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Blue-Light Regulation of the *Arabidopsis thaliana Cab*1 Gene[1]

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The steady-state level of *Cab* RNA in etiolated *Arabidopsis thaliana* increases as a result of a single pulse of blue light. The threshold for the response is at or below 10^0 μmol m^{-2} and begins within 1 h of irradiation. The response is not prevented by far-red treatment, and the blue-light source used does not elicit an observable very low fluence phytochrome response for *RbcS* RNA. The time course for blue-light-induced transcript accumulation differs from that of red, the blue beginning more quickly. Transcripts derived from the *Cab*1 (AB140; Lhcb1*3) member of the gene family are responsible in part for the blue-light-induced accumulation. This is the same member of the gene family that is responsible for phytochrome-induced *Cab* gene expression (G.A. Karlin-Neumann, L. Sun, E.M. Tobin [1988] Plant Physiol 88: 1323–1331). The mutant *hy*4, which lacks blue-light-induced suppression of hypocotyl elongation, retains the ability of *Cab* RNA to respond to blue light.

Higher plants are capable of sensing and responding to environmental light signals, discriminating among wavelength, intensity, and duration. These light signals serve to initiate or modify endogenous developmental programs.

At least three classes of photomorphogenic system are available for light perception as defined by the wavelengths absorbed by the photoreceptor: (a) phytochromes (responding primarily in the red and far red) (Furuya, 1993), (b) blue/UV-A receptors (Ahmed and Cashmore, 1993; Kaufman, 1993), and (c) UV-B receptors (Mohr, 1986). These three systems must integrate both the perceived environmental information and the final response. The situation becomes more complex when considering that the phytochrome and blue-light systems each define multiple systems; for example, the VLF, LF, and HIR responses for phytochrome (Mancinelli and Rubino, 1974; Kaufman et al., 1984, 1985), and LF and high-fluence system for blue light (Warpeha et al., 1989; Warpeha and Kaufman, 1990).

The UV-B-generated events are generally not inductive in that the response depends on long-term or continuous irradiation. These may represent developmental or cascade events removed from the light perception. This is in contrast to the phytochrome and blue-light systems, which are both capable of responding to a single, short pulse of light (seconds), with very low total fluences (less than 10^{-1} μmol m^{-2}). Such inductive events are not likely to depend on gene cascade or developmental events. Indeed, several events regulated by the blue-light and/or phytochrome systems are known to occur in the absence of protein synthesis (Marrs and Kaufman, 1991).

Inductive regulation of nuclear gene expression has been defined for both the phytochrome and the blue-light systems (Thompson and White, 1991). The signal transduction mechanisms leading to the altered expression of specific nuclear-coded genes by any of these systems remain unclear. However, regulation of specific genes or gene families in the absence of cytoplasmic protein synthesis has been observed for both systems, confirming that gene expression is unnecessary. This is true for blue-light regulation of the gene family encoding Chl *a*/*b*-binding proteins (*Cab*) in pea (Marrs and Kaufman, 1991) and phytochrome regulation of the *Cab* gene family in barley (Merkle and Schafer, 1988).

Several gene families are known to be regulated by more than one photomorphogenic system. This is the case for the *Cab* gene family in pea, in which there have been demonstrations of regulation by both the VLF and LF phytochrome responses (Kaufman et al., 1984, 1985) and the LF blue-light response (Warpeha and Kaufman, 1990; Marrs and Kaufman, 1991). Thus, a single pulse of either red or blue light, acting through independent photomorphogenic systems, will activate the *Cab* gene family in etiolated pea seedlings. It would be of interest to determine whether specific members of the *Cab* gene family have the potential for activation by both the phytochrome and blue-light systems or whether individual family members respond to individual photomorphogenic systems.

Arabidopsis thaliana, wherein several members of the *Cab* gene family have been cloned and sequenced (Leutwiler et al., 1986; McGrath et al., 1992), is a useful system for such a study. Furthermore, because *Arabidopsis* can be transformed and regenerated, it represents a useful system in which to study *cis*-acting elements crucial for blue-light-mediated effects on transcription. Karlin-Neumann et al. (1988) established that the *Cab*1 (also known as AB1140 or Lhcb1*3) gene and not the *Cab*2 (also known as AB165 or Lhcb1*1) or *Cab*3 (also known as AB180 or Lhcb1*2) genes is phytochrome responsive in etiolated seedlings. We have used this same system to determine which member(s) of the *Arabidopsis Cab* gene family (if any) is regulated through a blue-light system.

