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Both phyA and phyB Mediate Light-Imposed Repression of *PHYA* Gene Expression in Arabidopsis[1]

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The negatively photoregulated *PHYA* gene has a complex promoter structure in Arabidopsis, with three active transcription start sites. To identify the photoreceptors responsible for regulation of this gene, and to assess the relative roles of the three transcription start sites, we analyzed the changes in *PHYA* transcript levels in wild-type and photoreceptor mutant seedlings under various irradiation conditions. Continuous far-red or red light exposures each induced a significant decline in transcript levels in wild-type etiolated seedlings. Analysis of mutants specifically lacking either phyA or phyB protein demonstrated that these phytochromes are required for the negative regulation induced by far-red and red light, respectively. Ribonuclease protection experiments showed further that this negative regulation is confined almost exclusively to the shortest, most abundant *PHYA* transcript, and occurs predominantly in shoots. By contrast, both of the other minor transcripts in shoots, and all three transcripts in roots, exhibit near constitutive expression. This complex expression pattern indicates that the *PHYA* gene is subject to regulation by multiple signals, including environmental, developmental, and organ-specific signals.

In higher plants, phytochromes are a family of photoreceptor chromoproteins that monitor the red (R)/far-red (FR) region of the sunlight spectrum and regulate many photomorphogenic responses at all stages of the life cycle (Kendrick and Kronenberg, 1994). The phytochrome apoprotein is encoded by a small family of divergent genes, with five members (*PHYA–PHYE*) identified in Arabidopsis (Sharrock and Quail, 1989; Clack et al., 1994). Each type of phytochrome holoprotein exists in two interconvertible conformations: the inactive, R-absorbing form (Pr) and the active FR-absorbing form (Pfr). High levels of phyA are present in etiolated seedlings and after prolonged periods of darkness, whereas the abundance of this molecular species decreases drastically and rapidly following illumination with R or white (W) light. The photoregulation of phyA abundance occurs both at the protein level, by a rapid degradation of the unstable Pfr form, and at the mRNA level, by a light-induced reduction in *PHYA* transcript abundance (Quail, 1994). In contrast, the abundance of *PHYB*, *PHYC*, *PHYD*, and *PHYE* transcripts are relatively unaltered by light in Arabidopsis (Clack et al., 1994).

At the functional level, studies with phytochrome-deficient mutants have shown that phyA and phyB have distinct but overlapping roles in controlling seedling photomorphogenesis (Reed et al., 1994; Quail et al., 1995; Whitelam and Devlin, 1997). phyA is predominantly, if not exclusively, responsible for de-etiolation in continuous far-red (FRc) light, whereas phyB is predominantly responsible for de-etiolation in response to continuous red (Rc) light.

It has been shown in a variety of plant species that light induces an increase in the abundance of many different mRNAs, including those encoding the small subunit of Rubisco and chlorophyll *a/b*-binding protein (Silverthorne and Tobin, 1984), chloroplastic Gln synthetase (Edwards and Coruzzi, 1989) and Fd (Dobres et al., 1987), and others (Tobin and Kehoe, 1994; Terzaghi and Cashmore, 1995). Only a few identified genes have been demonstrated to be down-regulated in their expression by light, including genes encoding protochlorophyllide reductase (Forreiter et al., 1990), Asn synthetase (Tsai and Coruzzi, 1990), and *PHYA* itself (Colbert et al., 1983). The involvement of phytochromes in the light-induced changes in expression of some of these genes has been shown by reversibility of the effect of a single pulse of R light on the level of expression by a subsequent pulse of FR light, suggesting that this response has, at least, a low fluence response component (LFR). However, other genes show different fluence requirements (Terzaghi and Cashmore, 1995). For some of these light-regulated genes, including the oat *PHYA* gene, it has been demonstrated that phytochrome regulates their expression at the transcriptional level (Silverthorne and Tobin, 1987; Lissemore and Quail, 1988; Terzaghi and Cashmore, 1995).

All *PHYA* genes that have been investigated are strongly expressed in the dark and negatively regulated by light (Quail, 1991). However, significant differences have been shown between monocots and dicots: (a) the extent of light-induced down-regulation varies substantially between plant species, with the monocots in general appearing to respond more strongly than the dicots (Quail, 1994); (b) whereas monocot *PHYA* genes have only a single transcription start site (Hershey et al., 1987; Christensen and Quail, 1989; Kay et al., 1989), the dicot *PHYA* genes exam-

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ined, including that from Arabidopsis, have three transcription start sites (Sato, 1988; Dehesh et al., 1994; Adam et al., 1995), with evidence for differential control by phytochrome of the abundance of the transcripts initiated at each site in pea and tobacco (Tomizawa et al., 1989; Adam et al., 1995). The very low fluence (VLF) component shown by the light-imposed repression of the *PHYA* gene in oat suggests the action of at least phyA in this monocot (Quail, 1994). On the other hand, photobiological data have been interpreted as indicating that a stable phytochrome regulates the *PHYA* mRNA levels in pea seedlings through a LFR (Furuya et al., 1991), and a similar LFR pattern has been reported for *PHYA* mRNA levels in tobacco (Adam et al., 1994). These data raise the question of whether different phytochromes might be mediating this response in monocots and dicots.

