The Plant Cell, Vol. 5, 147–157, February 1993 © 1993 American Society of Plant Physiologists

RESEARCH ARTICLE

Mutations in the Gene for the Red/Far-Red Light Receptor Phytochrome B Alter Cell Elongation and Physiological Responses throughout Arabidopsis Development

Jason W. Reed,[a] Punita Nagpal,[a] Daniel S. Poole,[a,b] Masaki Furuya,[c,1] and Joanne Chory[a,2]

[a] Plant Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, California 92186-5800
[b] Department of Biology, University of California at San Diego, La Jolla, California 92093-0116
[c] Laboratory of Plant Biological Regulation, Frontier Research Programs, RIKEN Institute, Wako-shi, Saitama 351-01, Japan

Phytochromes are a family of plant photoreceptors that mediate physiological and developmental responses to changes in red and far-red light conditions. In Arabidopsis, there are genes for at least five phytochrome proteins. These photoreceptors control such responses as germination, stem elongation, flowering, gene expression, and chloroplast and leaf development. However, it is not known which red light responses are controlled by which phytochrome species, or whether the different phytochromes have overlapping functions. We report here that previously described *hy3* mutants have mutations in the gene coding for phytochrome B (PhyB). These are the first mutations shown to lie in a plant photoreceptor gene. A number of tissues are abnormally elongated in the *hy3*(*phyB*) mutants, including hypocotyls, stems, petioles, and root hairs. In addition, the mutants flower earlier than the wild type, and they accumulate less chlorophyll. PhyB thus controls Arabidopsis development at numerous stages and in multiple tissues.

INTRODUCTION

Plant development is distinguished by its plasticity, which arises from a close coupling of developmental responses to environmental stimuli. As the source of energy for photosynthesis, light is particularly important to plants, and plants modulate their physiology and their development according to prevailing light conditions. Examples of light-mediated responses include seed germination, stem elongation, phototropism of leaves and stems, development of leaves and chloroplasts, stomatal opening, and flowering. Incident red, blue, far-red, and UV light all elicit subsets of these responses (Cosgrove, 1986; Chory, 1991; Thompson and White, 1991).

The mechanisms of light signal transduction in plants are poorly understood. Responses to red and far-red light are controlled by a family of photoreceptor proteins called phytochromes (reviewed by Colbert, 1988; Furuya, 1989; Quail, 1991). Phytochromes are 120-kD soluble proteins that have a covalently linked linear tetrapyrrole chromophore, and exist in two photointerconvertible forms, Pr and Pfr. Pr, the red light–absorbing form, is converted to Pfr, the far-red light–absorbing form, upon absorption of red light. Pfr is thought to be the active form of phytochrome, and can be converted back to Pr by far-red light. Consequently, red light–induced responses mediated by phytochromes are typically reversible by far-red light. Most plants examined contain multiple phytochrome isoforms. In Arabidopsis, five phytochrome genes have been identified (Sharrock and Quail, 1989). Three of these, *PHYA*, *PHYB*, and *PHYC*, have been characterized in some detail. The sequences of PhyA, PhyB, and PhyC diverge significantly, having ~50% identity to each other at the amino acid level (Sharrock and Quail, 1989). The most abundant phytochrome in etiolated (dark-grown) seedlings is PhyA (Furuya, 1989; Somers et al., 1991). Whereas PhyA protein levels decrease in the light, PhyB and PhyC are present at equal levels in light- and dark-grown plants (Somers et al., 1991).

Based on their red/far-red reversibility, certain light responses have been hypothesized to be mediated by a phytochrome. These responses include red light–induced seed germination, inhibition of the rate of hypocotyl and stem elongation, induction of chloroplast and leaf development, and induction of expression of genes required for photosynthesis (Cosgrove, 1986; Mullet, 1988; Chory, 1991; Thompson and White, 1991). However, it is not known which of these responses is controlled by which phytochrome species, or to what extent the different phytochrome molecules have overlapping functions. It is also not known when, or in which tissues, the different phytochromes are active.

A number of researchers have taken a genetic approach to dissecting light responses. Mutants affected in red light inhibition of hypocotyl elongation have been isolated in several plants (reviewed in Chory, 1991; Kendrick and Nagatani, 1991;

[1] Current address: Advanced Research Laboratory, Hitachi Ltd., Hatoyama, Saitama 350-03, Japan.
[2] To whom correspondence should be addressed.

Reed et al., 1992). In Arabidopsis, these mutants are called *hy* (for long *hy*pocotyl) (Koornneef et al., 1980; Chory et al., 1989). Mutations in *hy1*, *hy2*, *hy3*, *hy5*, and *hy6* affect inhibition of hypocotyl elongation by red light. *hy1*, *hy2*, and *hy6* mutants also appear pale, as if affected in the greening response to red light. The *hy1*, *hy2*, and *hy6* mutants have PhyA apoprotein that is largely spectrally inactive, suggesting that they are deficient in phytochrome chromophore availability (Chory et al., 1989; Parks et al., 1989; Parks and Quail, 1991). These findings establish a correlation between deficiencies in red light–induced responses and a deficiency in active phytochrome photoreceptors.

hy3 mutations affect a number of responses that are mediated by red and far-red light. *hy3* mutants are deficient in elongation responses to supplementary far-red light (Nagatani et al., 1991a; Whitelam and Smith, 1991). Instead, they appear elongated without supplementary far-red light, as if showing a constitutive far-red elongation response. *hy3* mutant plants have also been found to flower earlier than wild-type plants (Goto et al., 1991; Whitelam and Smith, 1991; Chory, 1992), and to have a decreased germination response to red light (Spruit et al., 1980; Cone and Kendrick, 1985). In these respects, they are similar to the *hy1*, *hy2*, and *hy6* mutants; however, *hy3* mutants differ from the *hy1*, *hy2*, and *hy6* mutants in that they are less pale and have spectrally active PhyA (Chory et al., 1989; Parks et al., 1989).

That *hy3* mutations affect various red/far-red light responses suggests that they alter a phytochrome signaling pathway. In fact, immunological studies have shown that *hy3* mutants have a decreased level of PhyB relative to the wild type (Nagatani et al., 1991a; Somers et al., 1991). Like the *hy3* mutants, a cucumber mutant called *lh* (for *l*ong *h*ypocotyl) is defective in end-of-day far-red elongation, and this mutant also lacks a protein recognized by an anti-PhyB antibody (López-Juez et al., 1990, 1992). It therefore seems likely that PhyB mediates the responses that are deficient in these mutants.

However, it has not been determined whether the *hy3* mutations fall in the *PHYB* gene, whether they affect PhyB synthesis or stability, or whether they affect a downstream component of PhyB-mediated signal transduction. In this work, we demonstrate that the *HY3* locus encodes phytochrome B. In addition, we describe morphological and physiological phenotypes of *hy3* mutants. These phenotypes occur in a number of different tissues and throughout the life cycle of the plant, indicating that PhyB plays multiple roles in Arabidopsis development.

RESULTS

hy3 Mutations Fall in the *PHYB* Gene

Arabidopsis *hy3* mutants were isolated by screening for mutants having a long hypocotyl when grown in white light (Koornneef et al., 1980). Mutants Bo64, 4-117, 8-36, 548, 1053, and M4084 are shown in Figure 1, and were isolated previously after ethylmethane sulfonate (EMS) mutagenesis of ecotype Landsberg *erecta* seeds (Koornneef et al., 1980). Mutant EMS142 was obtained by EMS mutagenesis of ecotype Columbia seeds. In addition, we obtained one T-DNA insertion mutant, 464-19, in ecotype WS from K. Feldman (University of Arizona, Tucson) (Figure 1). The resistance to kanamycin conferred by this insertion segregated as a single locus in progeny of crosses with $HY3^+$ plants, and all long hypocotyl F_2 progeny were resistant to kanamycin (M. Koornneef, personal communication; data not shown). Genetic complementation analyses established that these mutations were in the *HY3* locus (data not shown).

