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Missense Mutations Define a Restricted Segment in the C-Terminal Domain of Phytochrome A Critical to Its Regulatory Activity

Yong Xu, Brian M. Parks,[1] Timothy W. Short,[2] and Peter H. Quail[3]

Department of Plant Biology, University of California at Berkeley, Berkeley, California 94720, and U.S. Department of Agriculture Plant Gene Expression Center, Albany, California 94710

The phytochrome family of photoreceptors has dual molecular functions: photosensory, involving light signal perception, and regulatory, involving signal transfer to downstream transduction components. To define residues necessary specifically for the regulatory activity of phytochrome A (phyA), we undertook a genetic screen to identify Arabidopsis mutants producing wild-type levels of biologically defective but photochemically active and dimeric phyA molecules. Of eight such mutants identified, six contain missense mutations (including three in the same residue, glycine 727) clustered within a restricted segment in the C-terminal domain of the polypeptide. Quantitative photobiological analysis revealed retention of varying degrees of partial activity among the different alleles—a result consistent with the extent of conservation at the position mutated. Together with additional data, these results indicate that the photoreceptor subdomain identified here is critical to the regulatory activity of both phyA and phyB.

INTRODUCTION

The phytochrome family of photoreceptors constantly monitors the light environment for informational signals that elicit photomorphogenic responses appropriate to the ambient conditions (Quail, 1991, 1994a, 1994b; Smith, 1992, 1994; Furuya, 1993; Kendrick and Kronenberg, 1994). These responses are evoked at all major phases of the plant life cycle and include seed germination, seedling deetiolation (inhibition of hypocotyl elongation, hook opening, cotyledon separation and expansion, and chloroplast development), shade avoidance, and flowering. Signal perception by the photoreceptor is facilitated by its unique capacity for reversible interconversion between the red light–absorbing (Pr) form and the far-red-light–absorbing (Pfr) form upon sequential photon absorption. Superimposed on this basic capacity is a sophisticated but not understood capacity to analyze and integrate information from multiple parameters in the light environment, including wavelength distribution, photon fluence rate, and the duration and periodicity of irradiation (Smith, 1994). Thus, the phytochrome molecule can be considered to have dual functions: a photosensory function involving perception and interpretation of incoming light signals, and a regulatory function involving transfer of the perceived information to downstream components of the signal transduction chain. Changes in gene expression underlying the various morphogenic changes induced by the photoreceptor are well documented (Batschauer et al., 1994; Millar et al., 1994; Tobin and Kehoe, 1994), but neither the biochemical mechanism of phytochrome action nor the primary steps in subsequent signal transduction have been defined. Efforts to elucidate these mechanisms include investigations of the photoreceptor molecules themselves (Quail, 1991, 1994a, 1994b; Furuya, 1993) and genetic and biochemical approaches to signaling component identification (Chory, 1993; Deng, 1994; Millar et al., 1994; Quail, 1994b).

The generic phytochrome molecule is dimeric, consisting of ~125-kD polypeptide subunits that fold into two major structural domains: the N-terminal domain that carries a single, covalently linked chromophore, and the C-terminal domain responsible for dimerization (Quail, 1991; Furuya, 1993; Furuya and Song, 1994). In Arabidopsis, five phytochromes, designated phyA, phyB, phyC, phyD, and phyE, have been defined (Sharrock and Quail, 1989; Clack et al., 1994), and sequences related to these have been identified in a range of plant species (Quail, 1994a; Schneider-Poetsch et al., 1994; Mathews et al., 1995; Pratt, 1995). Studies using photoreceptor mutants and phytochrome overexpression in transgenic plants have established that phyA and phyB have discrete photosensory functions in early seedling development (Smith, 1992, 1994; Whitelam and Harberd, 1994). phyA has been shown to be primarily, if not exclusively, responsible for the far-red high-irradiance response elicited by continuous, monochromatic

[1] Current address: Botany Department, Birge Hall, University of Wisconsin, Madison, WI 53706.
[2] Current address: Department of Biology, Queens College of the City University of New York, Flushing, NY 11367.
[3] To whom correspondence should be addressed.

far-red light (FRc) or by FRc-enriched environments (Dehesh et al., 1993; McCormac et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993; Reed et al., 1994). phyB has been shown to be responsible for the red high-irradiance response elicited by continuous, monochromatic red light (Rc) or by Rc-enriched environments as well as for the associated shade-avoidance and end-of-day far-red responses (Somers et al., 1991; McCormac et al., 1992, 1993; Parks and Quail, 1993; Reed et al., 1993). Recent domain-swapping experiments have shown that the sequences determining this photosensory specificity reside in the respective N-terminal domains of phyA and phyB (D. Wagner, R. Kuhn, and P. Quail, unpublished data).

The definition of determinants involved in the regulatory function, as distinct from the photosensory function, of the phytochrome molecule requires identification of mutant molecules that at minimum retain wild-type photochemical activity, quaternary structure, and expression levels. Such molecules are anticipated to be fully functional in photoperception but defective in signal transfer to downstream components. Previous studies have provided relatively limited information in this regard. These studies have involved either overexpression of site-directed mutant sequences in transgenic plants or sequencing of mutant photoreceptor genes identified in genetic screens. Evidence from use of an array of transgene-encoded deletion derivatives indicates that sequences within ~110 residues of the N and C termini of the phyA polypeptide are necessary for wild-type regulatory activity (Cherry et al., 1992, 1993; Cherry and Vierstra, 1994; Boylan et al., 1994). Equivalent studies with phyB have not been reported. Site-directed substitution of alanines for multiple serines at the N terminus of phyA was found to enhance rather than decrease activity in transgenic plants, indicating that these residues are not necessary for activity (Stockhaus et al., 1992). Although several point mutations have been identified in defective endogenous phyA and phyB sequences (Dehesh et al., 1993; Reed et al., 1993, 1994), only one missense mutation that fulfills the previously described criteria for selective loss of regulatory function has been described for phyA (Dehesh et al., 1993; Y. Xu and P. Quail, unpublished data). To begin to provide a comprehensive analysis of residues critical to the regulatory activity of phyA, we implemented a genetic screen designed to identify systematically Arabidopsis mutants that are defective in phyA function but that produce wild-type levels of photochemically active, dimeric phyA.

RESULTS

Arabidopsis *phyA* Mutants with the Wild-Type Level of Spectrally Active phyA Are Specifically Defective in phyA Regulatory Activity

A series of photobiological, genetic, and immunoblot procedures, as described by Parks and Quail (1993), was used to identify candidates for the desired class of mutants. Arabidopsis lines specifically defective in FRc perception were tested, using genetic complementation analysis, for allelism to the *phyA-101* (formerly *hy8-1*) allele shown to be null for phyA (Dehesh et al., 1993; Parks and Quail, 1993; Quail et al., 1994). Nine independently isolated mutants belonging to the *phyA* complementation group (data not shown) were chosen for detailed molecular characterization. New mutant allele designations are given according to the convention established by Quail et al. (1994).

Figure 1 shows an immunoblot analysis of PHYA protein levels in these new *phyA* mutants. Extracts were analyzed from seedlings that either were grown in darkness or were grown in darkness until exposed to Rc for 6 hr immediately preceding harvest. The wild type (RLD ecotype) and two previously isolated *phyA* mutant lines, *phyA-101* and *phyA-103-1* (previously *phyA-103*)(Parks and Quail, 1993; Quail et al., 1994), were included for comparison (Figure 1, lanes 1 to 6). For dark-grown wild-type seedlings, the PHYA polypeptide can be detected as a single, 116-kD band (Figure 1, lane 1). In *phyA-101*, as expected, there is no detectable PHYA band (Figure 1, lane 3). *phyA-103-1* and eight of the nine new *phyA* mutant lines (Figure 1, lanes 4, 6, 8, 10, 12, 14, 16, 18, and 22) contained levels of the PHYA protein approximately equal to that of the RLD wild-type plants, whereas *phyA-109* had barely detectable levels of the PHYA protein (Figure 1, lane 20). Figure 1 also shows that the immunochemically detectable polypeptide in all of the mutant lines declined in Rc-treated tissues in a manner similar to that of the RLD wild-type plants (Figure 1, lanes 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23). Because the photochemically active phyA molecule is light labile after its conversion to the Pfr form when exposed to Rc (Somers et al., 1991), this result suggests that the phyA molecules in these mutant lines are photoactive and are recognized by the cellular degradation machinery for normal turnover in vivo.

