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RED1 Is Necessary for Phytochrome B–Mediated Red Light–Specific Signal Transduction in Arabidopsis

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Seedlings of a transgenic Arabidopsis line (ABO) that overexpresses phytochrome B (phyB) display enhanced deetiolation specifically in red light. To identify genetic loci necessary for phytochrome signal transduction in red light, we chemically mutagenized ABO seeds and screened M_2 seedlings for revertants of the enhanced deetiolation response. One recessive, red light–specific extragenic revertant, designated *red1*, was isolated. The mutant phenotype was expressed in the original ABO background as well as in the nontransgenic Nossen (No-0) progenitor background. *red1* is also deficient in several other aspects of red light–induced responses known to be mediated by phyB, such as inhibition of petiole elongation and the shade avoidance response. *red1* was mapped to the bottom of chromosome 4 at a position distinct from all known photoreceptor loci. Together with complementation analysis, the data show that *red1* is a novel photomorphogenic mutant. The evidence suggests that *red1* represents a putative phytochrome signal transduction mutant potentially specific to the phyB pathway.

INTRODUCTION

Many developmental changes during early seedling development depend on the light environment. Plants have evolved a series of photoreceptors to sense the quality, quantity, periodicity, and direction of light (Kendrick and Kronenberg, 1994). Light-triggered deetiolation results in inhibition of hypocotyl elongation, opening of the hook and cotyledons, and stimulation of cotyledon expansion. Two major photoreceptor families that control deetiolation have been characterized molecularly in Arabidopsis: the blue light–responsive cryptochromes (Ahmad and Cashmore, 1993) and the red/far-red (R/FR) light–sensing phytochromes (Sharrock and Quail, 1989; Clack et al., 1994).

Structure and function are best understood for two members of the phytochrome family in Arabidopsis: phytochrome A (phyA) and phyB from experiments using overexpression in transgenic Arabidopsis (McCormac et al., 1991; Cherry et al., 1992, 1993; Boylan et al., 1994; Quail et al., 1995; Wagner and Quail, 1995; Wagner et al., 1996a, 1996b) and from characterization of mutations at both loci (Dehesh et al., 1993; Reed et al., 1993; Xu et al., 1995). phyA, which is light labile, is necessary for deetiolation in continuous FR light (FRc) in the so-called FR high irradiance response (FR-HIR) (Dehesh et al., 1993; McCormac et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993; Whitelam and Harberd, 1994). The light-stable phyB, on the other hand, is primarily responsible for deetiolation in continuous R light (Rc) via an R high irradiance response (Nagatani et al., 1991; Somers et al., 1991; McCormac et al., 1993; Reed et al., 1993). In addition, *phyB* mutants exhibit reduced cotyledon size, elongated petioles and leaves, stronger apical dominance, and early flowering (Reed et al., 1994). phyB has also been implicated in the so-called end-of-day FR (EOD FR) response as well as the shade avoidance response (Smith, 1994). Constitutive overexpression of phyB in transgenic Arabidopsis results not only in increased deetiolation but also causes an increase in cotyledon size, a reduction in petiole and leaf length, as well as reduced apical dominance (D. Wagner and P.H. Quail, unpublished data); these findings are consistent with the notion that these responses are dependent on the amount of phyB signal.

Despite recent progress in the effort to understand phytochrome signal transduction in Arabidopsis (reviewed in Chory, 1993, 1994; Pepper et al., 1993; Deng, 1994; Millar et al., 1994; Quail, 1994; Reed and Chory, 1994; Barnes et al., 1995; Quail et al., 1995), the initial events in this process are still poorly understood. In Arabidopsis, two general types of genetic screens have been performed to isolate phytochrome signal transduction mutants. In the first approach, mutants exhibiting constitutive deetiolation in the dark were isolated (Chory et al., 1989; Deng et al., 1991). Some of these have been defined molecularly (Deng et al., 1992; Castle and Meinke, 1994; Pepper et al., 1994; Wei et al.,

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1994; Takahashi et al., 1995; Li et al., 1996; Szekeres et al., 1996). These mutants show altered responses in R, FR, and blue light (for example, see Deng and Quail, 1992; Chory et al., 1994; Millar et al., 1994) and therefore are not likely to represent initial events specific to signal transduction from a single photoreceptor. Two novel, dominant mutants designated *shy1* and *shy2* (for suppressor of *hy2* mutation; Kim et al., 1996) may represent signal transduction mutants. However, they are at least partially constitutively active, exhibit phenotypic traits in addition to those due to loss of phytochrome activity, and are therefore more likely to be involved farther downstream in signal transduction.

The second approach focused on mutants that exhibit reduced deetiolation in the light. Screens for this type of mutation have been conducted mainly in continuous white light (WLc) and have resulted in the isolation of photoreceptor mutants (*hy4* [cryptochrome] and *hy3* [*phyB*]; Koornneef et al., 1980; Somers et al., 1991; Ahmad and Cashmore, 1993; Reed et al., 1993), mutants defective in phytochrome chromophore biosynthesis (*hy1* and *hy2*; Koornneef et al., 1980; Parks and Quail, 1991), as well as putative phytochrome signal transduction mutants (*hy5*; Koornneef et al., 1980). *hy5* is deficient in R, FR, and blue light signal transduction, is thought to interact with CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1; Ang and Deng, 1994), and is not likely to represent an early step in phytochrome signal transduction. The recently identified *elongated* (*elg*) mutant (Halliday et al., 1996) affects hypocotyl elongation in R, FR, and blue light and may act independently of phytochrome signal transduction.

Mutants affecting early steps in either phyA or phyB signal transduction should be selectively deficient in either FRc or Rc responsiveness. Thus, screening for loss of deetiolation in FRc or Rc should enhance the chance to recover mutants that may represent mutations in early steps of each of these phytochrome signal transduction pathways. Recently, a screen in FRc has led to the isolation of two putative FR light–specific signal transduction mutants, *fhy1* and *fhy3* (Whitelam et al., 1993). However, without evidence to the contrary, it cannot be ruled out that the *fhy1* and *fhy3* mutations affect one of the phytochrome photoreceptor loci of as-yet-unknown function (*PHYC*, *PHYD*, and *PHYE*).

No mutant affecting early, R light–specific phytochrome signal transduction has yet been isolated. To this end, we chemically mutagenized a transgenic Arabidopsis line that overexpresses phyB (designated ABO; Wagner et al., 1991) and screened in Rc for revertants of the overexpression phenotype. It was reasoned that this novel screen should enrich for mutations in transgenic phyB (intragenic revertants; Wagner and Quail, 1995) as well as for mutations in the phyB signal transduction pathway (extragenic revertants). Recovery of mutations in the endogenous phyB would not be expected in this screen because the deetiolation of ABO in the wild-type background is indistinguishable from that of an Arabidopsis line that overexpresses phyB (ABO) in a *phyB*-null background (D. Wagner and P. Quail, unpublished data).

