The Plant Cell, Vol. 5, 1081-1088, September 1993

Arabidopsis *HY8* Locus Encodes Phytochrome A

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hy8 **long hypocotyl mutants of Arabidopsis defective in responsiveness to prolonged far-red light (the so-called "far-red high-irradiance response") are selectively deficient in functional phytochrome A. To define the molecular lesion in these mutants, we sequenced the phytochrome A gene (*phyA*) in lines carrying one or other of two classes of *hy8* alleles. The *hy8-1* and *hy8-2* mutants that express no detectable phytochrome A each have a single nucleotide change that inserts a translational stop codon in the protein coding sequence. These results establish that *phyA* resides at the *HY8* locus. The *hy8-3* mutant that expresses wild-type levels of photochemically active phytochrome A has a glycine-to-glutamate missense mutation at residue 727 in the C-terminal domain of the *phyA* sequence. Quantitative fluence rate response analysis showed that the mutant phytochrome A molecule produced by *hy8-3* exhibited no detectable regulatory activity above that of the *phyA*-protein-deficient *hy8-2* mutant. This result indicates that glycine-727, which is invariant in all sequenced phytochromes, has a function important to the regulatory activity of phytochrome A but not to photoperception.**

INTRODUCTION

The phytochrome family of photoreceptors regulates a diverse array of plant developmental responses to light signals from the environment at all phases of the life cycle (Kendrick and Kronenberg, 1986). This photoreceptor family consists of five members, phytochromes A, B, C, D, and E, whose apoproteins are encoded by a corresponding number of divergent genes, designated *phyA*, *phyB*, *phyC*, *phyD*, and *phyE*, respectively, in Arabidopsis (Sharrock and Quail, 1989; R. A. Sharrock, personal communication) and rice (Dehesh et al., 1991; K. Dehesh, unpublished data), and possibly all angiosperms (Quail, 1991). Because of the complexity of the impinging light signals and the diversity of the plant responses to them, the proposition has arisen that individual members of the family might perform discrete photosensory functions in mediating responsiveness to the spectral environment (Smith and Whitelam, 1990; Smith, 1992). Studies with photomorphogenic mutants and transgenic plants overexpressing different phytochromes have begun to provide direct evidence that this proposition is correct (Somers et al., 1991; Whitelam and Smith, 1991; McCormac et al., 1992, 1993; Whitelam et al., 1992; Parks and Quail, 1993).

The *hy3* long hypocotyl mutants of Arabidopsis have been shown to be selectively deficient in phytochrome B (Somers et al., 1991), and recent data have established that this deficiency is caused by mutations directly in the *phyB* gene (Reed et al., 1993). These mutants are selectively defective in responsiveness to continuous red light (Rc) (Koornneef et al., 1980; McCormac et al., 1993) with the result that light-grown seedlings have long hypocotyls and lack "shade-avoidance" and "end-of-day far-red" responses (Nagatani et al., 1991; Whitelam and Smith, 1991; McCormac et al., 1992). These data provide evidence that phytochrome B is necessary for Rc perception in these responses and, conversely, that phytochromes A, C, D, and E cannot act alone or in concert to mediate Rc signals for these responses. In contrast, continuous far-red light (FRc) inhibits hypocotyl elongation in etiolated *hy3* seedlings and in transgenic phytochrome B overexpressers in a manner quantitatively indistinguishable from the wild type (Koornneef et al., 1980; Wagner et al., 1991; McCormac et al., 1993). These results indicate that phytochrome B is not involved in the "far-red high-irradiance response" (FR-HIR) of etiolated seedlings (Holmes and Schäfer, 1981; Smith and Whitelam, 1990). Consistent with the above observations, the elongated internode (*ein*) mutant of Brassica (Devlin et al., 1992) and the long hypocotyl (*lh*) mutant of cucumber (López-Juez et al., 1992), both of which have photoresponses similar to *hy3*, have also been shown to be deficient in phytochrome B. Together, the evidence indicates that phytochrome B is primarily responsible for Rc perception in light-grown seedlings.