Figure 2 shows the quantitative spectrophotometric analysis of phytochrome in these *phyA* mutants. Because phyA is the predominant phytochrome species in etiolated, wild-type Arabidopsis tissues (Quail, 1991, 1994a; Somers et al., 1991), essentially all the spectrally measurable phytochrome detected here represents phyA. Figure 2 shows that eight of the nine *phyA* mutant lines (*phyA-103-1*, *phyA-103-2*, *phyA-103-3*, *phyA-104*, *phyA-105*, *phyA-106*, *phyA-107*, and *phyA-108*) containing wild-type levels of the PHYA protein, as determined by immunoblot analysis (Figure 1), also contained spectrophotometrically detectable phytochrome activity similar to that of the wild type. Figure 3 shows that the phyA difference spectra determined for these mutant lines are also indistinguishable from that of the wild type, indicating the absence of any detectable perturbation of normal protein–chromophore interactions. By contrast, in a fashion similar to the phytochrome null mutant *phyA-101*, mutant line *phyA-110*, which had normal levels of PHYA polypeptide as determined by immunoblot analysis (Figure 1, lane 22), did not show the presence of spectrally active phytochrome in the dark-grown seedlings (Figure 2). Mutant line *phyA-109*, which had a low level of PHYA protein (Figure 1, lane 20), also had a comparably low level of spectrally active phyA (Figure 2).

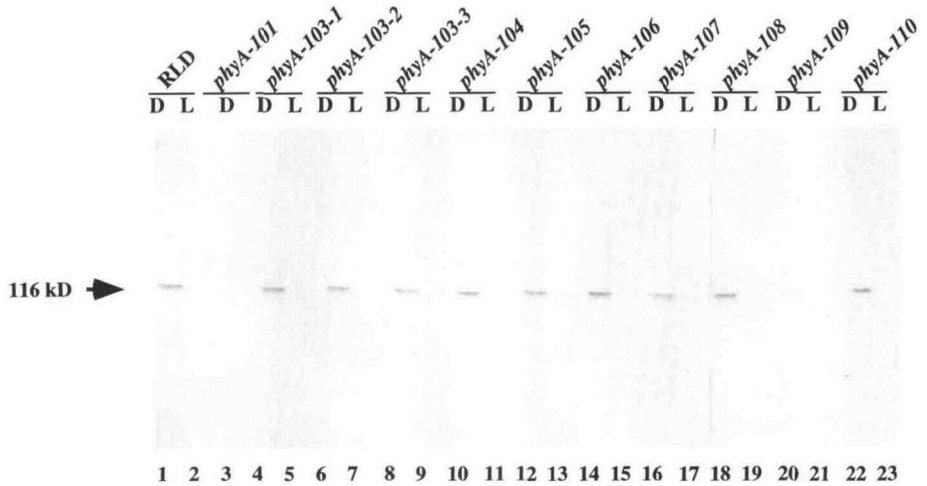


Figure 1. Immunoblot Analysis of PHYA Level and Light-Induced phyA Degradation in *phyA* Mutants.

Crude extracts were prepared from 7-day-old seedlings of wild type (RLD), *phyA-101* (*phyA* null mutant), *phyA-103-1*, and nine independently isolated new *phyA* mutants (nomenclature as described by Quail et al., 1994). Extracts from dark-grown tissues (D) and from dark-grown tissues treated with 6 hr of red light before harvesting (L) were run side by side, as indicated by their respective designations above the gel. Each lane contained 20 μg of protein from the crude extract. After SDS-PAGE and electroblotting, the membrane was probed with a type-selective monoclonal antibody (073D) raised against phyA. The size of the expected wild-type phyA polypeptide is indicated by an arrow. The results of two simultaneously run gels were combined to enable complete analysis of all the lines.

These initial analyses assessing the photochemical activity of phyA allow division of these new protein-positive *phyA* mutants into two groups. The first group contains seven mutant lines (*phyA-103-2*, *phyA-103-3*, *phyA-104*, *phyA-105*, *phyA-106*, *phyA-107*, and *phyA-108*) with wild-type levels of spectrally active phyA and with normal in vivo protein turnover properties. The previously isolated *phyA-103-1* mutant also belongs to this group. The second group contains two *phyA* mutants (*phyA-109* and *phyA-110*) that have significantly reduced levels of spectrally active phyA, although the PHYA polypeptide is still present to a greater or lesser extent in these mutants. The presence of wild-type levels of spectrophotochemically measurable phyA in the first group of mutants indicates that they contain fully photochemically active photoreceptors, which can properly perceive the FRc signal and undergo photoconversion but fail to elicit photoresponses (in this case, the FRc high-irradiance response). Because all of the mutants fall into the same complementation group as *phyA-101*, they are predicted to carry lesions in the *PHYA* structural genes that cause a selective defect in regulatory activity.

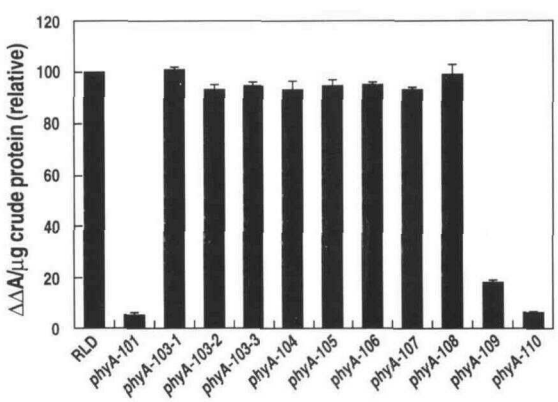


Figure 2. Spectrophotometric Detection of Phytochrome in Crude Extracts from the Etiolated Arabidopsis Wild Type and *phyA* Mutants.

Seeds of the wild type (RLD) and *phyA* mutants were germinated and grown in the dark for 7 days. Crude extracts were prepared from the etiolated seedlings. Spectrally active phytochrome content ($\Delta\Delta A$) was determined using a custom-built dual-wavelength spectrophotometer. The $\Delta\Delta A$ value of each sample was then normalized to the total protein in the crude extract used for the measurement. Relative phytochrome content ($\Delta\Delta A$ per microgram of crude protein) represents the spectrally active phytochrome content of each mutant line (designations are as given in Figure 1) compared with that of the wild type (RLD). The RLD value is set as 100.

