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Fluence and Wavelength Requirements for Arabidopsis *CAB* Gene Induction by Different Phytochromes[1]

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The roles of different phytochromes have been investigated in the photoinduction of several chlorophyll *a*/*b*-binding protein genes (*CAB*) of *Arabidopsis thaliana*. Etiolated seedlings of the wild type, a phytochrome A (PhyA) null mutant (*phyA*), a phytochrome B (PhyB) null mutant (*phyB*), and a *phyA/phyB* double mutant were exposed to monochromatic light to address the questions of the fluence and wavelength requirements for *CAB* induction by different phytochromes. In the wild type and the *phyB* mutant, PhyA photoirreversibly induced *CAB* expression upon irradiation with very-low-fluence light of 350 to 750 nm. In contrast, using the *phyA* mutant, PhyB photoreversibly induced *CAB* expression with low-fluence red light. The threshold fluences of red light for PhyA- and PhyB-specific induction were about 10 nmol m^{-2} and 10 μmol m^{-2}, respectively. In addition, *CAB* expression was photoreversibly induced with low-fluence red light in the *phyA/phyB* double mutant, revealing that another phytochrome(s) (PhyX) regulated *CAB* expression in a manner similar to PhyB. These data suggest that plants utilize different phytochromes to perceive light of varying wavelengths and fluence, and begin to explain how plants respond so exquisitely to changing light in their environment.

Plants sense many aspects of light in their environment, including its wavelength, duration, intensity, and direction. Photoregulations in plants have been classified into two categories known as the induction reaction and the HIR in terms of fluence and timing of irradiations, and the former is further divided into the VLF response and the LF response (Briggs et al., 1984; Furuya and Schäfer, 1996). VLF responses can be initiated by fluences as low as 0.1 nmol m^{-2}, whereas LF responses require fluences greater than 1 μmol m^{-2}. HIRs are elicited by prolonged or continuous irradiation that require exposure for hours, and fluences in excess of 10 mmol m^{-2}. Plants monitor their light environment using a number of photoreceptors, the best characterized of which are phytochromes.

Phytochromes are encoded by a small gene family in *Arabidopsis thaliana* (*PHYA* to *PHYE*; Sharrock and Quail, 1989; Clack et al., 1994). Recent progress using phytochrome-deficient mutants of Arabidopsis has shown that the HIR controlling stem extension growth is mediated by both PhyA and PhyB (Quail et al., 1995; Smith, 1995). In contrast, during the photoinduction of seed germination in Arabidopsis, PhyA is responsible for the VLF response, whereas PhyB controls the LF response (Shinomura et al., 1996): specifically, PhyA photoirreversibly induces seed germination upon irradiation with UV, visible, and far-red light of VLF (1–10^2 nmol m^{-2} for red light), whereas PhyB photoreversibly perceives LF red and far-red light (10–10^3 μmol m^{-2}). It was also shown that PhyA induces seed germination with a ratio of its Pfr to total PhyA of less than 0.1%, which corresponds to irradiation in the VLF range (Botto et al., 1996).

One well-analyzed and simple phytochrome-regulated response is the induction of nuclear genes by red-light pulses (Thompson and White, 1991). Among the best-characterized phytochrome-regulated genes are the *CAB*, which encode a structural component of the light-harvesting complex. *CAB* expression is regulated at the transcriptional level by phytochrome (Silverthorne and Tobin, 1984). The induction level of *CAB* versus photon fluence of red light has a biphasic fluence-response curve in pea (*Pisum sativum* L.; Kaufman et al., 1984), indicating that the level of *CAB* transcript is affected by light in the VLF and LF range. *CAB* expression is also regulated by HIR (Wehmeyer et al., 1990). The transcriptional activity of the tobacco *CAB* (*Lhcb1*2*) promoter is responsible for all of the VLF responses, LF responses, and HIRs; therefore, transcription of a single *CAB* is regulated by these three phytochrome action modes (Cerdán et al., 1997). On the other hand, each member of the *CAB* family in pea differs in fluence response, and only a subset possesses a VLF response (White et al., 1995). Both PhyA and PhyB mediate induction of *CAB* expression in Arabidopsis (Reed et al., 1994); however, the wavelength effects and fluence-response relationships for PhyA- and PhyB-specific induction of *CAB* expression are not known. This raises the question of whether PhyA and PhyB discriminate between

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Abbreviations: HIR, high-irradiance reaction; LF, low-fluence; PhyA (or B), spectrally active phytochrome A (or B); PhyX, spectrally active phytochrome species other than PhyA and PhyB; VLF, very-low-fluence.

VLF and LF light to regulate *CAB* expression, as was previously shown for seed germination (Shinomura et al., 1996).