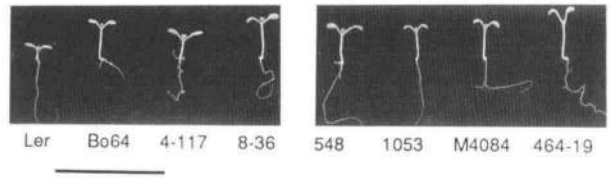

Figure 1. Ten-Day-Old Seedlings of Wild Type and Various *hy3* Mutants.

Shown are a wild-type Landsberg *erecta* (Ler) seedling at the left and seven *hy3* mutants. Scale bar = 2 cm.

Previous work has shown that a *hy3* mutation and the *PHYB* gene both map to chromosome 2 (Koornneef et al., 1980; R. Sharrock, personal communication). We tested whether a *hy3* mutation mapped to the *PHYB* locus by measuring the linkage between a *hy3* mutation and a restriction fragment length polymorphism detected by the cloned *PHYB* gene (see below). We crossed *hy3* strain Bo64 (in the Landsberg *erecta* ecotype) with wild-type plants of the Columbia ecotype, and analyzed the F_2 progeny of this cross having long hypocotyls for presence of the Landsberg or Columbia *PHYB* polymorphism. Of 103 such *hy3*/*hy3* progeny examined, all showed only the Landsberg-specific restriction fragment. Thus, none of the 206 chromatids tested showed a recombination event between the *hy3* mutation and the *PHYB* polymorphism. This result establishes that this *hy3* mutation is very closely linked to the *PHYB* gene.

The published cDNA sequence of Arabidopsis *PHYB* is from the Columbia ecotype (Sharrock and Quail, 1989), whereas most of our *hy3* mutants are in the Landsberg ecotype. To analyze our mutants molecularly, we cloned the wild-type Landsberg *PHYB* gene and sequenced it, as shown in Figure 2 and described in Methods. The sequence of the Landsberg gene is virtually identical to the Columbia cDNA sequence, differing only at one position in the coding sequence, at nucleotide 3177 (Figure 2), and at one position in the 5′ untranslated sequence, at nucleotide 2257 (Figure 2). The latter polymorphism creates a XhoI restriction site in the Landsberg gene, which we used for the linkage analysis above. Neither of these polymorphisms affected the amino acid sequence of PhyB. We found three introns in the coding sequence (Figure 2) at

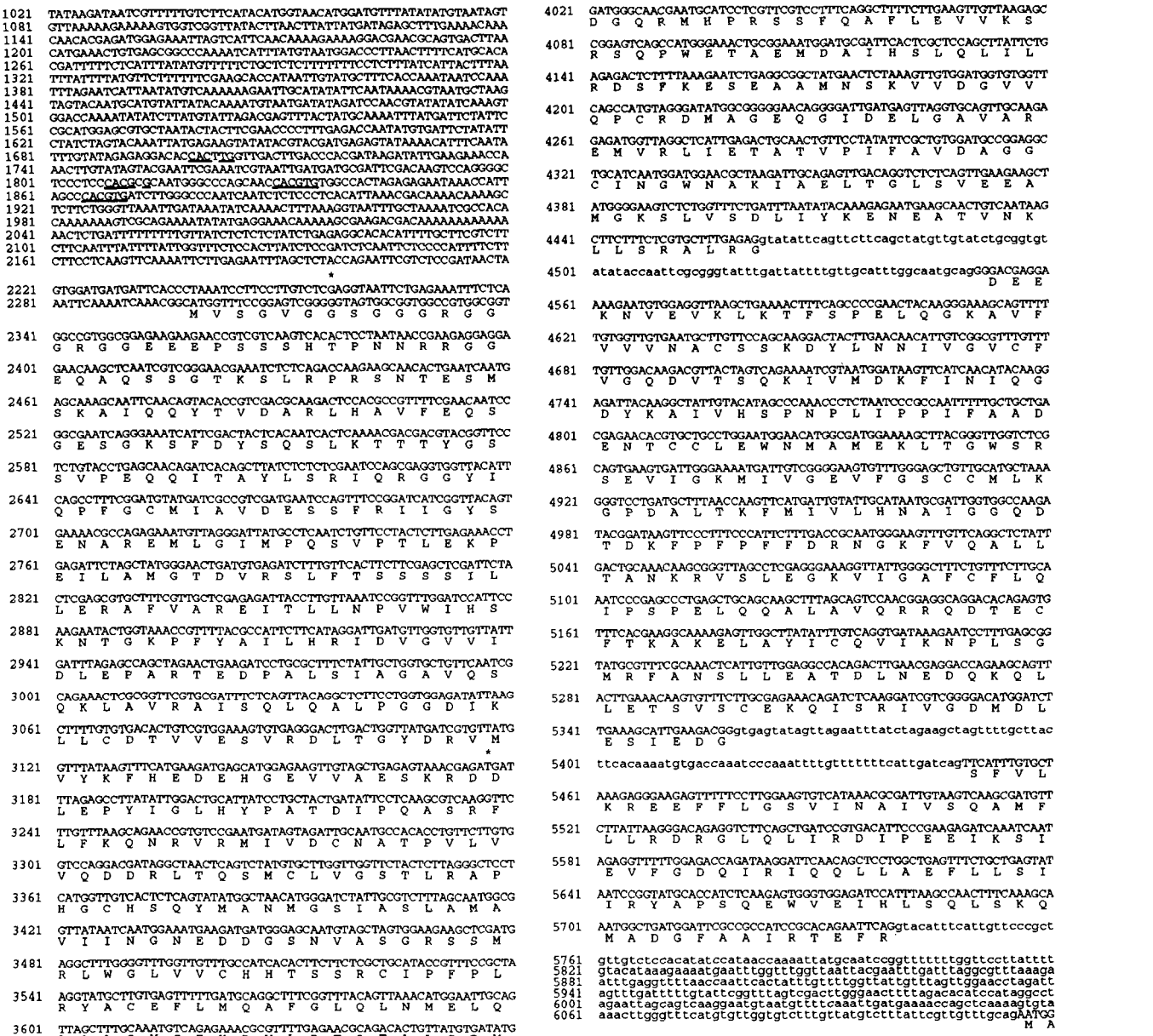

Figure 2. Sequence of the *phyB* Gene from Ecotype Landsberg *erecta*.

Introns are in lowercase letters, G-box consensus sequences are underlined, and polymorphisms compared to the published cDNA sequence from ecotype Columbia are marked with asterisks. One kilobase of sequence at the 5′ end has been omitted to conserve space. This sequence has been submitted to GenBank as accession number L09262.

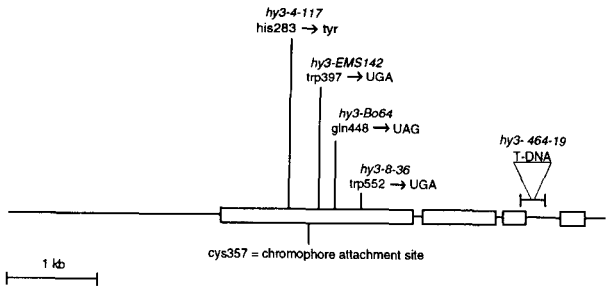

Figure 3. Mutations in the *phyB* Gene.

