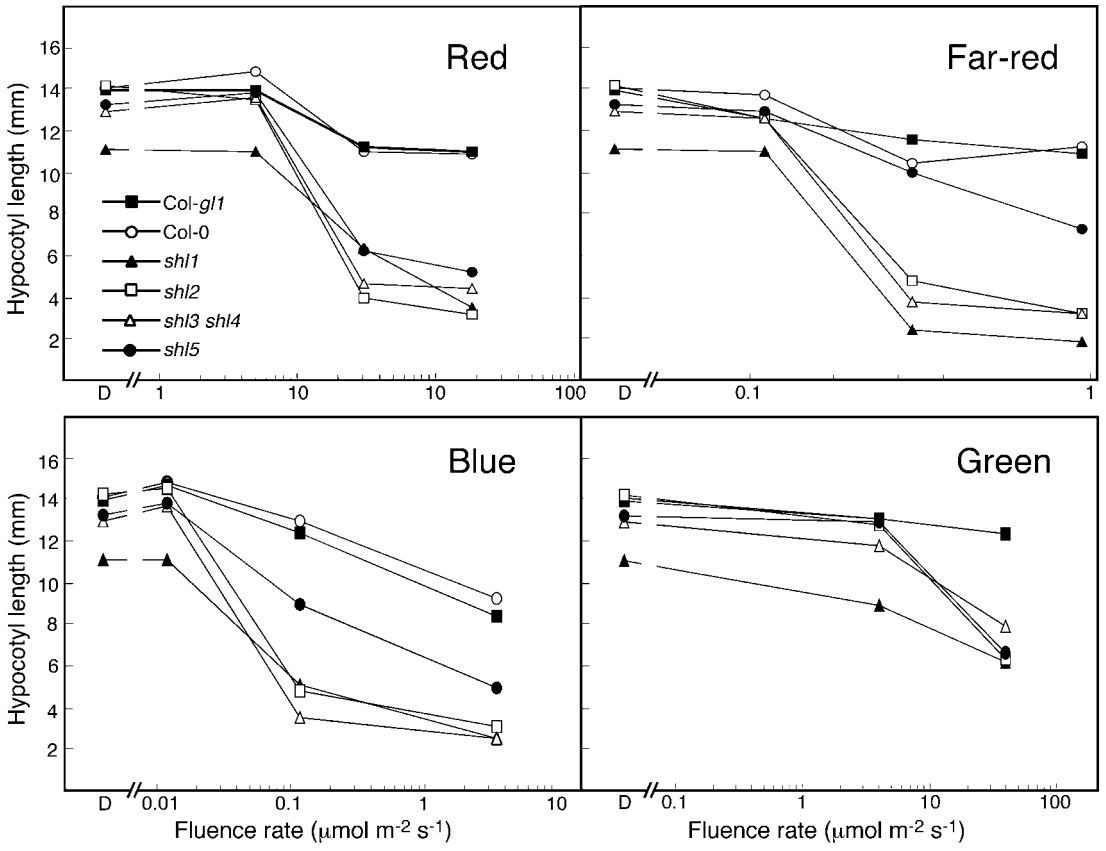

Figure 3. Hypocotyl length responses to various spectral conditions in WT and *shl* mutant seedlings. Hypocotyls were measured in seedlings grown for 6 d in R, FR, B, and G narrow-spectrum light sources at the range of fluences indicated. D, Dark condition.

phenotypic expression. *psi2* (Genoud et al., 1998) displays hypersensitivity to both R and FR light, but is dependent on *PHYB* and *PHYA*, respectively, for these effects and did not show a significant phenotype when tested in a range of B light intensities. Mutations in *SUB1*, a Ca^{2+} binding protein, show enhanced responsiveness to B and FR, but not to R (Guo et al., 2001). Finally, *shy1* (Kim et al., 1996) and *srl1* (Huq et al., 2000) have R-light-dependent phenotypes. *srl1* was located on chromosome 2 near the mapped location of *shl2*, but its phenotypic expression is strictly dependent on *PHYB*. In contrast, *shl2* was not strictly dependent on *PHYB* even for its R-light hypersensitivity. Furthermore, all four alleles of *shl2* showed clear hyperresponsive phenotypes in R, FR, B, and G light. Finally, although one of the *shl3* or *shl4* loci had a subtle hyperresponsive phenotype in FR (as a single mutant), we did not detect any linkage of either loci to nga168, which is linked to *SPA1* on chromosome 2 (Hoecker et al., 1998), or to nga8, which is linked to *EID1* on chromosome 4 (Buche et al., 2000).

