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RESEARCH ARTICLE

SPA1: A New Genetic Locus Involved in Phytochrome A–Specific Signal Transduction

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To identify mutants potentially defective in signaling intermediates specific to phytochrome A (phyA), we screened for extragenic mutations that suppress the morphological phenotype exhibited by a weak *phyA* mutant (*phyA-105*) of Arabidopsis. A new recessive mutant, designated *spa1* (for suppressor of *phyA-105*), was isolated and mapped to the bottom of chromosome 2. *spa1 phyA-105* double mutants exhibit restoration of several responses to limiting fluence rates of continuous far-red light that are absent in the parental *phyA-105* mutant, such as deetiolation, anthocyanin accumulation, and a far-red light–induced inability of seedlings to green upon subsequent transfer to continuous white light. *spa1* mutations do not cause a phenotype in darkness, indicating that the suppression phenotype is light dependent. Enhanced photoresponsiveness was observed in *spa1* seedlings in a wild-type *PHYA* background as well as in the mutant *phyA-105* background but not in a mutant *phyA* null background. These results indicate that phyA is necessary in a non-allele-specific fashion for the expression of the *spa1* mutant phenotype and that phyB to phyE are not sufficient for this effect. Taken together, the data suggest that *spa1* mutations specifically amplify phyA signaling and therefore that the *SPA1* locus encodes a component that acts negatively early in the phyA-specific signaling pathway.

INTRODUCTION

Through the process of photosynthesis, sunlight is the primary source of energy for most plants. Therefore, it is essential that plants adapt their growth and development to ambient light conditions. Several informational photoreceptors have evolved that allow constant monitoring of light intensity, light quality, the direction of the incoming light, and light periodicity (day length). There are three major classes of such photoreceptors: the red light (R)– and far-red light (FR)–sensing phytochromes, the blue/UV-A light–responsive cryptochromes, and the UV-B light–sensing UV-B receptors. Of these, the phytochromes are the most extensively characterized and are known to regulate many aspects of plant development, including induction of seed germination, seedling deetiolation (opening of apical hook and cotyledons, chloroplast biogenesis, and inhibition of hypocotyl elongation), stem elongation, and floral induction (Kendrick and Kronenberg, 1994).

The Arabidopsis phytochromes are encoded by a small family of five genes (*PHYA* to *PHYE*) (Sharrock and Quail, 1989; Clack et al., 1994). Physiological and mutational analyses have shown that two of these, phyA and phyB, have distinct yet overlapping functions in the plant (reviewed in Elich and Chory, 1994; Reed and Chory, 1994; Whitelam and Harberd, 1994; McNellis and Deng, 1995; Quail et al., 1995; Chory, 1997; Whitelam and Devlin, 1997).

phyA is highly abundant in dark-grown seedlings, accumulating to a level ~50-fold higher than that of phyB (Quail, 1991). Whereas the Pr form of phyA (PrA) is stable in the cell, the Pfr form (PfrA) is subject to rapid degradation, resulting in very low phyA levels under R (Quail, 1991; Clough and Vierstra, 1997). Analysis of phyA-deficient mutants has revealed that phyA is the primary, if not the only, phytochrome responsible for deetiolation in continuous FR (FRc) through the so-called FR high-irradiance response (Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). phyA is also required for anthocyanin accumulation in FRc (Kunkel et al., 1996). Although *phyA* mutants do not exhibit any apparent morphological phenotypes under high-irradiance conditions of continuous R (Rc) or white light (Wc), there is evidence that phyA plays a minor role in perception of R in Arabidopsis seedlings. *phyA phyB* double mutants exhibit reduced Rc-induced deetiolation and induction of chlorophyll *a*/*b* binding protein (*CAB*) gene expression compared with either single mutant, thus implicating phyA as well as phyB in mediating these responses to

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Rc (Reed et al., 1994). Also, phyA has been shown to be responsible for several very low fluence responses to R, such as seed germination (Botto et al., 1996; Shinomura et al., 1996), R-induced enhancement of phototropism (Parks et al., 1996; Janoudi et al., 1997), and seedling deetiolation (Mazzella et al., 1997). Moreover, transgenic Arabidopsis seedlings that overexpress phyA show enhanced deetiolation not only in FRc but also in Rc (Boylan and Quail, 1991).

The light-stable phyB, in contrast, is the primary phytochrome responsible for deetiolation in response to R (Nagatani et al., 1991; Somers et al., 1991; McCormack et al., 1993; Reed et al., 1993, 1994). In addition, there is evidence that phyB plays a role in other light-regulated responses, such as inhibition of petiole and stem elongation and delay of flowering (Koornneef et al., 1980; Reed et al., 1993, 1994; Neff and Van Volkenburgh, 1994).

Despite intense research efforts, it is not known by which molecular mechanism Pfr transduces the perceived light signal downstream. One powerful approach to this problem is the isolation of mutants defective in normal light signaling (reviewed in Deng, 1994; Millar et al., 1994; McNellis and Deng, 1995; Quail et al., 1995; Chory et al., 1996; von Arnim and Deng, 1996). Several types of screens for mutants have been used. One approach focused on isolating mutants that are constitutively photomorphogenic, namely, constitutively photomorphogenic (*cop*), deetiolated (*det*), and fusca (*fus*). *cop*, *det*, and *fus* dark-grown seedlings exhibit a light-grown morphology that includes short hypocotyls and open cotyledons. The recessive nature of these mutations suggests that these genes encode negative regulators of light signaling that normally are active in the dark and inactivated by light (McNellis et al., 1994; Misera et al., 1994). However, recently, dominant mutants at two apparently novel loci have been described as well (Kim et al., 1996). Because mutations at *COP*/*DET*/*FUS*–like loci do not require photoreceptor activation and are epistatic to mutations at several photoreceptor loci, they are thought to function in a common pathway downstream of phyA, phyB, and the blue light receptor CRY1 (McNellis and Deng, 1995; Chory et al., 1996).

An alternate strategy has been to isolate mutants that display reduced sensitivity to light. Apart from photoreceptor mutants, these screens identified a recessive signaling mutant, *hy5*, that is likely to be deficient in a positively acting component of light signaling (Koornneef et al., 1980). HY5 is required for responses to R, FR, and blue light, indicating that it functions downstream of more than one photoreceptor.

With the goal of identifying very early signaling intermediates, possibly specific to a single photoreceptor, screens for mutants have been conducted that selected for a defect in a phyA- or phyB-specific response, such as deetiolation in FRc or Rc or delay of flowering under short-day conditions. In these screens, mutants that appeared to be specifically affected in either phyA signal transduction (*fhy1* and *fhy3*; Whitelam et al., 1993) or phyB signal transduction (*pef2*, *pef3*, and *red1*; Ahmad and Cashmore, 1996; Wagner et al., 1997) were isolated.

We were interested in identifying additional phyA-specific signal transduction intermediates. To this end, we conducted a screen for extragenic mutations that are capable of suppressing the deficiencies exhibited by a *phyA* mutant. Because our goal was to recover suppressors that function in a phyA-dependent fashion rather than constitutively like mutations at *COP*/*DET*/*FUS*–like loci, we used a weak *phyA* mutant (*phyA-105*; Xu et al., 1995) as the progenitor for the screen. *phyA-105* produces photochemically active phyA at wild-type levels, with partial loss of function due to a missense mutation in the C-terminal half of the molecule. Phenotypic characterization of the *phyA-105* mutant indicates that it is capable of responding to FRc, albeit with reduced sensitivity (Xu et al., 1995). Hence, the *phyA-105* mutant appears to be partially defective in transferring the perceived light signal to downstream transduction components (Xu et al., 1995). We considered such a mutant to be a promising progenitor for identifying suppressor mutations that alter signaling from phyA.

RESULTS

Isolation of Extragenic, FRc-Dependent *phyA-105* Suppressors

Seeds homozygous for *phyA-105* were mutagenized, and M_2 seedlings were grown at an intermediate fluence rate of FRc (15 μmol m^{-2} sec^{-1}). At this fluence rate, *phyA-105* has long hypocotyls, whereas the wild type has short hypocotyls (Xu et al., 1995). We screened the M_2 seedlings for individuals that showed suppression of the *phyA-105* mutant phenotype and thereby appeared to be like wild-type seedlings. Thus, seedlings were sought that displayed a short hypocotyl and/or open cotyledons. We identified 62 suppressor lines representing 48 independent families. We expected to classify these lines into two types of suppressors: (1) those that display the suppressor phenotype in a FRc-dependent manner and (2) those that express the phenotype constitutively, that is, also in darkness. Mutants of the second class have been described for at least 12 loci and have been shown to be epistatic to null mutants in several photoreceptors (Chory et al., 1996). Therefore, they are not likely to be deficient in a phyA-specific signaling component. To eliminate such constitutive suppressors, we rescreened the identified lines in darkness. Fourteen of the 62 lines exhibited photomorphogenic features in darkness and therefore were not analyzed further. Rather, we concentrated on the remaining 48 lines that were indistinguishable from the wild type in darkness.