The absence of detectable photochemical activity in *phyA-110*, despite wild-type protein levels (Figures 1 and 2), indicates that the PHYA polypeptide carries a lesion that disrupts either chromophore attachment or reversible photoconversion between the Pr and Pfr forms. The retention of photo-induced in vivo degradation of phyA observed for this line (Figure 1) suggests that the latter is more likely. The data indicate that the phyA molecule of *phyA-110* can undergo photoconversion to the Pfr form, which is recognized by the degradation machinery, but that it is unable to undergo reverse photoconversion to the Pr form required for spectrophotometric detection in the assay used. Because the FRc high-irradiance response requires continuous cycling of phyA between the two photochemical

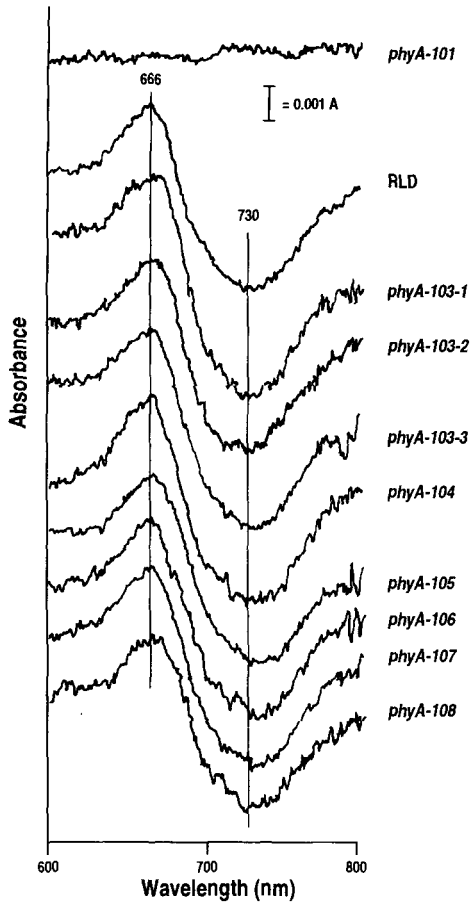


Figure 3. Phytochrome Difference Spectra of the Arabidopsis RLD Wild Type and *phyA* Mutants.

Extracts of 6-day-old dark-grown seedlings were concentrated ~12-fold by ammonium sulfate precipitation. Difference spectra were recorded for the RLD wild type, *phyA* null mutant (*phyA-101*), and eight *phyA* regulatory mutants (*phyA-103-1* to *phyA-103-3*; *phyA-104* to *phyA-108*). The peak positions of the Pr and Pfr forms of phyA in the wild type are indicated (666 and 730 nm, respectively). The bar represents 0.001 absorbance units. The spectra were derived from equal volumes of concentrated extract and were not adjusted for equal protein.

forms, the mutant molecule in *phyA-110* can be classified as a photosensory function mutant. It is uncertain whether the low levels of phyA in *phyA-109* (Figures 1 and 2) are sufficient to account for the phenotype or whether the phyA molecule is also intrinsically defective.

Regulatory Function Mutations Are Clustered in the C-Terminal Domain of the PHYA Polypeptide

The *phyA* genes from all nine new *phyA* mutants were completely sequenced. DNA sequence comparison with the wild-type RLD *PHYA* gene revealed that each mutant line carries a single nucleotide change within the respective *phyA* coding region, resulting in a single amino acid substitution relative to the wild-type PHYA polypeptide. Figure 4A shows schematically the location of the various missense mutations in the mutant *phyA* alleles, and Table 1 summarizes the allele designations according to the nomenclature of Quail et al. (1994). This result confirms that these mutants do indeed carry lesions in their respective *phyA* structural genes and establishes that, except for *phyA-109* and *phyA-110*, all are regulatory function mutants.

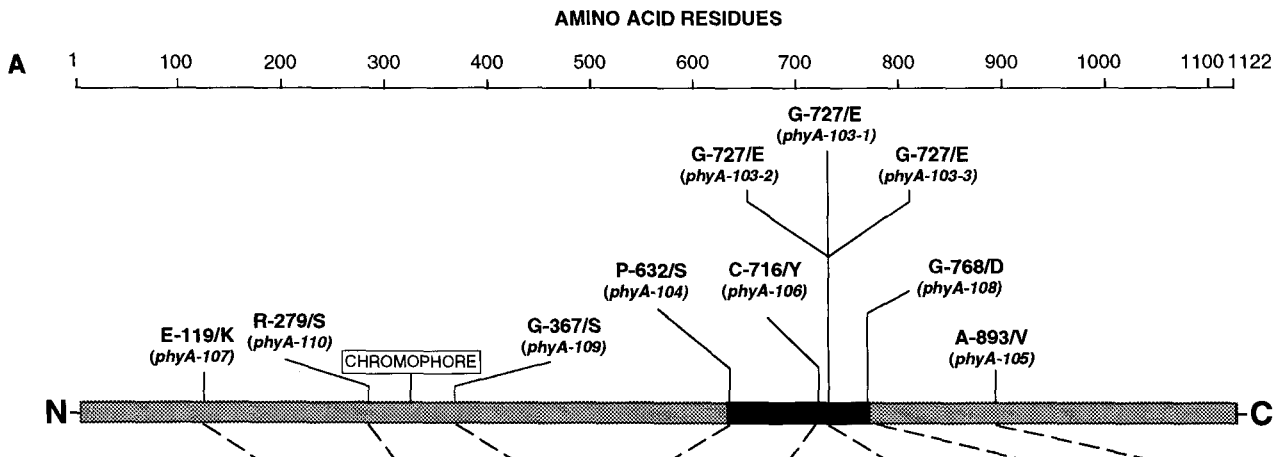
Although various point mutations were found across both N- and C-terminal domains of the PHYA polypeptide, it is intriguing that seven of the eight regulatory function mutations are located in the C-terminal domain of the molecule (Figure 4A). Moreover, six of the mutations are clustered within a region of 136 amino acids between P-632 and G-768. Only one regulatory function mutant (*phyA-107*) carries a missense mutation in the N-terminal domain. Two other mutant lines, *phyA-109* and *phyA-110*, which both had much lower levels of spectrally active phyA than did the wild-type plants, each carry a missense mutation in the N-terminal domain near the chromophore attachment site (Figure 4A).

Figure 4B compares the missense mutations in the Arabidopsis phyA molecule with a recently compiled alignment of all other phytochromes whose sequence data are available in the GenBank data base. Of the eight regulatory function mutations, five (*phyA-104* [P-632/S], *phyA-106* [C-716/Y], *phyA-103-1* [G-727/E], *phyA-103-2* [G-727/E], and *phyA-103-3* [G-727/E]; see figure legend for an explanation of mutant designations) occur at a residue that is 100% conserved among all phytochromes, including all of the identified phytochrome subfamilies in Arabidopsis (PHYB, PHYC, PHYD, and PHYE) and four lower plants (Selaginella, Ceratodon, Adiantum, and Physcomitrella), and one (*phyA-108* [G-768/D]) occurs at a residue that is 100% conserved among all PHYA sequences. The polypeptide segments surrounding these conserved amino acid residues are also well to moderately conserved among all phytochromes (Figure 4B). Regulatory mutant *phyA-107* (E-119/K) shows possible conservation of an acidic residue among the majority of phytochromes (depending on the chosen alignment), although overall conservation in the surrounding segment is not high. Mutant *phyA-105* (A-893/V) exhibits relatively less conservation. On the other hand, the two mutants that contained very little spectrally active phyA (*phyA-109* and *phyA-110*) contain mutations at residues (G-367/S and R-279/S, respectively) that are 100% conserved among all phytochromes, and the regions surrounding these two amino acid residues are well conserved among all of the phytochromes as well (Figure 4B).

Two of the new mutants contain the same missense mutation at amino acid 727, resulting in conversion of G-727 to E-727, as was found previously in the line formerly designated *phyA-103* (Dehesh et al., 1993; Quail et al., 1994). Because these two new lines were isolated independently from *phyA-103* (now *phyA-103-1*) and from each other (that is, from three separately generated M_2 populations screened at different times), they

have been named *phyA-103-2* and *phyA-103-3*, respectively (Table 1). Table 1 summarizes the designations, lesions, residue conservation, and mutant class for all of the *phyA* missense mutations identified so far.

To determine whether the missense mutations identified here are likely to cause localized structural changes in the protein, we subjected each sequence to secondary structure prediction analysis as described by Rost and Sander (1993a, 1993b).