The results herein demonstrate that the *Cab*1 gene of *Arabidopsis* is regulated by a blue-light system independently of the regulation by phytochrome. Therefore, this single

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Abbreviations: HIR, high irradiance; LF, low fluence; VLF, very low fluence.

member of the *Cab* gene family is co-regulated by both inductive photomorphogenic systems. Furthermore, the kinetics of induction differ between phytochrome and blue-light system excitation. The blue-light effect on the *Cab* gene persists in the *hy4* mutant, which is lacking in blue-light-induced suppression of hypocotyl elongation (Koornneef et al., 1980) and may represent a mutation in the photoreceptor controlling this blue-light-mediated event (Ahmed and Cashmore, 1993).

MATERIALS AND METHODS

Plant Growth Conditions

Stocks of *Arabidopsis thaliana* (L.) Hynh. (ecotype Columbia or Landsberg) seed collected from greenhouse-grown plants were stored at 4°C, surface sterilized with 40% commercial bleach for 10 min, rinsed three times with sterile water, and spread on filter paper on top of 0.5× Murashige-Skoog salt-saturated (Sigma), unbleached Kimpack (Kimberly-Clark, Neenah, WI). Sterilization and planting were performed under a green safelight. Light treatments were administered after 6 d of dark growth. Plants were returned to the dark after light treatments and harvested 4 h later, except as indicated for time-course experiments. Control seedlings received the same treatment as irradiated seedlings except the shutter on the light source was not opened.

Wild-type and mutant seedlings used in the *hy4* experiment were sown and kept in the dark at 4°C for 16 h and then exposed to a dim red light (0.2 μmol m^{-2} s^{-1}) for 3 h. This was done to overcome the poor germination rate of *hy4*. Seedlings were then transferred to the dark at 21°C for 5 d, after which light treatments were administered.

The light sources used are described elsewhere (Warpeha and Kaufman, 1990). The fluences and times of irradiation are described below in this paper.

Fluence-Response Experiments

Dark-grown, wild-type *Arabidopsis* seedlings (6 d old) were irradiated with a total fluence of 10^{-1}, 10^{0}, 10^{1}, 10^{2}, or 10^{3} μmol m^{-2} of blue light. Irradiations were for 10 s except for the 10^{3} μmol m^{-2} treatment, which was 100 s. Treated and control seedlings were harvested and quick frozen in liquid N_2 for RNA extraction.

Time-Course Experiments

Etiolated, wild-type seedlings (6 d old) were harvested 0, 0.5, and 1.0 h after irradiation with 10^{1} μmol m^{-2} of blue light. Unirradiated seedlings were harvested at each time point to serve as a control. Tissue was quick frozen for use in RNA extraction.

Phytochrome-Response Experiments

Separate trays of 6-d-old etiolated seedlings were treated with either red light (total fluence of 2.7×10^{3} μmol m^{-2} of red light delivered in 1 min), far-red light (total fluence of 5.4×10^{5} μmol m^{-2} delivered in 10 min), blue light (total fluence of 10^{1} μmol m^{-2} delivered in 10 s), red light followed immediately by far-red light, or blue light followed immediately by far-red light. Seedlings were harvested 4 h after the onset of irradiation and quick frozen for use in RNA extraction.

RNA Isolation and Northern Analysis

Total RNA was extracted as described previously (Marrs and Kaufman, 1989) except that aurintricarboxylic acid was omitted from the extraction and resuspension buffers. The concentration of the RNA samples was determined spectrophotometrically. RNA samples (10 μg) were separated on 1.5% agarose gels containing formaldehyde and transferred to GeneScreen (Dupont, Wilmington, DE). RNA concentration per lane was confirmed by ethidium bromide staining. Probes used for northern analysis were either a mixture of radiolabeled *Eco*RI/*Sst*I fragments from the *Arabidopsis Cab*1 and *Cab*3 genomic clones (Leutwiler et al., 1986) or the 500-bp *Hin*dIII fragment from the coding region of *Arabidopsis RbcS* genomic clone pATS-3 (Krebbers et al., 1988). Hybridization was performed at 42°C for 14 to 16 h in a solution containing 50% formamide. Membranes were washed three times in 0.6× SSC, 0.1% SDS solution at 65°C.