shl1, *shl2*, the *shl3 shl4* double mutant, and (to a lesser extent) *shl5* exhibit hyperresponsive phenotypes in FR, R, B, and G. One interpretation of this finding is that the *SHL* genes are acting in a downstream signaling pathway that is shared by *CRY1*, *PHYA*, and *PHYB* and possibly other photoreceptors (*CRY2*, *PHYC*, *PHYD*, *PHYE*). This downstream placement of the *SHL* genes would place them at or near the interface where light signal transduction elements are interacting with developmental regulators. The phenotypes of the *shl* mutants may be due to mutations in signaling molecules or other regulators that result in an increase in the sensitivity of a particular signaling process or amplify the developmental responses. In this respect, *SHL3* and *SHL4*

Table II. *Phenotypic analysis of shl mutant plants*

Plants were examined at the flowering stage, after ±35 d growth in long-day (16-h) conditions. A minimum of eight plants was examined for each determination. Anthocyanin content was measured as a ratio of $(A_{530} - A_{657})$/g fresh wt. The number of elongated inflorescence axes was used as an indicator of apical dominance. Total leaf number was used as a measure of flowering time.

Genotype	Anthocyanin	Inflorescence Axes	Leaf No.
Col-0	1.20 ± 0.47	1.0 ± 0.00	8.38 ± 0.52
Col-*gl1*	1.18 ± 0.34	1.25 ± 0.46	8.50 ± 0.76
shl1-1	1.06 ± 0.31	2.86 ± 1.67	10.86 ± 2.27
shl2-1	2.46 ± 0.34	1.42 ± 0.49	14.10 ± 1.55
shl3 shl4	3.06 ± 0.45	1.34 ± 0.66	9.49 ± 0.96
shl5-1	13.67 ± 3.29	1.0 ± 0.00	8.42 ± 0.53

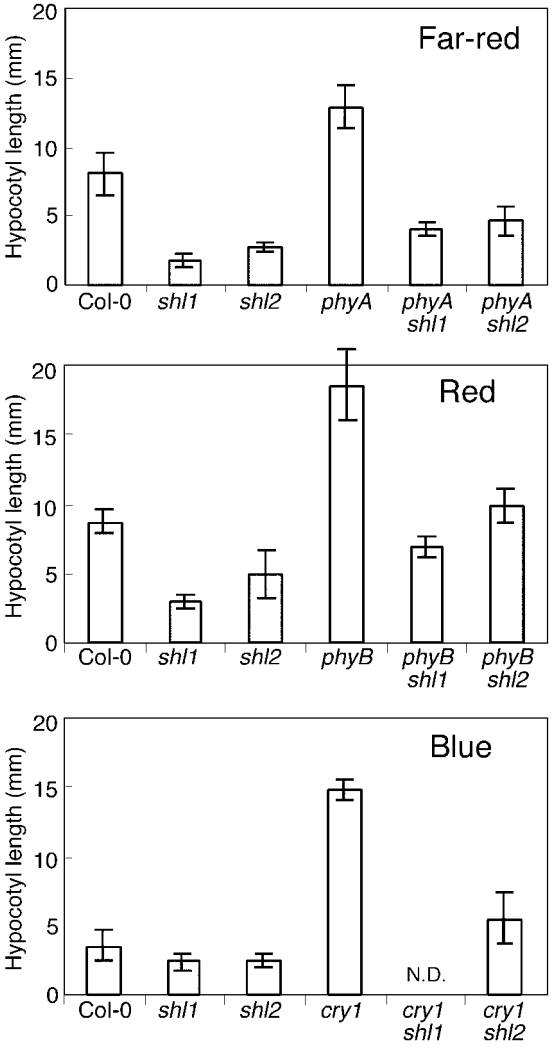

Figure 4. Genetic interactions between *shl-1* and *shl2-2,* and the photoreceptor mutants *phyA-211, phyB-9,* and *cry1-B36.* Hypocotyls were measured in seedlings grown for 7 d in R (64.4 μmol m^{-2} s^{-1}), FR (7.8 μmol m^{-2} s^{-1}), and B (2.78 μmol m^{-2} s^{-1}). These intensities were selected to provide effective phenotypic discrimination between WT and photoreceptor mutants. N.D., Not determined. Error bars = SD.