B

	119	279	367	632	716	727	768	893
Arabidopsis PHYA	PSV-GEH--P	IPQAARFLFM	KRLWGLVVCH	TATVPILAVD	VNACASRDLH	ENVVGVCFVAHD	PPIFGTDEFG	AERTAVKRL
soybean PHYA	PSV-GEH--P	IPQASRFLFR	ERLWGLVVCH	TATVPILAVD	VNRCASRDLR	DNVVGVCFVAHD	PPIFGTDEFG	SEQTASKRL
tobacco PHYA	PSV-GEL--P	IPQAARFLFM	KRLWGLVVCH	TASVPIFAVD	VNACASRDVG	DSVVGVCFIAQD	PPIFGTDQFG	SEQTALKRL
parsley PHYA	PSV-GEH--P	VPQAARFLFL	KKLWGLVVCH	TATVPIFAVD	VNACATRGLH	DNVVGVCFVAQD	PPIFGTDEFG	TEQTAMKRL
zucchini PHYA	PSM-GDY--P	IPQAARFLFM	KRLWGLVVCH	TATVPILAVD	VNACASRDLR	ENVVGVFFVAQD	PPIFGSDEFG	CEQTALKRL
pea PHYA	PSV-GDH--P	IPQAARFLFM	KRLWGLVVCH	TATVPILAVD	VNACASKDLR	ENVVGVCFVAQD	PPIFGTDEFG	SEQTALKRL
potato PHYA	PSV-GEH--P	IPQAARFLFM	KRLWGLVVSH	TASVPIFAVD	VNACASKDVR	DSVVGVCFIAQD	PPIFGTDQFG	SEQTALKRL
oat PHYA	PSV--D-DPP	IPQAARLLFM	KKLWGLLVCH	TATVPILAVD	VNACASRDLH	DHVVGVCFVAQD	PPIFGADEFG	SEQTSLKRL
rice PHYA	PSV--D-DPP	IPQAARFLFM	KKLWGLLVCH	TATVPILAVD	VNACASRDLH	DHVVGVCFVAQD	PPIFGADEFG	SQQNALTKL
maize PHYA	PNV--D-DPP	IPQAARFLFM	KRLWGLIVCH	TATVPILAVD	VNACASRDLH	DHVVGVCFVAQD	PPIFGADQFG	SEQTAQRKL
Arabidopsis PHYB	PTL--E-KPE	IPQASRFLFK	MRLWGLVVCH	TATVPIFAVD	VNACSSKDYL	NNIVGVCFVGQD	PPIFAADENT	QDTECFTKA
soybean PHYB	PSLDDKNDAA	IPQASRFLFK	MRLWGLVVCH	TATAPIFAVD	VNACSSKHFT	NNVVGVCFVGQN	PPIFASDDNT	QEKEFLGRM
tobacco PHYB	PSL--ER-PE	IPQASRFLFK	MRLWGLVVGH	TATAPIFAVD	VNACSSKDYT	NNIVGVCFVGQD	PPIFASDENT	QEKKCYSQM
potato PHYB	PSL--E-KCE	IPQASRFLFK	MRLWGLVVGH	TATAPIFAVD	VNACA-RDYT	NNIVGVCFVGQD	PPIFASDENT	QEKKCYSQM
rice PHYB	PSLDSSAVPP	IPQASRFLFR	MKLWGLVVCH	TATVPIFAVD	VNACSTRDYT	KNIVGVCFVGQD	PPIFASDENT	HEKKCYARM
Arabidopsis PHYC	PSM--E-QRE	IPQASRFLFM	RHLWGLVVCH	TAAVPIFAVD	VNTCCSRDMT	NNVLGVCFIGQD	PPIFITNENG	SEHAIACAL
Arabidopsis PHYD	PSIE-D-KSE	IPQASRFLFK	MRLWGLVVCH	TATVPIFAVD	VNACSSKDYL	NNIVGVCFVGQD	PPIFAADENT	QESEYFSRR
Arabidopsis PHYE	PHS-GEFDKV	IPQAARFLFK	SKLWGLVVGH	TATAPIFGVD	VNSCTSRDYT	ENIIGVCFVGQD	PPIFASDENA	SAQS----L
Selaginella	PSL-GSGQQD	IPQASRFLFM	RRLWGLVVCH	TATAPILAVD	VNACASRDVS	DNVVGVCFVGQD	PPIFGADEFG	TEKVALSKL
Ceratodon	PSM-GEM--D	IPQASRFLLM	RKLWGLVVCQ	TAAAPILAVD	VNACCSRDAS	DFVVGVFFVGQD	RPSFDGDEFG	---------
Adiantum	PTM-GQY--S	IPQAARFLFM	IKLWGLVVCH	TATAPILAVD	VNTCCSRDVS	NNVVGVCFVGQD	PPIFGADEYG	KEKV----D
Physcomitrella	PSV-GEM---	IPQASRFLFM	RKLWGLVVCH	TATAPILAVD	VDACSSIHVS	ENVVGVCFVGQD	PPIFGADEFG	TEKVAVAKL

Figure 4. Missense Mutations in Arabidopsis phyA Polypeptides.

(A) Schematic diagram of the locations of the various missense mutations in the Arabidopsis phyA polypeptide. These mutations were identified by DNA sequencing of *phyA* genes from different *phyA* mutants. The shaded bar represents the Arabidopsis PHYA polypeptide (1122 amino acid residues). Residues are numbered as described by Sharrock and Quail (1989). The location of the chromophore (C-323) is shown by an open box. Each missense mutation is shown at its location along the *phyA* polypeptide, with the wild-type residue (E, for example) followed by its residue number (119), which is followed by the mutant residue (K). The mutant designation (see Table 1) is shown in parentheses underneath the amino acid substitution. So, E-119/K denotes that the wild-type residue E at position 119 was changed into a K residue in mutant line *phyA-107*. Residue G-727 was changed into an E residue in three independently isolated mutant lines (*phyA-103-1*, *phyA-103-2*, and *phyA-103-3*). The solid black region within the bar represents a 136–amino acid region containing six of the eight regulatory mutants identified.

(B) Alignment of the Arabidopsis *phyA* missense mutations with all available phytochrome sequences. A region of 9 to 15 amino acid residues surrounding each missense mutation is compared with those from 18 higher plant and four lower plant phytochromes. The sequences are aligned as described by Mathews et al. (1995). The Arabidopsis PHYA sequence is shown in the first line, with each mutation indicated by its position. Boldface letters represent the locations of the missense mutations and their alignment with conserved residues in other phytochrome sequences. All amino acid residues are shown using the single-letter code. Dashes indicate gaps introduced in the alignment.

Table 1. Summary of *phyA* Missense Mutations

Isolate Name	New Name	Mutation[a]	Conservation[b]	Mutant Class[c]
hy8-3[d]	*phyA-103-1*[e]	G-727/E	+ +	Regulatory
2.1	*phyA-103-2*	G-727/E	+ +	Regulatory
30.1	*phyA-103-3*	G-727/E	+ +	Regulatory
2.3	*phyA-104*	P-632/S	+ +	Regulatory
13.3	*phyA-105*	A-893/V	*	Regulatory
16.1	*phyA-106*	C-716/Y	+ +	Regulatory
17.1	*phyA-107*	E-119/K	*	Regulatory
20.1	*phyA-108*	G-768/D	+	Regulatory
26.1	*phyA-109*	G-367/S	+ +	Low protein
21.5	*phyA-110*	R-279/S	+ +	Low $\Delta\Delta A$
m26[f]	*phyA-205*	V-631/M	+	Regulatory(?)

[a] The amino acid residue is shown using the single-letter code. The wild-type residue is shown first, followed by its position in PHYA and then by the mutant residue.

[b] + +, an amino acid residue that is 100% conserved among all phytochromes; +, an amino acid residue that is 100% conserved among all PHYA sequences; *, an amino acid residue that is not conserved even among PHYA sequences.