RESULTS

Isolation of Two Rc-Specific Extragenic Revertants of the phyB Overexpression Phenotype

Figure 1A outlines the primary and secondary screen used to isolate Rc-specific revertants of the enhanced deetiolation response in ABO. Four thousand to 8000 M_2 seedlings from each of 79 M_1 families comprising 500 to 1000 M_1 plants were screened for hypocotyls longer than those of the ABO parent after 5 days of growth in Rc (22 μmol m^{-2} sec^{-1}). We isolated 617 such long hypocotyl revertants and recovered seed from 500 of them. M_3 seed of a subset of lines (300) was rescreened in Rc and also tested for hypocotyl elongation in FRc (5.7 μmol m^{-2} sec^{-1}). Sixty-one percent of the M_3 progeny were tall in Rc only (class 1), whereas 39% showed elongated hypocotyls in Rc and FRc (class 2). Figure 1B shows a characteristic Petri plate from the primary screen. The phyB-overexpressing ABO line formed a lawn of short hypocotyl seedlings, above which longer hypocotyl revertants could be selected visually.

Table 1 summarizes the results obtained from the molecular characterization of a subset of class 1 revertants. These revertants include intragenic revertants (phyB truncations, loss of transgene expression, and single amino acid changes reducing phyB activity) as well as potential R light–specific extragenic revertants (candidate R light signal transduction mutants). We chose 101 class 1 revertants (maximally three per M_1 family) for immunochemical analysis to determine the presence or absence of full-length transgene-encoded phyB at parental levels. The presence of parental levels of full-length transgene-encoded phyB is indicative of either extragenic revertants or intragenic revertants carrying single amino acid substitutions. Of the 101 revertant lines analyzed on immunoblots (data not shown), only six showed parental levels of full-length transgene-encoded phyB. To distinguish between intragenic and extragenic revertants, we tested linkage of the mutation to the phyB-encoding transgene by backcrosses to the wild-type ecotype Nossen (No-0) and analysis of hypocotyl length in the F_1 and F_2 generations. Four mutants exhibited the revertant phenotype in the F_1 generation and in all kanamycin-resistant progeny in the F_2 generation. These mutations were linked to the phyB-encoding transgene and were found to be caused by single amino acid changes in the transgene-encoded phyB (Wagner and Quail, 1995). However, two mutants were not linked to the phyB-encoding transgene (below) and behaved as recessive, extragenic revertants. These mutants were therefore good candidates for R light–specific signal transduction mutants. The remaining 95 class 1 revertants carried phyB truncations or exhibited reductions in phyB levels and were linked to the transgene where tested (data not shown).

Class 2 revertants are likely to be extragenic revertants, because they affect deetiolation in both Rc and FRc. We chose 63 class 2 revertants for hypocotyl experiments with

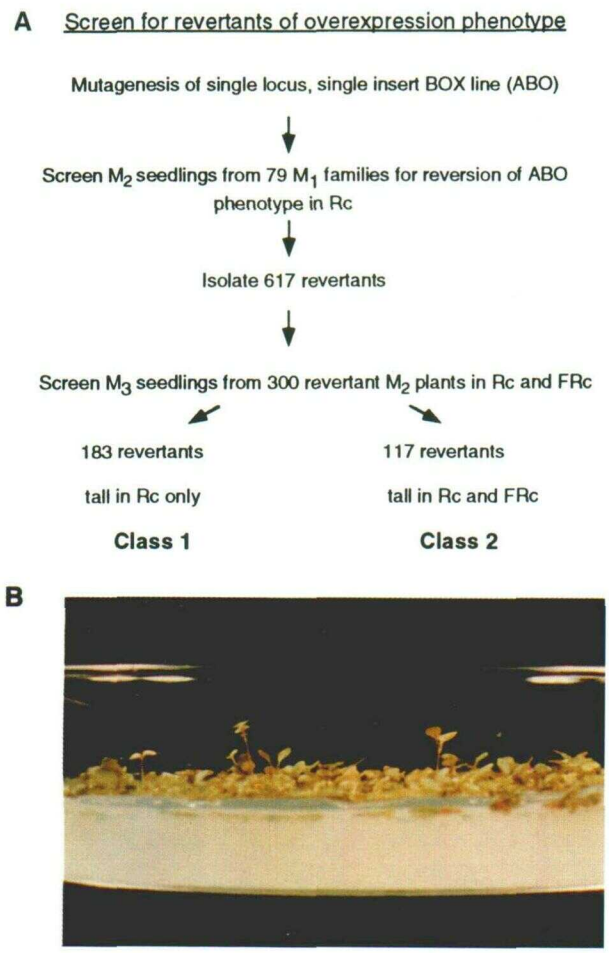


Figure 1. Summary of the Revertant Screen by Using the phyB Overexpression Phenotype.

(A) Schematic diagram of the screen employed to isolate revertants of the phyB overexpression phenotype. A phyB-overexpressing (BOX) transgenic Arabidopsis line, which contains a single phyB-encoding transgene at a single locus (ABO), was subjected to chemical mutagenesis. M_2 seedlings from 79 M_1 families were screened for hypocotyls longer than those of ABO in Rc (22 μmol m^{-2} sec^{-1}). A subset of the progeny of 617 tall revertants was again screened in Rc (22 μmol m^{-2} sec^{-1}) and FRc (5.7 μmol m^{-2} sec^{-1}) to identify Rc-specific revertants (class 1) and Rc/FRc revertants (class 2). Class 1 revertants were tested for extragenic revertants with unaltered phyB-protein levels.

(B) Visual screen for revertant M_2 Seedlings in Rc. M_2 population was grown in Rc (22 μmol m^{-2} sec^{-1}) for 5 days. phyB-overexpressing seedlings have short hypocotyls in Rc, and revertants with longer hypocotyls are easy to identify visually.

the exogenous chromophore precursor biliverdin (BV) and for analysis of the amount of spectrally active phytochrome, as shown in Table 2, to test for chromophore biosynthesis mutations (such as in *hy1* and *hy2*; Parks and Quail, 1991). The majority of the class 2 mutants could be rescued by the addition of BV, indicating that these mutants affect phytochrome chromophore biosynthesis (Table 2). Consistent with this interpretation, these revertants showed reduced levels of spectrally active phytochrome ($\Delta\Delta$A−) where tested. However, six class 2 mutants showed no reduction in the revertant phenotype when grown in the presence of BV, and they were fully spectrally active ($\Delta\Delta$A+) (Table 2). None of the six mutations was linked to the transgene, and all were recessive (data not shown). These may be allelic to the previously described putative Rc, FRc, and blue light signal transduction mutant *hy5* (Koornneef et al., 1980) or may represent novel signal transduction mutants. The six class 2 mutants were not characterized further. Rather, we focused on one of the two R light–specific extragenic revertants, which we named *red1* (for red elongated1).