We wished to define the photoregulation of the *PHYA* gene in Arabidopsis, with the intent of using the genetic advantages of this model organism to dissect the molecular mechanisms involved. Although single pulses of R light did not decrease *PHYA* mRNA levels in etiolated Arabidopsis and tomato seedlings, a significant reduction in transcript levels was detected after continuous irradiation with white light (Wc) (Sharrock et al., 1988; Sharrock and Quail, 1989). Moreover, the negative photoregulation of a *PHYA-GUS* transgene by Rc and FRc irradiation in Arabidopsis seedlings suggests that at least one light-stable phytochrome and phyA may mediate light regulation of *PHYA* gene expression in this species (Somers and Quail, 1995). We determined the role of phyA and phyB in mediating negative photoregulation of the *PHYA* gene in Arabidopsis using *phyA* and *phyB* null mutants and have quantitatively defined the kinetics and fluence-rate dependence of FRc-light-imposed repression of this gene. In addition, we examined the levels of the three nested *PHYA* transcripts in whole seedlings, shoots, and roots to determine their relative contributions to the light-induced changes in total transcript levels and whether there is organ-specific modulation of this response.

MATERIALS AND METHODS

Seedling Growth and Light Sources

Arabidopsis ecotype RLD was used in all experiments, except that for the analysis of *PHYA* mRNA levels in a *phyB* null background, in which the mutant *phyB-5* and the corresponding wild-type (WT) ecotype Landsberg *erecta* (L*er*) were used.

Seeds were surface-sterilized in 20% (v/v) commercial bleach and 0.03% (v/v) Triton X-100 for 10 min and then washed five times with sterile water. Seeds were plated on growth medium (Valvekens et al., 1988) without Suc. Germination was induced by keeping the plates at 4°C in the dark for 2 d followed by 3-h exposure to white light at 21°C. Plates were then returned to darkness at 21°C until the initiation of various continuous light treatments.

FRc and Rc light were supplied with LED light sources (Quantum Devices, Barnveld, WI) and Wc light with cool-white fluorescent lamps. The fluence rates used routinely were 390 μmol m^{-2} s^{-1} for FRc and Rc irradiations and 39 μmol m^{-2} s^{-1} for Wc light, unless otherwise stated.

RNA Isolation, Analysis, and Quantification

Total RNA was extracted as described previously (Cantón et al., 1993) or by using the RNasy purification kit from Qiagen (Valencia, CA).

For northern-blot analysis, equal amounts of total RNA samples were denatured at 65°C for 15 min in 1× MOPS buffer (20 mM 3-[*N*-Morpholino] propanesulfonic acid, pH 7.0, 1 mM EDTA, and 5 mM sodium acetate), 6.6% (v/v) formaldehyde, and 50% (v/v) formamide and separated by electrophoresis in 1.0% (w/v) agarose/6% (v/v) formaldehyde/1× MOPS buffer gels. After electrophoresis the RNA was transferred to nylon membranes (MAGNA, Micron Separations, Westborough, MA). The membranes were prehybridized and hybridized at 65°C in the hybridization buffer described by Church and Gilbert (1984). Specific ^{32}P-labeled probes were synthesized by the Multiprime DNA labeling system (Amersham-Pharmacia Biotech, Uppsala) using as a template a 642-bp DNA fragment from the 3′ end of an Arabidopsis *PHYA* cDNA clone (nucleotides [nt] 3,128–3,770) and a 262-bp DNA fragment from a *PHYB* cDNA clone (nt 3,607–3,868) (Sharrock and Quail, 1989). As a loading control membranes were rehybridized with a 400-bp fragment of a 17S rRNA from rice (nt 158–557) (Takaiwa et al., 1984), which hybridizes specifically with the 18S RNA from Arabidopsis.

For RNase protection experiments, a *Sca*I-*Kpn*I (position −259 to +125) fragment from the 5′-UTR and promoter region of the *PHYA* gene was cloned into the pBluescript plasmid (Stratagene, La Jolla, CA). Labeled antisense RNA was produced by in vitro transcription using the linearized plasmid as a template and [α-^{32}P]CTP to generate a probe 430 nt in length, in which the first 12 nt at the 5′ and the last 38 nt at the 3′ ends are derived from plasmid sequence. The RNase assay was performed using the RPA II Kit (Ambion, Austin, TX) following the instructions of the manufacturer. RNA size markers were obtained by in vitro transcription of the Century Marker template (Ambion). The expected sizes of the protected fragments from the *PHYA* transcripts were 121 nt for mRNA1, 204 nt for mRNA2, and 380 nt for mRNA3.

For quantitative analysis, hybridized blots and RNase protection gels were exposed to phosphor imager plates and images were subsequently obtained and analyzed with a Storm 860 system and ImageQuant version 1.1 software (Molecular Dynamics, Sunnyvale, CA). The radioactivity values for each different-length RNase protection fragment were corrected for the number of cytidines present in that probe fragment to permit direct quantitative comparison of the abundance of mRNA1, mRNA2, and mRNA3.