In 24 of the 48 lines, the *PHYA* gene was sequenced in the region of the *phyA-105* mutation to determine whether suppression of the *phyA-105* mutant phenotype occurred by reversion of the *phyA-105* mutation to the *PHYA* wild-type sequence. One true revertant was found and eliminated

from further analysis. To distinguish between intragenic and extragenic suppressors among the remaining 47 lines, we determined linkage of the suppressing mutation to the *phyA-105* gene: homozygous mutant suppressor plants were crossed to wild-type plants (ecotype RLD), and segregation in the F_2 generations was analyzed in FRc. Thirty-one of 36 lines tested segregated only short seedlings with fully opened cotyledons, indicating that the suppressor mutation is tightly linked to the *phyA-105* gene. These lines are therefore very likely to represent intragenic suppressors or revertants. This result was confirmed for five lines in which the *PHYA* gene was sequenced, and single missense mutations were detected in addition to the maintained *phyA-105* mutation. Five lines, in contrast, segregated tall seedlings in the F_2 generations derived from the respective crosses to the wild type, indicating that the *phyA-105* gene and the mutant suppressor gene segregated independently. These lines are therefore extragenic suppressors of *phyA-105.* In all five lines, the segregation ratio in the F_2 generation was in close agreement with a recessive, unlinked mutation conferring the suppressor phenotype (data not shown).

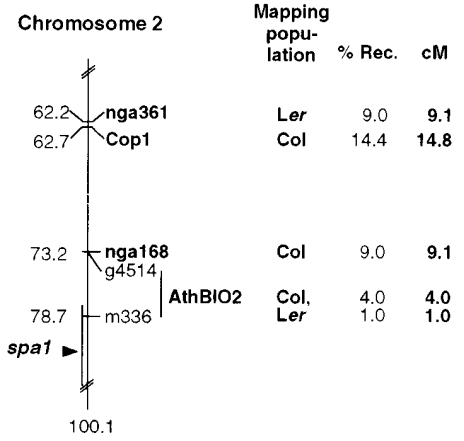

Figure 1. Map Position of the *spa1* Mutation on Chromosome 2.

To map *spa1,* we generated two mapping populations by crossing the *spa1-2 phyA-105* double mutant (ecotype RLD) to *phyA-211* (ecotype Col) and to *phyA-203* (ecotype L*er*). In the F_2 generation of each mapping population, 50 seedlings were selected for the suppressor phenotype under FRc. Pooled F_3 seedlings derived from the selected individual F_2 plants were used to determine recombination frequencies (% Rec) and map distances (in centimorgans [cM]) between *spa1* and the markers indicated in boldface. For each marker, the ecotype (Col and/or L*er*) that displays a polymorphism with RLD is indicated. Map position of markers on chromosome 2 (numbers at left) is based on Lister and Dean (1993; http://genome-www.stanford.edu/Arabidopsis/ww/Vol4i/home.html).

The Suppressor Mutations Are Recessive and Allelic

Backcrossing of the five extragenic *phyA-105* suppressor mutants to *phyA-105* and analysis of the phenotype in the F_1 generations and the segregation patterns in the F_2 generations confirmed that the suppression phenotype is caused by a monogenic recessive mutation in all five mutants identified (data not shown). Allelism tests revealed that these five mutants fall into one complementation group (data not shown). We designated this locus *SPA1* (for suppressor of *phyA-105*).

To determine the map position of *spa1*, two mapping populations were generated: *spa1* plants (RLD) were crossed to *phyA* plants of the Columbia (Col) or Landsberg *erecta* (L*er*) ecotypes, respectively (*phyA-211* or *phyA-203*). In the F_2 generations, linkage analysis of the *spa1* mutant phenotype to polymerase chain reaction (PCR)–based polymorphic markers mapped the *SPA1* locus to the bottom of chromosome 2 (Figure 1). This map position indicates that *spa1* mutants are not allelic to any previously isolated and mapped photomorphogenic mutants.

spa1 Mutations Partially Suppress Multiple Parameters of the Phenotype Caused by the *phyA-105* Missense Allele

Etiolated Growth in FRc

phyA-105 seedlings display an elongated hypocotyl with only partially opened cotyledons in intermediate fluence rates of FRc. In contrast, *spa1 phyA-105* double mutants deetiolated under these light conditions and exhibited short hypocotyls and fully opened cotyledons (Figure 2A). All five alleles of *spa1* altered growth of *phyA-105* to a similar extent (Figure 2B), indicating that there is little allele-specific variation among these mutants. When compared with wild-type seedlings (i.e., wild-type for *SPA1* and *PHYA*), *spa1 phyA-105* double mutants were slightly taller in FRc (Figure 2B). Thus, none of the *spa1* alleles completely suppressed the phenotype caused by the *phyA-105* allele.

In contrast, when grown in darkness, *spa1 phyA-105* double mutants were indistinguishable from *phyA-105* or the wild type: all genotypes exhibited an elongated hypocotyl of similar length, a closed apical hook, and closed, unexpanded cotyledons (Figures 2C and 2D). Hence, suppression of etiolated growth in *spa1 phyA-105* double mutants is FRc conditional.

Lack of an FRc-Preconditioned Block of Greening

Although wild-type Arabidopsis seedlings deetiolate morphologically in FRc, they fail to accumulate chlorophyll under these light conditions. Moreover, preillumination with FRc prevents greening when seedlings subsequently are exposed to Wc. In contrast, seedlings initially grown in darkness without FRc preillumination green within 1 or 2 days

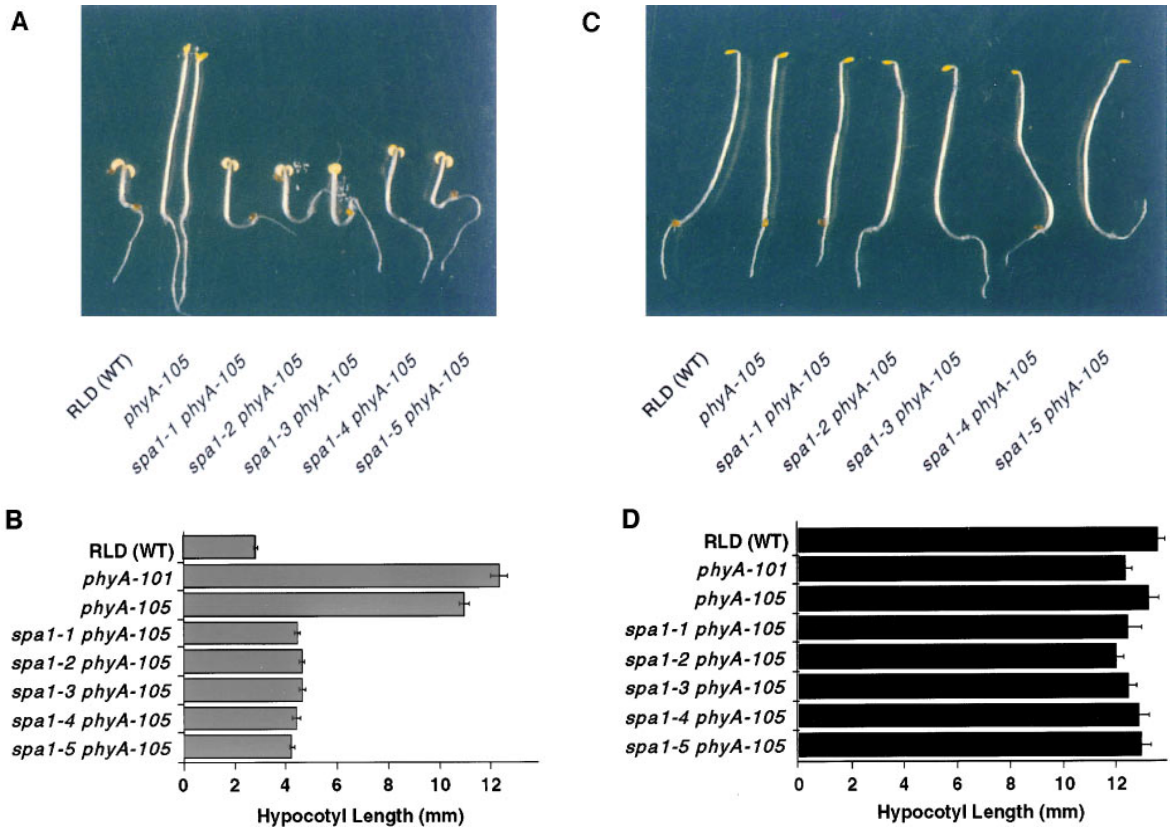

Figure 2. *spa1* Mutations Suppress the Morphological Phenotype in the *phyA-105* Mutant.

(A) Visual phenotype of FRc-grown seedlings.
(B) Hypocotyl length of FRc-grown seedlings.
(C) Visual phenotype of dark-grown seedlings.
(D) Hypocotyl length of dark-grown seedlings.
spa1 phyA-105 double mutants, the progenitor *phyA-105*, a *phyA* null mutant (*phyA-101*, shown in **[B]** and **[D]** only), and wild-type (WT) RLD were grown in FRc (7.5 μmol m^{-2} sec^{-1}) or darkness for 3 days. Error bars denote one standard error of the mean.

after the onset of illumination with Wc. Barnes et al. (1996) demonstrated that this FRc-preconditioned block of greening is dependent on both a functional phyA and an intact phyA signal transduction pathway. Hence, *phyA* null mutants are capable of greening after transfer from FRc to Wc.