Recently, we isolated a new class of Arabidopsis long hypocotyl mutants, designated *hy8*, selectively deficient in functional phytochrome A and showed that these mutants are in consequence defective in responsiveness to FRc (Parks and Quail, 1993). These data establish that phytochrome A is necessary for the FR-HIR of etiolated seedlings and that

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phytochromes B, C, D, and E are unable, alone or together, to substitute for phytochrome A in this photosensory capacity. Conversely, the *hy8* mutants appear to have wild-type responsiveness to Rc and to continuous white light throughout development, implying that phytochrome A does not have a major role in regulating photomorphogenesis under these conditions (Parks and Quail, 1993). Moreover, because transgenic Arabidopsis seedlings overexpressing phytochrome A exhibit enhanced sensitivity to FRc, in contrast to the phytochrome B overexpressers mentioned above, the capacity to mediate the FR-HIR appears to be an intrinsic property of the phytochrome A molecule not possessed by phytochrome B (Whitelam et al., 1992; McCormac et al., 1993). Taken together, the evidence indicates that phytochromes A and B have reciprocal responsivities to FRc and Rc, respectively, and, therefore, appear to perform complementary photosensory functions in mediating seedling responsiveness to the red and far-red regions of the spectrum.

Immunochemical and spectrophotometric analyses of several independent *hy8* mutant lines showed that two contrasting classes of *hy8* alleles have been identified: *hy8-1* and *hy8-2* that contain no detectable phytochrome A and *hy8-3* that contains wild-type levels of spectrally normal phytochrome A (Parks and Quail, 1993). These data are consistent with, but do not provide definitive evidence for, the possibility that the *HY8* locus corresponds to the *phyA* gene. We addressed this question in the present paper by direct sequencing of the *phyA* genes in the two classes of *hy8* mutants. In addition, we performed quantitative photoresponse analysis of the *hy8* mutants.

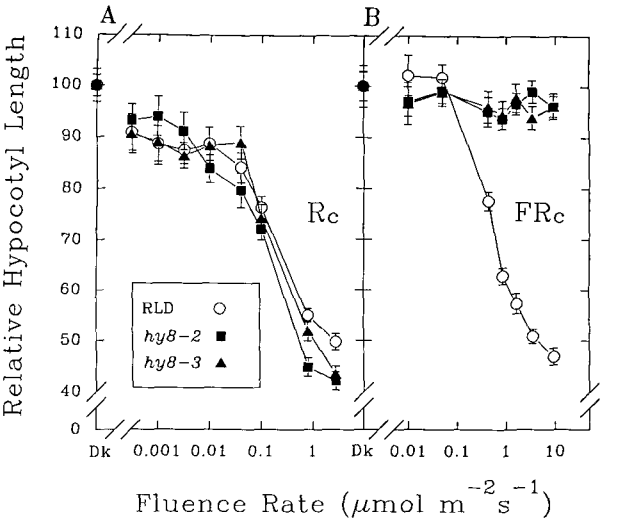

Figure 1. Photon Fluence Rate Response Curves for Inhibition of Hypocotyl Elongation in Etiolated Arabidopsis Seedlings.

(A) Hypocotyl lengths of seedlings grown either in constant darkness (Dk) or continuous red light (Rc) at the fluence rates indicated.
(B) Hypocotyl lengths of seedlings grown either in constant darkness (Dk) or continuous far-red light (FRc) at the fluence rates indicated. Wild-type (RLD), *hy8*-2, and *hy8-3* seedlings were grown for 5 days according to Parks and Quail (1993) under the conditions indicated, prior to measurement of hypocotyl lengths. Curves have been normalized to the respective dark-control seedlings for each Arabidopsis line. Error bars represent the standard error of the mean.

RESULTS

Quantitative Analysis of Photoresponsiveness in Etiolated Seedlings

Previous analysis of the effects of FRc and Rc light on hypocotyl elongation in the *hy8* mutants was performed at a single photon fluence rate for each wavelength (Parks and Quail, 1993). To determine whether these mutants might exhibit quantitative differences from the wild type in responsiveness to Rc and to examine for potential quantitative differences between *hy8* alleles in responsiveness to FRc, we performed fluence rate response experiments.

Figure 1A shows that the responsiveness of the *hy8* mutants to increasing fluence rates of Rc was essentially identical to the wild type. This result indicates that phytochrome A does not play a significant role in mediating Rc detection up to the highest fluence rate tested. Conversely, Figure 1B shows that these mutants exhibited no detectable responsiveness to FRc at any fluence rate tested. This result establishes that the phytochrome A molecule synthesized in *hy8-3* seedlings retains no detectable residual regulatory activity even when subjected to photocycling at the highest FRc excitation fluence rates used.