RESULTS

To more definitively determine whether phytochromes do indeed regulate *PHYA* mRNA abundance in Arabidopsis seedlings, and to identify the family members potentially involved, we analyzed the transcript steady-state

levels from wild-type and mutant 5-d-old etiolated seedlings and seedlings irradiated for 24 h before harvest with FRc, Rc, or Wc light (Fig. 1). For this purpose we used the mutants *phyA-101* (Dehesh et al., 1993) and *phyB-5* (Koornneef et al., 1980; Reed et al., 1993), and the relevant wild-type backgrounds RLD and L*er*. These mutants are null for phyA or phyB, respectively, as a result of point mutations introducing early stop codons into the protein coding regions of the genes. As a result of the mutation in *phyA-101*, the overall levels of *PHYA* mRNA were lower relative to the wild type under all conditions (Fig. 1A, left), but were still detectable by northern-blot hybridization. The intensities of radioactive signals were quantified and the results are summarized in Figure 1B.

All light qualities elicited a reduction in *PHYA* mRNA levels in wild-type etiolated seedlings compared with non-irradiated seedlings (Fig. 1B, RLD and Ler). However, the *phyA*-101 mutant failed specifically to respond to FRc light, while still showing a response to Rc and Wc light. In contrast, the *phyB* null mutant had full responsiveness to FRc light (compare levels of mRNA in FRc in L*er* and *phyB-5*), but lost most of its response to Rc and partially to Wc light. Therefore, the changes in the amount of *PHYA* mRNA levels induced by FRc and Rc light are independently mediated by different phytochromes. These data suggest that, as in the overall process of de-etiolation, phyA is necessary for changes in *PHYA* gene expression in FRc light, and no other phytochrome (phyB–E) seems to contribute to this response. In contrast, phyB is necessary for full control of *PHYA* gene expression in Rc light and no other phytochrome is able to account for this response to the same magnitude.

We focused on the effect of FRc light on *PHYA* expression, because this quality of light is non-photosynthetically

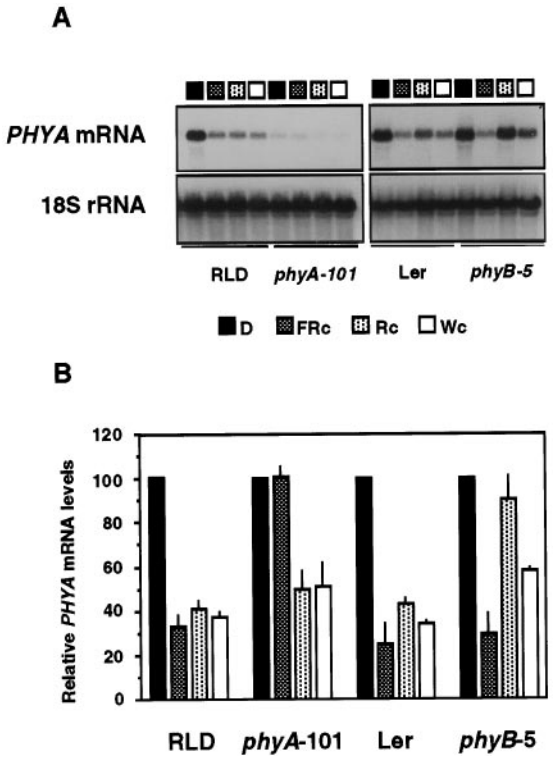

Figure 1. Phytochromes A and B control *PHYA* mRNA levels. A, Northern-blot analysis of 8 μg of total RNA isolated from seedlings of a *phyA* null mutant (*phyA-101*), a *phyB* null mutant (*phyB-5*), and the corresponding wild-types RLD and L*er*, respectively. Seedlings were grown for 5 d in continuous darkness (black bars) or transferred to FRc light (dark gray bars), Rc light (light gray bars), or Wc light (white bars) 24 h prior to harvest. The fluence rates used were 390 μmol m^{-2} s^{-1} for FRc and Rc light and 39 μmol m^{-2} s^{-1} for Wc light. B, *PHYA* transcript levels normalized to the 18S rRNA signal were expressed as a percentage of the corresponding value in the dark for each line. The mean of two completely independent experiments was determined, and the bars indicate the range of the two values.