The *red1* Mutant Is Novel, Extragenic, and Recessive

The *red1* revertant of the phyB overexpression phenotype (*red1* [ABO]) was tested for linkage of the mutation to the phyB-encoding transgene by outcrossing to the nontransgenic wild type (No-0), followed by analysis of hypocotyl length in the F_1 and F_2 generations. Figure 2A shows that the F_1 progeny of the *red1* (ABO) backcross to the wild type were very close in hypocotyl length to ABO; this observation is consistent with *red1* being a recessive, extragenic mutation. Segregation ratios in the F_2 generation are in agreement with this interpretation: the *red1* mutation segregated in a recessive manner in the transgenic (ABO) background as well as in the nontransgenic (No-0) background (Figures 2B and 2D). To confirm this result, homozygous *red1* mutants in the No-0 background were backcrossed to the wild type. We again observed a wild-type phenotype in the F_1 generation (data not shown) and segregation ratios in the F_2 generation that agreed well with a recessive mutation conferring the *red1* mutant phenotype (Figures 2C and 2E). As is conventional, the *red1* mutation in the nontransgenic No-0 background is henceforth referred to as *red1*.

red1 was mapped to the bottom of chromosome 4 by

Table 1. Molecular Analysis of Phytochrome in Rc-Specific (Class 1) Revertants

	phyB Protein[b]		
Number[a]	Size (kD)	Amount	Linked to phyB Transgene
2	122	Parental	No
4	122	Parental	Yes

[a] Number of class 1 revertants with the indicated characteristics. The total number of class 1 revertants screened was 101.

[b] Crude extracts of Rc-grown seedlings, showing unaltered size (122 kD) and amount (parental) of the transgene-encoded phyB polypeptide.

Table 2. Molecular Analysis of Phytochrome in Rc/FRc–Specific (Class 2) Revertants

Number[a]	Height + BV[b]	ΔΔA[c]
6	Tall	+
57	Short	−[d]

[a] Number of class 2 revertants with the indicated characteristics. The total number of class 2 revertants tested was 63.
[b] Hypocotyl length in Rc when supplemented with the phytochrome chromophore precursor (BV). Chromophore biosynthesis mutants are short (ABO parental height), whereas other mutants remain tall.
[c] Crude extracts of dark-grown seedlings, showing that endogenous phyA is spectrally active (ΔΔA+) or inactive (ΔΔA−) in the absence of BV.
[d] Based on random samples.

using polymerase chain reaction (PCR)–based markers (described in Methods), as shown in Figure 3. This map position rules out the possibility that *red1* represents a mutation in any of the phytochrome photoreceptors, the function of three of which (phyC, phyD, and phyE) is not yet understood. *PHYA, PHYB,* and *PHYC* map to other chromosomes and therefore are clearly separate loci from *red1.* Although *PHYD* and *PHYE* both map to chromosome 4, they map between positions 78.2 to 80.8 and 84.5 to 86.7, respectively (Schmidt et al., 1996), at least 18 centimorgans (cM) from *red1.* Thus, *red1* is a locus distinct from *PHYD* and *PHYE.*

The map position of *red1* also is distinct from all other known and mapped photomorphogenic mutants, with the exception of the mutant *elg* that maps 3 cM centromere proximal of the marker AP2 on chromosome 4 (Halliday et al., 1996). *elg* is a partially dominant mutant that displays a long hypocotyl in R as well as in FR light. This phenotype suggests that it represents a mutation at a locus distinct from *red1.* Complementation analysis of *red1* and *elg* confirmed this prediction, as shown in Figure 4. The F_1 progeny of a cross of *elg* to *red1* exhibited a hypocotyl length similar to that of the F_1 progeny of a cross of *elg* to No-0. *red1* is therefore not allelic to *elg.*

To rule out the possibility that *red1* acts simply by reducing the levels of one or more phytochromes, we used monoclonal antibodies specific for each phytochrome (J. Tepperman, R.A. Sharrock, and P.H. Quail, unpublished results) to analyze the amount of each phytochrome in concentrated protein extracts from wild-type and *red1* seedlings. No reduction in the level of any phytochromes was observed (data not shown), suggesting that *red1* is involved more directly in signal transduction.

Lastly, to exclude the possibility that *red1* may be a novel mutation defective in phytochrome chromophore biosynthesis like the previously described *hy1* and *hy2* mutants (Koornneef et al., 1980; Parks and Quail, 1991), we tested whether the elongated phenotype could be rescued by growth of the seedlings on 0.6 mM phytochrome chromophore precursor BV, as shown in Figure 5. This high level of BV was sufficient to completely rescue *hy1* and *hy2* compared with their wild type (Landsberg *erecta* [*Ler*]) in Rc. However, *red1* and *phyB* still showed elongated hypocotyls compared with their wild types (No-0) (Figure 5). Hence, we conclude that *red1* is a novel, R light–specific phytochrome signal transduction mutant.

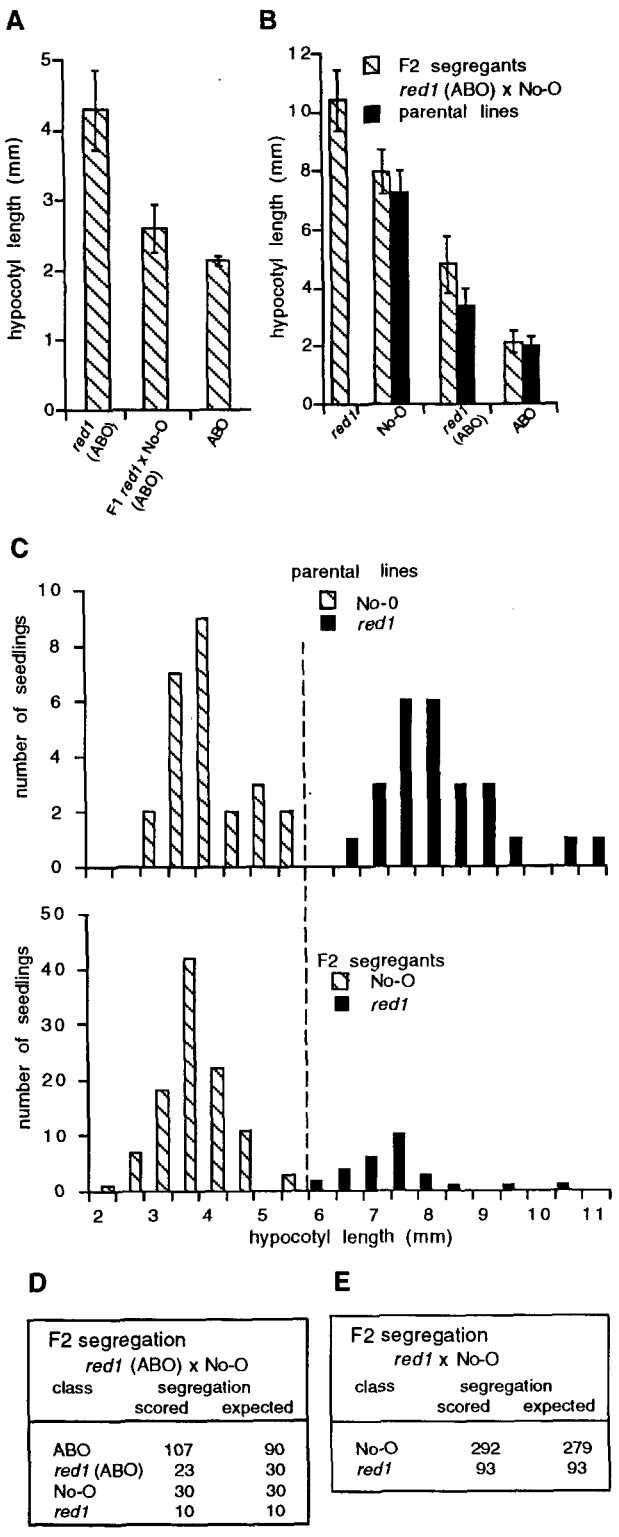
red1 Lacks Many Aspects of the phyB-Induced Response