phyA mRNA Levels in *hy8* Mutants

To assess the possibility that the absence of detectable phytochrome A protein in the *hy8-1* and *hy8-2* mutants (Parks and Quail, 1993) might be due to reduced *phyA* gene expression, we performed RNA gel blot analyses on these mutants. Figure 2 shows that *phyA* mRNA levels were significantly lower in *hy8-1* and marginally lower in *hy8-2* compared to the wild type and *hy8-3*, whereas *phyB* mRNA levels were equivalent in all four lines. However, because the phytochrome A protein is undetectable in the *hy8-1* and *hy8-2* mutants (Parks and Quail, 1993), the observed reduction in mRNA levels is insufficient to account for the apparent absence of the photoreceptor molecule. Therefore, these data argue against the possibility that differences in transcription or intrinsic transcript stability between *hy8-1* and *hy8-2* on the one hand, and the wild type and *hy8-3* on the other, account for the differences in phytochrome A levels. Similar observations have been made for the *hy3* mutants in which severe reductions in phytochrome B protein are accompanied by only a two- to threefold decrease in *phyB* mRNA levels (Somers et al., 1991). The suggestion that these latter results might reflect reduced *phyB* mRNA stability in the mutant resulting from premature termination of translation (Somers et al., 1991), as is observed in other systems (Daar and Maquat, 1988; Vancanneyt et al., 1990), is supported by

the recent demonstration that at least one of the *hy3* alleles examined (Bo64) contains a stop codon in the *phyB* gene coding sequence (Reed et al., 1993). Although the data in Figure 2 are also consistent with this interpretation for *phyA*, other possibilities are not ruled out by these results.

HY8 Locus Encodes Phytochrome A

To determine whether the *hy8* mutants contain aberrations in the *phyA* gene, we compared the *phyA* sequences of *hy8-1*, *hy8-2*, and *hy8-3* with that of the wild type. Because the previously determined Arabidopsis *phyA* cDNA sequence is from the Columbia ecotype (Sharrock and Quail, 1989) and our *hy8* mutants are in the RLD ecotype background (Parks and Quail, 1993), we first defined the sequence of the *phyA* gene in the RLD wild type. Figure 3 displays the RLD *phyA* sequence from just upstream of the ATG initiator codon to the presumptive 3′ end of the transcript. The sequence of this gene is almost identical to the corresponding regions of the Columbia cDNA, thus allowing identification of the intron sequences. Differences from the published Columbia sequence were detected at nucleotides 340 (T to C), 421 (G to C), 2710 (T to G), and 2789 (A to G). The changes at nucleotides 340 and 421 are translationally silent with the result that the phytochrome A polypeptide sequence remains identical in RLD and Columbia ecotypes in this region. We determined that the change at nucleotide 2710 is the result of a sequencing error in the published Columbia sequence (Sharrock and Quail, 1989). Correction of this error makes RLD and Columbia sequences identical and results in a conservative amino acid change in the published sequence from D to E at residue 835. The change at nucleotide 2789 causes a K-to-E change at residue 862. Whether this difference between the cDNA and genomic sequences reflects a genuine ecotype difference or a reverse transcriptase error has not been determined.

The overall structural organization of the Arabidopsis *phyA* gene determined here is the same as that of all available angiosperm *phy* genes, including *phyA* and *phyB*, in regard to exon/intron arrangements in the protein coding region (Quail, 1993). In addition, an intron is present in the 5′ untranslated region of the gene (data not shown) consistent with the structure of other *phyA* genes.

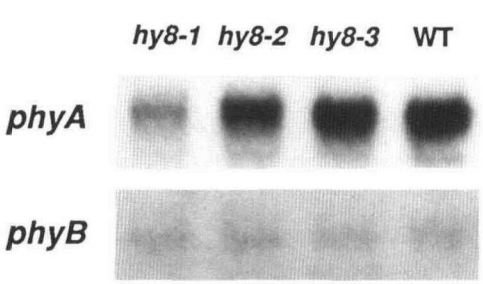

Figure 2. *phyA* and *phyB* mRNA Levels in Wild Type and Three *hy8* Long Hypocotyl Mutants of Arabidopsis.

Total RNA was extracted from wild type (WT) and *hy8-1*, *hy8-2*, and *hy8-3* 4-day-old dark-grown seedling samples, and 5 μg per lane was subjected to gel blot analysis according to Seeley et al. (1992) using gene-specific antisense RNA probes corresponding to the 3′ region of each transcript described in Sharrock and Quail (1989).

Direct comparison of the nucleotide sequences of the RLD wild-type, *hy8-1*, *hy8-2*, and *hy8-3 phyA* genes revealed single nucleotide changes in each of the three mutant lines. Figure 3 shows that the *hy8-1* mutant was found to contain a nonsense mutation at nucleotide 80 in the *phyA* sequence that converts Q-25 to a translational stop codon. This result is consistent with the absence of any detectable full-length phytochrome A polypeptide in this class of mutant (Parks and Quail, 1993).