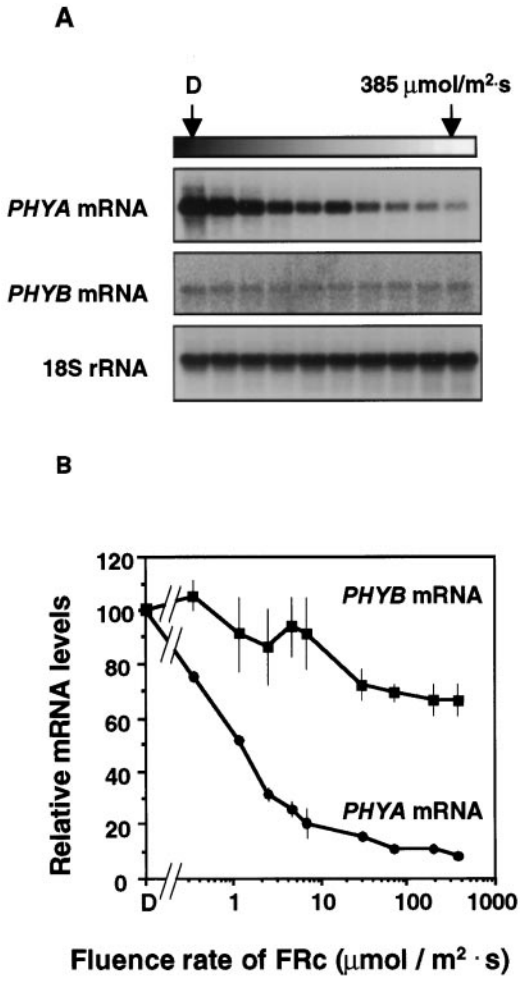

Figure 2. Fluence rate response curve of *PHYA* and *PHYB* mRNA levels in RLD seedlings irradiated with FRc light. A, Four-day-old dark-grown seedlings were exposed to increasing FRc fluence rates (0, 0.3, 1.2, 2.5, 4.7, 7.2, 30, 71, 200, and 385 μmol m^{-2} s^{-1}) for a 24-h period, and 4.5 μg of total RNA was analyzed by northern blot with specific *PHYA*, *PHYB*, and 18s rRNA probes and visualized by autoradiography. B, Relative *PHYA* and *PHYB* mRNA levels as a function of the fluence rate of FRc light. The relative transcript levels were expressed as a percentage of the corresponding value in dark after normalization to the 18S rRNA hybridization signal. The mean of two independent experiments was determined and the bars indicate the range of the two values.

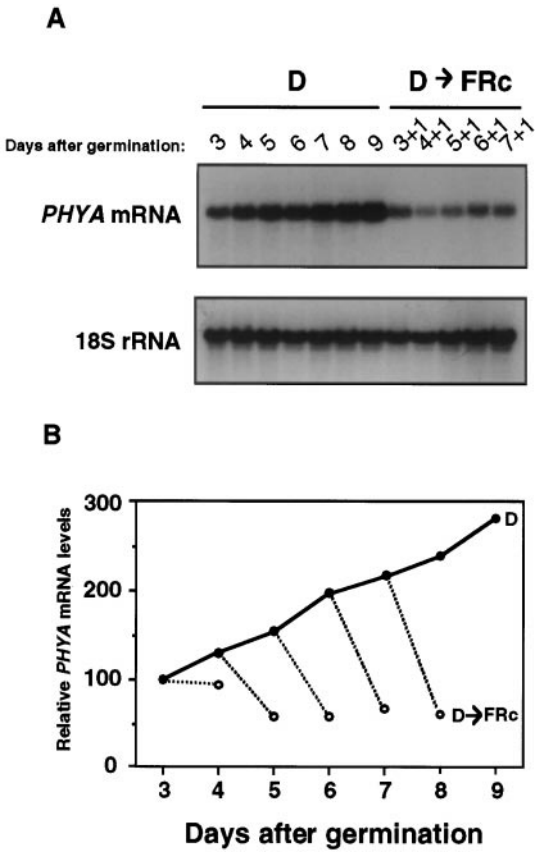

Figure 3. Responsiveness of *PHYA* mRNA levels to FRc illumination in dark-grown seedlings of increasing age. Total RNA was isolated from seedlings grown for increasing numbers of days in continuous dark (D) or irradiated with FRc light for 24 h before harvesting (D → Fc). A, Blot hybridization of 5 μg of total RNA with the *PHYA* and 18S rRNA specific probes. B, Relative *PHYA* mRNA levels at increasing days after germination expressed as a percentage of the transcript amount in 3-d-old dark-grown seedlings after normalization to the 18S rRNA hybridization signal. ●, Non-irradiated seedlings; ○, seedlings irradiated for 24 h before harvesting.

active and is a good indicator of phytochrome action. In addition, the results shown above for the regulation of *PHYA* gene expression are in agreement with the conclusion that only one molecular species of phytochrome (phyA) mediates the response to FRc light. Thus, this light quality provides us with the simplest situation involving a single photoreceptor and one responsive gene.

First, we measured the steady-state levels of *PHYA* mRNA as a function of FRc fluence rate (Fig. 2). *PHYB* mRNA levels were also determined as a control. The accumulation of *PHYA* mRNA was inversely related to fluence rate, with the highest levels in seedlings maintained in complete darkness. Fluence rates as low as 0.4 μmol m^{-2} s^{-1} induced a significant reduction in mRNA accumulation; however, maximal suppression of *PHYA* expression required FRc illumination of moderate intensity (70 μmol m^{-2} s^{-1}). On the contrary, fluence rates below 10 μmol m^{-2} s^{-1} did not have a significant effect on *PHYB* mRNA steady-state levels and only illumination of moderate and high intensities decreased mRNA levels to some extent (to 70% of the dark level). Therefore, the decrease in *PHYA* gene expression appears to be fluence rate dependent over a wide range of FRc fluence rates.

To determine whether the photoresponsiveness of *PHYA* expression to FRc light varied with the developmental age of the seedlings, we examined the time-course of *PHYA* mRNA accumulation in etiolated seedlings and its decline over a 24-h illumination period in seedlings of increasing age (Fig. 3). The quantification data summarized in Figure 3B show that the *PHYA* transcript levels increased during development of the seedlings in darkness at least up to 9 d old. However, irradiation of 4-d or older etiolated seedlings with FRc light for 24 h before harvest suppressed the accumulation of *PHYA* transcript, reducing the steady-state levels to a similar value at all these stages. By contrast, 3-d-old seedlings showed significantly lower relative responsiveness to the FRc light treatment. We also observed differences between seed batches in the relative amount of *PHYA* mRNA accumulated in darkness, possibly because of differences in the rates of seedling development. The resultant variability in levels at the start of the irradia-