Inhibition of Hypocotyl Elongation in Rc but Not FRc

To test whether *red1* specifically affects a range of phyB-mediated responses, we compared quantitatively the phenotype of *red1* to those of the two known phytochrome photoreceptor mutants *phyB* and *phyA.* Figures 6A and 6B show that although the *red1* mutant, like *phyB* mutants, displayed an elongated hypocotyl in R light, and although this phenotype was expressed in the original transgenic ABO background as well as after outcrossing into the nontransgenic No-0 background, this mutation did not cause full loss of Rc responsiveness in either genetic background: *red1* (ABO) is taller than ABO but not as tall as No-0, and *red1* is taller than No-0 but not as tall as *phyB.* This result suggests that *red1* may represent a leaky mutation or alternatively a null allele in a redundant pathway. In contrast to Rc, when seedlings were grown in FRc or in darkness, there was no significant difference in hypocotyl length between *red1* mutant and wild-type seedlings (Figures 6A and 6B). The Rc-specific defect in hypocotyl elongation was observed over a range of fluence rates, with stronger differences between *red1* and the wild type at higher fluence rates (Figure 6C). However, even at the highest fluence rates of FRc, no difference in hypocotyl length was observed between the wild type and *red1* (Figure 6D). Hence, *red1* mutant seedlings are specifically affected in their response to R light. *phyA,* on the other hand, was indistinguishable from its wild type (RLD) in Rc but significantly taller in FRc (Figure 6A), as previously reported (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993).

Cotyledon Expansion and Petiole Elongation

phyB in the active (Pfr) conformation is known to promote cotyledon expansion in R or white light (Neff and Van Volkenburgh, 1994). phyA, in contrast, plays a negligible role in this process under these light conditions (Reed and Chory, 1994). Consistent with these findings, cotyledon size of *phyB* seedlings grown in Rc was greatly reduced relative to the wild type (No-0), whereas cotyledons of the *phyA* mutant were indistinguishable from its wild type (RLD) (Figure 7A). Similar to the *phyB* mutation, the *red1* mutation caused a strong reduction in cotyledon size compared with the wild type



D

F2 segregation *red1* (ABO) x No-O		
class	segregation scored	expected
ABO	107	90
red1 (ABO)	23	30
No-O	30	30
red1	10	10

E

F2 segregation *red1* x No-O		
class	segregation scored	expected
No-O	292	279
red1	93	93

Figure 2. The *red1* Mutation Is Recessive and Unlinked to the phyB-Encoding Transgene.

(No-0) (Figure 7A). This phenotype was expressed in both genetic backgrounds, ABO and No-0. Significantly, the cotyledons of the *red1* mutant were almost as small as those of the *phyB* mutant. Hence, when compared with the role of phyB, the *RED1* gene product appears to play a stronger role in cotyledon expansion than in hypocotyl elongation.

Mutations in the *PHYB* gene result in increased petiole length (Reed et al., 1993). Figure 7 shows that the *red1* mutation also caused increased petiole elongation in WLc in both the transgenic ABO background and the nontransgenic No-0 background. In contrast, the *phyA* mutant did not exhibit elongated petioles, indicating that phyA does not play a role in inhibition of petiole elongation under these conditions (Figures 7A and 7B). In addition, leaf area and shape are altered in *red1* and *phyB* mutants but not in *phyA* mutants (Figure 7B). Taken together, these data suggest that *red1* behaves more like a mutant resulting from loss of phyB than of phyA activity, as predicted from the R light specificity of the hypocotyl phenotype.

(A) Mean hypocotyl length of the F_1 progeny of the backcross of *red1* (ABO) to the wild type (No-0) in Rc. Results using the original M_3 revertant in the transgenic phyB-overexpressing (ABO) background (*red1* [ABO]), the F_1 progeny of the backcross of *red1* (ABO) to the nontransgenic No-0 progenitor background, as well as the parental ABO line grown for 4 days in Rc (22 μmol m^{-2} sec^{-1}) are shown. The mean hypocotyl length was determined from 10 seedlings, and each error bar denotes 1 SD.

(B) Mean hypocotyl length of four visually identified subgroups in the F_2 generation of the cross in **(A)** compared with the parental lines. Hypocotyl length was determined for 4-day-old seedlings grown in Rc (22 μmol m^{-2} sec^{-1}). F_2 seedlings were scored visually as *red1* in the nontransgenic No-0 background (*red1*), No-0, *red1* in the transgenic ABO background (*red1* [ABO]), and ABO (striped bars), based on their hypocotyl length and cotyledon phenotype. Also shown for comparison is the mean hypocotyl length of the parental ABO, *red1* (ABO) M_3 seedlings, and No-0 (black bars), grown on the same plate as the F_2 population. Each error bar denotes 1 SD.

(C) Segregation of the F_2 population of *red1* crossed to No-0 shows the recessive nature of *red1*. Hypocotyl length distribution for the parental lines is shown at top. Plants were scored visually as *red1* or No-0 based on their cotyledon phenotype (bottom). Hypocotyl length distribution of the designated No-0 plants (striped bars) and the *red1* plants (black bars) separated by a vertical dashed line is shown.

(D) Segregation analysis of the F_2 population shown in **(B)** indicates that *red1* is a recessive unlinked revertant. The number of seedlings visually scored in the four subgroups identified in **(B)** as well as the number of seedlings expected for segregation of an unlinked, recessive mutation are indicated.

(E) Number of seedlings scored as *red1* and No-0 in the F_2 generation from a backcross of *red1* from three independent experiments. The number of seedlings scored as No-0 and *red1* as well as the number of seedlings expected in each group for a recessive mutation are indicated.

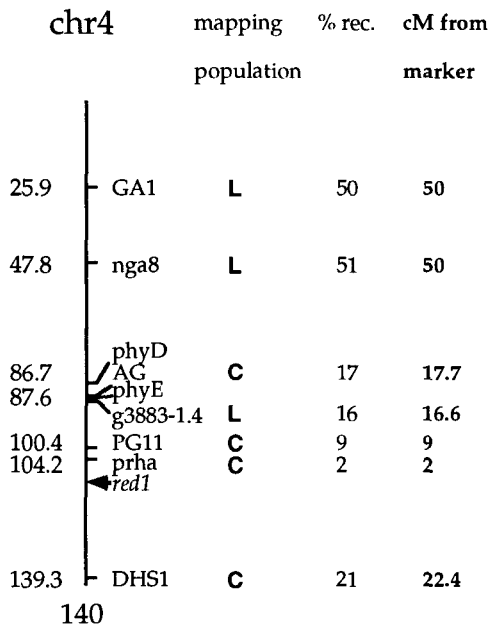


Figure 3. Map Position of the *red1* Mutation.

red1 was mapped using PCR-based cleaved amplified polymorphic sequences (CAPS; Konieczny and Ausubel, 1993) and simple sequence length polymorphisms (Bell and Ecker, 1994), as described in Methods. The two mapping populations used were F_3 seedlings derived from crossing *red1* (ABO) to L*er* (L) and to Columbia (C). The percentage of recombination (% rec.) was scored for seedlings derived from 50 independent F_2 parents. Map distance (in centimorgans [cM]) was calculated based on Kosambi, as described in Koornneef and Stam (1991). The map position at *chr.4* is shown relative to the markers used, and marker position is based on the method of Lister and Dean (1993; recombinant inbred lines, http://cbil.humgen.upenn.edu/~atgc/genetic-mapping/ListerFeb 95.html).