The purpose of this study was to begin to discover the role of individual phytochromes, especially PhyA and PhyB, in the photoregulation of *CAB* expression. We determined the wavelength dependency and fluence-response relationships for photoinduction of *CAB* expression in Arabidopsis wild type, a PhyA null mutant (*phyA*) (Reed et al., 1994), a PhyB null mutant (*phyB*) (Reed et al., 1993), and a *phyA/phyB* double mutant using monochromatic light irradiation generated at the Okazaki large spectrograph (Watanabe et al., 1982). We report differential regulation of *CAB* expression by at least three phytochromes in etiolated seedlings of Arabidopsis.

MATERIALS AND METHODS

Arabidopsis thaliana phytochrome-deficient mutant alleles were *phyA-201(fre1–1)* (Nagatani et al., 1993; Reed et al., 1994) and *phyB-1(hy3-Bo64)* (Koornneef et al., 1980; Reed et al., 1993). A *phyA/phyB* double mutant was newly obtained by crossing *phyA-201* with *phyB-1*, and neither PhyA apoprotein (PHYA) nor PhyB apoprotein (PHYB) were detectable in its 6-d-old etiolated seedling by immunochemical analysis (Fig. 1). The ecotype of the wild type and mutants is Landsberg *erecta*. Seeds were surface sterilized and plated on 0.7% agar containing Murashige-Skoog medium (Murashige and Skoog, 1962) and 2% Suc and then exposed to continuous white light with an intensity of 12 W m^{-2} (FL20SSW/18[G], Hitachi, Tokyo, Japan) for 16 h (for the wild type and the *phyA* and *phyB* mutants) or for 64 h (for the *phyA/phyB* double mutant) to induce germination. After light treatment seeds were transferred to total darkness, and then they were germinated and grown at 24 ± 1°C until light treatment. The 6-d-old seedlings treated in this way were etiolated, with closed, unexpanded cotyledons and an elongated hypocotyl.

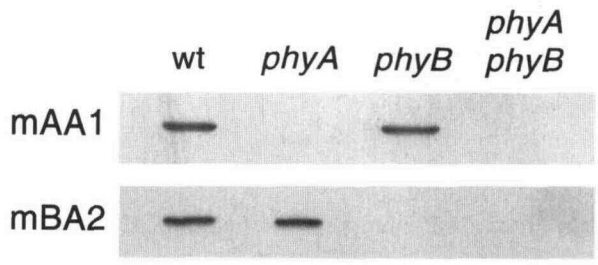

Figure 1. Immunoblot analysis of PHYA and PHYB in 6-d-old etiolated seedlings of Arabidopsis wild type and the phytochrome-deficient mutants. PHYA and PHYB were analyzed by immunoblot detection in 6-d-old-etiolated seedlings of wild-type Arabidopsis (wt), the *phyA* mutant (*phyA*), the *phyB* mutant (*phyB*), and the *phyA/phyB* double mutant (*phyA phyB*). mAA1 and mBA2 are monoclonal antibodies against Arabidopsis PHYA and PHYB, respectively. For the detection of PHYA lanes were loaded with 20 μg of total protein, and 100 μg was used for the detection of PHYB.

Immunochemical Analysis

PHYA and PHYB were analyzed by immunoblot in 6-d-old etiolated seedlings of the wild type and each phytochrome-deficient mutant strain with anti-Arabidopsis PHYA monoclonal antibody, mAA1, and anti-Arabidopsis PHYB monoclonal antibody, mBA2 (Shinomura et al., 1996; Fig. 1). Preparation of extracts from seedlings and immunochemical detection were performed as described previously (Nagatani et al., 1993).

Light Treatment

Etiolated 6-d-old seedlings were exposed to monochromatic light (wavelength 350–800 nm) using threshold boxes at the large spectrograph at the National Institute for Basic Biology (Okazaki, Japan; Watanabe et al., 1982). The duration of exposure and fluence rate differed according to the fluence of irradiation (see figure legends). Samples were maintained at 24 ± 1°C and manipulated using the safelight conditions reported previously (Shinomura et al., 1996). The seedlings were returned to darkness immediately after the light treatment. Four hours after the onset of irradiation, seedlings were harvested, frozen in liquid nitrogen, and stored at −80°C.