RNase Protection Assays

RNase protection assays were carried out essentially as described by Sambrook et al. (1989). Radiolabeled antisense RNA probes were hybridized to 20 μg of total RNA in 30 μL of hybridization buffer (80% formamide, 40 mM Pipes [pH 6.4], 1 mM EDTA, 400 mM NaCl) at 38 to 42°C overnight. Digestion was performed at 30°C for 1 h in 300 μL containing 3 μg/mL RNase T1, 55 μg/mL RNase A, 10 mM Tris-HCl (pH 7.5), 300 mM NaCl, 5 mM EDTA. The reaction was terminated by the addition of 20 μL of 10% SDS, 100 μg of proteinase K, followed by phenol/chloroform extraction. RNA was subsequently precipitated with ethanol, dissolved in 8 μL of 80% formamide, and separated by length in a 6.0% denaturing polyacrylamide gel.

Template Plasmid Construction and Antisense RNA Probes

The *Cab*1 antisense RNA probe was generated by RNA polymerase T3 from pCA1 (BlueScribe containing the *Cab*1 *Eco*RI/*Sca*I fragment from −1396 to +174) that had been linearized by *Eco*RV at base −195. The resulting RNA probe is approximately 400 bp long and is expected to protect a 237-bp fragment.

The *Cab*3 antisense RNA probe was generated by RNA polymerase T3 from pCA3 (BlueScribe containing the *Cab*3 genomic DNA *Eco*RI/*Bam*HI fragment from −958 to +157) that had been linearized with *Dra*I. The resulting RNA probe is approximately 500 bp long and is expected to protect a 209-bp fragment.

RESULTS

Fluence Response

A single pulse of blue light consisting of a defined fluence ranging from 10^{-1} to 10^{3} μmol m^{-2} was used to irradiate 6-

d-old, etiolated *Arabidopsis* seedlings. The response of *Cab* RNA was evaluated by measuring changes in the steady-state level of *Cab* RNA 4 h after the blue-light pulse (Fig. 1). Figure 1A shows the result of one experiment. Densitometry was used to quantify the relative amount of *Cab* RNA, and the results of the three independent experiments are presented in Figure 1B. The data demonstrate that the *Arabidopsis Cab* gene family is blue-light regulated and that the threshold fluence is at or below 10^0 μmol m^{-2}.

Time Course

The time course for accumulation of *Cab* RNA was measured in response to 10^1 μmol m^{-2} of blue light. Measurements were carried out at 0.5 and 1.0 h after the light treatment. The results (Fig. 2) indicate that the increase in *Cab* RNA begins between 0.5 and 1 h after a blue-light pulse.

The Blue-Light Response Is Independent of Phytochrome Excitation

Karlin-Neumann et al. (1988) have shown that the steady-state level of *Arabidopsis Cab* RNA increases in response phytochrome excitation. Blue light, which can be absorbed by phytochrome, is capable of eliciting VLF, LF, and HIR phytochrome responses. The single, short-pulse nature of the light treatment used herein precludes excitation of an HIR