[c] Regulatory mutant is defined as a mutant with a wild-type level of spectrally active, dimeric phytochrome; $\Delta\Delta A$, spectrophotometrically detectable phytochrome; (?), possible regulatory mutant based on wild-type levels of PHYA protein (Reed et al., 1994) and $\Delta\Delta A$ (Elich and Chory, 1994), but with an undetermined quaternary structure.

[d] Described by Parks and Quail (1993) and Dehesh et al. (1993).

[e] Formerly *phyA-103* (Quail et al., 1994).

[f] Described by Reed et al. (1994).

No perturbation of the predicted secondary structure of phyA was caused by any of the mutations (data not shown).

All Regulatory Mutant phyA Molecules Retain Dimerization Capacity

One possible cause of loss of phyA regulatory activity in these mutants would be disruption of the quaternary structure. This possibility is particularly pertinant to mutations in the C-terminal domain because this domain carries the determinants responsible for dimerization (Quail, 1991; Furuya, 1993; Furuya and Song, 1994). To determine whether these missense mutations interfere with the formation of functional phytochrome dimers, nondenaturing gradient PAGE followed by immunoblotting was used to analyze the native molecular weights of the eight fully photoactive mutant molecules. The *phyA-103-1* mutant was included in this analysis because, although this mutant had been shown previously to be expressed at wild-type levels and to be fully photoactive (Dehesh et al., 1993; Parks and Quail, 1993), the possibility remained that the mutation disrupted dimerization. Figure 5 shows that crude extracts from seven of the eight regulatory mutants yielded a single band with an apparent molecular mass of 340 kD, which is indistinguishable from that of the full-length, wild-type, dimeric phyA holoprotein (lane 1). Mutant *phyA-107* (E-119/K) gave a band with a slightly larger apparent molecular mass than the other lines for reasons that are not known, but the mutant molecule is still apparently dimeric (Figure 5, lane 9). The level of protein detected in these lines is also comparable with that of the wild-type plant. This result establishes that all the regulatory mutants retain normal dimerization capacity. In addition, because the rate of migration through the gel matrix is a function of molecular radius, the data indicate that no gross changes in overall molecular shape have resulted from the mutations. As expected, the null *phyA-101* mutant gave no detectable signal.

Photocontrol of Hypocotyl Elongation of *phyA* Mutants Demonstrates Differential Loss of Regulatory Function among Different Alleles

All of the *phyA* mutants were isolated based on their specific loss of function under FRc. Under the FRc fluence rate used for screening, all of the new *phyA* mutants isolated showed responses to FRc that were indistinguishable from that of the *phyA-101* null mutant, which displayed elongated hypocotyls with closed cotyledons. To assess the functional importance of individual missense mutations in the phyA molecule and to determine whether these mutants might exhibit potential quantitative differences in responsiveness to FRc, wild type (RLD) and all of the *phyA* mutants (null and missense) were grown under high-fluence-rate FRc.

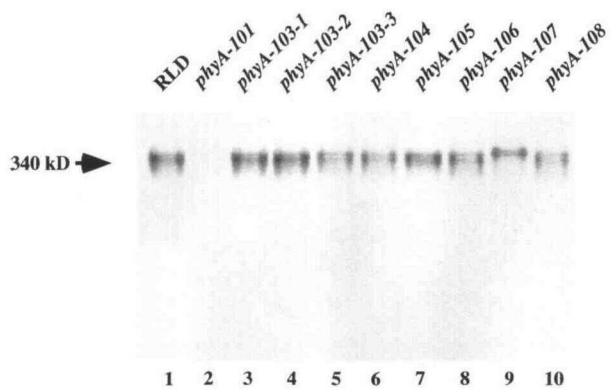


Figure 5. Immunoblot Determination of Native Molecular Mass of Mutant phyA Molecules.

Crude extracts were made from 7-day-old dark-grown seedlings of Arabidopsis wild type (RLD) and the *phyA* regulatory mutant lines. Nondenaturing gradient PAGE (4 to 20%) was used to analyze the native molecular mass of the phyA holoproteins. Each lane contained 20 μg of total protein from the crude extract of the wild-type plant (RLD) or each indicated *phyA* mutant (Table 1). After electrophoresis and electroblotting, the membrane was probed with a type-selective monoclonal antibody raised against phyA (073D). The arrow indicates the size of the wild-type phyA holoprotein. Molecular mass was determined using an electrophoresis calibration kit (Pharmacia).

Figure 6A, depicting the dark control, shows that the observed mutant responses are light dependent. Under low-fluence-rate FRc (2 W m^{-2}), which is the condition used for initial mutant screening, hypocotyl elongation of the wild-type plants was strongly inhibited, whereas all of the *phyA* mutant lines exhibited long hypocotyls approaching those of the dark controls (Figure 6B). There was no distinguishable difference between the *phyA* null mutant (*phyA-101*) and those with missense mutations. There was also no distinguishable difference in hypocotyl elongation among the different alleles carrying different missense mutations (Figure 6B).

Figure 6C shows, however, that under high-fluence-rate FRc, the *phyA* null mutant *phyA-101* was clearly distinguishable from several of the mutants with missense mutations. *phyA-101* displayed a very similar hypocotyl length under low- and high-fluence-rate FRc as well as in the dark, confirming that mutant plants do not show a detectable response to FRc when phyA is not present. In contrast, mutants with different missense mutations began to show differential responses in terms of inhibition of hypocotyl elongation, suggesting a range of allele severity (Figure 6C). Among the eight regulatory mutants, the highest response occurred in mutant *phyA-105*, which displayed a hypocotyl length similar to that of the wild-type plants. The smallest response occurred in *phyA-104*. This mutant exhibited a relatively long hypocotyl; however, the hypocotyl was shorter than that of the *phyA* null mutant. All other regulatory mutants fall between these two extremes (Figure 6C). Of the two mutants with low levels of spectral activity, *phyA-110* did not show a significant response to the high-fluence-rate FRc, whereas *phyA-109* was partially responsive (Figure 6C).

Figure 7 shows the seedling morphology of wild type, *phyA-101* (null), and two regulatory mutants under dark and low- and high-fluence-rate FRc conditions. It is clear that the cotyledon phenotype parallels the hypocotyl-length phenotype in that in high-fluence-rate FRc, *phyA-105* looks almost like wild type, which displayed fully expanded cotyledons, whereas *phyA-104* has much less expanded cotyledons, even under the high-fluence-rate FRc (Figure 7). Taken together, these analyses indicate that the different missense mutations in the *phyA* genes cause varying severity in terms of loss of regulatory activity.

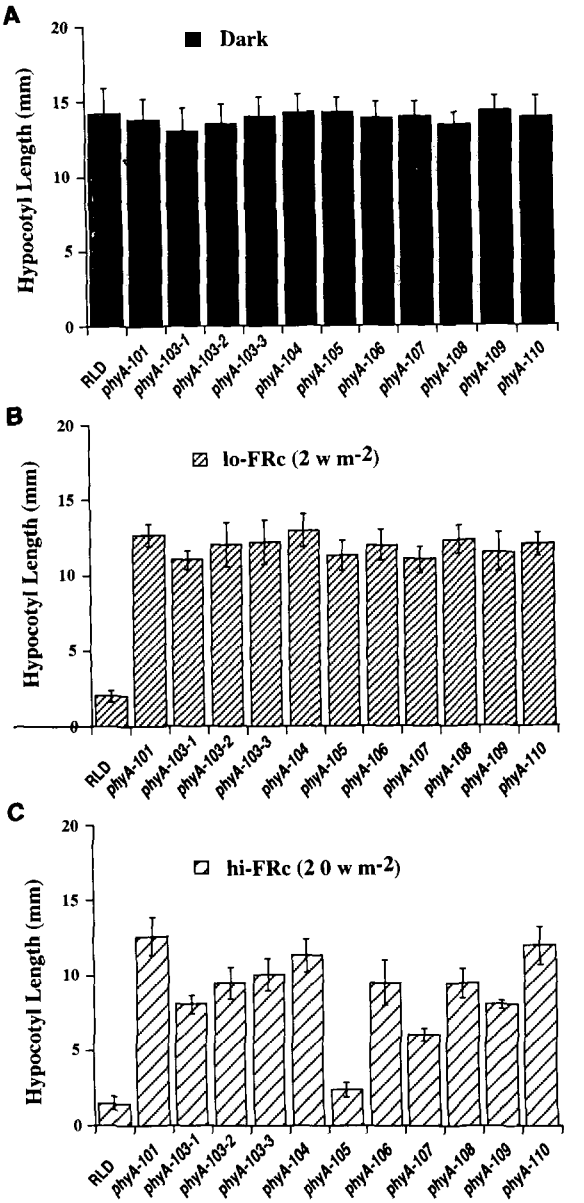


Figure 6. Photocontrol of Hypocotyl Elongation in *phyA* Mutants under Low- and High-Fluence-Rate FRc.