RNA Analysis

Total RNA was extracted from seedlings by the phenol/SDS/lithium chloride method (Shirzadegan et al., 1991). For northern-blot analysis, samples of 10 μg of total RNA were separated by electrophoresis on a formaldehyde-containing agarose gel and then transferred to a nylon membrane (Hybond-N, Amersham). The DNA probe was a 1.8-kb fragment of Arabidopsis *CAB3* cDNA labeled with ^{32}P to a specific activity of about 10^8 cpm μg^{-1} DNA by random priming. This probe hybridizes to *CAB1*, *CAB2*, and *CAB3* transcripts. The RNA blotted onto the membrane was hybridized with the probe for 16 to 20 h at 42°C in 50% (v/v) formamide, 5× Denhardt's solution (1× Denhardt's solution is 0.02% Ficoll, 0.02% PVP, and 0.02% BSA), 0.2% SDS, 4× SSC (1× SSC is 150 mM NaCl and 15 mM sodium citrate), and 0.12 mg mL^{-1} salmon-sperm DNA. After hybridization, the membrane was washed in 0.1× SSC at 55°C and then exposed to x-ray film at −80°C with an intensifying screen. The accumulation of *CAB* transcripts was quantified by measuring the radioactivity of each band with an image analyzer (BAS1000, Fujifilm, Tokyo, Japan). The S1 nuclease protection assay was performed as described previously (Millar and Kay, 1991), with the exception that the probe was end-labeled with the fluorescent dye Texas Red instead of radioisotope. Electrophoresis and detection were performed with a fluorescence-based DNA sequencer (SQ5500, Hitachi, Tokyo, Japan). Irradiations and RNA analyses were carried out twice for certain wavelengths and fluences, and reproducibility of the results was observed.

RESULTS

Wavelength Effects in Phytochrome-Deficient Mutants

To examine the effect of light irradiation on *CAB* expression in wild-type Arabidopsis, we exposed 6-d-old etiolated seedlings to blue (425 nm), red (667 nm), and far-red (726 nm) light of 1 or 10^3 μmol m^{-2} and then investigated the accumulation of *CAB* transcripts by northern-blot analysis (Fig. 2A). Exposure of the wild type to blue light of 10^3 μmol m^{-2}, red light of 1 and 10^3 μmol m^{-2}, and far-red light of 10^3 μmol m^{-2} clearly induced *CAB* expression.

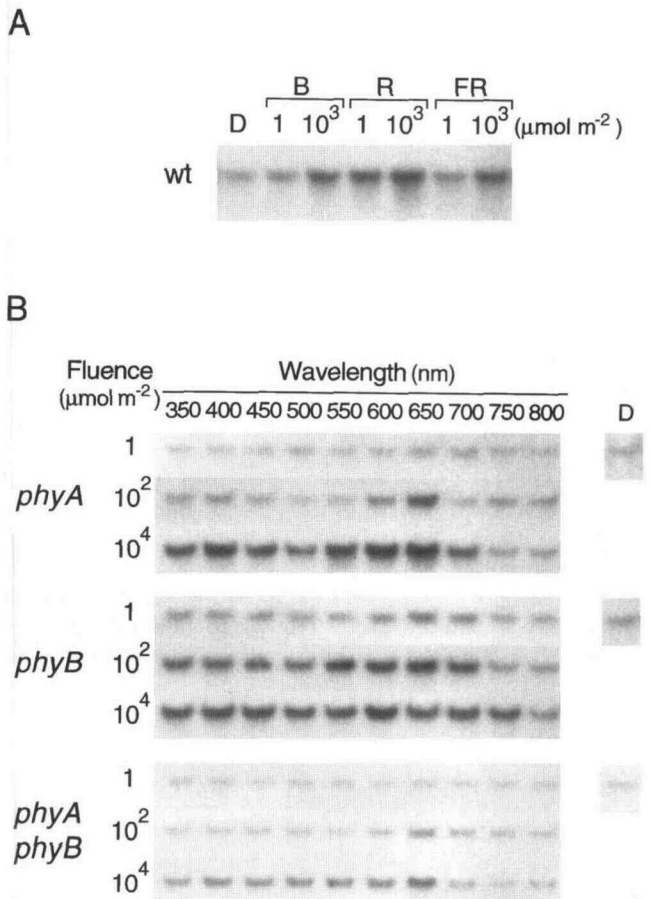

Figure 2. Effects of light irradiation on induction of *CAB* expression. A, Etiolated seedlings of the wild type (wt) were kept in the dark (D) or exposed to a monochromatic pulse of blue light at a wavelength of 425 nm (B), red light of 667 nm (R), and far-red light of 726 nm (FR). The fluences were 1 μmol m^{-2} (fluence rate: 1.0×10^{-1} μmol m^{-2} s^{-1}) or 10^3 μmol m^{-2} (2.5 μmol m^{-2} s^{-1}), respectively. The accumulation of *CAB* transcripts was determined by northern-blot analysis with 10 μg of total RNA in each lane. B, Wavelength effects on induction of *CAB* expression were determined in the *phyA* mutant (*phyA*), the *phyB* mutant (*phyB*), and the *phyA/phyB* double mutant (*phyA phyB*). Etiolated seedlings were kept in the dark (D) or exposed to a monochromatic pulse of light at 10 different wavelengths from 350 to 800 nm with 50-nm intervals. The fluences of irradiation were 1 μmol m^{-2} (fluence rate: 1.0×10^{-1} μmol m^{-2} s^{-1}), 10^2 μmol m^{-2} (2.5 μmol m^{-2} s^{-1}), and 10^4 μmol m^{-2} (2.5 μmol m^{-2} s^{-1}) for each wavelength. The accumulation of *CAB* transcripts was determined by northern-blot analysis with 10 μg of total RNA in each lane.