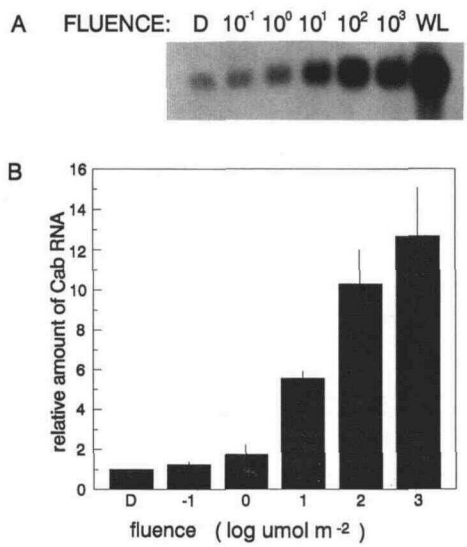

Figure 1. Fluence-response analysis of the steady-state level of *Arabidopsis Cab* RNA. Dark-grown seedlings (6 d old) were irradiated with a single pulse of blue light with a total fluence ranging from 10^{-1} to 10^3 μmol m^{-2} as indicated. Treatments were completed within 10 s except for the 10^3 μmol m^{-2} treatment, which was delivered in 100 s. Control seedlings (D) received a mock treatment. RNA was extracted from the control and blue-light-treated seedlings 4 h after the irradiation. Seedlings were also grown for 6 d in continuous white light (WL). Total RNA from each sample (10 μg) was used for northern analysis. A, A representative result from one experimental replicate. B, The quantified results of three independent replicate experiments. *Cab* RNA level in control seedlings (D) is set to 1.0. Error bars represent SE.

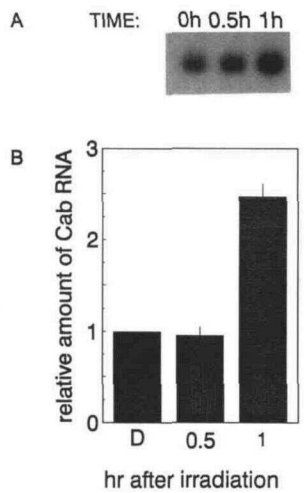

Figure 2. Kinetics of blue-light-induced accumulation of *Arabidopsis Cab* RNA. Dark-grown seedlings (6 d old) were irradiated with a single pulse of blue light (10^1 μmol m^{-2}, delivered in 10 s). Control (D) and blue-light-treated seedlings were harvested 0, 0.5, and 1.0 h after irradiation. The steady-state level of *Cab* RNA in each sample was quantified by northern analysis and densitometry. A and B are as described in Figure 1.

response. This still leaves the possibility of a VLF and/or LF phytochrome response.

Independence of phytochrome excitation could be established by demonstrating a response to blue light in *Arabidopsis* seedlings maintained in dim red light (Iino and Briggs, 1984). Unfortunately, growth in dim red light fully saturates the steady-state level of *Cab* RNA in *Arabidopsis* (data not shown). Consequently, two alternate experiments were carried out to distinguish the response of the *Arabidopsis Cab* gene family to blue light from a VLF and/or LF response to phytochrome.

First, the effects of a far-red light irradiation immediately following the blue-light treatment were examined. The results are shown in Figure 3A, wherein the lanes designated D, R, RFR, and FR represent the steady-state levels of *Cab* RNA in dark-grown seedlings, seedlings treated with a pulse of saturating red light, seedlings treated with a pulse of saturating red light followed immediately by far-red light, and seedlings treated with far-red light alone, respectively. The response to far-red light alone and the lack of full far-red light reversal indicate the presence of a VLF phytochrome response. Lanes designated B and BFR represent the steady-state levels of *Cab* RNA in response to a single pulse of blue light (10^1 μmol m^{-2}, delivered in 10 s) alone or followed immediately with a far-red pulse postirradiation, respectively. The far-red postirradiation results in accumulation of *Cab* RNA (lane BFR) at a level identical with that resulting from far-red alone, suggesting that the blue-light effect is independent of a phytochrome LF response but may elicit a subsaturating VLF effect.

To determine if the blue light is evoking a VLF response, we examined the effect of blue light (10^1 μmol m^{-2}) and far-red light on the expression of RNA transcribed from the gene encoding the small subunit of Rubisco (*RbcS*) (Fig. 3B). The *RbcS* gene family is known to have a phytochrome response