In an effort to characterize the effects of *spa1* mutations more fully, we tested whether these mutations restore the FRc-preconditioned block of greening in the *phyA-105* mutant. As shown in Figure 3A, *phyA-105* seedlings that were exposed to intermediate fluence rates of FRc for 3 days retained the ability to green upon subsequent illumination with Wc, resulting in accumulation of chlorophyll to amounts similar to those in a *phyA* null mutant (*phyA-101*). Hence, under these light conditions, *phyA-105* is insensitive to FRc. In contrast, *spa1 phyA-105* seedlings failed to green when transferred from FRc to Wc. These seedlings accumulated negligable amounts of chlorophyll that were indistinguishable from those produced by wild-type seedlings (Figure 3A). Thus, with respect to the FRc-preconditioned block of greening under the conditions used, *spa1* mutations appear to restore completely a wild-type phenotype in the *phyA-105* mutant. Restoration of a block of greening was not a constitutive response but was dependent on preillumination with FRc before transfer to Wc: *spa1 phyA-105* double mutants that were kept in darkness for 3 days before exposure to Wc accumulated chlorophyll at levels similar to the *phyA-105* mutant, although the accumulation was slightly lower than in the wild type grown under the same conditions (Figure 3B). Thus, *spa1 phyA-105* double mutants retain the competence to green when grown in darkness.

Lack of Anthocyanin Accumulation in FRc

Accumulation of anthocyanin in Arabidopsis seedlings grown in FRc has been shown to require the presence of functional

phyA (Kunkel et al., 1996). Therefore, we tested whether *spa1* mutations are capable of restoring anthocyanin accumulation in the *phyA-105* mutant. Seedlings were grown in FRc or darkness for 4 days, and anthocyanin levels were determined spectroscopically. FRc strongly induced the accumulation of anthocyanin in wild-type seedlings, whereas no significant amount of anthocyanin was detectable in the *phyA* null mutant *phyA-101* or in *phyA-105*, confirming that anthocyanin production in FRc is under strict control of phyA (Figure 4A). In contrast, *spa1 phyA-105* double mutants accumulated significant levels of anthocyanin ranging from ~25 to 60% of wild-type levels in the different alleles of *spa1* (Figure 4A). Hence, *spa1* mutations effectively restore the capacity to accumulate anthocyanin in response to intermediate fluence rates of FRc in the *phyA-105* mutant. *spa1* mutations did not elevate anthocyanin levels in dark-grown seedlings, indicating that expression of the suppressor phenotype is light dependent for this characteristic as well (Figure 4B).

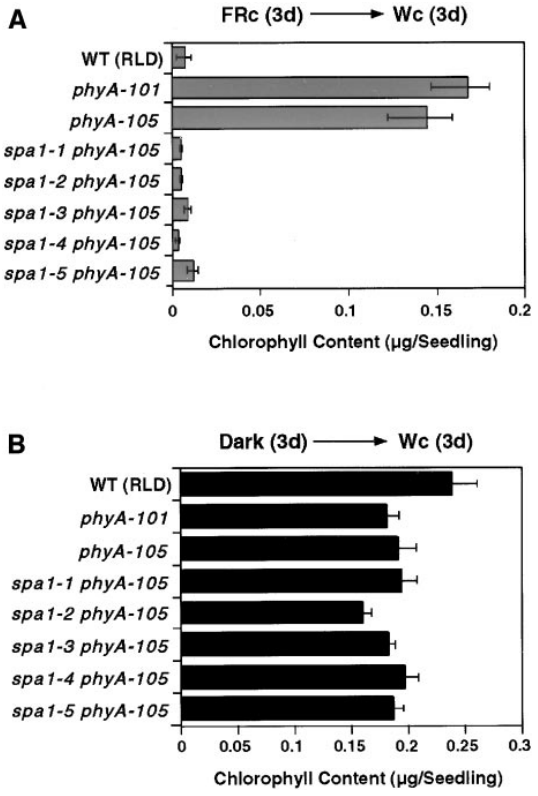

Figure 3. *spa1* Mutations Restore the FRc-Preconditioned Block of Greening in the *phyA-105* Mutant.

(A) Seedling chlorophyll content of FRc-preirradiated seedlings. Wild-type (WT), *phyA-101*, *phyA-105*, and *spa1 phyA-105* seedlings were grown in FRc for 3 days (3d) and were subsequently transferred to Wc for 3 days before determining chlorophyll content.
(B) Seedling chlorophyll content of dark-pretreated seedlings. Seedlings were grown in darkness for 3 days and transferred to Wc for 3 days before determining chlorophyll content. Genotypes are as described in **(A)**.
Error bars denote one standard error of the mean.

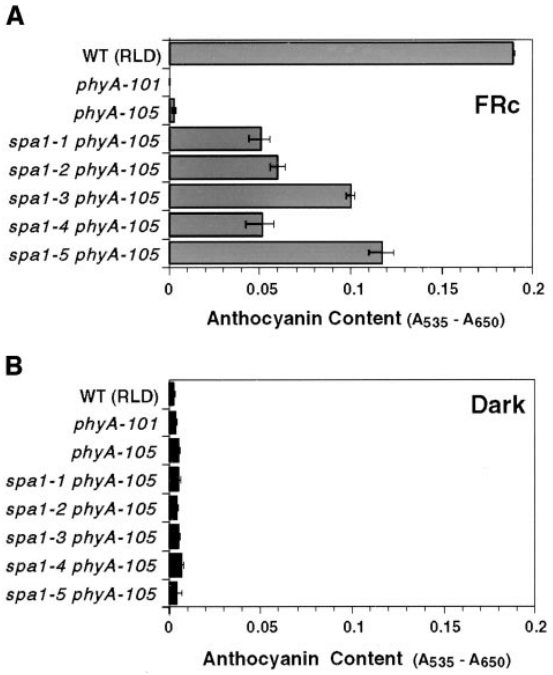

Figure 4. *spa1* Mutations Restore FRc-Induced Anthocyanin Accumulation in the *phyA-105* Mutant.

(A) Anthocyanin content of wild-type (WT), *phyA-101*, *phyA-105*, and *spa1 phyA-105* seedlings grown in FRc (7.5 µmol m^{-2} sec^{-1}) for 4 days.
(B) Anthocyanin content of seedlings grown in darkness for 4 days. Genotypes are as described in **(A)**.
Error bars denote one standard error of the mean.

Effect of *spa1* Mutations on Adult Growth

spa1 phyA-105 plants grew to maturity without displaying any apparent morphological changes compared with *phyA-105* or wild-type plants. Seedlings transplanted to soil and grown under greenhouse conditions developed into plants that showed normal, healthy rosettes, flowered at a time similar to that of wild-type and *phyA-105* plants, and showed normal seed set. No apparent dwarfed growth or reduction in petiole length was observed under greenhouse conditions (data not shown).

spa1 Mutations Confer Hypersensitivity to FRc in the Presence of Wild-Type phyA

The phenotypic analyses of *spa1 phyA-105* seedlings demonstrated that *spa1* mutations suppress various phenotypes

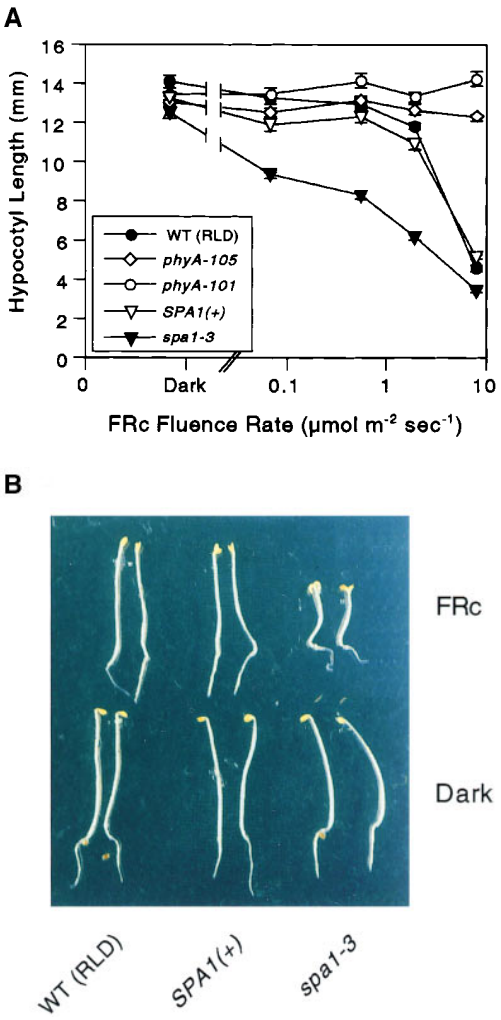

Figure 5. Deetiolation in the *spa1-3* Single Mutant Is Hypersensitive to FRc.

(A) FRc fluence rate response curves for hypocotyl length in wild-type (WT), *phyA-105*, and *phyA-101* seedlings and in seedlings that were homozygous mutant for *spa1-3* and homozygous wild type for *PHYA* (*spa1-3*). As an additional control, progeny of an F_2 segregant determined to be homozygous wild type for *SPA1* and *PHYA* (*SPA1(+)*) were included (see Methods). Error bars denote one standard error of the mean.

(B) Visual phenotype of seedlings grown in FRc (1 μmol m^{-2} sec^{-1}) or darkness for 3 days. Genotypes in the wild-type *PHYA* background are as described in **(A)**. Most seedlings of the *spa1-3* single mutant exhibited fully opened cotyledons in FRc (seedling at left in each pair). However, a few seedlings showed only partially opened cotyledons (seedling at right in each pair).

of the *phyA-105* mutant. To test whether this effect is allele specific or could also be observed with wild-type phyA, we crossed *spa1* mutations into a wild-type *PHYA* background (to clarify this point, we continue to refer to these lines as *spa1* single mutants). This allowed us to assess the effect of *spa1* mutations on FRc responsiveness in the presence of fully functional phyA.