These results suggest that *CAB* expression was induced by VLF light in wild-type Arabidopsis seedlings.

To find the role of individual phytochromes in photoinduction of *CAB* expression, we determined the wavelength effects for induction of *CAB* expression in the *phyA* mutant, the *phyB* mutant, and the *phyA/phyB* double mutant. The difference in the amount of *CAB* mRNA accumulation in the *phyB* mutant and in the *phyA/phyB* double mutant is expected to be a reflection of the contribution of PhyA. Likewise, the role of PhyB in a particular light treatment is assessed by comparing the *CAB* mRNA accumulation in the *phyA* mutant and in the *phyA/phyB* double mutant. *CAB* mRNA accumulation in the double null mutant suggests whether additional phytochromes are involved in a particular response. We exposed 6-d-old etiolated seedlings of each of the three mutant backgrounds to monochromatic light of wavelengths from 350 to 800 nm, with 50-nm intervals using fluences of 1, 10^2, and 10^4 μmol m^{-2}. The accumulation of *CAB* transcripts was then assessed by northern-blot analysis (Fig. 2B). Overall, the wavelength effects on *CAB* mRNA accumulation can be categorized into two patterns: one that is characteristic of the *phyB* mutant and the other seen in either the *phyA* mutant or the *phyA/phyB* double mutant. In the *phyB* mutant *CAB* expression was induced by irradiation with VLF light of wavelengths from 350 to 750 nm (Fig. 2B). The *phyB* mutant was particularly sensitive to red light (650 nm), with a response seen with a treatment of 1 μmol m^{-2}. In contrast, in the *phyA* mutant or the *phyA/phyB* double mutant, *CAB* expression was induced by light of wavelengths from 350 to 700 nm but not by far-red light of 750 and 800 nm (Fig. 2B). In addition, an estimated 3-orders-of-magnitude higher-fluence light was required to induce an equivalent level of *CAB* mRNA, as seen in the *phyB* mutant. When the wavelength effects were compared in the *phyB* mutant and the *phyA/phyB* double mutant, the results suggested that PhyA is responsible for the response of *CAB* to VLF light in Arabidopsis. From the fluence response in the *phyB* mutant, we can deduce that the threshold fluence for PhyA-specific induction was about 10 nmol m^{-2} for red light. These light treatments also show that PhyA acts over a broad spectrum of light, including the near far red. Moreover, PhyA was the most sensitive photoreceptor for induction of *CAB* accumulation at all wavelengths tested.

In either the *phyA* mutant or the *phyA/phyB* double mutant, *CAB* mRNA accumulation showed the same wavelength and fluence dependency. However, the level of *CAB* mRNA accumulation in the *phyA* mutant was higher than in the *phyA/phyB* double mutant for a specific light treatment. Since the only difference between these two genotypes is the presence or the absence of PhyB, this suggests that PhyB contributes to *CAB* induction upon irradiation with LF light of wavelengths from 350 to 700 nm.

Photoreversible Effects on Induction

The classical view of phytochromes is that they are photochromic molecules that result in red-light induction of *CAB* expression and far-red-light reversal of this induction (Apel, 1979). To determine whether this is true for all

phytochromes, we investigated the red/far-red reversible effects on *CAB* expression in the wild type, *phyA*, *phyB*, and the *phyA/phyB* double mutant by northern-blot analysis (Fig. 3).

In the wild type and the *phyB* mutant, *CAB* expression was induced by either red or far-red light (Fig. 3, lanes R and FR); the induction by red light was not reversed by subsequent far-red-light irradiation (Fig. 3, lane R/FR). In contrast, in either the *phyA* mutant or the *phyA/phyB* double mutant, *CAB* expression was induced by red but not by far-red-light irradiation (Fig. 3, lanes R and FR), and the induction showed red/far-red reversibility (Fig. 3, lanes R/FR and R/FR/R). Partial reversion observed in the *phyA* mutant might be caused by an escape of the signal from phytochrome before far-red-light irradiation. These studies show that in the presence of PhyA induction of *CAB* expression was photoirreversible, but in the absence of PhyA induction was photoreversible. Considering the induction by far-red-light irradiation, this result suggests that PhyA-specific induction of *CAB* expression is photoirreversible. Moreover, in the *phyA/phyB* double mutant, *CAB* expression was still induced by red-light irradiation and this induction was reversed by subsequent far-red-light irradiation, suggesting that a phytochrome in addition to PhyA and PhyB is involved in photoregulation of *CAB* expression. Since we do not know the identity of this phytochrome (e.g. PhyC, PhyD, or PhyE), we refer to it as PhyX. Therefore, PhyX-specific induction is photoreversible. The level of induction in the *phyA* mutant was higher than in the *phyA/phyB* double mutant, showing that PhyB and PhyX have an additive effect on induction in the *phyA* mutant. This suggests that PhyB-specific induction is photoreversible. As a result, both PhyB and PhyX appear to induce *CAB* expression by a photoreversible mechanism.