appear to have at least partially overlapping functions. Several of the mutants also had light-related phenotypes as adult plants, displaying short petioles, elevated anthocyanin (*shl2*, *shl3 shl4,* and *shl5*), and in the case of *shl2*, a moderate late-flowering phenotype similar to that seen in plants overexpressing *CRY1* (Lin et al., 1996).

The photoreceptors PHYA, PHYB, and CRY1 play the dominant roles in seedling photomorphogenetic responses to FR, R, and B, respectively (Reed et al., 1994; Ahmad and Cashmore, 1997; Casal and Mazzella, 1998; Neff and Chory, 1998). It is interesting to note that the FR, R, and B phenotypes of *shl1* and *shl2* were only partially dependent on *PHYA*, *PHYB*, and *CRY1*, respectively. However, the roles played by these major photoreceptors are not exclusive. For example, both Pr and Pfr absorb in the B region of the spectrum (Smith, 1986). *PHYA* plays a subsidiary role in B inhibition of hypocotyl elongation (Whitelam et al., 1993; Ahmad and Cashmore, 1997; Casal and Mazzella, 1998; Neff and Chory, 1998). CRY2 plays a significant role in B-dependent inhibition of hypocotyl elongation at low fluence levels (<10 μmol m^{-2} s^{-1}), similar to those used in our phenotypic analyses. Finally, PHYB plays a minor role in FR-stimulated opening of the apical hook (Neff and Chory, 1998), and other phytochromes (PHYC, PHYD, and PHYE) are either known to, or presumed to, play subsidiary roles in various photomorphogenetic responses to R and FR (Aukerman et al., 1997; Poppe and Schäfer, 1997; Devlin et al., 1999). Thus, the phenotypes of the *shl* mutants in R, FR, and B may be dependent on signals generated by a larger set of photoreceptors with partially overlapping, and often synergistic, activities that may include PHYA through PHYE and both CRY1 and CRY2.

Although PHYA and PHYB are required for full activity of CRY1 (Ahmad and Cashmore, 1997; Casal and Mazzella, 1998; Neff and Chory, 1998), CRY1 can also act independently of PHYA and PHYB (Casal and Mazzella, 1998; Neff and Chory, 1998). All of the *shl* mutants showed substantial hyperresponsiveness to G light. Both Pr and Pfr have absorption minima in the green region of the spectrum (Smith, 1986), and hypersensitivity to G light has previously only been observed in transgenic plants overexpressing *CRY1* (Lin et al., 1996). This result strongly indicates that the *shl* mutations affect pathways that are downstream from cryptochrome(s), as well as the phytochromes. In this respect, it is interesting to note the possible dependence of *shl1* on *CRY1* for expression of its B-hyperresponsive phenotype. This finding would suggest a direct interaction between *SHL1* and *CRY1* in B signaling.

Unlike *sub1*, which is entirely dependent on the activity of *HY5* for the expression of its B and FR hyper-responsive phenotype (Guo et al., 2001), both the *shl1* and *shl2* mutant phenotypes were only par-

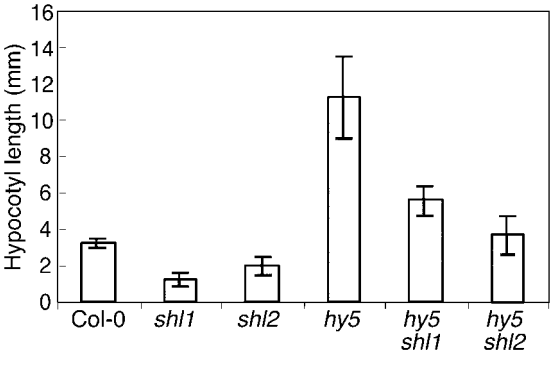

Figure 5. Genetic interactions between *shl-1* and *shl2-2* and the *hy5-5C* mutant. Hypocotyls were measured in seedlings grown for 7 d in white light at an intensity of 45 μmol m^{-2} s^{-1}. Error bars = SD.

tially independent of *HY5*. Since we do not know for certain that *shl1-1* and *shl2-2* are null alleles, all we can conclude is that signals generated by these mutations do act through *HY5*, but also act through alternate pathways. *shl1-1* was more dependent on *HY5* for its phenotypic effect than was *shl2-2*, suggesting that a significant portion of the photomorphogenetic signaling generated in the *shl1* mutant exerts its effect through *HY5* and that *SHL1* may act in a pathway that is upstream from *HY5* and other regulators.