We sequenced 6509 bp of the wild-type gene. The published cDNA sequence begins at nucleotide 2207 (Figure 2), and coding sequences are drawn as blocks. The chromophore attachment site is at cysteine-357. Mutation *hy3-4-117* is a C-to-T mutation at nucleotide position 3145 that changes a histidine codon to a tyrosine codon at amino acid residue 283. Mutation *hy3-EMS142* is a G-to-A mutation at nucleotide 3489 that changes a tryptophan codon to a stop codon at amino acid position 397. Mutation *hy3-Bo64* is a C-to-T mutation at nucleotide position 3640 that changes a glutamine codon to a stop codon at amino acid residue 448. Mutation *hy3-8-36* is a G-to-A mutation at nucleotide 3954 that changes a tryptophan codon to a stop codon at amino acid position 552. Mutation *hy3-464-19* is a T-DNA insertion in a 0.15-kb EcoRI-DraI fragment encompassing nucleotides 5732 to 5877.

amino acid positions corresponding exactly to the introns in genomic *PHYA* genes previously sequenced from oat, maize, rice, and pea (Hershey et al., 1987; Sato, 1988; Christensen and Quail, 1989; Kay et al., 1989), and to the introns in a *PHYB* gene from potato (Heyer and Gatz, 1992). It is interesting that *PHYA* and *PHYB* genes each have the same conserved exon/intron structure.

We also sequenced ~2 kb of DNA upstream of the previously reported *PHYB* sequence. We found that the published cDNA sequence starts immediately after a natural EcoRI site in our genomic sequence. Because the library from which the cDNA clone was obtained was constructed by adding EcoRI linkers to the cDNA molecules and then digesting these with EcoRI before ligating to a vector (Crawford et al., 1988; Sharrock and Quail, 1989), it seems likely that the actual transcriptional start site lies upstream of the previously reported cDNA sequence. In the presumptive promoter sequence, we found two G-box sequences (CACGTG) (Williams et al., 1992) at nucleotide positions 1830 to 1835 and 1865 to 1870 (Figure 2). Two other sequences, at nucleotide positions 1699 to 1704 and 1808 to 1813, had a single change from this palindromic consensus motif (Figure 2). We sequenced just 158 bp of the 3′ noncoding region, because our clone extended no further (Methods). The polyadenylation site of the published cDNA sequence is 239 nucleotides downstream of the stop codon (Sharrock and Quail, 1989).

By DNA sequencing, we found point mutations in the *PHYB* gene from four EMS-generated *hy3* mutants, diagrammed in Figure 3. Three alleles, *hy3-Bo64*, *hy3-8-36*, and *hy3-EMS142*, have stop codons in the coding sequence (Figure 3). A fourth allele, *hy3-4-117*, has a missense mutation that causes a histidine-to-tyrosine change at amino acid residue 283 (Figure 3). Gel blot hybridization analysis of genomic DNA from the T-DNA–generated allele *hy3-464-19* indicated that it contains a T-DNA insertion in the *PHYB* gene (Figure 3). These results establish unequivocally that the *HY3* locus encodes phytochrome B. Henceforth, we refer to the mutations as *phyB*.

Phenotypes of *phyB* Mutants

We found that some *phyB* mutants had longer hypocotyls than others, as shown in Figure 1 and Table 1. Mutants Bo64, 8-36, M4084, EMS142, and 464-19 had the longest hypocotyls, whereas mutants 4-117, 548, and 1053 had hypocotyl lengths intermediate between those of the wild type and the mutants having the longest hypocotyls (Table 1).

This long hypocotyl phenotype was caused by increased cell elongation. Hypocotyl epidermal peels from 13-day-old seedlings of the wild type and two *phyB* mutants are shown in Figure 4. The epidermal cells from the hypocotyl of null mutant 8-36 (Figure 4C) are ~2.5 times as long as the same cells from the wild-type plant (Figure 4A; data not shown). Epidermal cells from the hypocotyl of partial loss-of-function mutant 4-117 are intermediate in length (Figure 4B), ~1.75 times the mean length of cells of the wild type (data not shown). Cell division in the hypocotyl was unaffected by the mutations, as the wild-type and mutant plants all had approximately the same number of cells along the length of the hypocotyl (data not shown).

Many other tissues in *phyB* mutant plants also appeared elongated. As shown in Figure 5, *phyB* mutant plants had more elongated petioles and smaller leaf areas than did wild-type plants (Nagatani et al., 1991a). In addition, we found that *phyB* plants were taller than wild-type plants, as shown in Figure

Table 1. Hypocotyl Lengths and Chlorophyll Contents of *phyB* Mutant Seedlings[a]

Strain	Hypocotyl Length (mm)	μg Chlorophyll/ Seedling
Wild type (Ler)	2.7 ± 0.5	3.9
hy1	8.4 ± 1.2	1.1
phyB-464-19	8.1 ± 1.3	1.4
phyB-8-36	8.3 ± 0.5	2.2
phyB-Bo64	8.1 ± 0.6	2.0
phyB-M4084	8.0 ± 1.1	2.7
phyB-548	6.2 ± 0.7	2.0
phyB-1053	5.5 ± 0.6	2.0
phyB-4-117	5.1 ± 0.5	2.6

[a] Hypocotyls and chlorophyll contents were measured 12 days after sowing. Hypocotyl lengths are based on measurements of 20 plants, and chlorophyll levels are based on measurements of four plants.

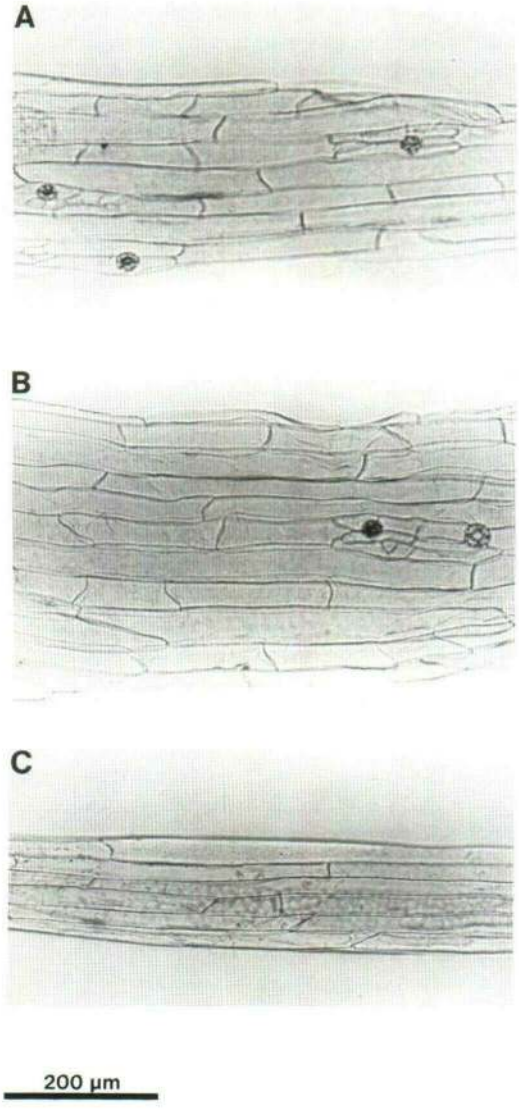

Figure 4. Hypocotyl Epidermal Peels of Wild-Type and *phyB* Mutant Plants.

(A) Wild-type Landsberg *erecta*.
(B) *phyB* mutant 4-117.
(C) *phyB* mutant 8-36.
Scale bar = 200 μm.

6. Measurements of 6-week-old plants, summarized in Table 2, revealed that *phyB* plants had longer main stems than wild-type plants, fewer secondary stems, fewer stem termini (as a result of the decreased number of stems), and fewer siliques (fruits) on the main stem. These phenotypes indicate that the *phyB* mutants have greater apical dominance than the wild type.