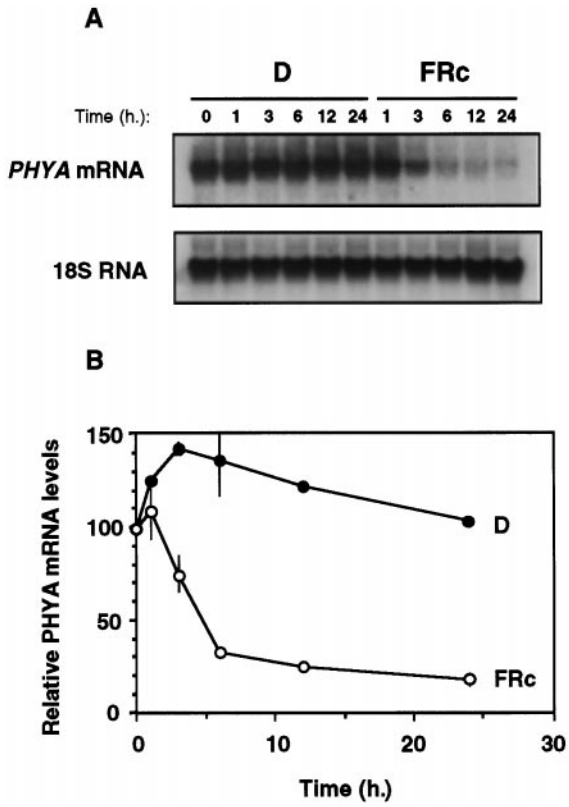

Figure 4. Time course of repression of *PHYA* mRNA levels in response to FRc irradiation. A, Northern blot of total RNA (2.5 μg) isolated from 7-d-old seedlings grown in continuous darkness and transferred to FRc light 0, 1, 3, 6, 12, and 24 h prior to harvest. As a control, samples were harvested at the same time points from seedlings maintained in darkness (D). B, Relative *PHYA* mRNA levels are shown as a percentage of the value at time zero after normalization to the 18S rRNA hybridization signal. The mean of two independent experiments was determined and the bars indicate the range of the two values.

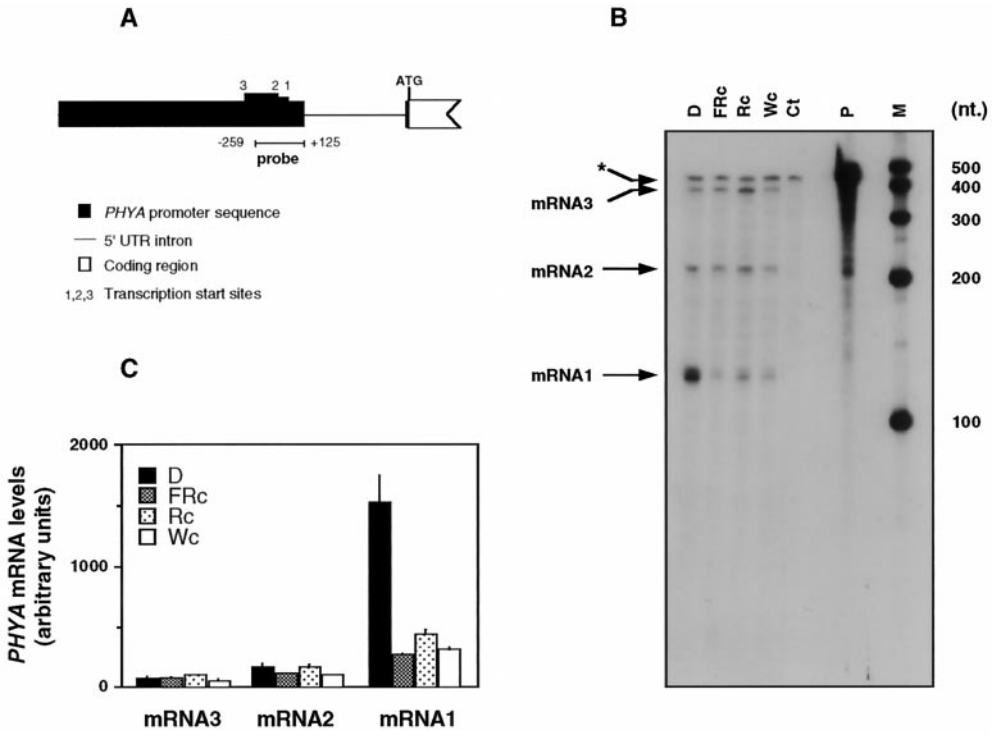

Figure 5. Differential response of the three nested *PHYA* transcript levels to continuous light. Total RNA (8 μg) extracted from 5-d-old seedlings grown in continuous dark (D) or transferred to FRc, Rc, or Wc light 24 h prior to harvest were analyzed by RNase protection assay. A, Schematic of *PHYA* promoter and surrounding DNA and fragment used as a template for riboprobe synthesis. B, Autoradiograph: Ct, 8 μg of yeast RNA; P, full-length undigested probe; M, labeled molecular mass markers; asterisk, full-length undigested probe; nt., nucleotide. C, The hybridization signal for every protected fragment was quantified and corrected for the differences in length. The mean of two independent experiments was determined and the bars indicate the range of the two values.

tion treatments could explain the differences in final relative levels after irradiation observed between Figures 1, 2, and 4.