Wild type (RLD), *phyA* null mutant *phyA-101*, and the various *phyA* missense mutants, as indicated in Table 1, were sown on a single plate containing germination media without sucrose and grown under the indicated continuous irradiation regimes as described in Methods.
(A) Hypocotyl lengths of seedlings grown in the dark.
(B) and **(C)** Hypocotyl lengths of seedlings grown under continuous FRc. Lengths were determined by measuring the mean hypocotyl length of 20 seedlings of each line from each growth regime. Two fluence rates of FRc (low-fluence rate [lo-FRc] at 2 W m^{-2}, and high-fluence rate [hi-FRc] at 20 W m^{-2}) were used. Error bars represent the standard deviation of the mean.

DISCUSSION

The multistep genetic screen described here has permitted identification of mutant phyA molecules that are selectively compromised in their regulatory function. Eight of the 10 mutant molecules characterized are full length, are expressed at wild-type levels, are fully photochemically active with normal difference spectra, and are dimeric. The missense mutations identified also do not appear to have altered the intracellular stability of the protein. Likewise, because spectral properties are a sensitive indicator of the fidelity of protein-chromophore interactions, these mutations have not caused any apparent

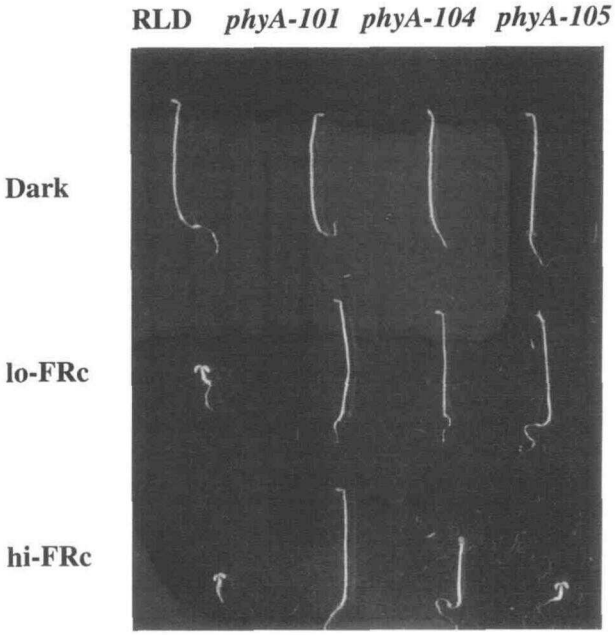


Figure 7. Partial Activity of *phyA* Regulatory Mutants under High–Fluence-Rate FRc.

Representative seedlings from the wild type (RLD), *phyA* null mutant *phyA-101*, and two *phyA* regulatory mutants (*phyA-104* and *phyA-105*) are compared in terms of seedling morphology when grown under the indicated light regimes. Seeds from each line were sown on germination plates without sucrose. The seedlings were grown for 3 days in darkness or under continuous FRc (low-fluence rate [lo-FRc] at 2 W m^{-2} and high-fluence rate [hi-FRc] at 20 W m^{-2}).

structural disruptions in the N-terminal domain surrounding the chromophore. The apparently normal light-induced turnover of the mutant phytochromes is evidence that the Pfr form of these molecules is recognized in normal fashion by the cellular degradation machinery responsible for this process. The apparently wild-type behavior of the mutant phyA molecules under nondenaturing gel electrophoresis conditions indicates not only that the capacity for dimerization has been retained, but also that gross conformational alterations affecting overall molecular shape do not appear to have been induced (with the possible exception of the N-terminal domain mutant *phyA-107*). The data indicate, therefore, that these mutant phyA molecules are fully competent in photoperception but are defective in further propagation of the perceived signal. The molecular basis for this loss of function is unclear. In principle, it could result from abrogation of intramolecular signal transfer, inability to recognize or interact correctly with target molecules, or inability to induce a productive change in target molecules.

The concentration of missense mutations recovered in the C-terminal domain of phyA indicates the importance of this domain to the regulatory function of the photoreceptor. Previous studies, showing that overexpression of only the N-terminal domain of either phyA (Boylan et al., 1994) or phyB (D. Wagner and P. Quail, unpublished data) in transgenic Arabidopsis resulted in photochemically active but biologically defective chromoproteins, also indicated that the C-terminal domain is necessary for normal regulatory activity. However, these data did not distinguish between a purely structural or other role for this domain in this activity. The observation that the missense mutations identified here do not cause perturbation of predicted local secondary structure or gross structural alterations detectable as disruption of dimerization or changed overall molecular shape may favor the possibility that the C-terminal domain has a more direct role in signal propagation.

Despite the small number of regulatory mutants obtained thus far, the clustering of amino acid substitutions in a relatively restricted polypeptide segment is striking. Six of the eight such mutants characterized here fall within a 136-residue segment of the C-terminal domain (~12% of the PHYA polypeptide). In addition, the previously described mutant *phyA-205* in which PHYA is expressed at wild-type levels (Reed et al., 1994) and is photochemically active (Elich and Chory, 1994) has a missense mutation (V-631/M) in the residue immediately adjacent to that described here in mutant *phyA-104* (P-632/S). Although examination of dimerization capacity has not yet been reported for *phyA-205*, it is a potential regulatory mutant.

Even more compelling, however, is that in two separate genetic screens for regulatory mutations using transgenic overexpressor lines of Arabidopsis, the majority of missense mutations identified in transgene-encoded phyA (M. Boylan and P. Quail, unpublished data) and phyB (Wagner and Quail, 1995) were found to fall between residues 624 and 777 (Quail et al., 1995). This region is coincident with but extends slightly beyond that depicted in Figure 4. In consequence, of the combined total of 21 regulatory missense mutations thus far identified in phyA and phyB, 16 (76%) fall within this ~160-residue region. Moreover, these mutations include multiple, independent substitutions in each of only four separate amino acids clustered in a segment of just 18 residues surrounding G-727 within this larger region. These latter mutations account for a remarkable 43% of all regulatory missense mutations thus far detected. Taken together, the data suggest that the restricted 160-residue C-terminal region, and in particular the residues in the smaller 18-residue segment, may have a direct mechanistic role in the biochemical process responsible for signal transfer to downstream transduction components. On the other hand, the present data do not preclude a more indirect role of this region in this process.

Recent domain-swapping experiments have provided evidence that the N-terminal domains of phyA and phyB determine the photosensory specificity of each molecule and that the C-terminal domains are functionally interchangeable (D. Wagner, R. Kuhn, and P. Quail, unpublished data). These data imply that photoreceptor activity requires some form of intramolecular communication of the perceived signal from the N-terminal to the C-terminal domain. In addition, evidence indicates that the C-terminal domains of phyA and phyB carry common determinants necessary for regulatory activity (D. Wagner, R.