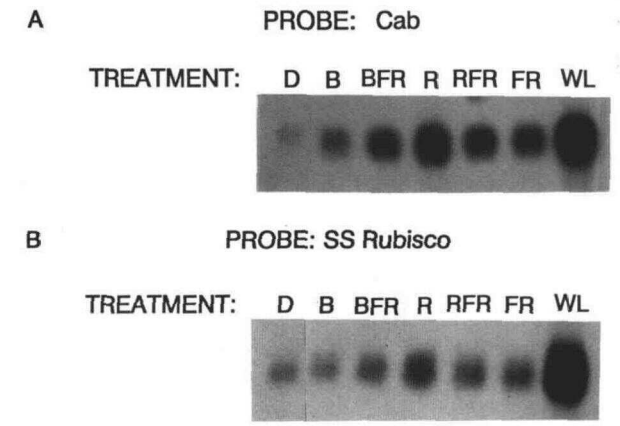

Figure 3. The role of phytochrome in the blue-light responses of dark-grown *Arabidopsis* seedlings. Dark-grown seedlings (6 d old) were treated with blue light (B; 10^1 μmol m^{-2}, delivered in 10 s) or blue light followed immediately by far-red light (5.4×10^5 μmol m^{-2}, delivered in 10 min; BFR). Control seedlings received either no light (D), red light (R; 2.7×10^3 μmol m^{-2}, delivered in 1 min), red-light followed immediately by far-red light (RFR), or far-red light alone (FR). The seedlings were harvested 4 h after treatment, and RNA was extracted for northern analysis. A, Northern analysis indicating the steady-state levels of *Cab* RNA. B, Northern analysis indicating the steady-state levels of *RbcS* RNA. WL, White light.

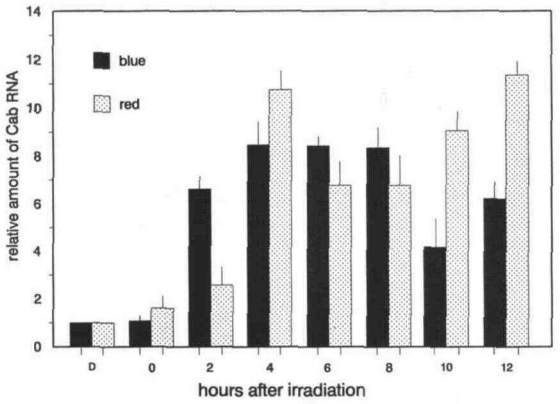

Figure 4. Comparison of kinetics for *Cab* RNA accumulation in response to a single pulse of blue or red light. Separate samples of 6-d-old, dark-grown seedlings were treated with either no light (D), a single pulse of blue light (10^2 μmol m^{-2}, delivered in 10 s), or a single pulse of red light (2.7×10^3 μmol m^{-2}, delivered in 60 s). Seedlings were harvested and analyzed for *Cab* RNA level 0, 2, 4, 6, 8, 10, and 12 h after irradiation. Data represent the average of three independent replicate experiments. Error bars represent SE. The dark levels of the respective controls have been set to 1.0.

in *Arabidopsis* (Dedonder et al., 1993). The light treatments and lane designation are the same as in Figure 3A. The steady-state level of *RbcS* RNA has a VLF response as indicated by the response to far-red alone and the red/far-red light treatment. However, blue light alone does not result in the accumulation of *RbcS* RNA. These data suggest that the blue light does not elicit the VLF phytochrome response for *RbcS*, and, therefore, the response of *Cab* RNA to blue light is probably independent of the VLF phytochrome response.

The second experiment compares the kinetics of blue-light-induced *Cab* RNA accumulation with phytochrome-induced *Cab* RNA accumulation. The blue-light fluence is 10^2 μmol m^{-2}, and the red light is saturating (2.7×10^3 μmol m^{-2}). The results (Fig. 4) indicate that the kinetics of *Cab* RNA accumulation differ in response to the two, different light treatments, suggesting two separate signaling pathways. Saturating red light results in accumulation beginning between 2 and 4 h after treatment, whereas the response to blue light was observed within 1 h after the light treatment. Furthermore, the long-term accumulation differs between the two treatments.

Individual *Cab* Gene Members Are Differentially Regulated

Three members of the *Cab* gene family, the *Cab*1, *Cab*2, and *Cab*3, form a cluster on a single 11-kb genomic fragment. Sequence comparison of coding and flanking regions between *Cab*2 and *Cab*3 suggests that these two genes probably represent a gene duplication event. RNase protection assays suitable for differentiating the transcripts deriving from the individual *Cab* gene members were used to determine the role of each gene and the response to blue light. Two antisense RNA probes, specific for *Cab*1 and *Cab*3, were transcribed in vitro. Fully protected fragments corresponding to *Cab*1 and *Cab*3 RNAs are expected to be 237 and 209 nucleotides, respectively. *Cab*1 and *Cab*2 RNAs will result in protection of approximately 90 and 180 nucleotides, respectively, when using the *Cab*3 probe.