The *hy8-2 phyA* gene was determined to contain a G-to-A mutation at nucleotide 3524 in the 3′ splice-junction dinucleotide of the fourth intron. This mutation is predicted to produce a stop codon in the mature transcript at either residue 1057 or 1066 (Figure 3), depending on the form of aberrant splicing caused by the loss of the natural 3′ splice junction (Green, 1986). Preliminary polymerase chain reaction (PCR) analysis suggests that the intron is not spliced from the transcript (data not shown), resulting in a TAA stop codon immediately at the first in-frame triplet inside the 5′ border of the intron, at a position equivalent to residue 1057 (Figure 3). The increase in transcript size caused by failure to excise this 82-bp intron is below the limits of resolution of the RNA gel blot analysis in Figure 2. This result is consistent with the lack of any measurable full-length phytochrome A protein in this class of mutant (Parks and Quail, 1993). Although a very faint band approximately the size of the predicted premature termination product was detected on some highly overdeveloped immunoblots (data not shown), we have no independent evidence that this represents the authentic phytochrome A polypeptide because no photochemical activity above background was measurable (Parks and Quail, 1993). We estimate that if this band represents the truncated product, it is present at less than 1% of wild-type phytochrome A levels.

By contrast, the *hy8-3* mutant was found to contain a missense mutation at nucleotide 2385 in the *phyA* sequence, with the result that G-727 is converted to an E residue (Figure 3). This result is also consistent with the observation that normal levels of full-length phytochrome A are detected in this mutant class (Parks and Quail, 1993). Together, the above data provide strong evidence that the *HY8* locus corresponds to the *phyA* structural gene.

Figure 4 shows that G-727, which is located in the C-terminal domain of the protein, is strictly conserved in all available phytochrome sequences, including members of all three subfamilies, A, B, and C, and two lower plant (Selaginella and Ceratodon) phytochromes. This amino acid is embedded in a strongly hydrophobic segment of the polypeptide, as indicated by hydropathy analysis (Figure 4C). As expected, substitution of the charged E residue for the neutral G-727 causes a decrease in the predicted hydrophobicity of the surrounding segment. However, the extent of this decrease appears to be relatively small and may not result in a major