Kuhn, and P. Quail, unpublished data). The coincidence of clustered missense mutations within the same restricted region of the phyA and phyB polypeptides indicates that this region represents such a common determinant and that the biochemical mechanism of intermolecular signal transfer to downstream signaling intermediates may be common to both photoreceptors.

It has been proposed, based on limited sequence similarity with bacterial two-component systems, that the phytochromes may function as histidine kinases (Schneider-Poetsch and Braun, 1991; Schneider-Poetsch et al., 1991). None of the missense mutations identified here fall within the postulated kinase domain and so do not directly address this possibility. On the other hand, site-directed substitution of the three residues in the proposed domain that are completely conserved in both the bacterial proteins and all sequenced phytochromes failed to reduce the activity of overexpressed oat phytochrome in transgenic Arabidopsis (M. Boylan and P. Quail, unpublished). Together with the absence of any compelling sequence similarity between the phytochromes and other proteins currently in the data bases, these results leave open the possibility that the biochemical mechanism of phytochrome action may be novel.

The severity of the phenotype induced by the regulatory mutations recovered here is correlated with the location and conservation in the wild-type sequences of the mutated residue. The mutations in the 136–amino acid C-terminal region of phyA involve residues that are invariant among either all sequenced phytochromes (P-632, C-716, and G-727) or all phyA sequences (G-768), and these cause the most severe loss of regulatory activity. The less severe mutations are outside the 136-residue region and involve less well conserved amino acids (E-119 and A-893). These data are consistent with this region's critical role in phyA regulatory activity. In addition, the availability of phyA mutants retaining partial activity may facilitate the recovery of second-site suppressor mutants involving loci important to early steps in phyA signal transduction.

Although most of the regulatory mutations characterized here are in the C-terminal domain, the recovery of the E-119/K mutation in the N-terminal domain has significant implications. This mutation suggests that the N-terminal domain has determinants necessary for regulatory as well as photosensory activity. Previous data are consistent with this observation. When the N-terminal domain of oat phyA was overexpressed in transgenic Arabidopsis, it interfered, in dominant negative fashion, with the activity of the endogenous Arabidopsis phyA molecule (Boylan et al., 1994). The data are consistent with the conclusion that the N-terminal domain carries determinants facilitating interaction with endogenous transduction components, but that without the C-terminal domain, the interaction is not only nonproductive but results in unavailability of these components to the endogenous Arabidopsis phyA. Based on the assembled data, we speculate that the phytochrome molecule may carry twin surfaces in separate domains for contact with its primary signaling reaction partner: one surface in the N-terminal domain specifying partner selection, and the other in the C-terminal domain facilitating the biochemical modification of the reaction partner responsible for intermolecular signal transfer.

METHODS

Plant Growth and Screening for Mutants

Arabidopsis thaliana ecotype RLD was used throughout this study. Genetic selection for the phytochrome *phyA* mutants by a two-step screen was described in detail by Parks and Quail (1993). In brief, ethyl methanesulfonate–mutagenized M_2 seeds (RLD; Lehle Seed Co., Tucson, AZ) were grown under continuous far-red light (FRc), and seedlings with very long hypocotyls and closed, unexpanded cotyledons (that is, no response to FRc) were selected and grown to produce M_3 seed. M_3 seedlings were grown under continuous red light (Rc), and individuals with short hypocotyls and fully expanded cotyledons (that is, normal response to Rc) were selected as putative *phyA*-specific mutants. These lines were tested for allelism to the *phyA* null mutant *phyA-101* (Dehesh et al., 1993; Parks and Quail, 1993; Quail et al., 1994) by genetic complementation, as described by Parks and Quail (1993). All lines selected in the *phyA* complementation group were recessive when backcrossed to the RLD wild type. These lines were tested for PHYA protein by immunoblot analysis (Somers et al., 1991; Parks and Quail, 1993), and those selected for further characterization were backcrossed to RLD for two to three generations to obtain the homozygous lines used here, except for *phyA-110*, which was backcrossed only once.

For hypocotyl measurements, seeds were surface sterilized in 20% bleach and 0.03% Triton X-100 and sown on plates containing germination media (Valvekens et al., 1988) without sucrose. Plates were kept at 4°C for 5 days in the dark (stratification), followed by treatment in white light for 3 hr at 21°C (to induce germination). Plates were then put in the dark at 21°C for 24 hr (for seed germination and initial growth until shoots were just beginning to emerge from seed coats) and then either kept in the dark or transferred to appropriate irradiation conditions at 21°C for 3 more days. Rc and FRc were produced using either special fluorescent tubes plus filters (fluence rate: Rc, 2 W m^{-2}; FRc, 2 W m^{-2}), as described previously (Parks and Quail, 1993), or light-emitting diode light sources (Quantum Devices, Barneveld, WI) (FRc, 20 W m^{-2}).

Protein Extraction, Immunoblot Analysis, and Spectrophotometry

Crude extracts were prepared from 7-day-old dark-grown seedlings as described by Somers et al. (1991). Extracted protein samples were subjected to either SDS-PAGE (4%/6% stacking/resolving gel; Laemmli, 1970) or nondenaturing gradient gel PAGE (4% stacking gel, pH 6.8; 4 to 20% gradient gel, pH 8.0). Nondenaturing gel electrophoresis was performed using 0.025 M Tris, 0.19 M glycine, pH 8.6, running buffer. Protein concentration was determined using the method described by Bradford (1976). All extracts were loaded on an equal crude protein basis. Immunoblotting and detection of PHYA protein by a type-selective monoclonal antibody prepared against PHYA were performed as described by Somers et al. (1991), except that 2% milk buffer was used during blocking and primary and secondary antibody incubations.

Quantitative spectrophotometric measurements of phytochrome content ($\Delta\Delta A$) were performed using a custom-built dual-wavelength spectrophotometer (Pratt et al., 1985), as described by Parks and Quail (1993). Crude extract (550 μL) was added to 450 mg of $CaCO_3$ to increase light scatter. The $\Delta\Delta A$ value was then normalized by the total amount of crude protein used in each measurement. Difference spectra were measured as previously described (Boylan et al., 1994).

DNA Sequencing of *phyA* Mutant Alleles

Genomic DNA was isolated from all nine putative *phyA* mutant lines, according to the miniprep procedure described by Dellaporta et al. (1983). A series of primers was synthesized based on the published RLD *PHYA* sequence (Dehesh et al., 1993, 1994), and polymerase chain reaction (PCR) was used to amplify segments of the *phyA* gene from the mutant genomic DNA (Dehesh et al., 1993). PCR products were purified using a PCR purification kit (Qiagen, Chatsworth, CA) before sequencing. Both strands of the mutant *phyA* genes were sequenced using primers internal to the PCR product, with either a *fmol* PCR sequencing kit (Promega) or an automated DNA sequencer (Applied Biosystems, Inc., Sunnyvale, CA).

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REFERENCES

Batschauer, A., Gilmartin, P.M., Nagy, F., and Schäfer, E. (1994). The molecular biology of photoregulated genes. In Photomorphogenesis in Plants, 2nd ed, R.E. Kendrick and G.H.M. Kronenberg, eds (Dordrecht, Netherlands: Kluwer), pp. 559–599.

Boylan, M., Douglas, N., and Quail, P.H. (1994). Dominant negative suppression of Arabidopsis photoresponses by mutant phytochrome A sequences identifies spatially discrete regulatory domains in the photoreceptor. Plant Cell **6,** 449–460.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72,** 248–254.

Cherry, J.R., and Vierstra, R.D. (1994). The use of transgenic plants to examine phytochrome structure/function. In Photomorphogenesis in Plants, 2nd ed, R.E. Kendrick and G.H.M. Kronenberg, eds (Dordrecht, Netherlands: Kluwer), pp. 271–300.

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.