EOD FR Response and Shade Avoidance Response

To test further the hypothesis that *red1* is deficient in phyB signal transduction, we performed additional photobiological analyses. *phyB* is defective in the so-called EOD FR response and the shade avoidance response. Figure 8A summarizes the responses of *red1* and *phyA* and *phyB* to EOD FR treatment. The EOD FR response is based on the conversion of the majority of the light-stable phytochrome (presumably phyB) into its inactive Pr form before the night period. During the night, all lines should therefore show increased growth rates (equivalent to those seen in the phyB null mutant in Rc). Both wild types (No-0 and RLD) and the *phyA* mutant showed a typical EOD FR response: hypocotyl length in white light with a predark FR pulse (WL plus FRp) is longer than in white light without a predark FR pulse (WL minus FRp). No increase in hypocotyl length was seen in the *phyB* mutant under EOD FR conditions as expected. *red1* showed a decrease in the EOD FR response, which on the surface is consistent with a role of phyB in this response. However, higher growth rates during the day period (such as in *red1*) should theoretically always result in a reduced difference between the day growth rate and the maximal night growth rate, thus resulting in a smaller difference between hypocotyl length in WL minus FRp compared with WL plus FRp (less derepression). Thus, although suggestive, it is not clear whether the reduced EOD FR response in *red1* is a direct result of a loss in phyB-like activity during the dark period.

The effect of *red1* on the shade avoidance response, which is thought to be predominantly mediated by phyB, is illustrated in Figure 8B. The shade avoidance response is manifested as increased growth under lower R/FR ratios of light (i.e., under a leaf canopy that filters out R light). In the laboratory, this situation has been mimicked by adding different fluence rates of FRc to WLc (WLc plus FRc; Smith, 1994). The addition of very high FRc fluence rates, however, also activates the FR-HIR mediated by phyA (Quail et al., 1995), which results in a reduction of hypocotyl elongation in etiolated seedlings. Under these extreme WLc plus FRc conditions, the *phyB* mutant showed the opposite response of the *phyA* mutant (Figure 8B). The *phyA* null mutant exhibited a very strong shade avoidance response (elongated hypocotyls in WLc plus FRc) compared with the wild type (RLD), presumably due to reduction of the amount of phyB in the active (Pfr) form. No FR-HIR was observed due to the lack of phyA. The *phyB* null mutant, on the other hand, showed a strong reduction in height in WLc plus FRc, most likely the result of the FR-HIR mediated by phyA, whereas lack of phyB precluded the shade avoidance response.

In the wild type, we did not observe a typical shade avoid-

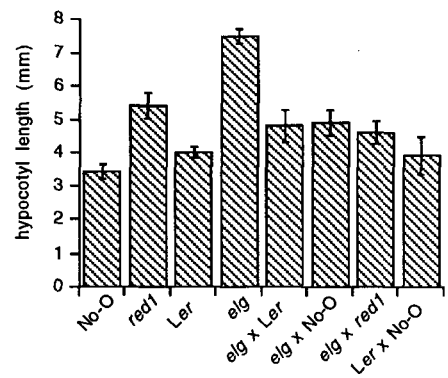


Figure 4. Complementation Analysis of *red1* and *elg*.

Mean hypocotyl length was determined for at least 10 4-day-old seedlings of the parental lines (*red1* in the nontransgenic No-0 background [*red1*] and *elg*) as well as the F_1 generation of each cross. All seedlings were grown on a single large Petri plate at 80 μmol m^{-2} sec^{-1} Rc. Error bars denote 1 SD. Crosses of *elg* to both wild-type ecotypes, No-0 and L*er*, are included for comparison.

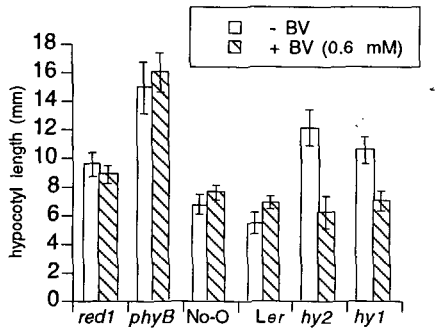


Figure 5. *red1* Is Not Rescued by the Phytochrome Chromophore Precursor BV.

red1 in the nontransgenic No-0 background (*red1*) was grown in the absence (−BV) or presence (+BV) of 0.6 mM BV, a phytochrome chromophore precursor. *phyB* and wild-type No-0 as well as two chromophore biosynthesis mutants (*hy1* and *hy2*) and their wild type (*Ler*) were included for comparison. Mean hypocotyl length was determined from >20 seedlings, and each error bar denotes 1 SD.

ance response under the conditions used here. Instead, the hypocotyl length remained the same as in WLc alone. We hypothesize that the opposite effects caused by reduction of PfrB and the phyA-mediated FR-HIR balance each other out in the wild type under the conditions used here such that the hypocotyl length in WLc and in WLc plus FRc are similar. This conclusion is supported by more extensive quantitative studies (Smith et al., 1997). The *red1* revertant showed a significant reduction in hypocotyl length in WLc plus FRc. This reduction was similar to although smaller than that seen in the *phyB* null mutant. This phenotype is consistent with a partial loss of phyB activity. In sum, it is difficult to determine phyB involvement in the EOD FR response; however, the shade avoidance response conditions used here clearly distinguish between the activity of phyA and phyB and demonstrate that *red1* behaves like a mutant, resulting in selective partial loss of phyB activity.

DISCUSSION

We have utilized the enhanced deetiolation response of a transgenic line, ABO, overexpressing phyB to screen for extragenic revertants in monochromatic Rc. This screen was designed to enhance recovery of R light–specific signal transduction mutants and resulted in the identification of a novel, recessive mutant, *red1*, with a phenotype consistent with this goal. *red1* seedlings exhibit an elongated hypocotyl in Rc, whereas their appearance is indistinguishable from that of the wild type in FRc or in darkness. Hence, the phyA-mediated response to FRc is normal in the *red1* mutant, whereas the response to Rc is diminished. This phenotype is reminiscent of that of a *phyB* mutant. Further characterization of *red1* indicated that *red1* is also affected in other phyB-mediated responses: *red1* seedlings display severely reduced cotyledon expansion in Rc, elongated petioles, and a reduced shade avoidance response.