The *shl* mutants may also be defective in elements of phytohormone signaling and perception pathways—a finding that is not mutually exclusive with their involvement in light perception. Cytokinin, giberellins, and brassinolides have all been implicated in the regulation of seedling development by light (Chory et al., 1994; Chin-Atkins et al., 1996; Chory and Li, 1997). Recently, several lines of evidence have directed attention towards the interplay of light and auxin signaling. *shy2*, an extragenic suppressor of *phyB* and *hy2*, is mutated in an auxin regulatory gene *IAA3* (Tian and Reed, 1999; Soh et al., 1999). Expression of *FIN219*, a phytochrome A signaling molecule, is also regulated by auxin (Hsieh et al., 2000). It is of particular interest to note that napthylphthalamic acid, an inhibitor of auxin transport, is also a potent inhibitor of hypocotyl elongation in light-grown, but not dark-grown, seedlings (Jenson et al., 1998). This effect was stimulated by R, FR, and B, and was fluence dependent. This finding suggests that *shl* phenotypes could be generated through mutations that perturb auxin transport or signaling.

shl1 in particular displays several of the phenotypic hallmarks of a phytohormone signaling defect (Chory and Li, 1997; Leyser, 1998), including reduced fertility, reduced leaf elongation, and reduced apical dominance. Although *shl1* maps to the top of chromosome 1, where *AXR1* and *AXR3* are located, it complements a recessive allele of *axr1*, and was separated from the location of the cloned *AXR1* gene (Leyser et al., 1993) and the mapped position of *axr3* by multiple recombination breakpoints. It is interesting to note that *shl1* did not show elevated levels of anthocyanin, indicating that only a subset of light-dependent responses are effected. Thus, *SHL1* may play a role in the regulation of only a subset of developmental responses affecting growth and morphology.

MATERIALS AND METHODS

Plant Materials, Growth Conditions, and Mutant Isolation

Arabidopsis ecotype Col-*gl1* seeds were obtained from Lehle Seeds (Round Rock, TX). Null mutants *phyA-211*, *phyB-9*, and *cry1-B36* (in the Col-0 ecotypic background) were obtained from Jason Reed (Reed et al., 1994). The *hy5-5C* null allele was isolated in the Col-0 background as a suppressor of *det1-1* (Pepper and Chory, 1997). This *hy5* allele was back-crossed twice to WT Col-0 and was homozygous for the WT *DET1* allele. Col-*gl1* seeds were mutagenized by imbibation in 0.3% (v/v) ethyl methane sulfonate for 12 h, followed by extensive washing with H_20. M_1 seeds were sown on soil to achieve a final density of 0.25 plants cm^{-2}, grown under an 8-h day-length regime for 40 d, then transferred to a 16-h day length in order to stimulate flowering. This protocol produced mature plants with a stout, erect inflorescence, thus preventing entanglement and greatly facilitating the harvest of independent M_2 seed pools from individual M_1 plants. Aliquots of ±80 seeds from each M_2 seed pool were surface sterilized (Chory et al., 1989), resuspended in sterile 0.1% (w/v) phytagar, then cold treated at 4°C for 40 h. Seed pools were then dispersed onto duplicate plates containing Murashige and Skoog/phytagar/2% (w/v) Suc media. Seeds were illuminated for 4 h with white light (100 μmol m^{-2} s^{-1}) to ensure optimal germination, then screened simultaneously in darkness and in either low-intensity white light (4 μmol m^{-2} s^{-1}) or under a yellow-green acrylic filter (24 μmol m^{-2} s^{-1}). Mutants were identified after 7 to 8 d. Unless stated otherwise, experiments were performed at 23°C ± 0.5°C under a 16-h day-length regime.