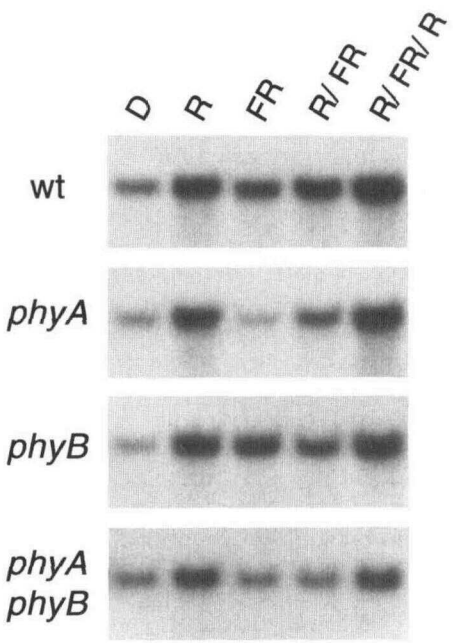

Figure 3. Photoreversible effects on induction of *CAB* expression. Etiolated seedlings of the wild type (wt), the *phyA* mutant (*phyA*), the *phyB* mutant (*phyB*), and the *phyA/phyB* double mutant (*phyA phyB*) were kept in the dark (D) or exposed to red light (wavelength: 667 nm) with a fluence of 3×10^2 μmol m^{-2} (R), far-red light (726 nm) with a fluence of 10^3 μmol m^{-2} (FR), red light followed by far-red light (R/FR), and red light followed by far-red light, and then red light again (R/FR/R). The fluence rates of irradiation were 3.0×10^1 μmol m^{-2} s^{-1} for red light and 3.5×10^1 μmol m^{-2} s^{-1} for far-red light; therefore, the duration of red light irradiation and far-red light irradiation was 10 and 29 s, respectively. The time interval between initial red light and the following far-red light was about 20 s in red light/far-red light and red light/far-red light/red light; similarly the time interval between far-red light and the second red light was also about 20 s in red light/far-red light/red light. The accumulation of *CAB* transcripts was determined by northern-blot analysis with 10 μg of total RNA in each lane.

Table I. *Photoinduction of CAB1 and CAB2/CAB3 expression in Arabidopsis wild type and the phytochrome-deficient mutants*

Photoinduction of *CAB1* and *CAB2/CAB3* expression was investigated in the wild type (wt), the *phyA* mutant (*phyA*), the *phyB* mutant (*phyB*), and the *phyA/phyB* double mutant (*phyA phyB*). Their etiolated seedlings were kept in the dark (D) or exposed to pulses of blue light at a wavelength of 425 nm (B), red light at a wavelength of 667 nm (R), and far-red light at a wavelength of 726 nm (FR) with a fluence of 10^3 μmol m^{-2} (fluence rate: 2.5 μmol m^{-2} s^{-1}).

Strain	Gene	Relative Amounts of Transcripts			
		Light treatment			
		D	B	R	FR
wt	*CAB1*	51	82	100*	67
	CAB2/3	9	15	20	14
phyA	*CAB1*	48	48	100*	51
	CAB2/3	6	6	20	6
phyB	*CAB1*	55	79	100*	76
	CAB2/3	5	8	14	10
phyA	*CAB1*	47	49	100*	43
phyB	*CAB2/3*	7	5	11	6

* The amounts of *CAB* transcripts were standardized with the level of the accumulation of *CAB1* transcript by red light irradiation as 100 in each strain.

Regulation of Individual *CAB*s by Phytochromes

In Arabidopsis *CAB* constitutes a gene family, and *CAB1*, *CAB2*, and *CAB3* are the most conserved, having 96% DNA sequence identity within the translated region (Leutwiler et al., 1986). In the RNA gel-blot experiments described above, we used *CAB3* cDNA as a probe, which simultaneously detects *CAB1*, *CAB2*, and *CAB3* transcripts. To analyze the photoregulation of individual *CAB*s by different phytochromes, we investigated the accumulation of *CAB1* transcript versus *CAB2/CAB3* transcripts in the wild type, *phyA*, *phyB*, and the *phyA/phyB* double mutant by S1 nuclease protection assay (Table I). Unfortunately, it is impossible to separate *CAB2* and *CAB3* transcripts by this method because of the extremely high homology between these genes (Leutwiler et al., 1986).