The similarity in phenotype between the mutants *red1* and *phyB* strongly suggests that the *RED1* gene product plays a role specifically in phyB signal transduction. However, because the functions of the photoreceptors phyC, phyD, and phyE are so far not known, *red1* could simply be deficient in functional phyC, phyD, or phyE. Our data argue against this possibility: the *RED1* locus maps to a chromosomal location that is distinct from all loci encoding phytochrome apoproteins. Moreover, protein gel blot analysis using antibodies specific to each phytochrome indicated that the *red1* mutant produces all five phytochromes at wild-type levels. Hence, *red1* is likely to be a phytochrome signal transduction mutant. Whether this defect is truly phyB specific, as the data suggest, or also affects signaling from phyC, phyD, and/or phyE cannot be determined before mutants at these phytochrome loci are isolated and characterized.

red1 shows partial loss of phyB-mediated activity in the transgenic ABO background and in the nontransgenic No-0 background. This partial loss of phyB activity could be due to the fact that *red1* is either a leaky mutation or alternatively a null mutation in a redundant pathway. The isolation of an allelic series of *red1* mutants and their molecular characterization will help clarify this question.

Previous genetic analysis of the phytochrome signal transduction pathway in Arabidopsis has resulted in isolation of constitutively active and loss-of-function mutants (*deetiolated* [*det*] loci and *cop* loci and *hy5*, *shy1*, *shy2*, and *elg*; Koornneef et al., 1980; Chory et al., 1989; Deng et al., 1991; Halliday et al., 1996; Kim et al., 1996). These mutants are either constitutively active, are affected in Rc, FRc, and blue light signal transduction together, or show other phenotypes not associated with loss of a phytochrome response. Because of their pleiotropic phenotypes, these mutants are not likely to be involved in the initial steps of a phytochrome-specific signal transduction pathway. Recently, however, two putative FR light–specific signal transduction mutants have been isolated (*fhy1* and *fhy3*), thus suggesting a possible phyA-specific pathway (Whitelam et al., 1993). One caveat with these mutants is that they have not yet been reported to be separate loci from the phytochrome photoreceptors of unknown function. Early events in phyB signal transduction should specifically affect phyB-mediated responses. *red1* is a good candidate for an early phyB signal transduction pathway component, based on the finding that it specifically reduces sensitivity to Rc and selectively affects several responses known to be mediated by phyB. Hence, the data reported here now support the possibility of a phyB-specific pathway. Thus, the early events in phyA and phyB signal transduction may indeed be mediated by transduction pathway components that are specific for either phyA (*fhy1* and *fhy3*) or phyB (*red1*). The FRc and Rc signaling

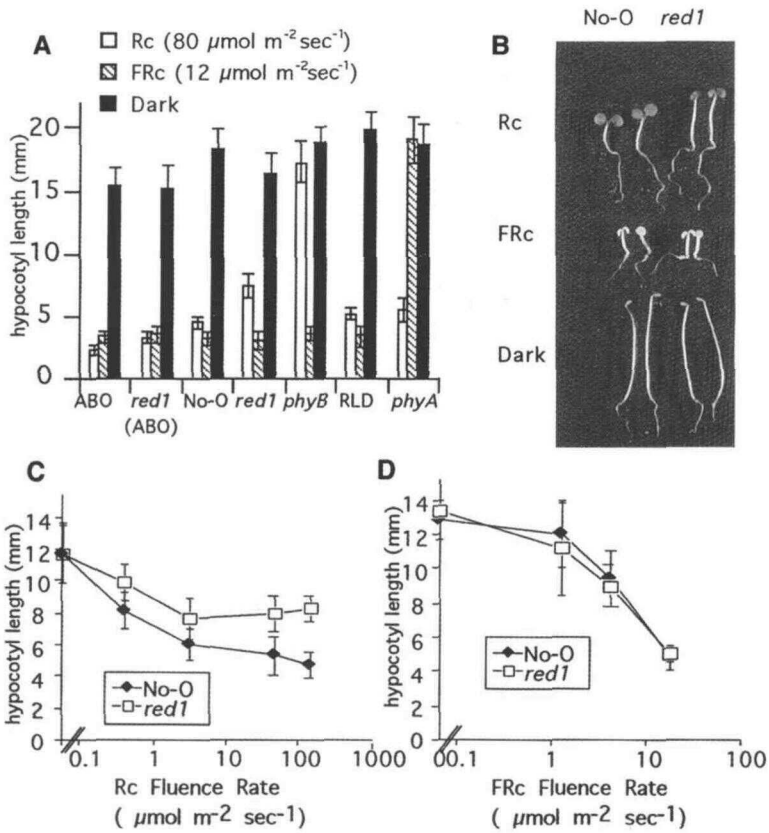


Figure 6. Hypocotyl Length Responsiveness of *red1* to Rc and FRc Compared with the Wild Type, *phyB*, and *phyA*.

(A) Hypocotyl length of *red1*, parental lines (ABO, No-0, and RLD), *phyB*, and *phyA* in different light qualities. The hypocotyl length of *red1* in the transgenic ABO background (*red1* [ABO]) and in the nontransgenic No-0 background (*red1*) was compared with the parental lines (ABO and No-0) and with the *phyB-1* null mutant allele in the No-0 background (*phyB*) as well as with the *phyA-101* null mutant in the RLD background (*phyA*) and its wild type (RLD). Mean hypocotyl length was determined from >20 4-day-old seedlings grown in Rc (80 μmol m^{-2} sec^{-1}), FRc (12 μmol m^{-2} sec^{-1}), or darkness. Each error bar denotes 1 SD.

(B) The phenotype of wild-type (No-0) and *red1* seedlings grown in Rc (80 μmol m^{-2} sec^{-1}), FRc (12 μmol m^{-2} sec^{-1}), or darkness for 3 days.

(C) Fluence rate response of *red1* and the wild type (No-0) to Rc. Growth and measurements are as described in **(A)**.

(D) Fluence rate response of *red1* and the wild type (No-0) to FRc. Growth and measurements are as described in **(A)**.

pathways would then merge farther downstream (before or at the positions defined by *det*, *cop*, and *hy5* mutants).

Since this paper was submitted, three novel mutants have been described (phytochrome signaling, early flowering; *pef1*, *pef2*, and *pef3*), two of which also appear to show defects in inhibition of hypocotyl elongation in Rc only (Ahmad and Cashmore, 1996). These mutants flower early and are thus unlikely to be allelic to *red1*, for which no such phenotype was observed.

Recent mutational analysis of the phyA and phyB photoreceptors has indicated that a small region on the C-terminal half of both photoreceptors is important for regulatory activity (signal transfer to downstream components; Quail et al., 1995; Wagner and Quail, 1995; Xu et al., 1995). In addition, using chimeric phyA/phyB photoreceptors in transgenic Arabidopsis, we have determined that the C-terminal halves of phyA and phyB are reciprocally interchangeable and thus functionally equivalent, whereas the N-terminal domains determine specificity to FRc and Rc in the context of a full-length molecule (Wagner et al., 1996a). These findings suggest the possibility of a similar biochemical mechanism of signal transfer to downstream transduction components for both photoreceptors. Based on this proposal, conservation of some features of the components of the R/FR light–specific signal transduction pathway would be predicted.