For comparison, we measured the same parameters for adult *hy1* plants, probably deficient in multiple phytochromes (Chory et al., 1989; Parks et al., 1989; Parks and Quail, 1991) (Table 2). We found that they had even greater apical dominance than *phyB* mutant plants, each *hy1* plant having just a single stem. However, this stem was shorter than wild-type (and *phyB*) stems, and the number of siliques was lower. These phenotypes could have arisen because the *hy1* mutant has a very low chlorophyll content (see below), which may limit the growth of this mutant.

Interestingly, we also found morphological differences between roots of wild-type and *phyB* plants. As illustrated in Figure 7, root hairs of 1-week-old seedlings of a *phyB* mutant were longer than root hairs of the wild-type strain. Dark-grown seedlings had very few root hairs, but the root hairs present were approximately the same length in the mutant and the wild type (data not shown). This result suggests that perception of light by PhyB normally inhibits root hair elongation. In contrast, the roots of *phyB* mutant seedlings were shorter than the roots of wild-type seedlings, as shown in Table 3. The mean root length of *phyB* seedlings was approximately three-fourths that of the mean root length of wild-type seedlings (Table 3). This was true even in the dark (Table 3). This observation suggests that the shorter roots of *phyB* mutants did not depend on absorption of light, and raises the possibility that PhyB may have some activity in the absence of red light activation. Alternatively, the light treatment given prior to dark incubation to induce germination of seeds (see Methods) may have been sufficient to affect subsequent root growth.

Figure 5. Three-Week-Old Wild-Type and *phyB* Plants.

(A) Wild-type Landsberg *erecta* (Ler).
(B) *phyB* mutant Bo64.
(C) *phyB* mutant 4-117.
(D) *phyB* mutant 8-36.
The mutants have elongated petioles and flower early.

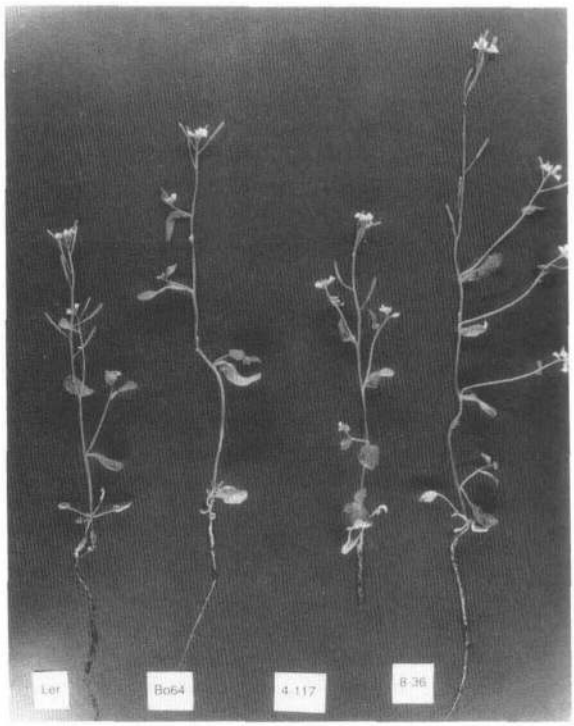

Figure 6. Five-Week-Old Adult Plants of Wild Type and Three *phyB* Mutants.

The wild-type Landsberg *erecta* (Ler) plant is at left, and *phyB* mutants Bo64, 4-117, and 8-36 are at right.

In addition to these morphological phenotypes, *phyB* mutants exhibited physiological differences from wild-type plants. In agreement with others (Goto et al., 1991; Whitelam and Smith, 1991), we found that they flowered earlier than the wild type, during both long and short days (data not shown). Consistent with their accelerated flowering, *phyB* mutant plants had fewer rosette leaves than wild-type plants (Table 2; Whitelam and Smith, 1991; Chory, 1992). We also found that *phyB* seedlings had less chlorophyll per seedling than wild-type seedlings (Table 1; Lifschitz et al., 1990). Furthermore, *phyB* leaf mesophyll cells have fewer chloroplasts per cell than wild-type mesophyll cells (J. Chory, unpublished result), suggesting that the reduced chlorophyll level may arise partly from a decreased number of chloroplasts.

We observed a rough correlation between the chlorophyll levels of the mutants and their hypocotyl lengths. Thus, the presumed null mutants described above, Bo64, 8-36, EMS142, and 464-19, which had the most extreme hypocotyl phenotypes, all had low chlorophyll levels, whereas mutant 4-117, which we hypothesized to carry a partial loss-of-function mutation, had an intermediate chlorophyll level (Table 1 and data not shown). In no case, however, was the chlorophyll content of a *phyB* mutant as low as that of the *hy1* mutant (Table 1).

Expression of the *PHYB* Gene

We analyzed *PHYB* mRNA in the mutants by RNA gel blotting. As shown in Figure 8A, *PHYB* mRNA could be detected in all of the mutants. In the EMS-generated mutants, the mRNA was the same size as the wild-type mRNA, ~4 kb. In the T-DNA insertion mutant 464-19, however, the mRNA was substantially larger, ~7 kb. Presumably, the greater length of the *PHYB* transcript in this strain is caused by T-DNA sequences fused to the *PHYB* sequences. In addition, we observed that the level of *PHYB* mRNA in the mutants was slightly lower than the wild-type level, except in mutant 4-117, where we observed no obvious difference from the wild-type level (Figure 8A). All of the mutants had levels of *PHYA* (phytochrome A) mRNA approximately equal to the wild-type level (Figure 8A), showing that the *phyB* mutations do not affect expression of the *PHYA* gene.

We examined PhyB protein levels in these mutants using a monoclonal antibody raised against the C terminus of the presumptive PhyB protein from tobacco. It was found previously that this antibody recognizes Arabidopsis PhyB protein selectively (Nagatani et al., 1991a). Protein gel blots using this antibody failed to reveal PhyB protein in any of the *phyB* mutants except for mutant 4-117, which had a level approximately equal to the wild-type level (Figure 8B). Because the antibody used was raised against the C-terminal portion of the protein, it would not have detected a truncated fragment of PhyB, as might exist in mutants Bo64 and 8-36 (nonsense mutations) or 464-19 (T-DNA insertion allele).

DISCUSSION

We have shown here that Arabidopsis *hy3* mutations fall in the *PHYB* gene, encoding one of the five red/far-red light–

Table 2. Characteristics of 6-Week-Old Wild-Type, *hy1*, and *phyB* Plants[a]

Strain	Length of Main Stem	Number of Siliques (main stem)	Number of Stems	Number of Termini	Number of Rosette Leaves
Ler	22.7 ± 2.2	23.3 ± 1.9	2.7 ± 0.6	10.7 ± 1.6	7.9 ± 1.0
hy1-21.84N	19.9 ± 1.6	14.3 ± 2.0	1.0 ± 0	4.8 ± 1.5	4.0 ± 1.2
phyB-Bo64	27.8 ± 2.3	16.7 ± 2.2	1.3 ± 0.6	7.3 ± 1.3	5.3 ± 1.6

[a] Numbers are means (± standard deviation) of measurements of 10 (for *hy1*) or 15 (for Ler and *phyB*) plants.