As shown in Figure 5A, the *spa1-3* single mutant exhibited a significantly reduced hypocotyl length compared with the RLD wild type over a range of FRc fluence rates tested. Moreover, *spa1-3* seedlings opened their cotyledons at a lower FRc fluence rate than did RLD wild-type seedlings. At an FRc fluence rate of 1 μmol m^{-2} sec^{-1}, cotyledons of wild-type seedlings were closed or only partially opened, whereas most *spa1-3* seedlings exhibited fully opened and expanded cotyledons at this fluence rate (Figure 5B). In dark-grown seedlings, in contrast, the *spa1-3* mutation had no effect on hypocotyl length or cotyledon phenotype (Figures 5A and 5B). The alleles *spa1-1* and *spa1-2* were outcrossed into a wild-type *PHYA* background as well and caused a phenotype very similar to that caused by the *spa1-3* allele (data not shown).

These results indicate that *spa1* single mutants show an increased sensitivity to FRc. This FRc-hypersensitive phenotype segregated in F_2 generations at the expected Mendelian ratio (data not shown), indicating that the phenotype is caused by a monogenic recessive mutation at the *SPA1* locus. Consistent with this result, progeny of a homozygous wild-type segregant (*SPA1(+)*) that was identified in a population segregating for the *spa1-3* mutation exhibited a hypocotyl length and a cotyledon phenotype similar to that of the RLD wild type (Figures 5A and 5B).

We further tested whether FRc-induced accumulation of anthocyanins is affected in *spa1* single mutants. As shown in Figure 6A, *spa1* single mutants (*spa1-1*, *spa1-2*, and *spa1-3*) accumulated higher levels of anthocyanins in FRc than did the RLD wild type or available progeny of respective homozygous wild-type control segregants (*SPA1-2(+)* and *SPA1-3(+)*). In dark-grown seedlings, in contrast, *spa1* mutations did not cause a significant increase in anthocyanin levels (Figure 6B). These results indicate that *spa1* single mutants are hypersensitive to FRc with respect to anthocyanin accumulation in addition to deetiolation.

Because *spa1* mutations did not cause any apparent phenotype in dark-grown seedlings, we predicted that expression of FRc hypersensitivity in *spa1* single mutants is strictly dependent on the presence of *phyA*, the only phytochrome that senses FRc. Indeed, *spa1-2 phyA-101* seedlings, which produce no phyA, exhibited a hypocotyl length similar to that of the *phyA* null mutant *phyA-101* and progeny of a homozygous *SPA1(+) phyA-101* control segregant when grown under FRc (Figure 7A). Also, *spa1-2 phyA-101* seedlings had closed cotyledons in FRc and thus were indistinguishable in appearance from *phyA-101* seedlings (Figure 7B). Hence, in contrast to the *spa1-2* single mutant that is hypersensitive to FRc (Figures 7A and 7B), the *spa1-2 phyA-101* double mu-

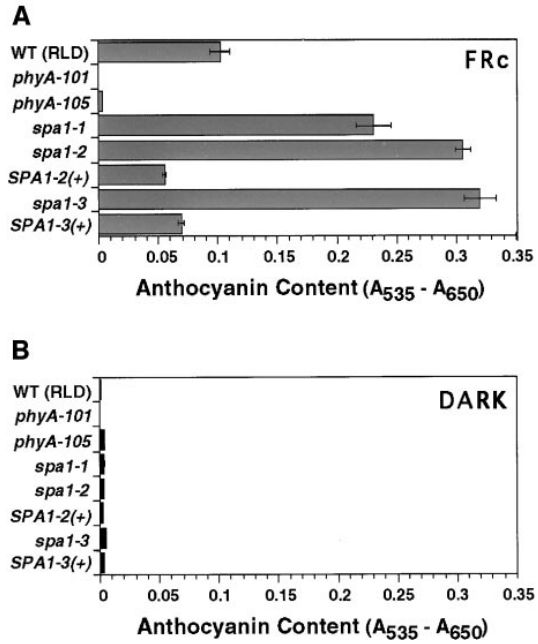

Figure 6. *spa1* Single Mutants Show Enhanced Anthocyanin Accumulation in FRc.

(A) Anthocyanin content of seedlings grown in FRc (7.5 μmol m^{-2} sec^{-1}) for 3 days. The following genotypes were analyzed: wild type (WT), *phyA-101*, *phyA-105*, three *spa1* single mutants (*spa1-1*, *spa1-2*, and *spa1-3*), and, as an additional control, progeny of two wild-type F_2 segregants derived from the crosses of *spa1-2 phyA-105* or *spa1-3 phyA-105* to the wild type (WT RLD) (*SPA1-2(+)* and *SPA1-3(+)*; see Methods). From the cross of *spa1-1 phyA-105* to the wild type (RLD), no progeny of a wild-type F_2 segregant was available.
(B) Anthocyanin content of seedlings grown in darkness for 3 days. Genotypes are as described in **(A)**.
Error bars denote one standard error of the mean.

tant was fully insensitive to FRc. The increase in sensitivity to FRc caused by *spa1* mutations therefore requires light perception through the photoreceptor phyA.

Strictly Dependent on phyA, *spa1* Mutations Also Confer Hypersensitivity to Rc

To characterize further the *spa1* mutants, we tested whether *spa1* mutations alter responsiveness to Rc. Figure 8A shows that *spa1 phyA-105* seedlings exhibited shorter hypocotyls in Rc than did wild-type or *phyA* seedlings. As shown for the *spa1-2 phyA-105* double mutant, this Rc effect of *spa1* mutations was evident under all Rc fluence rates tested (Figure 8B). These results indicate that *spa1* mutations increase the sensitivity of seedlings not only to FRc but also to Rc. Rc hypersensitivity was also observed in the *spa1-2* single mutant, and conspiciously, it was expressed more strongly in the *spa1-2* single mutant than in the *spa1-2 phyA-105* double mutant (Figure 8B).

We considered at least two possibilities to explain the observed lack of wavelength specificity. First, *spa1* mutations might alter the function or signal transduction capacity of other phytochromes, such as phyB, in addition to phyA. Second, *spa1* mutations might specifically affect phyA sensory specificity or signal transduction capacity. In this latter scenario, the increased sensitivity of *spa1* mutants to FRc as well as to Rc would be caused by enhancing the light signal perceived and transduced by phyA.

To distinguish between these two possibilities for SPA1 function, we tested the effect of *spa1* mutations on Rc

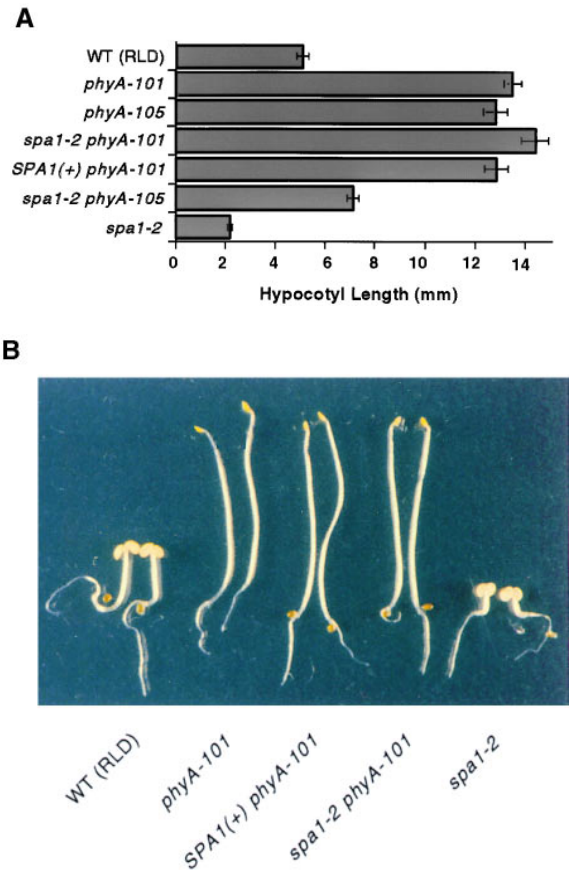

Figure 7. *spa1* Mutations Cause No Apparent Phenotype in a phyA-Deficient Background in FRc.

(A) Hypocotyl length of seedlings grown in FRc (7.5 μmol m^{-2} sec^{-1}) for 3 days. The following genotypes were tested: wild type (WT), *phyA-101* (null for *PHYA*), *phyA-105*, *spa1-2 phyA-101* double mutant, progeny of an F_2 segregant that was determined to be homozygous wild type for *SPA1* and mutant for *phyA-101* (*SPA1(+) phyA-101*; see Methods), *spa1-2 phyA-105* double mutant, and *spa1-2* single mutant. Error bars denote one standard error of the mean.
(B) Visual phenotype of seedlings grown in FRc (7.5 μmol m^{-2} sec^{-1}) for 3 days. Genotypes are as described in **(A)**.