Genetic Analysis

The genetic methods employed have been described previously (Chory et al., 1989; Pepper and Chory, 1997). Routine phenotyping for complementation, segregation, and mapping experiments was performed under low white light or under a yellow-green acrylic filter. Genomic DNAs were isolated using the micropreparation method described by Pepper and Chory (1997). Mapping of *shl* mutants was performed using PCR-based CAPS (Konieczny and Ausubel, 1993) and SSLP (Bell and Ecker, 1994; Lukowitz et al., 2000) markers. Mutants were back-crossed to WT Col-0 or Col-*gl1* at least three times prior to comprehensive phenotypic analysis.

Our strategy for the identification of *shl phyA*, *shl phyB*, *shl cry1*, and *shl hy5* double mutants was partially dependent on an assumption that the *shl* mutations acted in a fully recessive manner. Alleles *shl1–1* and *shl2–2* were crossed with *phyA-211*, *phyB-9*, *cry1-B36*, and *hy5-5C*. We phenotyped the F_2 generation under conditions that gave excellent discrimination between the WT and *phyA* (7.8 μmol m^{-2} s^{-1} FR), *phyB* (64.4 μmol m^{-2} s^{-1} R), *cry1* (2.78 μmol m^{-2} s^{-1} B), and *hy5* (45 μmol m^{-2} s^{-1} white light) and identified individuals with phenotypes that were similar to *phyA-211*, *phyB-9*, *cry1-B36*, and *hy5-5C* controls. These F_2 individuals, assumed to be homozygous for their respective photoperception-deficient alleles, were then examined in the F_3 generation for the appearance of distinct short hypocotyl progeny at a frequency consistent with the segregation of the recessive *shl* mutant (1, short; 3, long). In the absence of such progeny, the phenotype of the double mutant could not be conclusively determined. F_2 and F_3 individuals homozygous for the *cry1-B36* mutant allele were identified by a PCR-based assay: oligonucleotide

primers CRY1-F2 (5′-GATCAAACAGGTCGCGTGG-3′) and CRY1-R2 (5′-TTTCATGCCACTTGGTTAGACC-3′) failed to produce an amplification product in the homozygous *cry1-B36* mutant.

Analytical Methods

Occasional seedlings with obvious severe developmental defects were omitted from any phenotypic analyses. For measurements of hypocotyl length, 30 seeds of each genotype were evenly dispersed onto Murashige and Skoog/phytager/2% (w/v) Suc media in a 7-mm grid pattern. All seeds were subjected to 4 h of white light (100 μmol m^{-2} s^{-1}) prior to placement in the dark or in various light regimes for 6 d. Hypocotyls were straightened using forceps if necessary, then measured under a stereo dissecting microscope using a 0.5-mm ruler. Hypocotyls of seedlings growing appressed to the agar media were not measured. Analyses of anthocyanin content (by an acid-methanol extraction), flowering time, and apical dominance were performed as described in Pepper and Chory (1997).

Light Sources

Narrow-spectrum R and FR light were supplied by light-emitting diode arrays (models SL515-670 [670-nm maximum] and SL515-735 [735-nm maximum], respectively; Quantum Devices, Inc., Barneveld, WI). Narrow-spectrum B light (420-nm maximum) was supplied by Coralife Actinic 03 fluorescent aquarium bulbs (Energy Savers Unlimited, Inc., Carson, CA) filtered through a Kopp 5-57 blue glass filter (Kopp Glass, Inc., Swissvale, PA). White light was supplied by an equal mixture of cool-white and Growlux wide-spectrum fluorescent bulbs (Sylvania, Danvers, MA). A 2472 yellow-green acrylic filter (Polycast Technology, Stamford, CT) with a transmission maximum of ±550 produced light that was partially depleted in the photomorphogenetically active UV, B, R, and FR regions of the spectrum. Narrow-spectrum G light (±520-nm maximum) was produced by a 2092 green acrylic filter (Polycast Technology), as described previously (Ahmad and Cashmore, 1993; Lin et al., 1996). Dark experiments were performed in a passively ventilated dark box. Fluence rates of white, R, B, yellow, and G light were measured with a quantum photometer (model LI-189, LI-COR, Lincoln, NE). Fluence rates of FR light were measured using a radiometer (model IL1400, International Light, Newburyport, MA) with FR probe (model SEL033, International Light).

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