Reconciliation of the photosensor-specific mutants described above (*fhy1*, *fhy3*, and *red1*) with the postulated biochemically common features of the proposed phyA and phyB regulatory region is possible by invoking modular reaction partners for the two phytochromes. The reaction

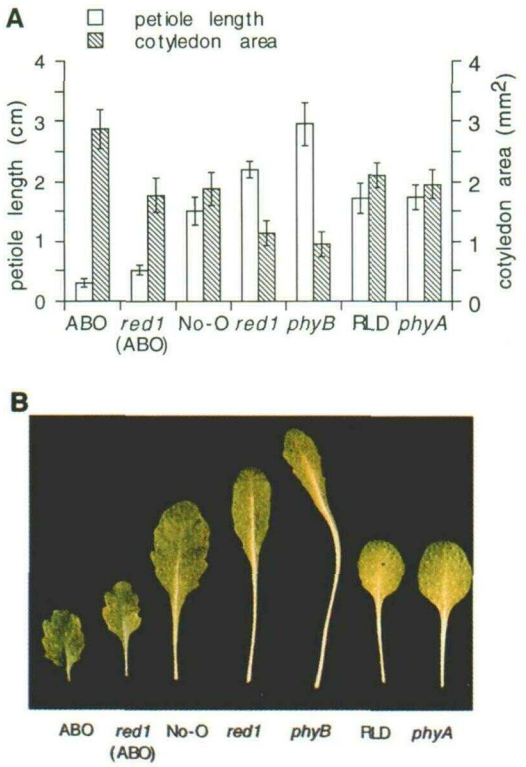


Figure 7. Petiole Length and Cotyledon Area Responsiveness of *red1* Compared with the Wild Type, *phyB*, and *phyA*.

(A) Petiole length and cotyledon area of *red1*, parental lines (ABO, No-0, and RLD), *phyB*, and *phyA*. Petiole length and cotyledon area of *red1* in the transgenic ABO background (*red1* [ABO]) and in the nontransgenic No-0 background (*red1*) were compared with the parental lines (ABO and No-0) and with the *phyB-1* null mutant allele in the No-0 background (*phyB*) as well as with the *phyA-102* null mutant in the RLD background (*phyA*) and its wild type (RLD). The petiole length of the longest leaves from eight plants was determined after 3 weeks in WLc (2.2 μmol m^{-2} sec^{-1}). Cotyledon area was measured on >10 4-day-old seedlings grown in Rc (22 μmol m^{-2} sec^{-1}). Each error bar denotes 1 SD.

(B) Rosette leaves from *red1*, parental lines, *phyB*, and *phyA*. Representative leaves were excised from 3-week-old plants grown in WLc (2.2 μmol m^{-2} sec^{-1}) to demonstrate differences in petiole length as well as in leaf size and shape.

partners of phyA and phyB could have a domain/recognition sequence that interacts specifically with either phyA or phyB (presumably interacting with their respective N-terminal domains) and a second domain capable of biochemical interaction with both phytochromes at the postulated regulatory region in the C-terminal domain (similar biochemical activation mechanism), as suggested in the model in Figure 9. *red1* as well as *fhy1* and *fhy3* are candidates for genes encoding such molecules. Alternatively, two types of molecules could be involved in early phytochrome signal transduction:

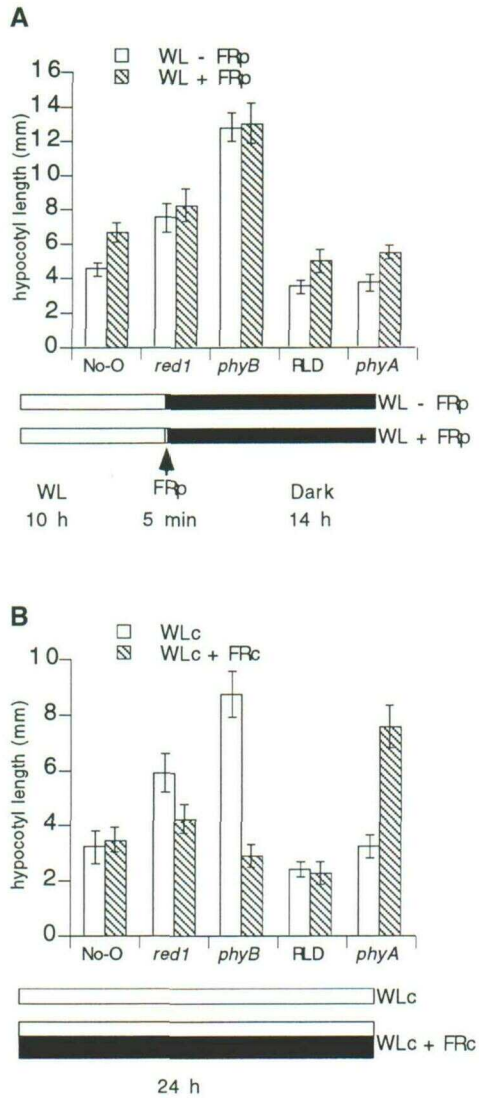


Figure 8. Hypocotyl Elongation in *red1* under EOD FR and Shade Avoidance Conditions.

(A) EOD FR analysis of *red1* and known photoreceptor mutants. Seedlings of *red1* in the nontransgenic No-0 background (*red1*) and photoreceptor mutants and their wild-type progenitor lines were grown for 2 days in 10 hr of white light (WL) (39 μmol m^{-2} sec^{-1}) *plus 14-hr dark cycles, followed by 4 days in the same light regime* (WL without a predark FRp [WL − FRp]) or by 4 days with a 5-min FRp (76 μmol m^{-2} sec^{-1}) at the beginning of the dark period (WL + FRp). The bars represent means ± SD of hypocotyl lengths measured after 6 days of growth. The diagram below the plot illustrates the irradiation regimes (one light–dark cycle). h, hour.

(B) Shade avoidance analysis of *red1* and known photoreceptor mutants. Seedlings for all lines described in **(A)** were grown for 2 days in WLc (39 μmol m^{-2} sec^{-1}), followed by 4 days in the same light regime (WLc) or by 4 days in WLc supplemented with FRc (76 μmol m^{-2} sec^{-1}) (WLc + FRc). The bars represent means ± SD of hypocotyl length measured after 6 days of growth. The diagram below the plot illustrates the irradiation regimes.

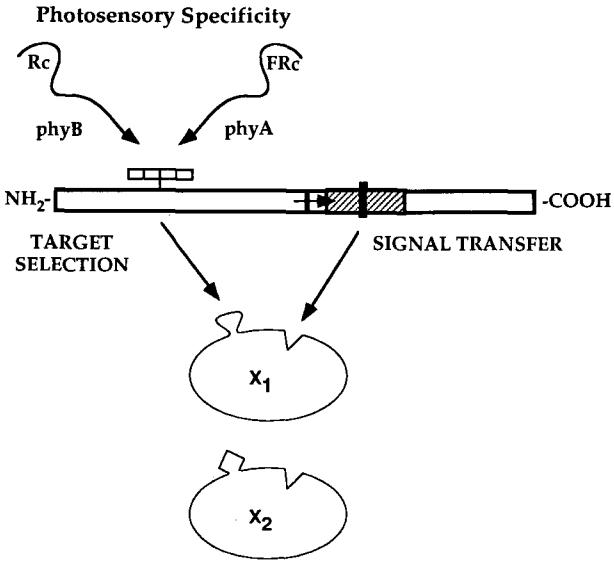


Figure 9. Model for Activity of FRc- and Rc-Specific Signal Transduction Mutants.