Etiolated seedlings were exposed to blue (425 nm) or far-red (726 nm) light of 10^3 μmol m^{-2} for PhyA-specific induction and to red (667 nm) light of 10^3 μmol m^{-2} for PhyA-, PhyB-, and PhyX-specific inductions. The results showed that the patterns of induction of *CAB1* and *CAB2/*

CAB3 were similar, irrespective of the light treatments. However, the amount of *CAB1* transcript was 5- to 10-fold higher than that of *CAB2/CAB3* transcripts, indicating that *CAB1* transcript constituted the bulk of *CAB* transcripts in our previous experiments. The patterns for induction in the wild type, *phyA*, *phyB*, and the *phyA/phyB* double mutant showed that PhyA, PhyB, and PhyX all contribute to the photoinduction of *CAB1* expression, as well as to that of *CAB2/CAB3*.

Blue/Far-Red Reversible Effects on Induction

In wild-type Arabidopsis *CAB* expression was induced by blue light of 10^3 μmol m^{-2} (Fig. 2A). Based on the mutant analysis, PhyA appears to be the most sensitive receptor for blue light, with a threshold fluence of about 1 μmol m^{-2}. In contrast, blue light of 10^4 μmol m^{-2} was required to induce *CAB* expression in the *phyA* mutant and the *phyA/phyB* double mutant (Fig. 2B). The level of induction was higher in the *phyA* mutant than in the *phyA/phyB* double mutant, suggesting that PhyB is involved in the *CAB* induction by blue light. To define this phenomenon more precisely, we investigated the blue/far-red light reversible effect on *CAB* expression in the *phyA* mutant and the *phyA/phyB* double mutant by northern-blot analysis (Fig. 4).

In either the *phyA* mutant or the *phyA/phyB* double mutant, *CAB* expression was induced by blue light (400 nm) of 10^4 μmol m^{-2} but not by far-red light (726 nm) of the same fluence (Fig. 4, lanes B and FR). These inductions by blue light were cancelled by subsequent far-red-light irradiation (Fig. 4, lane B/FR); however, expression could be induced again with another blue-light pulse (Fig. 4, lane B/FR/B). Therefore, both in the *phyA* mutant and *phyA/phyB* double mutant, induction of *CAB* expression showed blue/far-red reversibility. Moreover, *CAB* mRNA accumulation by blue-light irradiation was higher in the *phyA* mutant than in the *phyA/phyB* double mutant (Fig. 4, lane B); therefore, PhyB and PhyX appear to have an additive effect on induction. We conclude that conversion of PhyB and/or PhyX to Pfr is essential for blue-light induction of *CAB* expression.

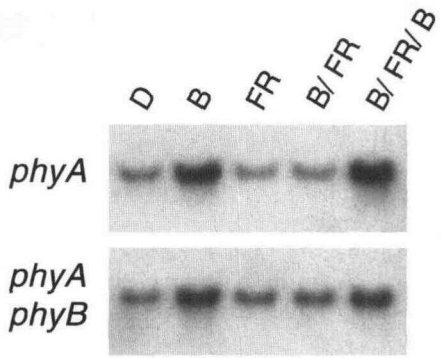

Figure 4. Blue/far-red reversible effects on induction of *CAB* expression. Etiolated seedlings of the *phyA* mutant (*phyA*) and the *phyA/phyB* double mutant (*phyA phyB*) were kept in the dark (D) or exposed to blue light (wavelength: 400 nm) with a fluence of 10^4 μmol m^{-2} (B) or far-red light (726 nm) with a fluence of 10^4 μmol m^{-2} (FR), blue light followed by far-red light (B/FR), and blue light followed by far-red light, and then blue light again (B/FR/B). The fluence rates of irradiation were 7.0×10^1 μmol m^{-2} s^{-1} for both blue light and far-red light; therefore, the duration of each irradiation was 143 s. The time interval between the initial blue light and the following far-red light was about 20 s in blue light/far-red light and blue light/far-red light/blue light; similarly, the time interval between far-red light and the second blue light was also about 20 s in blue light/far-red light/blue light. The accumulation of *CAB* transcripts was determined by northern-blot analysis with 10 μg of total RNA in each lane.

DISCUSSION

The Role of Different Phytochromes

We addressed the wavelength and fluence requirements for induction of *CAB* expression by different phytochromes using Arabidopsis phytochrome-deficient mutants and the Okazaki large spectrograph. Our results allow us to conclude the following: First, PhyA acts to induce *CAB* expression in the VLF range of light over a broad range of wavelengths (350–750 nm). The threshold fluence was about 10 nmol m^{-2} for red light. Moreover, PhyA-specific induction of *CAB* expression is photoirreversible by far-red light. Second, in contrast, PhyB induces *CAB* expression in red light (650 and 667 nm) but requires about 3-orders-of-magnitude higher fluences than PhyA for an equal level of induction. PhyB-specific induction of *CAB* expression is photoreversible by far-red light. Third, at least one other phytochrome in addition to PhyA and PhyB (PhyX) controls *CAB* induction by red light in a photoreversible manner. Furthermore, PhyA, PhyB, and PhyX also contribute to the blue-light induction of *CAB* expression. Together, these data suggest that at least in this system multiple phytochromes contribute to the induction of even this simple molecular response to light.