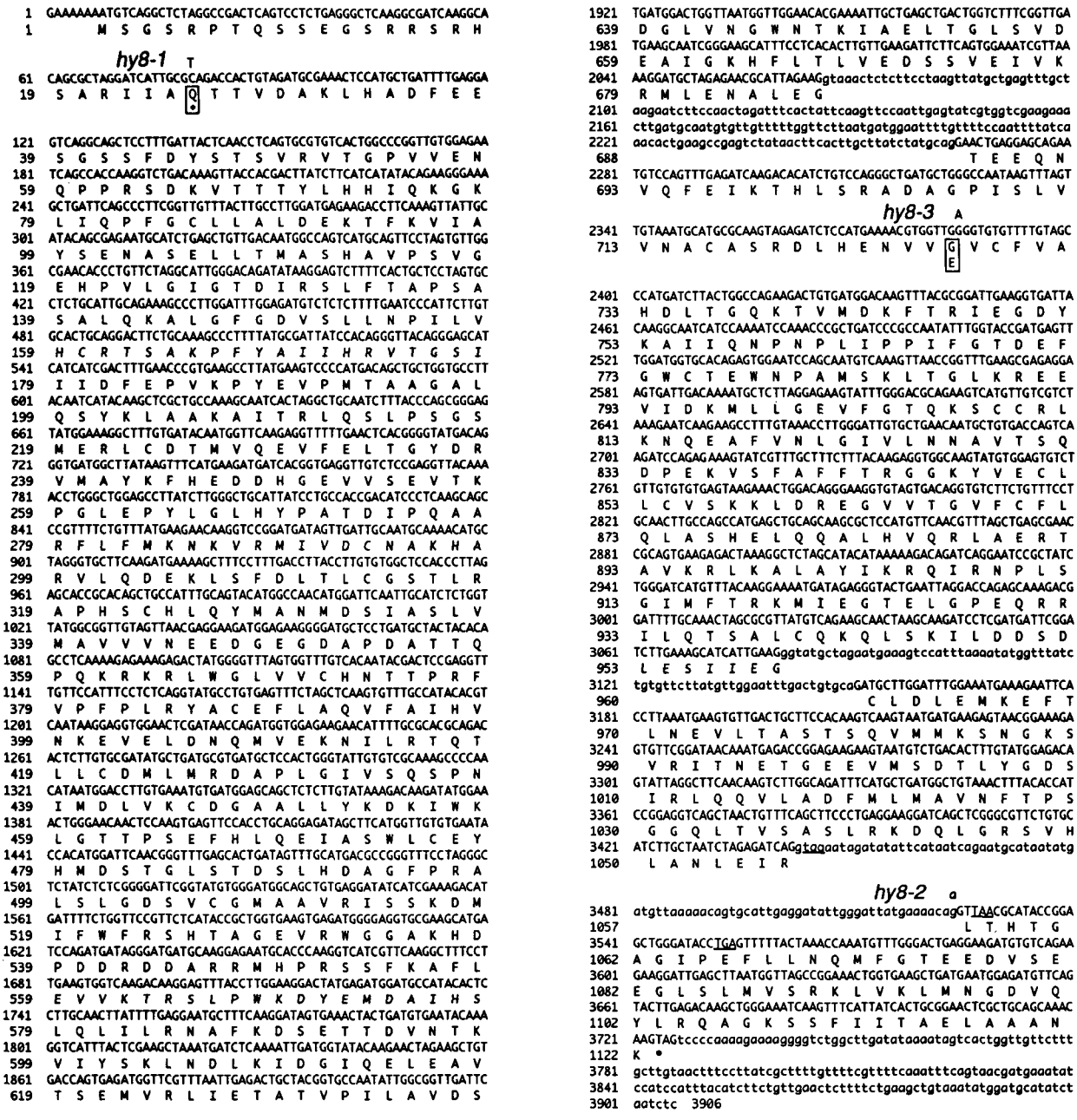

Figure 3. Nucleotide and Derived Amino Acid Sequences of RLD Wild-Type and *hy8* Mutant Arabidopsis *phyA* Genes.

Exons are indicated in the nucleotide sequence as uppercase letters, and introns and untranslated flanking DNA are in lowercase letters. The amino acid sequence is presented in single-letter code below the nucleotide sequence starting at the first in-frame methionine and ending at the first stop codon in the wild-type sequence, indicated as an asterisk at residue position 1123. Differences in the wild-type sequence from the published Columbia ecotype *phyA* cDNA sequence (Sharrock and Quail, 1989) are as follows: T to C at nucleotide 340, translationally silent; G to C at nucleotide 421, translationally silent; T to G at nucleotide 2710, causing a D-to-E change at residue 835 in the amino acid sequence; and A to G at nucleotide 2789, causing a K-to-E change at residue 862 in the amino acid sequence. Mutations present in the *hy8* alleles are indicated above the nucleotide sequence and are boxed below the amino acid sequence of the wild type: *hy8-1*, C to T at nucleotide 80, causing a Q-to-* (stop) change at residue 25; *hy8-3*, G to A at nucleotide 2385 causing a G-to-E change at residue 727; *hy8-2*, G to A at nucleotide 3524, creating a defective 3′ splice junction in intron 4. Three new, alternative in-frame stop codons that would result from three alternative possible defective splicing products (Green, 1986) in the *hy8-2* mutant are underlined. From 5′ to 3′, first TAA would result from failure to splice out the intron and is at the equivalent of position 1057 in the wild-type polypeptide; second TAA would result from splicing at the new AG dinucleotide created 1 bp down at the original 3′ splice junction and is also at the equivalent of position 1057 in the wild-type polypeptide; TGA would result from splicing at the next AG further downstream and is at the equivalent of residue 1066 in the wild-type protein. The wild-type RLD sequence has been submitted to GenBank as accession number L21154.