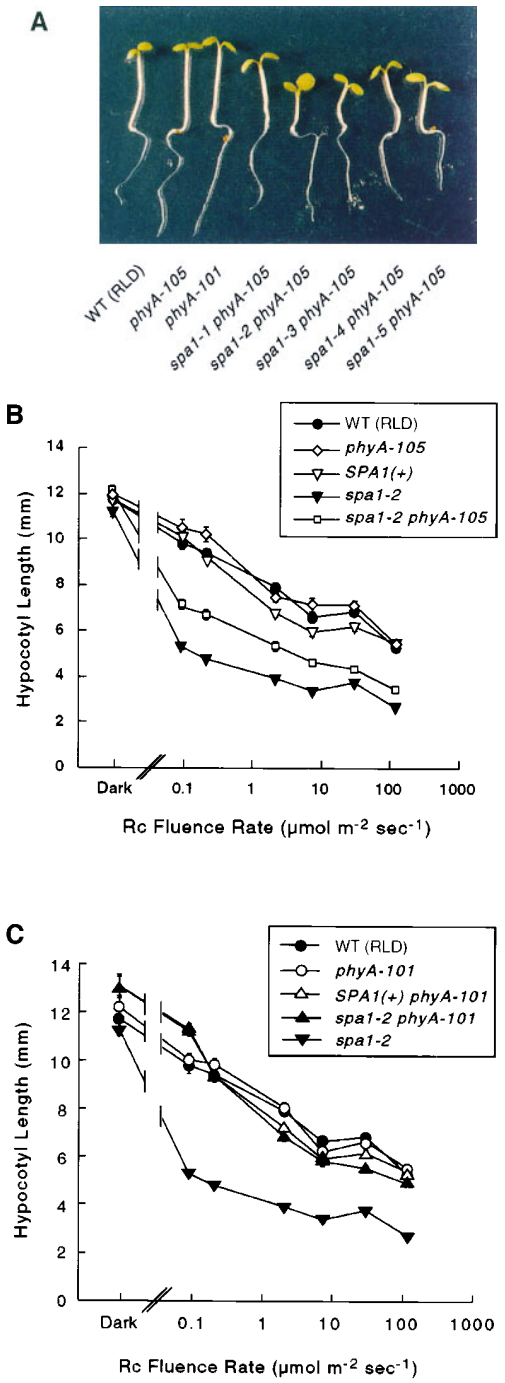

Figure 8. In a phyA-Dependent Manner, *spa1* Mutations Also Confer Hypersensitivity to Rc.

(A) Visual phenotype of *spa1 phyA-105* double mutants grown in Rc (3 μmol m^{-2} sec^{-1}) for 3 days compared with the wild type (WT), *phyA-105*, and *phyA-101*.

(B) Rc fluence rate response curves for hypocotyl length of seedlings of the following genotypes: wild type (WT), *phyA-105*, *spa1-2 phyA-105* double mutant, *spa1-2* single mutant, and, as an additional control, progeny of an F_2 segregant determined to be homozygous wild type for both *SPA1* and *PHYA* (*SPA1(+)*; see Methods). Seedlings were grown in Rc for 3 days. Error bars denote one standard error of the mean.

(C) Rc fluence rate response curves for hypocotyl length of seedlings of the following genotypes: wild type (WT), *phyA-101*, *spa1-2* single mutant, *spa1-2 phyA-101* double mutant, and, as an additional control, progeny of an F_2 segregant determined to be homozygous wild type for *SPA1* and mutant for *phyA-101* (*SPA1(+) phyA-101*; see Methods). Seedlings were grown in Rc for 3 days. Error bars denote one standard error of the mean.

responsiveness in a *phyA* null (*phyA-101*) background. This allowed us to determine whether phyA is required for the Rc hypersensitive phenotype in the *spa1* mutants. Figure 8C shows that no difference in hypocotyl length was observed between the *spa1-2 phyA-101* double mutant and the genotypes *phyA-101* and RLD wild type. As an additional control, we determined hypocotyl length of the progeny of a segregant that was determined to be homozygous wild type at the *SPA1* locus and homozygous *phyA-101* at the *PHYA* locus. Seedlings of this line (*SPA1(+) phyA-101*) also exhibited a hypocotyl length in Rc similar to that of *spa1-2 phyA-101* seedlings (Figure 8C). Thus, no Rc hypersensitive phenotype was observed in the *spa1-2 phyA-101* double mutant. In contrast, the *spa1-2* single mutant exhibited the previously described significant reduction in hypocotyl length in Rc (Figure 8C). Similar results were obtained for the *spa1-3 phyA-101* double mutant (data not shown). Hence, expression of increased sensitivity to Rc in *spa1* mutants strictly depended on the presence of wild-type phyA or the partially functional phyA-105. These results suggest therefore that *spa1* mutations specifically alter phyA function or signal transduction.

spa1 Mutations Do Not Alter phyA Protein Levels

The hypersensitivity to both Rc and FRc observed in *spa1* single mutants is reminiscent of the phenotype displayed by transgenic seedlings that overexpress phyA (Boylan and Quail, 1991). Therefore, it is possible that *spa1* mutations increase the sensitivity of seedlings to FRc and Rc by elevating phyA protein levels. We tested this possibility by determining the levels of phyA or phyA-105, respectively, in seedlings of *spa1* single mutants or *spa1 phyA-105* double mutants relative to respective control seedlings. Figure 9A shows that in neither dark-grown nor FRc-grown seedlings did *spa1* mutations cause an increase in phyA or phyA-105 protein levels, respectively. These results indicate that the phenotypic effects of *spa1* mutations in FRc are not caused by an increase in phyA or phyA-105 protein levels per se.

We further tested the possibility that the Rc hypersensitive phenotype observed in *spa1* mutant seedlings might be

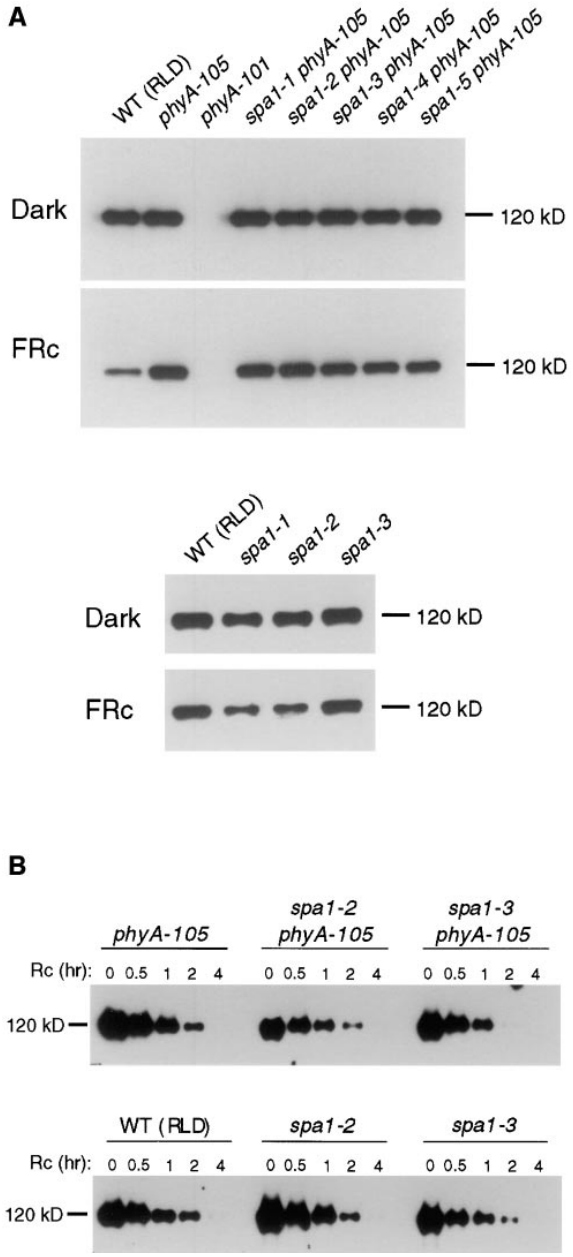

Figure 9. *spa1* Mutations Do Not Increase phyA or phyA-105 Protein Levels in FRc or Darkness and Do Not Alter phyA or phyA-105 Degradation Kinetics in Rc.

(A) Immunoblots of crude protein extracts of seedlings grown in darkness or FRc (7.5 μmol m^{-2} sec^{-1}) for 3 days were probed with a phyA-specific monoclonal antibody. The top pair of immunoblots provide a comparison of phyA-105 protein levels in *spa1 phyA-105* double mutants with those in *phyA-101*, *phyA-105*, and wild type (WT). The bottom pair of immunoblots provide a comparison of phyA protein levels in *spa1* single mutants with those in the wild type. The 120-kD markers indicate the expected size for phyA.

(B) Immunoblots of crude protein extracts of seedlings grown in darkness for 3 days followed by exposure to Rc (30 μmol m^{-2} sec^{-1}) for 0 to 4 hr were probed with a phyA-specific monoclonal antibody. The top immunoblot provides a comparison of phyA-105 protein levels in *spa1 phyA-105* double mutants with those in the *phyA-105* mutant. The bottom immunoblot provides a comparison of phyA protein levels in *spa1* single mutants with those in the wild type (WT). The 120-kD markers indicate the expected size for phyA.

caused by an increase in protein stability of PfrA. As shown in Figure 9B, illumination of seedlings with Rc caused a rapid decrease in phyA levels that is similar in the *SPA1* wild type and all tested *spa1* mutants, both in the mutant *phyA-105* and the wild-type *PHYA* backgrounds. Hence, *spa1* mutations did not cause a detectable increase in phyA protein levels in Rc-grown seedlings.