To determine how rapidly *PHYA* expression is suppressed in FRc light, we determined the short-term kinetics of *PHYA* mRNA levels in 7-d-old etiolated seedlings transferred to FRc light for 24 h. As a control, *PHYA* mRNA levels were also monitored in seedlings maintained in darkness. Figure 4A shows the results of blot hybridization with specific *PHYA* and 18S rRNA probes for seedlings illuminated with FRc light or kept in darkness for the period indicated. The quantification of the hybridization signals showed a significant reduction in *PHYA* mRNA levels within 1 to 3 h following the transfer from dark to light, with only 30% of the zero-time dark levels being detected after 6 h of exposure to FRc light (Fig. 4B). Longer periods of illumination up to 24 h induced a relatively small further decrease in *PHYA* transcript levels, suggesting that the response was almost saturated after 6 h of continuous irradiation.

We wished to determine the relative contributions of the three previously defined Arabidopsis *PHYA* transcripts (Dehesh et al., 1994) to the overall differences in *PHYA* mRNA levels between etiolated and irradiated seedlings. For this purpose, 4-d-old etiolated seedlings were exposed to different light qualities for 24 h and total RNA was analyzed by RNase protection assay using a [α-^{32}P]CTP uniformly labeled riboprobe derived from the 5′-untranslated region of the Arabidopsis *PHYA* gene (Fig. 5A). The autoradiograph (Fig. 5B) shows the three protected fragments derived from the *PHYA* transcripts with the expected sizes (121, 204, and 380 nt) only in the samples from Arabidopsis seedlings. No similar bands were detected in the yeast RNA control. The extra fourth band at the top of all the samples (labeled with an asterisk), including the yeast RNA control, represents the full-length probe remaining after digestion with RNase.

As shown in Figure 5C, the three transcripts contributed quantitatively to different extents to the total pool of *PHYA* mRNA in the seedlings. The shortest transcript (mRNA1) was the most abundant, accounting for most of the *PHYA* transcript levels in the dark, and the abundance of the other two transcripts decreased with length. In addition, most of the quantitative differences observed in *PHYA* mRNA abundance between irradiated and non-irradiated seedlings were the result of the decrease in mRNA1 levels. Although some minor changes were observed in the levels of mRNA2 and mRNA3, their relative contributions to the changes in the total pool of *PHYA* transcript levels were relatively insignificant, because their absolute levels were low in both conditions relative to mRNA1, and the effect of light on their relative levels was small.

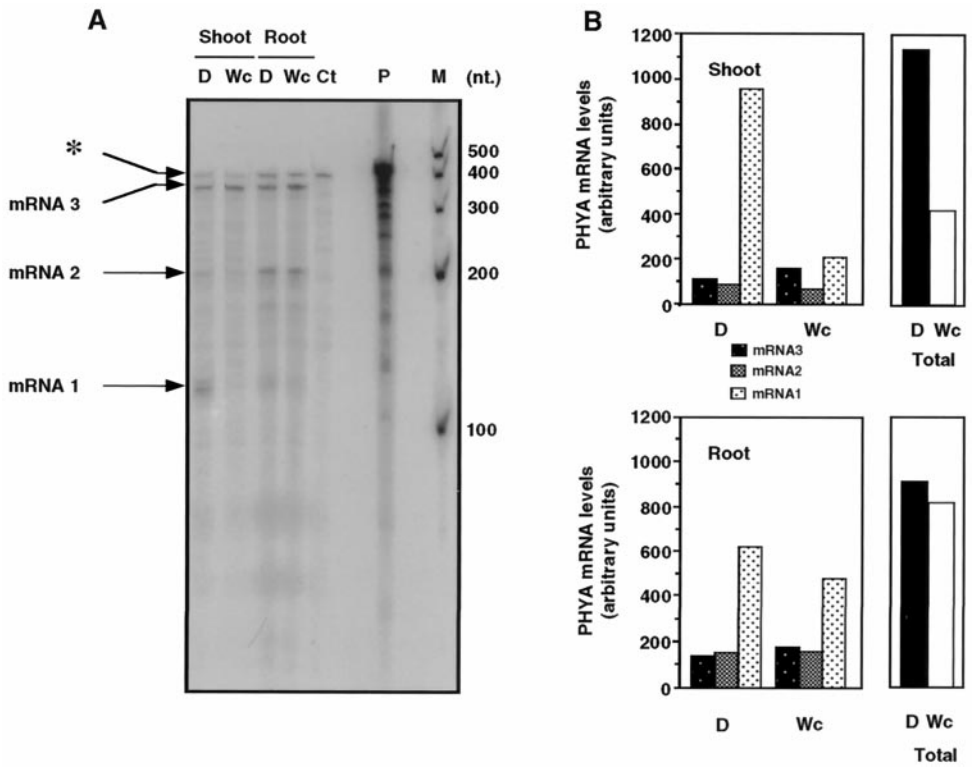

Figure 6. Differential photoresponsiveness of *PHYA* mRNA1 levels in shoots and roots of Arabidopsis seedlings. A, Total RNA (5 μg) from shoots and roots of 7-d-old seedlings grown either in darkness (D) or under 39 μmol m^{-2} s^{-1} Wc light were analyzed by RNase protection assay. Ct, Yeast RNA (5 μg), P, full-length undigested probe; M, labeled molecular mass marker. The size of the RNA markers are indicated in nucleotides on the right. The asterisk indicates full-length undigested probe. B, The hybridization signal for every protected fragment was quantified and corrected for the differences in length. The abundance of each transcript in shoot (top) and roots (bottom) is shown in the left panels. The right panels show the sum of the levels of the three transcripts in each treatment for shoots (top) and roots (bottom). Note that *PHYA* mRNA levels represent relative abundance per mass of total RNA (5 μg) extracted from each organ, so differences between root and shoot do not reflect comparative absolute abundance per organ.