The globular N-terminal chromophore-bearing domain of phyA and phyB is depicted at left, and the extended C-terminal domain is depicted at right. The four small open boxes represent the chromophore. The small striped box in the C-terminal domain indicates an area important for phyA and phyB regulatory activity (Wagner and Quail, 1995; Xu et al., 1995). phyA and phyB photosensory specificity is apparently mediated by the N-terminal domain of each species (Wagner et al., 1996a). Photoreceptor binding polypeptides (X_1 and X_2) are postulated to have a recognition signal specific for phyA or phyB (inverted triangle or diamond protruding from X_1 and X_2), respectively. This signal is used for target selection, and recognition of it is a prerequisite for binding. X_1 and X_2 are also proposed to have a subunit or domain that can react with either photoreceptor (indented triangle in X_1 and X_2). Interaction at this latter site proposedly leads to signal transfer through biochemical modification of X_1 and X_2.

one subset of interaction partners binding to the N-terminal domain of phyA or phyB selectively after light activation, followed by binding of a second, nonselective type of interaction partner recruited to the C-terminal domain of both photoreceptors by the first partner. Here, *red1* as well as *fhy1* and *fhy3* could represent the selective interaction partners, whereas a nonselective interaction partner could be *hy5*.

red1 should be useful in our attempts to understand the initial events in phyB signal transduction. Genetic epistasis analysis will test whether the *red1* mutation affects an early step in the signaling pathway, because all pleiotropic photomorphogenic mutants should be epistatic to *red1*. Molecular characterization of *red1* will potentially shed light on the biochemical mechanism of phyB action and signal transfer, which are as yet not understood. In addition, molecular identification of proteins that interact directly with phyA and phyB will test the proposed modular nature of phytochrome signal transduction.

METHODS

Mutagenesis and Revertant Screen

Seed (10 g) of a transgenic *Arabidopsis thaliana* line overexpressing Arabidopsis phytochrome B (phyB) from a single insert at a single locus (ABO; Wagner et al., 1991; Wagner and Quail, 1995) was mutagenized by agitation in 0.3% ethyl methanesulfonate for 12 to 16 hr. Approximately 2000 seeds were sown directly into soil in individual flats. Flats were harvested independently and averaged 500 to 1000 M_1 plants. Four thousand to 8000 M_2 seedlings were screened per M_1 family for the revertant phenotype (long hypocotyls) in continuous red light (Rc; 22 μmol m^{-2} sec^{-1}). Before plating, M_2 seeds were sterilized for 10 min in 1.6% Na hypochlorite and 0.03% Triton X-100. After sowing on growth medium (Valvekens et al., 1988) without sucrose, seeds were cold treated (4°C) in the dark for 5 days (stratification). Germination was induced by 3 hr of continuous white light (WLc; 39 μmol m^{-2} sec^{-1}) at 21°C and allowed to continue for 24 hr in the dark at 21°C, after which time M_2 seedlings were transferred to Rc (22 μmol m^{-2} sec^{-1}) at 21°C. Revertants were identified by visual inspection after 5 days in Rc, allowed to grow for 7 days in WLc (22 μmol m^{-2} sec^{-1}), and then planted into soil and transferred to the greenhouse. M_3 seed was harvested from individual M_2 plants, and a subset was screened again in Rc as well as in continuous far-red light (FRc; 5.7 μmol m^{-2} sec^{-1}).

Seedling and Plant Growth and Measurement

For hypocotyl and cotyledon measurements, seedlings were grown as described above, except that after 24 hr of darkness, growth was continued in the light for 3 days. Light sources were as described previously (Wagner et al., 1991), except for high-intensity Rc and FRc (LED light sources; Quantum Devices, Barnveld, WI). For end-of-day far-red (EOD FR) treatment and shade avoidance (WLc plus FRc), stratified seed was germinated directly in white light (39 μmol m^{-2} sec^{-1}) for 2 days before the treatment with FR light pulses (EOD FR) or simultaneous FRc irradiation (shade avoidance). Measurements of hypocotyl length were performed as described in Wagner et al. (1996b). For cotyledon measurements, the cotyledons were photographed; their perimeters were traced, and the cotyledon area was determined using National Institutes of Health image software (public domain; Bethesda, MD). Petiole length was measured with a ruler on the longest leaf of eight plants after growth for 3 weeks in WLc (2.2 μmol m^{-2} sec^{-1}). Plates for biliverdin (BV) rescue were prepared as described in Parks and Quail (1991), except that seedlings were grown in wells of 95-well microtiter plates.

Alleles of Photomorphogenic and Photoreceptor Mutants Used

The *hy1*, *hy2*, and *hy5* alleles used were those identified in the original screen (Koornneef et al., 1980). The *phyB* allele used was derived from the original B064 (Koornneef et al., 1980) allele (now *phyB-1*; Quail et al., 1994), which is null for phyB (Reed et al., 1993) and was integrated into the ecotype Nossen (No-0) (Wester et al., 1994). The

phyA alleles used (*phyA-101* and *phyA-102*) are also a null allele (Dehesh et al., 1993; Parks and Quail, 1993).

Mapping of *red1*

Because the *red1* (for red elongated1) revertant was isolated in the No-0 ecotype, two mapping populations were generated. One consisted of *red1* (ABO) crossed to the Landsberg *erecta* (Ler) ecotype; the other was generated by crossing *red1* (ABO) to the Columbia ecotype. *red1* (ABO) revertants were identified in the F_2 generation on kanamycin plates. To confirm that these revertants indeed carried the mutant phenotype, F_3 seed was harvested from all *red1* (ABO) F_2 plants individually and again scored for the *red1* mutant phenotype. DNA was prepared by the method of Edwards et al. (1991) from populations of F_3 seedlings derived from each of 50 independent F_2 revertant plants. Polymerase chain reactions (PCRs) were performed with KTLA polymerase (Barnes, 1994) and analyzed directly (simple sequence length polymorphisms) or after overnight digestion with the appropriate restriction enzyme (cleaved amplified polymorphic sequences [CAPS]) on agarose gels ranging from 1 to 4%. *red1* was mapped using the following PCR-based CAPS (Konieczny and Ausubel, 1993; E.P. Drenkard, J. Morris, and F.M. Ausubel, Arabidopsis CAPS markers, Arabidopsis Research Companion Gopher, <Url gopher://weeds.mgh.edu) and simple sequence length polymorphism (Bell and Ecker, 1994) markers: *chr.1*, nga63, nga248, nga289, and nga111; *chr.2*, m246, GPA1, and m249; *chr.3*, GAPC, GAPA, and BGL1; *chr.4*, GA1, nga8, AG, g3883-1.4, PG11, PRHA, and DHS1; and *chr.5*, nga225, nga106, DFR, and LFY. Map distance (centimorgans) was calculated based on Kosambi, as described in Koornneef and Stam (1991). Two positive controls were included in each experiment: DNA from each parental line and a 1:1 mix of both DNAs (used to test for differential amplification in the heterozygotes).

Protein Analysis

Ammonium sulfate–concentrated extracts and immunoblots were prepared as described by Wagner et al. (1991). Monoclonal antibodies specific to phyA, phyB, and phyC were identified, and monoclonal antibodies raised against phyD and phyE were generated (J. Tepperman, R.A. Sharrock, and P.H. Quail, unpublished data).

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