DISCUSSION

It is well established that phytochromes A and B exhibit contrasting photosensory specificity in the control of seedling deetiolation (Elich and Chory, 1994; Quail et al., 1995; Whitelam and Devlin, 1997), and there is increasing evidence that this specificity may involve separate, perhaps early signal transduction pathway segments specific to phyA or phyB (Whitelam et al., 1993; Ahmad and Cashmore, 1996; Wagner et al., 1997). Our phenotypic and genetic analyses of the *spa1* mutants, isolated here in a screen for extragenic mutations that suppress the phenotype of a partial loss-of-function *phyA* mutant (*phyA-105*), support this notion. The data provide evidence for a new locus encoding a novel component that is specifically involved in phyA signal transduction.

The Effects of *spa1* Mutations Are Light Dependent

Because it was our goal to identify mutationally early signaling intermediates potentially specific to the phyA photoreceptor, we screened for extragenic *phyA-105* suppressors that exhibited the suppression phenotype in a light-dependent fashion rather than constitutively in darkness. Mutations conferring constitutive photomorphogenesis have been described for many loci (designated *cop*/*det*/*fus*) and are thought to affect signaling from multiple photoreceptors, including phyA, phyB, and CRY1 (McNellis and Deng, 1995; Chory et al., 1996). A secondary screen in darkness enabled us to eliminate this class of mutants. Thus, whereas the five isolated mutant *spa1* alleles suppressed several facets of the phenotype caused by the *phyA-105* mutation in FRc-grown seedlings, they did not cause any apparent phenotype in dark-grown seedlings. Similarly, *spa1* mutations outcrossed into a *PHYA* wild-type background also did not confer a mutant phenotype in dark-grown seedlings. Hence, *spa1*

mutations cause effects in a light-conditional fashion and are therefore clearly distinct from mutations conferring constitutive photomorphogenesis.

The Enhanced Photoresponsiveness Caused by the *spa1* Mutations Is phyA Dependent

Characterization of *spa1* mutants in the original *phyA-105* background as well as in a *PHYA* wild-type background indicated that *spa1* mutations increased the responsiveness of seedlings to FRc. *spa1* mutations not only enhanced FRc-induced deetiolation, which is the phenotype used for mutant selection, but they also increased FRc-induced anthocyanin accumulation and the sensitivity of seedlings to the effect of FRc preillumination on subsequent greening in Wc. Although the initial screen for suppressors was performed in FRc, *spa1* mutations also caused enhanced responsiveness to Rc, whether present in the mutant *phyA-105* or wild-type *PHYA* genetic background. The complete elimination of this enhanced responsiveness to Rc as well as to FRc in a *phyA* null mutant background indicates that the effectiveness of both wavelengths requires phyA. Conversely, these data indicate that phyB to phyE are not sufficient for mediating either photoresponse.

Although epistatic interactions between phyA and phyC, phyD, or phyE are not known, these findings argue against an involvement of phytochromes other than phyA in the *spa1* mutant phenotype. In particular, the observation that no detectable Rc-induced seedling hypocotyl phenotype was observed in *spa1 phyA* null double mutants strongly suggests that phyB function and signal transduction are not affected by *spa1* mutations. That *spa1* mutations did not cause any clearly visible effects on additional phyB-mediated responses, such as petiole elongation and flowering time, is consistent with this interpretation.

spa1 Mutations Amplify phyA Signal Transduction

It is well established that overexpression of phyA in transgenic Arabidopsis seedlings causes hypersensitivity to FRc and to Rc (Boylan and Quail, 1991). Hence, one possible mechanism by which the enhanced light response could occur in the *spa1* mutants is that *spa1* mutations could lead to the accumulation of elevated levels of phyA. However, immunoblot analyses clearly showed that phyA protein levels were not increased by *spa1* mutations. Moreover, phyA degradation kinetics in Rc were similar in *spa1* and wild-type seedlings. Hence, these results eliminate the possibility that *spa1* mutations simply increase the abundance of phyA. These findings together with the phyA dependence of the *spa1* single mutant phenotype suggest that increased transduction specifically of the phyA-perceived light signal is the likely cause of the FRc/Rc hypersensitive phenotype in *spa1* mutants. Moreover, the pleiotropic nature of the *spa1* mutant phenotype suggests that SPA1 functions at an early step in the cascade of phyA-specific signaling events. Together with evidence for other transduction components, such as FHY1 and FHY3 (Whitelam et al., 1993) that are specific to phyA signaling and RED1 (Wagner et al., 1997) and PEF2 and PEF3 (Ahmad and Cashmore, 1996) that are specific to phyB signaling, these observations support the hypothesis that early events in phyA and phyB signal transduction are distinct and specific to either phyA or phyB.

Because *spa1* mutations increased light responsiveness in both mutant *phyA-105* and wild-type *PHYA* backgrounds, it is evident that the effects of *spa1* mutations are not specific to the *phyA-105* allele. These findings suggest that *spa1* mutations are likely to increase phyA signaling in the *PHYA* and *phyA-105* backgrounds via the same mechanism and that this mechanism does not specifically rescue the defect caused by the *phyA-105* mutation. Moreover, *spa1* mutations caused a higher FRc and Rc responsiveness in the wild-type *PHYA* background than in the mutant *phyA-105* background, in which only a partially functional phyA is expressed. Hence, it appears that *spa1* mutations cause a general amplification of signaling through the phyA pathway at a magnitude that is proportional to the signal–strength input from the phyA photoreceptor. An amplification of phytochrome responsiveness also appears to occur in the high pigment (*hp*) mutants in tomato (Peters et al., 1992; Kendrick et al., 1997).

Regulation of phyA Signaling by SPA1

The recessive nature of the *spa1* mutations suggests that SPA1 functions as a negative regulator of phyA signal transduction. We suggest that at least two models for the regulation of phyA signaling by SPA1 are possible. In the first scenario (Figure 10A), SPA1 could interact directly with the phyA molecule and thereby reduce the efficiency with which the perceived light signal is transduced to the signaling cascade. For example, SPA1 could compete with a positive regulator for binding to phyA. It is also possible that SPA1 post-translationally modifies the phyA molecule and thereby downregulates the signaling activity of phyA. Such a scenario was suggested as a function of the serine-rich region at the extreme N terminus of phyA. When a mutant monocot phyA construct having this region deleted or replaced by alanine residues was expressed in transgenic tobacco plants, it produced a hyperactive photoreceptor (Stockhaus et al., 1992; Emmler et al., 1995; Jordan et al., 1995). In an attempt to explain this phenotype, it was hypothesized that phosphorylation of these serine residues might reduce the signaling activity of wild-type phyA. Thus, for example, SPA1 could be a kinase responsible for this phosphorylation event.

In an alternative scenario (Figure 10B), SPA1 could downregulate the activity or expression of a positive regulator of phyA signal transduction. FHY1 and FHY3 are potential can-

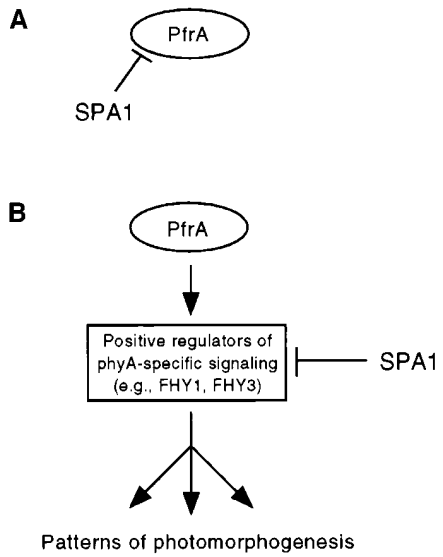

Figure 10. Alternative Models for Mode of SPA1 Function.

(A) SPA1 acts on phyA directly and thereby reduces transmission of light signal from phyA to signal transduction cascade.
(B) SPA1 downregulates activity or expression of positive regulators of phyA-specific signal transduction.

didates for an SPA1 target. *fhy1* and *fhy3* mutations cause a reduction in responsiveness specifically to FRc and are therefore also potential phyA-specific signal transduction intermediates (Whitelam et al., 1993). Because these mutations are recessive, FHY1 and FHY3 are most likely positive regulators of phyA signaling (Whitelam et al., 1993).

There are several possible purposes for SPA1-mediated inhibition of phyA signal transduction. It is possible that the evolution of a negative regulatory factor, such as SPA1, in addition to positively acting factors may have allowed fine-tuning of plant responses to ambient light and environmental conditions. For example, SPA1-mediated inhibition of phyA signaling may be a mechanism permitting negative feedback control of phyA signal transduction by downstream phyA action. It is also possible that SPA1 mediates cross-talk from other signaling cascades that sense, for example, other environmental stimuli that lead to modulation of phyA signaling. Alternatively, SPA1 may function as a constitutive repressor of phyA signaling. In this scenario, SPA1 evolution may have been advantageous in the adaptation to a possibly changed environment.