Figure 6 shows the analysis of *PHYA* transcript levels in total RNA extracted separately from shoots and roots of seedlings grown for 7 d completely in darkness or Wc light. Three protected fragments with the same sizes as in Figure 5A were present in all the samples (Fig. 6A), indicating that the three transcripts are accumulated to some extent in both organs. Figure 6B shows quantitatively the relative abundance of each transcript in each sample (left) and the result of totaling the abundance of the three mRNAs (right). mRNA1 is clearly the most abundant transcript in the shoots of dark-grown seedlings. Although for the root samples mRNA1 appears visually to be less abundant than mRNA2 or mRNA3 (Fig. 6A), after integration of the radioactive counts in the more diffuse mRNA1 band (actually three closely spaced bands), and correction for the number of labeled cytidines in the different-length probe fragments, mRNA1 was in fact the most abundant transcript in roots as well (see Fig. 6B, root).

Like in the RNA samples isolated from whole seedlings, the main difference in the overall *PHYA* mRNA levels between shoots of dark-grown and light-grown seedlings was as a result of changes in mRNA1 levels (with Wc-light-grown shoots having 21% of the dark value; Fig. 6B, top). The differences in levels of mRNA2 and mRNA3 contributed little to the overall changes in *PHYA* transcript abundance. Similarly, because mRNA1 was the most abundant transcript in roots, this was also the main transcript accounting for the overall differences in *PHYA* mRNA levels between darkness and light in this organ (Fig. 6B, bottom). However, the extent to which the levels of mRNA1 were reduced by light in roots was comparatively low (78% of the dark value) relative to shoots. As a result, the overall levels of *PHYA* transcripts were nearly the same in light- and dark-grown roots. It is important to note that the levels of mRNAs shown in shoots and roots represent relative abundance per mass of total RNA (5 μg) extracted from each organ. Thus, the levels shown do not correspond to absolute differences in *PHYA* transcript abundance between the two organs in a single seedling because they are not directly comparable.

DISCUSSION

The decrease in *PHYA* mRNA levels in wild-type Arabidopsis seedlings in response to irradiation, not only with Wc light, but also with Rc light and FRc light (Fig. 1),

suggests the involvement of one or more phytochromes. This observation is in close agreement with the previously reported photoregulation of a *PHYA-GUS* transgene in Arabidopsis (Somers and Quail, 1995), suggesting that the changes in the level of *PHYA* transcript shown here may be determined primarily by photocontrol of the transcription rate. Moreover, the data in Figure 1 provide genetic evidence that at least two molecular species of phytochromes (phyA and phyB) are involved in the light-induced decrease of *PHYA* mRNA levels and that, like in the regulation of de-etiolation, phyA and phyB mediate the response of *PHYA* gene expression to FRc and Rc light, respectively. The significant decrease of *PHYA* transcript levels under Wc light compared with that under Rc light in the *phyB* mutant suggests additional regulation by other photoreceptors, such as the cryptochromes.

Previous photobiological studies using pulse R and FR irradiation were interpreted as indicating that *PHYA* expression is regulated by phyA in monocots such as oats (Quail, 1994) and by a stable phytochrome in dicots such as pea and tobacco (Furuya et al., 1991; Adam et al., 1994). The present data were obtained under continuous irradiation conditions, indicating that both phyA and phyB can regulate Arabidopsis PHYA expression via FR and R high-irradiance response modes, respectively (Fig. 1 and 2). Whether other dicots exhibit similar control of *PHYA* remains to be determined. Likewise, it remains to be seen whether monocots null for phyA would exhibit a cryptic R-high-irradiance response under phyB control, once the dominant influence of phyA has been removed.

In terms of kinetics, the response of the Arabidopsis *PHYA* gene to FRc light (Fig. 4) is broadly similar to the response to Wc light in *Cucurbita* (Lissemore et al., 1987), pea (Sato, 1988), and the monocot oat (Colbert et al., 1985). In these four species the level of transcript reached a minimum a few hours after starting the irradiation, suggesting a similar rate of degradation of the transcripts in the different species. On the other hand, significant differences are observed in the extent of the decrease, with Arabidopsis and other dicots showing a quantitatively lower extent of repression than the monocot.

One caveat to this observation is illustrated in Figure 3B, which shows that the responsiveness of *PHYA* mRNA levels to FRc illumination in etiolated seedlings of increasing age in the range of 4 to 8 d was very similar in terms of absolute basal levels of transcript reached after the light treatment, but different in relative levels when expressed as a percentage of the corresponding dark level in seedlings of the same age. This effect was even more pronounced in younger (3-d-old) seedlings (Fig. 3B). This pattern is reminiscent of the decline in spectroscopically detectable phytochrome in mustard seedlings cotyledons in response to FRc light, where the same stable plateau level was reached independent of the starting level (Schäfer et al., 1972). These observations indicate that the extent of repression observed relative to the dark levels depends on the stage of development examined, and raise the possibility that absolute basal levels of *PHYA* transcript in light-grown plants could be more similar in monocots and dicots than was previously thought. The relatively small response of 3-d-old Arabidopsis seedlings to FRc light (Fig. 3B) emphasizes that the response is subject to developmental control.