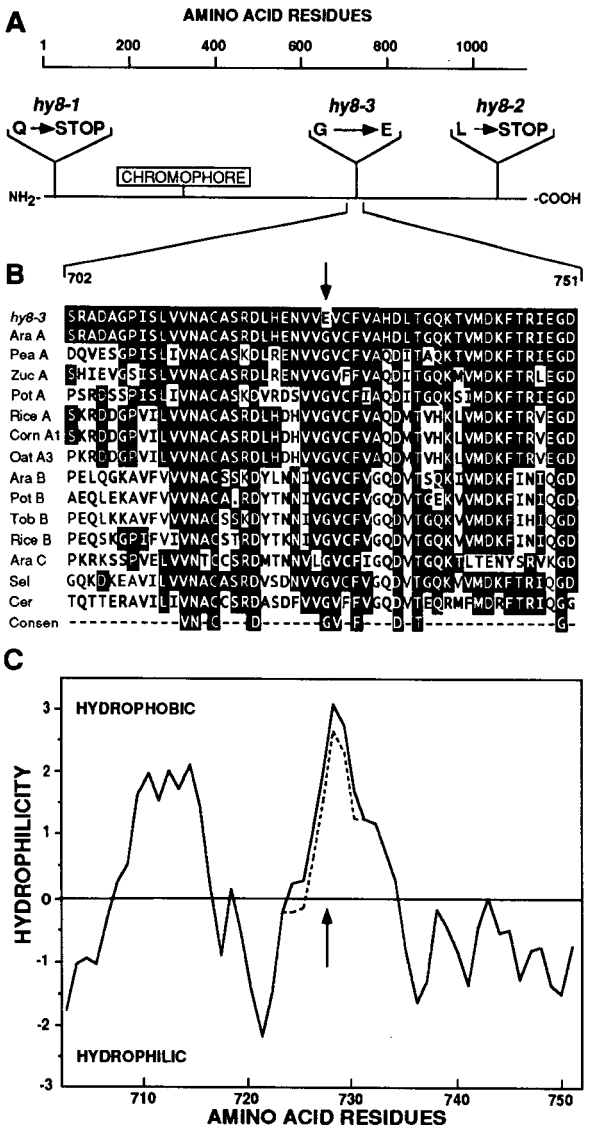

Figure 4. Mutations in Arabidopsis Phytochrome A.

(A) Physical map of the phytochrome A polypeptide indicating the locations of the chromophore (covalently attached at C-323), the Q-25–to–stop nonsense mutation in *hy8-1*, the G-727–to–E missense mutation in *hy8-3*, and the stop mutation at amino acid residue position 1057 in *hy8-2* that would result either from failure to splice intron 4 out of the mature transcript or from splicing at the new AG dinucleotide created immediately at the 3′ end of the intron by the G-to-A mutation (Figure 3). The alternative not shown here would be a stop codon at residue position 1066 that would result from splicing at the next available AG dinucleotide further downstream of the original 3′ site (Green, 1986).

(B) Alignment of all available phytochrome amino acid sequences in the region between residues 702 and 751 surrounding the G-727–to–E mutation in *hy8-3*. Phytochrome A sequences: *hy8-3*, mutant phytochrome A polypeptide from Arabidopsis; Ara A, wild-type Arabidopsis sequence (Sharrock and Quail, 1989) (Figure 3); Pea A, pea (Sato, 1988); Zuc A, zucchini (Sharrock et al., 1986); Pot A, potato (Heyer and Gatz, 1992a); Rice A, rice (Kay et al., 1989); Corn A1, maize (Christensen and Quail, 1989); Oat A3, oat (Hershey et al., 1985). Phytochrome B sequences: Ara B, Arabidopsis (Sharrock and Quail, 1989); Pot B, potato (Heyer and Gatz, 1992b); Tob B, tobacco (López-Juez et al., 1992); Rice B, rice (Dehesh et al., 1991). Phytochrome C sequence: Ara C, Arabidopsis (Sharrock and Quail, 1989). Selaginella phytochrome sequence: Sel (Hanelt et al., 1992). Ceratodon phytochrome sequence: Cerat (Thümmler et al., 1992). Consen, consensus sequence showing residues completely conserved in all phytochromes by letter and nonconserved residues as dashes. Residues identical to those in the same position in Arabidopsis wild-type phytochrome A are shown as reverse contrast characters, and the mutated E residue in the *hy8-3* sequence is indicated by the arrow.

(C) Hydropathy plot of Arabidopsis phytochrome A sequence between residues 702 and 751 according to Kyte and Doolittle (1982). Solid curve, wild-type phytochrome A; dotted line, *hy8-3*–encoded protein; arrow, location of G-727–to–E mutation in *hy8-3*.

(D) Secondary structure predictions for Arabidopsis phytochrome A sequence between residues 702 and 751 according to Garnier et al. (1978) and Chou and Fasman (1978). H, α helix; B, β sheet; T, β turn; dots, random coil; uppercase letters, strongly predicted structure; lowercase letters, weakly predicted structure.

shift in location of the polypeptide segment toward the protein surface. Secondary structure predictions obtained with the Garnier algorithm (Garnier et al., 1978) indicated that the G-727–to–E substitution would convert a β turn and a short segment of β sheet surrounding this residue position to an α helix (Figure 4D). By contrast, the local secondary structure predicted by the Chou–Fasman algorithm (Chou and Fasman, 1978) is intrinsically different from that of the Garnier prediction and is completely unchanged by the G-727–to–E substitution (Figure 4D). Thus, evidence of a substantial change in local structure induced by the *hy8-3* mutation is inconclusive.