SPA1 Plays an Important Role in Determining Photosensory Specificity of phyA

The data presented here clearly demonstrate that in *spa1* seedlings, there is a strong phyA-dependent component in the seedling deetiolation response to Rc. In wild-type seedlings, in contrast, phyA plays only a minor role in Rc-induced deetiolation: in comparison with the wild type, phyA-deficient seedlings appear to exhibit a deetiolation defect only under very low fluence rates of Rc or in a phyB-deficient background (Reed et al., 1994; Mazzella et al., 1997). Hence, SPA1 plays a crucial role in reducing phyA responsiveness to Rc and thereby in determining the FRc photosensory specificity of phyA function in the seedling deetiolation response. This indicates that at least three mechanisms exist in the emerging seedling that normally confine phyA responsiveness primarily to FRc rather than Rc with respect to deetiolation: first, transcription of the *PHYA* gene is downregulated by Rc (Somers and Quail, 1995); second, the Pfr conformation of phyA, most abundant in Rc, is rapidly degraded (Clough and Vierstra, 1997); and third, signaling from phyA appears to be inhibited by an SPA1-dependent process. Thus, phyA function in Rc is reduced by mechanisms that affect the gene, protein, and signal transduction. With respect to the observed Rc responsiveness in the *spa1* mutants, it remains to be determined whether *spa1* mutations specifically enhance the very low fluence response to Rc or, alternatively, cause an expansion of phyA-mediated R responsiveness to higher fluence rates of Rc.

In conclusion, we have genetically identified a new locus, *SPA1*, that is likely to encode a negative regulator of phyA-specific signal transduction. Genetic epistasis analysis of the *spa1* mutations with *fhy1* and *fhy3* will help to place SPA1 within the phyA signaling network. Cloning and molecular characterization of *SPA1* will shed light on the mechanisms involved in phyA signaling that are as yet not understood. Moreover, further analysis of the Rc responsiveness of phyA in the *spa1* mutant background should enhance our knowledge of phyA function.

METHODS

Alleles of Photomorphogenic Mutants Used

As the progenitor for the *phyA* mutant suppressor screen, the *phyA-105* allele (*Arabidopsis thaliana* ecotype RLD) was used (Xu et al., 1995). The *phyA* null allele used was *phyA-101*, which was also isolated in the ecotype RLD (Dehesh et al., 1993). For mapping of *spa1*, the alleles *phyA-211* (ecotype Columbia [Col]) and *phyA-203* (ecotype Landsberg *erecta* [L*er*]) were used (Nagatani et al., 1993).

Seedling Growth and Screen for Mutants

To grow seedlings in different light qualities, seeds were surface sterilized in 20% bleach (1.05% sodium hypochlorite) and 0.03% Triton X-100 for 10 min and plated on growth medium (Valvekens et al., 1988) without sucrose. To induce seed germination, plates were kept at 4°C in the dark for 5 days, followed by a 3-hr exposure to white light at 21°C. Plates were then returned to darkness at 21°C for 21 hr. For light treatments, plates were subsequently transferred to

continuous red light (Rc), continuous far-red light (FRc), or darkness for 3 days. Hypocotyl length was determined at the end of this irradiation regime as described in Wagner et al. (1996). Light sources used were described previously (Wagner et al., 1991, 1997). Fluence rates were determined using a spectroradiometer (model LI-1800; Li-Cor, Lincoln, NE).

For mutagenesis, ~50,000 seeds homozygous for *phyA-105* were exposed to 0.25% ethyl methanesulfonate for 16 hr and subsequently sown on soil in pots. M_2 seeds were harvested in bulk for each of the 205 pots and considered independent families. M_2 seeds (800 to 1000 per family) were treated and plated on growth medium supplemented with 2% sucrose, as described above, and screened in FRc (13 μmol m^{-2} sec^{-1}) for seedlings with open cotyledons and/or short hypocotyls. Selected individuals were transferred to fresh plates and kept in the dark at 21°C for 3 days (FR rescue). Seedlings were then exposed to continuous white light (Wc) for several days and subsequently transferred to soil to produce M_3 seeds. M_3 seeds were rescreened in FRc (13 μmol m^{-2} sec^{-1}) and in darkness.

To distinguish between intragenic and extragenic suppressors, we determined linkage of the suppressor mutations to the *phyA-105* gene: duplicate crosses between homozygous suppressor lines and wild type (RLD) were conducted, and three F_1 plants per cross were grown to produce F_2 seed. At least 200 F_2 seeds obtained from each F_1 plant were plated and grown in FRc (13 μmol m^{-2} sec^{-1}) for 3 days. Lines that segregated tall seedlings were considered extragenic suppressors.

DNA Sequencing of *phyA-105* Suppressors

The *PHYA* gene was amplified by polymerase chain reaction (PCR) (Advantage cDNA PCR kit; Clontech, Palo Alto, CA), and the PCR product was used to sequence a region of the *PHYA* gene containing the site of the *phyA-105* mutation. Sequencing was performed with an automated sequencer (model ABI373; Perkin Elmer, Foster City, CA).

Construction of *spa1* Single Mutants and *spa1 phyA-101* Double Mutants

To construct *spa1* single mutants, we crossed *spa1 phyA-105* double mutants to the wild type (RLD). In the F_2 generation, plants with the genotypes *spa1*/*spa1 PHYA*/*PHYA* and *SPA1*/*SPA1 PHYA*/*PHYA* (wild-type control segregants) were identified by the following procedure. To determine the genotype at the *PHYA* locus, we took advantage of a polymorphism generated by the *phyA-105* mutation (elimination of an AciI restriction site). Genomic DNA from 50 randomly chosen F_2 plants was isolated (Edwards et al., 1991) and used as a template to PCR amplify an ~500-bp region flanking the site of the *phyA-105* mutation. The PCR products were subsequently digested with AciI, and the restriction fragments obtained were resolved by agarose gel electrophoresis to display the polymorphism. F_2 plants thus identified as homozygous for the wild-type allele of *PHYA* were testcrossed to the progenitor suppressor line, and F_2 seeds harvested from at least eight F_1 plants derived from each cross were plated and grown in FRc (13 μmol m^{-2} sec^{-1}). Progeny that did not segregate tall seedlings in any of the eight F_2 families were considered to be derived from a plant that was homozygous mutant at the *SPA1* locus (referred to as *spa1* single mutant). In contrast, progeny that segregated tall seedlings in all F_2 families were considered to be derived from a plant that was homozygous wild type at the *SPA1* locus.

To construct *spa1 phyA-101* double mutants, homozygous *spa1 phyA-105* plants were crossed to *phyA-101.* In the F_2 generation, 50 seedlings were randomly chosen and assayed for their genotype at the *PHYA* locus by using the PCR-based polymorphism of *phyA-105* described above. F_2 plants thus identified as homozygous mutant for *phyA-101* were then testcrossed to the progenitor suppressor line to determine the genotype at the *SPA1* locus. Hence, all F_1 seeds produced were heterozygous at the *PHYA* locus carrying one allele each of *phyA-105* and *phyA-101.* In contrast, F_1 seeds segregated at the *SPA1* locus. To detect segregation at the *SPA1* locus, F_1 seeds were plated and grown in FRc (7.5 μmol m^{-2} sec^{-1}) for 3 days. Because *spa1* mutations suppress the phenotype of homozygous mutant *phyA-105*, it was expected that *spa1* mutations also cause a phenotype in a *phyA-105*/*phyA-101* heterozygous background, which should produce half as much phyA-105 protein as a *phyA-105* homozygote. Indeed, this was observed. When grown in FRc, F_1 progenies of the testcrosses fell into three groups: those that segregated 100% seedlings with fully opened cotyledons and hypocotyls shorter than *phyA-105*, those that segregated 100% tall seedlings with almost fully closed cotyledons, and those that segregated these two phenotypes at a ratio of ~1:1. Hence, progeny in which all or no F_1 seedlings displayed open cotyledons and hypocotyls shorter than *phyA-105* were considered to be derived from a plant that was homozygous mutant or homozygous wild type, respectively, at the *SPA1* locus.

Mapping of the *spa1* Mutation

To generate mapping populations, homozygous *spa1-2 phyA-105* plants were crossed to *phyA* mutants in the ecotypes Col and L*er* (*phyA-211* and *phyA-203*, respectively). F_2 seeds were plated and grown in FRc (7.5 μmol m^{-2} sec^{-1}) for 3 days, and individuals that displayed the suppressor phenotype were selected and grown to produce F_3 seed. Segregation ratios in the F_2 generation derived from the cross of *spa1-2 phyA-105* to *phyA-203* were consistent with a 1:16 (short to tall) segregation ratio, suggesting that only seedlings that were homozygous mutant for *phyA-105* and *spa1* displayed a clear suppressor phenotype. Analysis using the PCR-based polymorphism for *phyA-105* described in the previous section confirmed that all identified suppressors in this mapping population were homozygous for *phyA-105.*

In contrast, the F_2 generation derived from the cross of *spa1-2 phyA-105* to *phyA-211* segregated seedlings displaying phenotypes ranging from full to intermediate suppression at ratios that were consistent with 3:16 (short plus intermediate to tall). PCR analysis showed that F_2 seedlings were either homozygous for *phyA-105* or heterozygous *phyA-105*/*phyA-211.* No F_2 seedling selected for the suppressor phenotype was found to be homozygous *phyA-211.*

To confirm that selected F_2 plants in both mapping populations were indeed homozygous mutant for *spa1*, F_3 seed was rescored for the suppressor phenotype. Only those F_3 seeds that showed 100% short seedlings in FRc were used for mapping of *spa1.* Hence, F_3 seeds of the *spa1-2 phyA-105* × *phyA-211* mapping population that segregated tall seedlings because of *phyA-105*/*phyA-211* heterozygosity were not used. Genomic DNA was isolated from populations of F_3 seedlings derived from 50 selected F_2 plants per mapping population, according to the method of Edwards et al. (1991), and used for mapping of *spa1* with simple sequence length polymorphism

markers and cleaved amplified polymorphic sequence markers (Konieczny and Ausubel, 1993; Bell and Ecker, 1994; web sites at http://cbil.humgen.upenn.edu/~atgc/SSLP_info/coming-soon.html; http://genome-www.stanford.edu/Arabidopsis/aboutcaps.html). Map distances were calculated based on the Kosambi function, as described by Koornneef and Stam (1991).