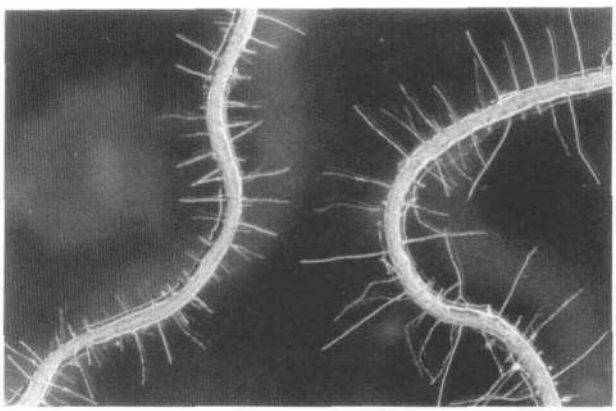

Figure 7. Root Hairs of Wild-Type and *phyB* Mutant Seedlings.

A root from a wild-type plant is at left, and a root from a *phyB-8-36* plant is at right. Seedlings were grown for 1 week in white light.

sensing phytochrome proteins in this plant. The various phenotypes of *hy3*(*phyB*) plants are thus the downstream manifestations of alterations in a light signal transduction pathway (or pathways) having PhyB as the initial receptor. These are the only mutations shown to lie in a phytochrome gene, and they allow us to assess directly the role of this phytochrome in Arabidopsis development.

The very long hypocotyls and the molecular phenotypes of mutants 8-36, EMS142, Bo64, and 464-19 suggest that these strains have null or near null mutations. Mutant 4-117, on the other hand, appears to carry a partial loss-of-function mutation because it has an intermediate hypocotyl length. Since mutant 4-117 contains the normal amount of PhyB protein, mutation *phyB-4-117* probably changes the functional properties of the PhyB molecule. The molecular defect in this mutant, a histidine-to-tyrosine missense mutation, is consistent with this hypothesis. We do not know the biochemical function affected by this mutation, but it is intriguing that it occurs in a charged region that is highly conserved among different phytochromes from both monocots and dicots (Hershey et al., 1987; Sato, 1988; Christensen and Quail, 1989; Kay et al., 1989; Sharrock and Quail, 1989; Dehesh et al., 1991; Heyer and Gatz, 1992; J. C. Lagarias, personal communication), as well as lower plants (Hanelt et al., 1992; Maucher et al., 1992; Winands et al., 1992). The His-283 residue is conserved in all sequenced phytochromes, including PhyA and PhyC (Sharrock and Quail, 1989).

The multiplicity of phenotypes of *phyB* mutants underscores the importance of light quality in controlling plant development. Shade-avoiding plants inhibit elongation of hypocotyl and other tissues and promote leaf and chloroplast development in response to red light. In high fluences of far-red light, they grow tall, show increased apical dominance, flower early, and make less chlorophyll (Brown and Klein, 1971; Smith, 1982; Corré, 1983; Martínez-Zapater and Somerville, 1990; Chory, 1991; Thompson and White, 1991; Whitelam and Smith, 1991). Far-red growth patterns are constitutive in the *phyB* mutants because the relevant photoreceptor (PhyB) is missing. Since plant shade is richer in far-red light than direct sunlight, the various phenotypes of the *phyB* mutants may represent components of the plant's response to excessive shade (Smith, 1982). Other environmental states affecting red/far-red light ratios, such as the onset of twilight or the latitude at which an individual plant grows, might have similar effects on plant development (Smith, 1982).

Our observation that root hairs are longer in the *phyB* mutants than in the wild type is particularly intriguing, because roots are normally hidden from light. This phenotype may indicate that red light normally inhibits root hair growth. Other researchers have noted phytochrome effects in roots: for example, in regulating expression of the asparagine synthetase gene (Tsai and Corruzzi, 1991), and in promotion of gravitropism (Lake and Slack, 1961; Feldman, 1984; Kelly and Leopold, 1992). Phytochrome has been found to be expressed in root caps of maize (Johnson et al., 1992). In preliminary experiments, we failed to observe any effect of light on root gravitropism in Arabidopsis, and we did not notice any defect in gravitropism in the *phyB* mutants (J. W. Reed, unpublished results).

Comparison of *hy3*(*phyB*) mutant phenotypes to *hy1*, *hy2*, and *hy6* mutant phenotypes shows that the relative contribution of PhyB to phytochrome-mediated responses depends on the particular response. The *hy1*, *hy2*, and *hy6* mutants are deficient in phytochrome chromophore attachment or availability (Chory et al., 1989; Parks et al., 1989; Parks and Quail, 1991). Presumably, they are deficient in multiple phytochrome species. These mutants have hypocotyls only slightly longer than *phyB* mutant hypocotyls (Table 1; Koornneef et al., 1980; Chory, 1992; J. W. Reed, unpublished results), flower slightly earlier than *phyB* mutants (Table 2; Chory, 1992), have substantially less chlorophyll (Table 1; Koornneef et al., 1980; Lifschitz et al., 1990; Chory, 1992), and have defects in red light induction of gene expression not exhibited by *phyB* mutants (Chory et al., 1989; Sun and Tobin, 1990). Thus, PhyB appears to play a major role in inhibition of hypocotyl elongation by red light, significant roles in controlling flowering and inducing chlorophyll accumulation, and no part in red light induction of gene expression in dark-grown plants. Therefore, phytochromes seem to have distinct but partially overlapping

Table 3. Lengths of Wild-Type and *phyB* Seedling Roots[a]

Strain	Root Length (light)	Root Length (dark)
Ler	25.7 ± 7.1 mm	22.9 ± 6.9 mm
phyB-8-36	17.9 ± 6.2 mm	16.2 ± 6.8 mm

[a] Root lengths were measured 8 days after sowing on plates incubated vertically. Ler, wild type Landsberg *erecta*.

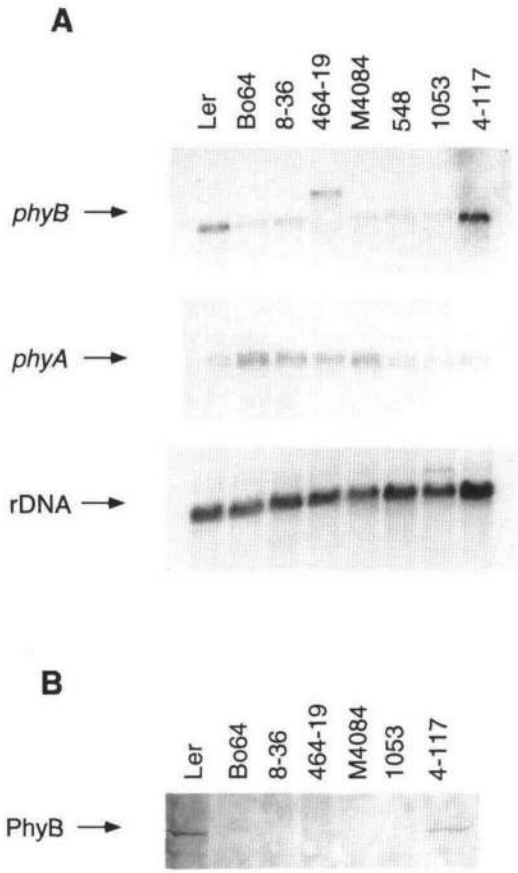

Figure 8. RNA and Protein Gel Blot Analysis of Wild-Type and *phyB* Mutant Plants.

(A) Blots of RNA from wild-type (Ler) and *phyB* mutant plants probed with a *PHYB*-specific fragment, a *PHYA*-specific fragment, and a ribosomal DNA (rDNA) fragment. The data are from two RNA gel blots, one probed with the *PHYB*-specific fragment and the rDNA fragment, and the second probed with the *PHYA*-specific fragment. When the blot used for *PHYA* was reprobed with an rDNA fragment, the apparent variation in *PHYA* transcript levels among strains was found to be due to unequal amounts of RNA in different lanes (data not shown). The same RNA preparations were used for both blots.
(B) Gel blot of proteins from wild-type (Ler) and *phyB* mutants probed with the anti-PhyB monoclonal antibody.

functions. Differences in the biochemistry of signal transduction mediated by the various phytochromes might account for the apparently distinct developmental roles of the different phytochrome isoforms, or they might be expressed in different tissues of the plant or at different times during development.