The polypeptide segment surrounding the conserved G-727 residue is reasonably well conserved among phytochrome A species but only moderately so across all phytochromes (Figure 4B). On the premise that this region of the polypeptide might be involved in a biological activity that had been defined in a previously identified protein, we searched the GenBank and EMBL databases for sequences similar to the 50–amino acid stretch of Arabidopsis phytochrome A shown in Figure 4B. No nonphytochrome proteins with significant sequence similarity to this polypeptide segment were detected.

DISCUSSION

The data presented here establish that the *HY8* locus of Arabidopsis corresponds to the *phyA* gene. The nonsense mutation causing conversion of Q-25 to a stop codon in the *hy8-1 phyA* gene (Figures 3 and 4) provides a molecular explanation for the observed lack of a detectable *phyA* polypeptide by immunoblot analysis of this mutant (Parks and Quail, 1993). The lower level of *phyA* mRNA in the *hy8-1* mutant than in the wild type (Figure 2) might also be accounted for by this mutation. Transcripts containing stop codons that cause premature translational termination have been shown to have reduced intracellular stability, possibly due to reduced protection from degradation by traversing ribosomes (Daar and Maquat, 1988; Vancanneyt et al., 1990). The extremely short length of the encoded N-terminal peptide fragment in *hy8-1 phyA* and the absence of the chromophore attachment site (Figure 4) render this fragment, if synthesized, photochemically inactive. The *hy8-1* mutant would appear, therefore, to be truly null for phytochrome A. The stop codon present toward the C terminus of the *phyA* polypeptide in the *hy8-2* mutant (Figures 3 and 4) has the potential to produce a photochemically active, truncated phytochrome A molecule. However, the absence or very low levels of an immunodetectable band of the predicted size in *hy8-2* seedlings suggest that the truncated polypeptide may be unstable. Together, these findings verify the predicted chromosomal colocation of *phyA* and *HY8* based on the identification of both protein-positive and protein-negative *hy8* alleles (Parks and Quail, 1993) and provide conclusive evidence that the missense mutation in the *hy8-3 phyA* allele is responsible for the loss of regulatory activity by the encoded phytochrome A molecule.

The molecular basis for the loss of phytochrome A function resulting from the G-727–to–E change in *hy8-3* is yet to be determined. As reported previously, this mutation has no detectable effect on photoreversibility and, therefore, does not affect the photoperception function of the molecule (Parks and Quail, 1993). This result is not unexpected in retrospect given that the mutation has been determined to lie in the C-terminal domain of the photoreceptor (Figures 3 and 4). It has been shown that the entire C-terminal domain can be removed proteolytically without affecting the spectral properties of the molecule (Jones et al., 1985), thus indicating that this domain is not directly involved in interactions with the chromophore that is covalently bound in the N-terminal domain.

The *hy8-3* phytochrome A molecule also appears unaltered in regard to the rapid intracellular turnover of the Pfr form observed for this photoreceptor species (Parks and Quail, 1993). This result indicates that the mutation does not affect the molecular determinants selectively recognized by the cellular degradative machinery and that the *hy8-3* phenotype, therefore, is not an indirect consequence of aberrant Pfr turnover.

There is substantial evidence that the phytochrome A molecule dimerizes through interactions in the C-terminal domain (Jones and Quail, 1986; Jones and Erickson, 1989; Quail, 1991). Therefore, the location of the *hy8-3* mutation in the C-terminal domain raises the possibility that the amino acid change disrupts normal dimerization. However, residue 727 lies outside two regions (residues 623 to 673 and 1049 to 1129) that have recently been reported to be involved in dimerization, and it lies within a region (residues 685 to 815) that showed no dimerization activity (Edgerton and Jones, 1992). Quaternary structure analysis of phytochrome A extracted from *hy8-3* is needed to address this question directly.