The fluence and wavelength requirements for PhyA- and PhyB-specific induction of *CAB* expression are similar to our previous results for the photoinduction of seed germination (Shinomura et al., 1996), showing that phytochromes can act over a broad spectrum of light. Moreover, PhyA is responsible for the VLF induction of *CAB* expression, and PhyA is not a classical phytochrome in terms of red/far-red photoreversibility. Although it had been shown that *CAB* expression is induced by VLF red light (Kaufman et al., 1984) and by far-red light (Kaufman et al., 1984; Karlin-Neumann et al., 1988; Wehmeyer et al., 1990; Gao and Kaufman, 1994), our data now allow us to assign these responses to PhyA. The induction by red light (650 and 667 nm) in either the *phyA* mutant or the *phyA/phyB* double mutant showed a similar threshold fluence of 10 μmol m^{-2}, implying that PhyB- and PhyX-specific induction have a similar threshold fluence, 10 μmol m^{-2}. Furthermore, both PhyB- and PhyX-specific induction of *CAB* expression are photoreversible, allowing us to conclude that PhyB and PhyX induce *CAB* expression in LF light by a similar mode of action and fluence requirement. In addition, in either the *phyA* mutant or the *phyA/phyB* double mutant, light treatments of 350 to 700 nm were capable of inducing *CAB* expression. However, far-red light was in-

effective in these mutant backgrounds. The higher level of induction in the *phyA* mutant suggests that PhyB is involved in the induction of *CAB* expression by irradiation with LF light from the near UV to red.

To our knowledge, this study is the first in which the mode of action and the fluence requirements of a phytochrome other than PhyA and PhyB has been precisely determined. Although Reed et al. (1994) inferred the possible contribution of additional phytochromes in *CAB* mRNA accumulation, they could not make the sophisticated measurements that are possible at the Okazaki large spectrograph. There have been reports of phenomena that are regulated by phytochromes other than PhyA and PhyB. For instance, far-red induction of the homeotic gene *Athb-2* is mediated mainly by phytochrome(s) other than PhyA and PhyB (Carabelli et al., 1996). Bagnall et al. (1995) suggested the possibility of the involvement of phytochrome(s) other than PhyA and PhyB in the end-of-day far-red response of flowering. It was also reported that the *phyA/phyB* double mutant displays an inhibition of petiole elongation accompanied by elongated internodes and an early-flowering response to end-of-day far-red treatment. Internode elongation and early flowering were abolished by a subsequent red-light treatment, suggesting regulation by a phytochrome(s) other than PhyA and PhyB (Devlin et al., 1996). We do not know how this phytochrome(s) relates to the role that PhyX plays in regulation of *CAB* expression in etiolated seedlings.

In the blue-light irradiation of 10^4 μmol m^{-2}, the induction level by irradiation with an intensity of 2.5 μmol m^{-2} s^{-1} was slightly higher than the induction level by irradiation with an intensity of 7.0×10^1 μmol m^{-2} s^{-1} in the *phyA* mutant and the *phyA/phyB* double mutant, suggesting an effect of HIR. In red-light irradiations in the LF range tested in the present study, the level of induction was in proportion to the logarithm of fluence, and reciprocity of induction was observed.

In addition to photoregulation, *CAB* expression is regulated by circadian rhythm (Millar and Kay, 1991). In dark-grown seedlings circadian oscillation was not clearly distinguishable in accumulation of *CAB1* mRNA (Brusslan and Tobin, 1992) and in the transcriptional activity of the *CAB2* promoter (Millar and Kay, 1996), suggesting that there may be little or no influence of the circadian clock on the induction level of *CAB* expression in our experiment. However, the effect of germination treatments on circadian rhythm is still unknown. Therefore, there is a possibility that phytochrome responsiveness of *CAB* induction is affected by light treatments for induction of seed germination.

This study shows that *CAB* expression is differentially regulated by at least three phytochromes: PhyA, PhyB, and PhyX. Taken together with our previous studies of seed germination, we propose that phytochromes play discrete roles in controlling plant responses to light fluence and wavelength. In etiolated seedlings the major phytochrome, PhyA (Somers et al., 1991), induces *CAB* expression upon irradiation with VLF light in a wide range of wavelengths, from the near UV to far-red, and the effects of PhyB- and PhyX-specific inductions are masked by the dominating effect of PhyA. Since *CAB*s encode structural components of the light-harvesting complex that are essential for photosynthesis, it makes sense that VLF light of any wavelength should trigger de-etiolation, thereby allowing the accumulation of the photosynthetic apparatus and preparing the seedlings for life above the ground. After exposure to light, PhyA is degraded rapidly (Somers et al., 1991), and PhyB and PhyX regulate *CAB* expression in a photoreversible manner by monitoring the ratio between near-UV and visible light versus far-red light in the environment.