The finding that overexpression of *PHYB* in transgenic Arabidopsis plants caused a short hypocotyl phenotype is consistent with the conclusion that PhyB is the principal phytochrome involved in controlling hypocotyl elongation (Wagner et al., 1991). However, overexpression of *PHYA* genes in tobacco, tomato, or Arabidopsis can also inhibit stem or hypocotyl elongation (Boylan and Quail, 1989, 1991; Keller et al., 1989; Nagatani et al., 1991b; Cherry et al., 1992). These results suggest that PhyA can mediate inhibition of cell elongation, at least when overproduced substantially. More careful characterization of the light responses of such transgenic plants has suggested that the responses seen reflect the unusually high levels of PhyA present, and that these responses are therefore aberrant (McCormac et al., 1992). In the *PHYA* overexpression studies, PhyA appears to be able to play a role normally performed by PhyB. Mutants defective specifically in PhyA have not been described, but if PhyB really is the most important phytochrome in mediating inhibition of hypocotyl elongation, we would expect that *phyA* mutants would not have elongated hypocotyls in white light screens. These experiments underscore the importance of considering the phenotypes of mutants lacking a particular phytochrome, as well as phenotypes caused by overexpression of a gene for that phytochrome, to reach conclusions regarding biological function.

The biochemical targets of PhyB signal transduction are not known. Different tissues exhibit different types of deficiencies in the *phyB* mutants. Thus, leaf mesophyll cells produce fewer chloroplasts and less chlorophyll in the mutant; apical meristematic cells undergo the switch from vegetative to inflorescence meristem growth prematurely; and hypocotyl, stem, petiole, and root hair cells elongate more. Perhaps levels of (or sensitivities to) some hormone or metabolite that controls these processes are affected in the mutants. It has been shown that an increase in cytosolic calcium levels precedes, and can induce, swelling of etiolated wheat protoplasts (Shacklock et al., 1992), implicating calcium in phytochrome signal transduction. Auxins, gibberellins, and brassinosteroids are known to cause cell elongation (Cleland, 1987; Métraux, 1987; Reid, 1987; Mandava, 1988), and *phyB* mutations may well affect synthesis or action of one of these plant hormones. Other researchers have found that a protein corresponding to a phytochrome is missing in a sorghum variant that overproduces gibberellins (Beall et al., 1991; Childs et al., 1991, 1992). This variant, which carries the $ma3^R$ allele at the *MA3* maturity locus, flowers early and has elongated leaf sheaths and leaf blades (Pao and Morgan, 1986). These phenotypes are reminiscent of those of the *phyB* mutants described here. Similarly, an oilseed rape mutant that overproduces gibberellins is missing a phytochrome (Rood et al., 1990; Devlin et al., 1992). Farther down the pathway, changes in cell wall properties probably account for the increased cell elongation in hypocotyls, root hairs, and other tissues (Taiz, 1984; Casal et al., 1990; Kigel and Cosgrove, 1991).

The identification of mutations in the *PHYB* gene will facilitate more detailed investigation of light signal transduction and light-regulated development in Arabidopsis. By characterizing additional point mutations, it will be possible to learn more about the biochemical activity of this unique light-absorbing protein. The mutants will also allow further biochemical and physiological studies aimed at unraveling the downstream signal transduction pathways initiated by absorption of light by phytochrome.

METHODS

Plant Growth and Phenotypic Analysis

Seeds were surface sterilized and plated on Murashige-Skoog/sucrose/phytagar plates (1 × Murashige-Skoog salts, 2% sucrose, 0.8% phytagar [GIBCO]), stored overnight at 4°C, and grown in light chambers (16-hr day/8-hr night) kept at 22°C. Seedlings were transferred to soil 10 days to 2 weeks after sowing and grown in a greenhouse. For root observations, the seeds were sown in a line across MS/sucrose/phytagar plates, and the plates were placed vertically instead of horizontally. Dark-grown seedlings were first induced to germinate by treatment with white light for 16 to 24 hr. Chlorophyll measurements were as described by Chory (1992).

Cloning of *phyB* and Sequencing of Mutant Alleles

A clone containing phytochrome B (*PHYB*) gene sequences was detected in a library of *Arabidopsis thaliana* ecotype Landsberg *erecta* genomic DNA sequences (Voytas et al., 1990) cloned in λ Fix (Stratagene), using a probe obtained by polymerase chain reaction (PCR) amplification from genomic DNA. The primers used to amplify the PCR probe were 5′-GACTCATATGATGGCGGGGGAACAG-3′ (contains an introduced NdeI restriction site followed by nucleotides 4216 to 4230; Figure 2) and 5′-GCTCAAAGGATTCTTTATCACCTGACAAAT-3′ (nucleotides 5218 to 5189; Figure 2), and were designed based on the published cDNA sequence of *PHYB* from ecotype Columbia (Sharrock and Quail, 1989). The *PHYB* clone obtained was found to lack the 3′ end of the gene, so we amplified a fragment of Landsberg DNA by PCR using primers complementary to the 3′ end of the published Columbia cDNA sequences. The primers used were 5′-TCTGTTTCT-TGCAAATCCCGAGC-3′ (from nucleotide 5088 to nucleotide 5110 in Figure 2) and 5′-GCTCTAGAGCTGAACGCAAATAATCTCCC-3′ (containing an XbaI site near the 5′ end, and nucleotides 6509 to 6487 of the *PHYB* gene sequence; Figure 2). The clones were sequenced by standard methods. A portion of the gene, including the promoter, was sequenced by Lark Sequencing Technologies, Inc. (Houston, TX). Mutant *phyB* alleles were sequenced by amplifying segments of the gene by PCR, reamplifying these fragments by asymmetric PCR, and sequencing these products directly as described by Beitel et al. (1990). The mutation in strain 8-36 was localized before sequencing by denaturing gradient gel electrophoresis (Myers et al., 1987, 1989).

RNA and Protein Analyses

RNA was purified as described previously by Chory et al. (1989). For RNA gel blot hybridizations, a purified 1.1-kb XbaI fragment containing nucleotides 5380 to 6509 (Figure 2; the latter XbaI site came from the PCR primer used to clone the 3′ end of the gene, see above) was used as a *PHYB* probe; a PCR product, made by amplifying Landsberg genomic DNA with two primers designed from the published *PHYA* cDNA sequence (Sharrock and Quail, 1989), was used as a *PHYA* probe. The primers used for the *PHYA* amplification were 5′-TGTTGACAATGG-CCAGTCATGCAGTTCC-3′ (positions 535 to 562) and 5′-GTCATGCAA-ACTATCAGTGCTCAAACCC-3′ (positions 1694 to 1665). The ribosomal DNA probe was described previously (Chory et al., 1989). Proteins were extracted by the method of Chory et al. (1989). Protein gel blots were visualized using an alkaline phosphatase–conjugated goat anti–mouse antibody kit from Bio-Rad.

ACKNOWLEDGMENTS

We thank Daniel Voytas for the Landsberg *erecta* chromosomal library; Maarten Koornneef, Janny Peters, Richard Kendrick, and Ken Feldman for identifying and making available the T-DNA insertion allele of *hy3*; Alan Pepper for designing the PCR primers used to amplify a probe for the *phyB* gene; Akira Nagatani for supplying anti-PhyB antibody; Nathan Aguilar for help with plant DNA preps; and Marc Lieberman for plant photography. This research was supported by a grant from the Samuel Roberts Noble Foundation (Ardmore, OK) to the Salk Institute (San Diego, CA), and by grants from the U.S. Department of Energy (DOE), the U.S. Department of Agriculture, and the National Science Foundation to J. C. Part of the sequencing work was supported by funds from the Frontier Research Programs, RIKEN Institute. J. W. R. is a DOE–Energy Biosciences Research Fellow of the Life Sciences Research Foundation (Baltimore, MD), and P. N. was partially supported by a grant from Schering-Plough (Kenilworth, NJ). The cover photograph was taken by Marc Lieberman, and the cover artwork was by Liz Grabowski and Cynthia Doane.