The levels of the three mRNAs transcribed from the *PHYA* gene in Arabidopsis are differentially regulated by Rc, FRc, and Wc irradiation (Fig. 5). mRNA1 is the major transcript in the dark and the most strongly light-regulated. In contrast, the contribution of the minor mRNA2 and mRNA3 to the overall changes in *PHYA* transcript level is limited. The differential effect of the light treatments on the levels of the three *PHYA* transcripts could be the result of differences in the responsiveness of the transcriptional activities driven from the three different TATA boxes. Alternatively, light could differentially affect the stability of the three mRNAs, mainly altering the degradation rate of the shorter transcript. Although the determinants for RNA stability are considered to reside usually in the 3′ end of transcripts (Gallie, 1993), differences in stability among the three *PHYA* mRNAs determined by elements present in the non-shared region of the 5′-UTRs cannot be ruled out.

The relative position of TATA box 1 in the Arabidopsis promoter, as well as a sequence with homology to the functionally defined *cis* repressor element (Dehesh et al., 1994), are very similar to those in the oat *PHYA* gene (Bruce et al., 1991), suggesting a similar basic promoter structure and repression mechanism in different species. The mRNA1s from the pea *PHYA* and the tobacco *PHYA1* genes are also the major transcripts in these species and the most strongly light regulated (Tomizawa et al., 1989; Adam et al., 1995). However, in the tobacco *PHYA2* gene, mRNA2 instead of mRNA1 is the major and most light-regulated transcript (Adam et al., 1995). A sequence similar to the repressor element identified in oat is also present in the *PHYA* promoter of pea (Sato, 1988; Dehesh et al., 1994), and the discovery that a sequence with homology to this element is also required for light-imposed repression of the Asn synthetase gene promoter from pea (Neuhaus et al., 1997; Ngai et al., 1997) suggests the possibility that this sequence is a common element mediating negative light regulation of plant promoters. However, the functionality of this sequence in dicot *PHYA* genes remains to be determined.

Previous work using transgenic Arabidopsis seedlings harboring a *PHYA-GUS* gene fusion showed differential light regulation of the *PHYA* promoter activity in shoots and roots (Somers and Quail, 1995). A differential organ-specific distribution of the three mRNAs could have explained the differences in photoregulation of *PHYA* between the two organs. In particular, the absence of expression of the light-regulated mRNA1 in roots could have potentially accounted for these observed differences. However, the three transcripts are accumulated to some extent in both organs, and mRNA1 is the major transcript in roots as well as shoots (Fig. 6B). Nevertheless, a significant difference in the extent of the repression imposed by light on the levels of mRNA1 was observed between organs. Whereas only 21% of the dark value of mRNA1 remained in Wc-light-grown shoots, the levels of this transcript in roots were as high as 78% relative to dark-grown

roots. These data suggest that the specific light control of expression driven from TATA box 1 of *PHYA* is strongly modulated by organ-specific factors.

The presence of alternative promoters is postulated to allow more flexibility in the regulation of the gene. In higher eukaryotes, such multiple promoters are frequently associated with genes that are expressed in a tissue-specific and/or developmentally specific manner (Schibler and Sierra, 1987; Ayoubi and Van De Ven, 1996; Myers et al., 1998; Holzfeind et al., 1999). In plants, differential developmental regulation of alternative promoters in the nuclear gene encoding the chloroplast ribosomal protein L21 of spinach (Lagrange et al., 1993) and the use of two alternative promoters from the chalcone flavanone isomerase A gene in various flower tissues of *Petunia hybrida* (Tunen et al., 1989) have been reported.

To our knowledge, dicot *PHYA* is the first plant gene reported with multiple promoters that are differentially regulated by an environmental factor. In this gene the occurrence of two TATA boxes weakly regulated or not regulated by light allows a certain persistent level of *PHYA* expression in light. This could be required to maintain basal levels of phyA protein in the light, where it has an active role in fully de-etiolated plants, as indicated from physiological studies with *phyA* and *phyB* mutants (Johnson et al., 1994; Reed et al., 1994; Whitelam and Devlin, 1997). By contrast, the mRNA1 promoter unit that is strongly expressed in the dark and negatively regulated in the light allows the modulation of *PHYA* expression in response to variable light conditions, and has the capacity to generate high levels of phyA during development in darkness or dark transitions. This pool of phyA generated in the dark and added to the basal pool may be critical for phyA function in dark-to-light transitions and FR-enriched environments in fully green plants. If this is so, it is possible that the monocots evolved a different strategy to compensate for the absence of the two extra TATA boxes. On the other hand, we do not know how mRNA2 and mRNA3 contribute to the cellular phyA protein pool in Arabidopsis, and the presence of three additional short open reading frames in the 5′-UTR of mRNA3 could diminish its rate of translation (Mueller and Hinnebusch, 1986; Oliveira and McCarthy, 1995).

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