FRc-Preconditioned Block of Greening Experiments and Chlorophyll Determinations

Fifty seeds per genotype and per light treatment were plated in triplicate on growth medium without sucrose and induced to germinate as described above. Plates were kept in either FRc (7.5 μmol m^{-2} sec^{-1}) or darkness at 21°C for 3 days. Subsequently, all plates were transferred to Wc (25 μmol m^{-2} sec^{-1}) for 3 days. At the end of this illumination regime, seedlings were homogenized in 80% acetone. After centrifugation to remove debris, the chlorophyll concentration in the supernatants was determined spectroscopically, as described by Chory et al. (1991).

Anthocyanin Accumulation

Fifty seeds per genotype were plated in duplicate on growth medium supplemented with 2% sucrose and induced to germinate as described above. Subsequently, plates were kept in either FRc (7.5 μmol m^{-2} sec^{-1}) or darkness for 3 or 4 days. Anthocyanins were extracted under dim green safelight, and anthocyanin content was determined spectroscopically, as described by Schmidt and Mohr (1981).

Immunoblot Analysis of phyA

Crude extracts from seedlings were prepared as described by Wagner et al. (1991). Ten or 25 μg of crude protein prepared from dark- or FRc-grown seedlings, respectively, was loaded on an SDS–polyacrylamide gel. For analysis of phyA or phyA-105 levels in Rc, 20 μg of crude protein was loaded. Immunoblot analysis was performed as described by Wagner et al. (1991), with the exception that membranes were blocked in 0.1% Tween 20 in PBS overnight. This allowed staining of the membrane with Coomassie Brilliant Blue R 250 after antibody development to confirm equal loading of crude protein. Membranes were probed with a monoclonal antibody specific for phyA as the primary antibody and an anti–mouse horseradish peroxidase–conjugated antibody as a secondary antibody. Antibodies were detected by using chemilluminescence (SuperSignal; Pierce, Rockford, IL).

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REFERENCES

Ahmad, M., and Cashmore, A.R. (1996). The *pef* mutants of *Arabidopsis thaliana* define lesions early in the phytochrome signaling pathway. Plant J. **10,** 1103–1110.

Barnes, S.A., Kishizawa, N.K., Quaggio, R.B., Whitelam, G.C., and Chua, N.-H. (1996). Far-red light blocks greening of Arabidopsis seedlings via a phytochrome A–mediated change in plastid development. Plant Cell **8,** 601–615.

Bell, C.J., and Ecker, J.R. (1994). Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis.* Genomics **19,** 137–144.

Botto, J.F., Sanchez, R.A., Whitelam, G.C., and Casal, J.J. (1996). Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade light in *Arabidopsis*. Plant Physiol. **110,** 439–444.

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.

Chory, J. (1997). Light modulation of vegetative development. Plant Cell **9,** 1225–1234.

Chory, J., Nagpal, P., and Peto, C.A. (1991). Phenotypic and genetic analysis of *det*2, a new mutant that affects light-regulated seedling development in *Arabidopsis.* Plant Cell **3,** 445–459.

Chory, J., Chatterjee, M., Cook, R.K., Elich, T., Fankhauser, C., Li, J., Neff, M., Pepper, A., Poole, D., Reed, J., and Vitart, V. (1996). From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc. Natl. Acad. Sci. USA **93,** 12066–12071.

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.

Clough, R.C., and Vierstra, R.D. (1997). Phytochrome degradation. Plant Cell Environ. **20,** 713–721.

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.

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

Edwards, K., Johnstone, C., and Thompson, C. (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. **19,** 1349.

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

Emmler, K., Stockhaus, J., Chua, N.-H., and Schäfer, E. (1995). An amino-terminal deletion of rice phytochrome A results in a dominant negative suppression of tobacco phytochrome A activity in transgenic tobacco seedlings. Planta **197,** 103–110.

Janoudi, A.K., Gordon, W.R., Wagner, D., Quail, P., and Poff, K. (1997). Multiple phytochromes are involved in red-light–induced enhancement of first-positive phototropism in *Arabidopsis thaliana.* Plant Physiol. **113,** 975–979.

Jordan, E.T., Cherry, J.R., Walker, J.M., and Vierstra, R.D. (1995). The amino-terminus of phytochrome A contains two functional domains. Plant J. **9,** 243–257.

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

Kendrick, R.E., Kerckhoffs, L.H.J., Van Tuinen, A., and Koornneef, M. (1997). Photomorphogenic mutants of tomato. Plant Cell Environ. **20,** 746–751.

Kim, B.C., Soh, M.S., Kang, B.J., Furuya, M., and Nam, H.G. (1996). Two dominant photomorphogenic mutations of *Arabidopsis thaliana* identified as suppressor mutations of *hy2.* Plant J. **9,** 441–456.

Konieczny, A., and Ausubel, F.M. (1993). A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J. **4,** 403–410.

Koornneef, M., and Stam, P. (1991). Genetic Analysis. In Methods in Arabidopsis Research, C. Koncz, N.-H. Chua, and J. Schell, eds (Singapore: World Scientific), pp. 83–99.

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

Kunkel, T., Neuhaus, G., Batschauer, A., Chua, N.-H., and Schäfer, E. (1996). Functional analysis of yeast-derived phytochrome A and B phycocyanobilin adducts. Plant J. **10,** 625–636.

Lister, C., and Dean, C. (1993). Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana.* Plant J. **4,** 745–750.

Mazzella, M.A., Alconoda Magliano, T.M., and Casal, J.J. (1997). Dual effect of phytochrome A on hypocotyl growth under continuous red light. Plant Cell Environ. **20,** 261–267.

McCormac, A.C., Wagner, D., Boylan, T.B., 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.

McNellis, T.W., and Deng, X.-W. (1995). Light control of seedling morphogenetic pattern. Plant Cell **7,** 1749–1761.

McNellis, T.W., von Arnim, A.G., and Deng, X.-W. (1994). Overexpression of Arabidopsis COP1 results in partial suppression of light-mediated development: Evidence for a light-inactivable repressor of photomorphogenesis. Plant Cell **6,** 1391–1400.

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

Misera, S., Mueller, A.J., Weiland-Heidecker, U., and Juergens, G. (1994). The *FUSCA* genes of *Arabidopsis*: Negative regulators of light responses. Mol. Gen. Genet. **244,** 242–252.

Nagatani, A., Chory, J., and Furuya, M. (1991). 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., 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.

Neff, M.M., and Van Volkenburgh, E. (1994). Light-stimulated cotyledon expansion in *Arabidopsis* seedlings. Plant Physiol. **104,** 1027–1032.

Parks, B.M., and Quail, P.H. (1993). *hy*8, a new class of Arabidopsis long hypocotyl mutants deficient in functional phytochrome A. Plant Cell **5,** 39–48.

Parks, B.M., Quail, P.H., and Hangarter, R.P. (1996). Phytochrome A regulates red-light induction of phototropic enhancement in *Arabidopsis.* Plant Physiol. **110,** 155–162.

Peters, J.L., Schreuder, M.E., Verduin, S.J.W., and Kendrick, R.E. (1992). Physiological characterization of a high-pigment mutant of tomato. Photochem. Photobiol. **56,** 75–82.

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

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., and Chory, J. (1994). Mutational analysis of light-controlled seedling development in *Arabidopsis.* Semin. Cell Biol. **5,** 327–334.

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.

Schmidt, R., and Mohr, H. (1981). Time-dependent changes in the responsiveness to light of phytochrome-mediated anthocyanin synthesis. Plant Cell Environ. **4,** 433–437.

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.

Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M., and Furuya, M. (1996). Action spectra for phytochrome A– and B–specific photoinduction of seed germination in *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. USA **93,** 8129–8133.

Somers, D.E., and Quail, P.H. (1995). Phytochrome-mediated light regulation of *PHYA*– and *PHYB–GUS* transgenes in *Arabidopsis thaliana* seedlings. Plant Physiol. **107,** 523–534.

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.

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.

von Arnim, A., and Deng, X.-W. (1996). Light control of seedling development. Annu. Rev. Plant Physiol. Plant Mol. Biol. **47,** 215–243.

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.

Wagner, D., Koloszvari, M., and Quail, P.H. (1996). Two small spatially distinct regions of phytochrome B are required for efficient signaling rates. Plant Cell **8,** 859-871.

Wagner, D., Hoecker, U., and Quail, P.H. (1997). *Red1* is necessary for phytochrome B–mediated red light–specific signal transduction in Arabidopsis. Plant Cell **9,** 731–743.

Whitelam, G.C., and Devlin, P.F. (1997). Roles of different phytochromes in *Arabidopsis* photomorphogenesis. Plant Cell Environ. **20,** 752–758.

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.

Xu, Y., Parks, B.M., Short, T.W., and Quail, P.H. (1995). Missense mutations define a restricted segment in the C-terminal domain of phytochrome A critical to its regulatory activity. Plant Cell **7,** 1433–1443.