Received November 19, 1992; accepted December 21, 1992.

REFERENCES

Beall, F.D., Morgan, P.W., Mander, L.N., Miller, F.R., and Babb, K.H. (1991). Genetic regulation of development in *Sorghum bicolor.* V. The ma_3^R allele results in gibberellin enrichment. Plant Physiol. **95,** 116–125.

Beitel, G.J., Clark, S.G., and Horvitz, H.R. (1990). *Caenorhabditis elegans ras* gene *let-60* acts as a switch in the pathway of vulval induction. Nature **348,** 503–509.

Boylan, M.T., and Quail, P.H. (1989). Oat phytochrome is biologically active in transgenic tomatoes. Plant Cell **1,** 765–773.

Boylan, M.T., and Quail, P.H. (1991). Phytochrome A overexpression inhibits hypocotyl elongation in transgenic *Arabidopsis.* Proc. Natl. Acad. Sci. USA **88,** 10806–10810.

Brown, J.A.M., and Klein, W.H. (1971). Photomorphogenesis in *Arabidopsis thaliana* (L.) Heynh: Threshold intensities and blue-far red synergism in floral induction. Plant Physiol. **47,** 393–399.

Casal, J.J., Whitelam, G.C., and Smith, H. (1990). Phytochrome control of extracellular peroxidase activity in mustard internodes: Correlation with growth, and comparison with the effect of wounding. Photochem. Photobiol. **52,** 165–172.

Cherry, J.R., Hondred, D., Walker, J.M., and Vierstra, R.D. (1992). Phytochrome requires the 6-kDa N-terminal domain for full biological activity. Proc. Natl. Acad. Sci. USA **89,** 5039–5043.

Childs, K.L., Pratt, L.H., and Morgan, P.W. (1991). Genetic regulation of development in *Sorghum bicolor.* VI. The ma_3^R allele results in abnormal phytochrome physiology. Plant Physiol. **97,** 714–719.

Childs, K.L., Cordonnier-Pratt, M.-M., Pratt, L.H., and Morgan, P.W. (1992). Genetic regulation of development in *Sorghum bicolor.* VII.

ma_3^R flowering mutant lacks a phytochrome that predominates in green tissue. Plant Physiol. **99,** 765–770.

Chory, J. (1991). Light signals in leaf and chloroplast development: Photoreceptors and downstream responses in search of a transduction pathway. New Biologist **3,** 538–548.

Chory, J. (1992). A genetic model for light-regulated seedling development in *Arabidopsis.* Development **115,** 337–354.

Chory, J., Peto, C.A., Ashbaugh, M., Saganich, R., Pratt, L., and Ausubel, F. (1989). Different roles for phytochrome in etiolated and green plants deduced from characterization of *Arabidopsis thaliana* mutants. Plant Cell **1,** 867–880.

Christensen, A.H., and Quail, P.H. (1989). Structure and expression of a maize phytochrome-encoding gene. Gene **85,** 381–390.

Cleland, R.E. (1987). Auxin and cell elongation. In Plant Hormones and Their Role in Plant Growth and Development, P.J. Davies, ed (Dordrecht: Martinus Nijhoff), pp. 132–148.

Colbert, J.T. (1988). Molecular biology of phytochrome. Plant Cell Environ. **11,** 305–318.

Cone, J.W., and Kendrick, R.E. (1985). Fluence-response curves and action spectra for promotion and inhibition of seed germination in wild-type and long-hypocotyl mutants of *Arabidopsis thaliana.* Planta **163,** 43–54.

Corré, W.J. (1983). Growth and morphogenesis of sun and shade plants. II. The influence of light quality. Acta Bot. Neerland. **32,** 185–202.

Cosgrove, D.J. (1986). Photomodulation of growth. In Photomorphogenesis in Plants, R.E. Kendrick and G.H.M. Kronenberg, eds (Dordrecht: Martinus Nijhoff), pp. 341–366.

Crawford, N.M., Smith, M., Bellissimo, D., and Davis, R.W. (1988). Sequence and nitrate regulation of the *Arabidopsis thaliana* mRNA encoding nitrate reductase, a metalloflavoprotein with three functional domains. Proc. Natl. Acad. Sci. USA **85,** 5006–5010.

Dehesh, K., Tepperman, J., Christensen, A.H., and Quail, P.H. (1991). *phyB* is evolutionarily conserved and constitutively expressed in rice seedling shoots. Mol. Gen. Genet. **225,** 305–313.

Devlin, P.F., Rood, S.B., Somers, D.E., Quail, P.H., and Whitelam, G.C. (1992). Photophysiology of the elongated internode (*ein*) mutant of *Brassica rapa*: *ein* mutant lacks a detectable phytochrome B-like polypeptide. Plant Physiol. **100,** 1442–1447.

Feldman, L.J. (1984). Regulation of root development. Annu. Rev. Plant Physiol. **35,** 223–242.

Furuya, M. (1989). Molecular properties and biogenesis of phytochrome I and II. Adv. Biophys. **25,** 133–167.

Goto, N., Kumagai, T., and Koornneef, M. (1991). Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. Physiol. Plant. **83,** 209–215.

Hanelt, S., Braun, B., Marx, S., and Schneider-Poetsch, H.A.W. (1992). Phytochrome evolution: A phylogenetic tree with the first complete sequence of phytochrome from a cryptogamic plant (*Selaginella martensii* Spring). Photochem. Photobiol. **56,** 751–758.

Hershey, H.P., Barker, R.F., Idler, K.B., Murray, M.G., and Quail, P.H. (1987). Nucleotide sequence and characterization of a gene encoding the phytochrome polypeptide from *Avena.* Gene **61,** 339–348.

Heyer, A., and Gatz, C. (1992). Isolation and characterization of a cDNA clone for potato type B phytochrome. Plant Mol. Biol. **20,** 589–600.

Johnson, E.M., Pao, L.I., and Feldman, L.J. (1991). Regulation of phytochrome message abundance in root caps of maize: Spatial, environmental, and genetic specificity. Plant Physiol. **95,** 544–550.

Kay, S.A., Keith, B., Shinozaki, K., and Chua, N.-H. (1989). The sequence of the rice phytochrome gene. Nucl. Acids Res. **17,** 2865–2866.

Keller, J.M., Shanklin, J., Vierstra, R.D., and Hershey, H.P. (1989). Expression of a functional monocotyledonous phytochrome in transgenic tobacco. EMBO J. **8,** 1005–1012.

Kelly, M.O., and Leopold, A.C. (1992). Light regulation of the growth response in corn root gravitropism. Plant Physiol. **98,** 835–839.

Kendrick, R.E., and Nagatani, A. (1991). Phytochrome mutants. Plant J. **1,** 133–139.

Kigel, J., and Cosgrove, D.J. (1991). Photoinhibition of stem elongation by blue and red light: Effects on hydraulic and cell wall properties. Plant Physiol. **95,** 1049–1056.

Koornneef, M., Rolff, E., and Spruit, C.J.P. (1980). Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z. Pflanzenphysiol. **100,** 147–160.

Lake, J.V., and Slack, G. (1961). Dependence on light of geotropism in plant roots. Nature **191,** 300–302.