Cherry, J.R., Hondred, D., Walker, J.M., Keller, J.M., Hershey, H.P., and Vierstra, R.D. (1993). Carboxy-terminal deletion analysis of oat phytochrome A reveals the presence of separate domains required for structure and biological activity. Plant Cell **5,** 565–575.

Chory, J. (1993). Out of darkness: Mutants reveal pathways controlling light-regulated development in plants. Trends Genet. **9,** 167–172.

Clack, T., Mathews, S., and Sharrock, R.A. (1994). The phytochrome apoprotein family in Arabidopsis is encoded by five genes: The sequences and expression of *PHYD* and *PHYE*. Plant Mol. Biol. **25,** 413–427.

Dehesh, K., Franci, C., Parks, B.M., Seeley, K.A., Short, T.W., Tepperman, J.M., and Quail, P.H. (1993). Arabidopsis *HY8* locus encodes phytochrome A. Plant Cell **5,** 1081–1088.

Dehesh, K., Franci, C., Sharrock, R.A., Somers, D.E., Welsch, J.A., and Quail, P.H. (1994). The *Arabidopsis* phytochrome A gene has multiple transcription start sites and a promoter sequence motif homologous to the repressor element of monocot phytochrome A genes. Photochem. Photobiol. **59,** 379–384.

Dellaporta, S., Wood, J., and Hicks, J.B. (1983). A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. **1,** 19–21.

Deng, X.W. (1994). Fresh view of light signal transduction in plants. Cell **76,** 423–426.

Elich, T.D., and Chory, J. (1994). Initial events in phytochrome signaling: Still in the dark. Plant. Mol. Biol. **26,** 1315–1327.

Furuya, M. (1993). Phytochromes: Their molecular species, gene families, and functions. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44,** 617–645.

Furuya, M., and Song, P.S. (1994). Assembly and properties of holophytochrome. In Photomorphogenesis in Plants, 2nd ed, R.E. Kendrick and G.H.M. Kronenberg, eds (Dordrecht, Netherlands: Kluwer), pp. 105–140.

Kendrick, R.E., and Kronenberg, G.H.M., eds (1994). Photomorphogenesis in Plants, 2nd ed. (Dordrecht, Netherlands: Kluwer).

Laemmli, U.K. (1970). Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature **227,** 680–685.

Mathews, S., Lavin, M., and Sharrock, R.A. (1995). Evolution of the phytochrome gene family and its utility for phylogenetic analyses of angiosperms. Anal. Missouri Bot. Garden **82,** 296–321.

McCormac, A.C., Whitelam, G.C., Boylan, M.T., Quail, P.H., and Smith, H. (1992). Contrasting responses of etiolated and light-adapted seedlings to red:far-red ratio: A comparison of wild type, mutant and transgenic plants has revealed differential functions of members of the phytochrome family. J. Plant Physiol. **140,** 707–714.

McCormac, A.C., Wagner, D., Boylan, M.T., Quail, P.H., Smith, H., and Whitelam, G.C. (1993). Photoresponses of transgenic Arabidopsis seedlings expressing introduced phytochrome B-encoding cDNAs: Evidence that phytochrome A and phytochrome B have distinct photoregulatory functions. Plant J. **4,** 19–27.

Millar, A.J., McGrath, R.B., and Chua, N.-H. (1994). Phytochrome phototransduction pathways. Annu. Rev. Genet. **28,** 325–349.

Nagatani, A., Reed, J.W., and Chory, J. (1993). Isolation and initial characterization of Arabidopsis mutants that are deficient in phytochrome A. Plant Physiol. **102,** 269–277.

Parks, B.M., and Quail, P.H. (1993). *hy8*, a new class of Arabidopsis long hypocotyl mutants deficient in functional phytochrome A. Plant Cell **5,** 39–48.

Pratt, L.H. (1995). Phytochromes: Differential properties, expression patterns and molecular evolution. Photochem. Photobiol. **61,** 10–21.

Pratt, L.H., Wampler, J.E., and Rich, E.S.J. (1985). An automated dual-wavelength spectrophotometer optimized for phytochrome assay. Anal. Instrum. **13,** 269–287.

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

Quail, P.H. (1994a). Phytochrome genes and their expression. In Photomorphogenesis in Plants, 2nd ed, R.E. Kendrick and G.H.M. Kronenberg, eds (Dordrecht, Netherlands: Kluwer), pp. 71–104.

Quail, P.H. (1994b). Photosensory perception and signal transduction in plants. Curr. Opin. Gen. Dev. **4,** 652–661.

Quail, P.H., Briggs, W.R., Chory, J., Hangarter, R.P., Harberd, N.P., Kendrick, R.E., Koornneef, M., Parks, B., Sharrock, R.A., Schäfer, E., Thompson, W.F., and Whitelam, G.C. (1994). Spotlight on phytochrome nomenclature. Plant Cell **6,** 468–471.

Quail, P.H., Boylan, M.T., Parks, B.M., Short, T.W., Xu, Y., and Wagner, D. (1995). Phytochromes: Photosensory perception and signal transduction. Science **268,** 675–680.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., and Chory, J. (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell **5,** 147–157.

Reed, J.W., Nagatani, A., Elich, T.D., Fagan, M., and Chory, J. (1994). Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol. **104,** 1139–1149.

Rost, B., and Sander, C. (1993a). Improved prediction of protein secondary structure by use of sequence profiles and neural networks. Proc. Natl. Acad. Sci. USA **90,** 7558–7562.

Rost, B., and Sander, C. (1993b). Prediction of protein structure at better than 70% accuracy. J. Mol. Biol. **232,** 584–599.

Schneider-Poetsch, H.A.W., and Braun, B. (1991). Proposal on the nature of phytochrome action based on the C-terminal sequences of phytochrome. J. Plant Physiol. **137,** 576–580.

Schneider-Poetsch, H.A.W., Braun, B., Marx, S., and Schaumburg, A. (1991). Phytochromes and bacterial sensor proteins are related by structural and functional homologies. FEBS Lett. **281,** 245–249.

Schneider-Poetsch, H.A.W., Marx, S., Kolukisaoglu, H.U., Hanelt, S., and Braun, B. (1994). Phytochrome evolution: Phytochrome genes in ferns and mosses. Physiol. Plant. **91,** 241–250.

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.

Smith, H. (1992). The ecological functions of the phytochrome family. Clues to a transgenic programme of crop improvement. Photochem. Photobiol. **56,** 815–822.

Smith, H. (1994). Sensing the light environment: The functions of the phytochrome family. In Photomorphogenesis in Plants, 2nd ed, R.E. Kendrick and G.H.M. Kronenberg, eds (Dordrecht, Netherlands: Kluwer), pp. 377–416.

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

Stockhaus, J., Nagatani, A., Halfter, U., Kay, S., Furuya, M., and Chua, N.-H. (1992). Serine-to-alanine substitutions at the amino-terminal region of phytochrome A result in an increase in biological activity. Genes Dev. **6,** 2364–2372.

Tobin, E., and Kehoe, D.M. (1994). Phytochrome regulated gene expression. Semin. Cell Biol. **5,** 335–346.

Valvekens, D., Van Montagu, M., and van Lijsebettens, M. (1988). *Agrobacterium tumefaciens*–mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. Proc. Natl. Acad. Sci. USA **85,** 5536–5540.

Wagner, D., and Quail, P.H. (1995). Mutational analysis of phytochrome B identifies a small COOH-terminal-domain region critical for regulatory activity. Proc. Natl. Acad. Sci. USA, in press.

Whitelam, G.C., and Harberd, N.P. (1994). Action and function of phytochrome family members revealed through the study of mutant and transgenic plants. Plant Cell Environ. **17,** 615–625.

Whitelam, G.C., Johnson, E., Peng, J., Carol, P., Anderson, M.L., Cowl, J.S., and Harberd, N.P. (1993). Phytochrome A null mutants of Arabidopsis display a wild-type phenotype in white light. Plant Cell **5,** 757–768.