The level of sequence conservation surrounding the *hy8-3* mutation is consistent with this polypeptide segment having an important function in all phytochrome As and possibly all phytochromes sequenced to date (Figure 4B). It is possible that this segment and even the conserved G-727 residue itself are directly involved in the regulatory action of the photoreceptor. At the other extreme, however, it is equally possible at this point that the effects of the G-to-E substitution are entirely indirect as a result of induced structural or conformational changes either locally or at remote locations in the molecule. Nevertheless, the relatively small change in hydrophilicity, the ambiguous nature of potential changes in local secondary structure (Figure 4), and the absence of detectable perturbations in protein–chromophore interactions in the N-terminal domain (Parks and Quail, 1993) caused by the G-727–to–E substitution might argue against major structural disruptions and for the likelihood that the C-terminal domain has a direct role in the regulatory action of phytochrome A.

Data similar to those described above for *hy8* were obtained recently for the *hy3* mutants of Arabidopsis. Direct evidence that the *hy3* phenotype is due to a selective deficiency in phytochrome B was provided initially by immunoblot analysis. With monoclonal antibodies selective for phytochrome A, phytochrome B, or phytochrome C, it was shown that whereas phytochromes A and C are at wild-type levels in *hy3*, phytochrome B is severely deficient (Somers et al., 1991). Direct sequencing of the *phyB* gene from several independent *hy3* lines revealed that each carries a mutation in the *phyB* gene (Reed et al., 1993), thus providing a molecular explanation for the observed phytochrome B deficiency. The majority of the point mutations reported result in generation of a stop codon in the predicted protein coding sequence. However, a single allele, *hy3-4-117*, contains a H-to-Y missense mutation at residue 283. This mutant produces wild-type levels of the phytochrome B polypeptide (Reed et al., 1993). However, because no data have been reported on the photochemical activity of this molecule, it is unknown whether the mutation causes a loss of the photoperception or regulatory functions of the photoreceptor.

Quantitative comparison of the responsiveness of the *hy8* (Figure 1) and *hy3* (McCormac et al., 1993) mutants to increasing fluence rates of Rc and FRc light illustrates the striking photosensory specificity of phytochromes A and B. Although both phytochromes control the deetiolation process in young seedlings, each monitors a different parameter of the spectral environment. This finding verifies earlier predictions made on the basis of photophysiological studies (McCormac et al., 1992).

It is now of prime interest to determine at what level these two photosensory pathways converge to control common photomorphogenic events in the plant.

METHODS

Plant Material and Growth Analysis

Arabidopsis thaliana ecotype RLD (wild type and *hy8* mutants) (Parks and Quail, 1993) was used throughout. Seedlings for photon fluence rate response analysis were grown for 5 days according to the method of Parks and Quail (1993) under the conditions indicated prior to measurement of hypocotyl lengths. Data points represent the means of 50 to 100 seedlings at each fluence rate. Seedlings for RNA gel blot analysis and genomic DNA isolation were grown for 4 days in the dark as described by Parks and Quail (1993) and harvested into liquid nitrogen.

RNA Isolation and Gel Blot Analysis

Total RNA was extracted from frozen dark-grown seedling samples, and 5 μg per lane was subjected to electrophoresis and gel blot analysis according to the method of Seeley et al. (1992). Gene-specific antisense RNA probes corresponding to the 3′ region of each transcript described by Sharrock and Quail (1989) were used for mRNA detection.

Symmetrical and Asymmetrical Polymerase Chain Reaction Amplification of *phyA* from Genomic DNA

The phytochrome A gene (*phyA*) sequence was determined for wild-type RLD, *hy8-1*, *hy8-2*, and *hy8-3* long hypocotyl mutants using polymerase chain reaction (PCR)–amplified genomic DNA. DNA was isolated with the miniprep procedure of Dellaporta et al. (1983) and used as a template for symmetrical PCR amplification. Based on published Arabidopsis *phyA* cDNA sequence (Sharrock and Quail, 1989), a series of primers were designed to amplify all exons and introns from upstream of the translational start site to the 3′ end of the published sequence. The products of these reactions were purified by agarose gel electrophoresis, the desired bands were excised, and the DNA fragments were eluted. Two microliters (~10 ng) of this eluted material was then used in an asymmetric PCR reaction as described by Beitel et al. (1990). For sequencing, the products of two 100 μL of asymmetric PCR reactions were pooled and purified using a PCR purification kit (Qiagen; Studio City, CA). DNA sequencing was performed using primers internal to the PCR products and a Sequenase kit (U.S. Biochemicals).

ACKNOWLEDGMENTS

We thank members of our laboratory for helpful discussions and Ron Wells for preparing and editing the manuscript. This research was supported by National Institute of General Medical Sciences, National Institutes of Health Grant No. GM47475 and U.S. Department of Agriculture, Current Research Information System Grant No. 5335-21000-006-00D.

Received May 21, 1993; accepted July 6, 1993.

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