Blue-Light Effects on Induction of *CAB* Expression

There are at least three major photoreceptor systems in plants: phytochromes, blue/UV-A receptors, and UV-B receptors (Kendrick and Kronenberg, 1994). Several blue-light responses have been described in higher plants, and they have been categorized as either photoirreversible or photoreversible. It is thought that the photoirreversible responses are mediated by a unique, blue/UV-A receptor (Kaufman, 1993) or PhyA (Shinomura et al., 1996). Blue/far-red reversible regulation of coleoptile elongation in rice (Paul and Furuya, 1973) and adventitious shoot formation in horseradish (Saitou et al., 1993) have also been attributed to phytochrome.

Previous studies have shown that the expression of *CAB* is induced by blue light in pea (Sasaki et al., 1988; Marrs and Kaufman, 1989), tomato (Oelmüller et al., 1989; Wehmeyer et al., 1990), tobacco (Wehmeyer et al., 1990), and Arabidopsis (Gao and Kaufman, 1994). What is the receptor for blue-light induction of *CAB* expression? These studies have concluded that blue-light induction of *CAB* expression is regulated by the blue/UV-A photoreceptor system (Marrs and Kaufman, 1989; Oelmüller et al., 1989; Wehmeyer et al., 1990; Gao and Kaufman, 1994) or that there is a co-action between the blue/UV-A receptor and phytochrome (Oelmüller et al., 1989). In this report we demonstrated that PhyA, PhyB, and PhyX also contribute to the blue-light regulation of *CAB* expression in etiolated seedlings of Arabidopsis. PhyA induced *CAB* expression by blue light with a threshold fluence of about 1 μmol m^{-2}, suggesting that PhyA is the most sensitive blue-light receptor for induction of *CAB* expression. Furthermore, in either the *phyA* mutant or the *phyA/phyB* double mutant, *CAB* expression was induced by blue light with a threshold fluence of 10^3 μmol m^{-2}; this induction showed blue/far-red reversibility. Therefore, conversion of PhyB and/or PhyX to Pfr is essential for the blue-light induction of *CAB* expression.

There are at least two explanations for the blue/far-red reversible regulation of *CAB* expression. One is that signals from PhyB and PhyX directly regulate *CAB* expression in a blue/far-red reversible manner. The other is that there is an interaction between a blue/UV-A receptor and phytochrome(s) (Oelmüller et al., 1989; Mohr, 1994), and a signal from this blue/UV-A receptor is interrupted by PhyB and/or PhyX in a blue/far-red reversible manner. Our studies do not address the possible involvement of the blue/UV-A receptors. Gao and Kaufman (1994) investigated the involvement of blue light in induction of *CAB* expression and reported that *CAB1* was regulated by blue light inde-

pendently of the action of phytochrome. In other studies, however, it was shown that the *CAB1* promoter is regulated in a blue/far-red reversible manner via CA-1 DNA-binding activity, which is essential for phytochrome responsiveness in vivo (Kenigsbuch and Tobin, 1995). Thus, it appears that blue-light-induced *CAB* expression is controlled by a blue/UV-A photoreceptor, as well as by multiple phytochromes. The induction is regulated primarily by PhyA.

Regulation of Individual *CAB*s by Phytochromes

In Arabidopsis light-harvesting chlorophyll *a/b*-binding proteins are encoded by a family of *CAB*s (Leutwiler et al., 1986; Zhang et al., 1991; Jensen et al., 1992; McGrath et al., 1992), the best studied of which are *CAB1*, *CAB2*, and *CAB3*. These three *CAB*s are highly similar and are tandemly arrayed in one chromosomal cluster (Leutwiler et al., 1986), and both *CAB1* and *CAB2/CAB3* are regulated by phytochrome (Karlin-Neumann et al., 1988). Here we show that expression of *CAB1* and *CAB2/CAB3* was regulated similarly by PhyA, PhyB, and PhyX, although the major transcript is *CAB1*. It was previously reported that *CAB1* mRNA is highly expressed in etiolated seedlings after light treatments and that the *CAB1* mRNA level is at least 5 times higher than that of *CAB2/CAB3* mRNA (Karlin-Neumann et al., 1988), but to our knowledge, this is the first study in which expression of both *CAB1* and *CAB2/CAB3* was shown to be regulated by multiple phytochromes. Millar and Kay (1991) reported that the transcription rate of both *CAB1* and *CAB2* are circadian regulated, but posttranscriptional events stabilize the *CAB1* transcript. This may account for the higher amount of *CAB1* mRNA seen in our studies.

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