Lifschitz, S., Gepstein, S., and Horwitz, B.A. (1990). Phytochrome regulation of greening in wild type and long-hypocotyl mutants of *Arabidopsis thaliana.* Planta **181,** 234–238.

López-Juez, E., Buurmeijer, W.F., Heeringa, G.H., Kendrick, R.E., and Wesselius, J.C. (1990). Response of light-grown wild-type and long hypocotyl mutant cucumber plants to end-of-day far red light. Photochem. Photobiol. **52,** 143–149.

López-Juez, E., Nagatani, A., Tomizawa, K.-I., Deak, M., Kern, R., Kendrick, R.E., and Furuya, M. (1992). The cucumber long hypocotyl mutant lacks a light-stable PHYB-like phytochrome. Plant Cell **4,** 241–251.

Mandava, N.B. (1988). Plant growth-promoting brassinosteroids. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39,** 23–52.

Martínez-Zapater, J.M., and Somerville, C.R. (1990). Effect of light quality and vernalization on late-flowering mutants of *Arabidopsis thaliana.* Plant Physiol. **92,** 770–776.

Maucher, H.P., Scheuerlein, R., and Schraudolf, H. (1992). Detection and partial sequence of phytochrome genes in the ferns *Anemia phyllitidis* (L.) Sw (Schizaeaceae) and *Dryopteris filix-mas* L. (Polypodiaceae) by using polymerase-chain reaction technology. Photochem. Photobiol. **56,** 759–763.

McCormac, A., Whitelam, G., and Smith, H. (1992). Light-grown plants of transgenic tobacco expressing an introduced oat phytochrome A gene under the control of a constitutive viral promoter exhibit persistent growth inhibition by far red light. Planta **188,** 173–181.

Métraux, J.-P. (1987). Gibberellins and plant cell elongation. In Plant Hormones and Their Role in Plant Growth and Development, P.J. Davies, ed (Dordrecht: Martinus Nijhoff), pp. 296–317.

Mullet, J.E. (1988). Chloroplast development and gene expression. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39,** 475–502.

Myers, R.M., Maniatis, T., and Lerman, L.S. (1987). Detection and localization of single base changes by denaturing gradient electrophoresis. Methods Enzymol. **155,** 501–527.

Myers, R.M., Sheffield, V.C., and Cox, D.R. (1989). Mutation detection by PCR, GC-clamps, and denaturing gradient gel electrophoresis. In PCR Technology: Principles and Applications for DNA Amplification, H. Ehrlich, ed (New York: Stockton Press), pp. 71–88.

Nagatani, A., Chory, J., and Furuya, M. (1991a). Phytochrome B is not detectable in the hy3 mutant of *Arabidopsis*, which is deficient

in responding to end-of-day far red light treatments. Plant Cell Physiol. **32,** 1119–1122.

Nagatani, A., Kay, S.A., Deak, M., Chua, N.-H., and Furuya, M. (1991b). Rice type I phytochrome regulates hypocotyl elongation in transgenic tobacco seedlings. Proc. Natl. Acad. Sci. USA **88,** 5207–5211.

Pao, C.-I., and Morgan, P.W. (1986). Genetic regulation of development in *Sorghum bicolor.* I. Role of the maturity genes. Plant Physiol. **82,** 575–580.

Parks, B.M., and Quail, P.H. (1991). Phytochrome-deficient *hy1* and *hy2* long hypocotyl mutants of Arabidopsis are defective in phytochrome chromophore biosynthesis. Plant Cell **3,** 1177–1186.

Parks, B.M., Shanklin, J., Koornneef, M., Kendrick, R.E., and Quail, P.H. (1989). Immunochemically detectable phytochrome is present at normal levels but is photochemically nonfunctional in the *hy1* and *hy2* long hypocotyl mutants of *Arabidopsis.* Plant Mol. Biol. **12,** 425–437.

Quail, P.H. (1991). Phytochrome: A light-activated molecular switch that regulates plant gene expression. Annu. Rev. Genet. **25,** 389–409.

Reed, J.W., Nagpal, P., and Chory, J. (1992). Commentary: Searching for phytochrome mutants. Photochem. Photobiol. **56,** 833–838.

Reid, J.B. (1987). The genetic control of growth via hormones. In Plant Hormones and Their Role in Plant Growth and Development, P.J. Davies, ed (Dordrecht: Martinus Nijhoff), pp. 318–340.

Rood, S.B., Williams, P.H., Pearce, D., Murofushi, N., Mander, L.N., and Pharis, R.P. (1990). A mutant gene that increases gibberellin production in *Brassica.* Plant Physiol. **93,** 1168–1174.

Sato, N. (1988). Nucleotide sequence and expression of the phytochrome gene in *Pisum sativum*: Differential regulation by light of multiple transcripts. Plant Mol. Biol. **11,** 697–710.

Shacklock, P.S., Read, N.D., and Trewavas, A.J. (1992). Cytosolic free calcium mediates red light-induced photomorphogenesis. Nature **358,** 753–755.

Sharrock, R.A., and Quail, P.H. (1989). Novel phytochrome sequences in *Arabidopsis thaliana*: Structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Dev. **3,** 1745–1757.

Somers, D.E., Sharrock, R.A., Tepperman, J.M., and Quail, P.H. (1991). The *hy3* long hypocotyl mutant of Arabidopsis is deficient in phytochrome B. Plant Cell **3,** 1263–1274.

Smith, H. (1982). Light quality, photoperception, and plant strategy. Annu. Rev. Plant Physiol. **33,** 481–518.

Spruit, C.J.P., Van den Bom, A., and Koornneef, M. (1980). Light induced germination and phytochrome content of seeds of some mutants of Arabidopsis. Arabidopsis Info. Serv. **17,** 137–141.

Sun, L., and Tobin, E.M. (1990). Phytochrome-regulated expression of genes encoding light-harvesting chlorophyll a/b-protein in two long hypocotyl mutants and wild type plants of *Arabidopsis thaliana.* Photochem. Photobiol. **52,** 51–56.

Taiz, L. (1984). Plant cell expansion: Regulation of cell wall mechanical properties. Annu. Rev. Plant Physiol. **35,** 585–657.

Thompson, W.F., and White, M.J. (1991). Physiological and molecular studies of light-regulated nuclear genes in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42,** 423–466.

Tsai, F.-Y., and Coruzzi, G. (1991). Light represses transcription of asparagine synthetase genes in photosynthetic and nonphotosynthetic organs of plants. Mol. Cell. Biol. **11,** 4966–4972.

Voytas, D.F., Konieczny, A., Cummings, M.P., and Ausubel, F.A. (1990). The structure, distribution, and evolution of the *Ta1* retrotransposable element family of *Arabidopsis thaliana.* Genetics **126,** 713–721.

Wagner, D., Tepperman, J.M., and Quail, P.H. (1991). Overexpression of phytochrome B induces a short hypocotyl phenotype in transgenic Arabidopsis. Plant Cell **3,** 1275–1288.

Whitelam, G.C., and Smith, H. (1991). Retention of phytochrome-mediated shade avoidance responses in phytochrome-deficient mutants of *Arabidopsis*, cucumber, and tomato. J. Plant Physiol. **139,** 119–125.

Williams, M.E., Foster, R., and Chua, N.-H. (1992). Sequences flanking the hexameric G-box core CACGTG affect the specificity of protein binding. Plant Cell **4,** 485–496.

Winands, A., Wagner, G., Marx, S., and Schneider-Poetsch, H.A.W. (1992). Partial nucleotide sequence of phytochrome from the zygnematophycean green alga *Mougeotia*. Photochem. Photobiol. **56,** 765–770.