Figure 5 shows the results using the *Cab*1 probe to detect the changes of *Cab*1 RNA in blue-light-treated *Arabidopsis* seedlings. The lane designated "P" represents no substrate RNA, and the lane designated "tRNA" represents the use of *Escherichia coli* tRNA as substrate. Protection is not observed in either lane. Lane D represents the use of RNA from untreated seedlings, and lanes 10^{-1} through 10^3 represent RNA from seedlings treated with 10^{-1}, 10^0, 10^1, 10^2, and 10^3 μmol m^{-2} of blue light, respectively. The fully protected fragments (237 nucleotides) in lane D indicate the presence

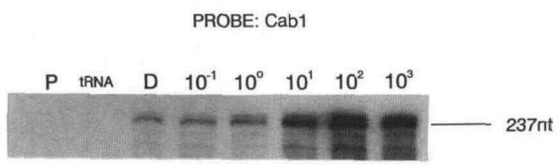

Figure 5. Fluence response analysis of the steady-state level of *Cab*1 RNA. A radiolabeled *Cab*1 antisense RNA probe was used in an RNase protection assay to define the level of RNA derived from the *Cab*1 gene. Samples tested in the assay include no RNA (P), 20 μg of *E. coli* tRNA (tRNA), 20 μg of total RNA extracted from 6-d-old dark-grown seedlings (D), and 20 μg of total RNA from 6-d-old dark-grown seedlings treated with various fluences of blue light as described in Figure 1. Subsequent to annealing and RNase A and RNase T1 digestion, protected fragments were visualized by separation on denaturing acrylamide gels and autoradiography. Full protection for transcripts derived from the *Cab*1 gene results in a 237-nucleotide (nt) fragment.

of *Cab*1 RNA in dark-grown seedlings. The increase of the fully protected fragment in lanes 10^{-1} through 10^{3} indicates that the steady-state level of *Cab*1 RNA increases as a function of increasing blue-light fluence.

Figure 6 represents an identical experiment using the *Cab*3-specific probe. Full protection was not detected either in RNA from dark-grown seedlings (lane D) or in RNA from seedlings receiving blue-light treatment, suggesting that *Cab*3 is not responsive to blue light. Furthermore, no protection at 180 nucleotides is observed, indicating a lack *Cab*2 RNA accumulation in response to blue light. Fluence-dependent protection is observed at 90 nucleotides, confirming the *Cab*1 expression observed in Figure 5 (lane D).

Cab RNA Accumulation in the *hy*4 Mutant Shows a Wild-Type Blue-Light Response

The *hy*4 mutant of *Arabidopsis* lacks blue-light-induced suppression of hypocotyl elongation but retains both the red- and far-red light-induced suppression responses (Koornneef et al., 1980). The maintenance of the phytochrome-induced suppression confirms that *hy*4 is not lacking in the ability to be suppressed and suggests that the lesion is within the blue-light-mediated signal transduction mechanism. To determine whether this lesion also affects blue-light-induced *Cab* RNA accumulation, the level of *Cab* RNA was examined in response to a single pulse of blue light (total fluence of 10^{0} μmol/m^{2}). The results (Fig. 7) indicate that *Cab* RNA increases with the same magnitude as in the Landsberg wild-type *Arabidopsis* from which the mutant was originally derived.

DISCUSSION

We have demonstrated that the *Cab*1 member of the *Arabidopsis Cab* gene family is responsive to excitation of a blue-light photomorphogenic system. RNase protection assays using a *Cab*1-specific probe demonstrate that a single, 10-s pulse of blue light, with fluences as low as 10^{-1} μmol m^{-2}, results in an increase in the steady-state level of *Cab*1 RNA (Figs. 5 and 6), which has been shown by northern analysis to begin soon after the light treatment (Fig. 2).

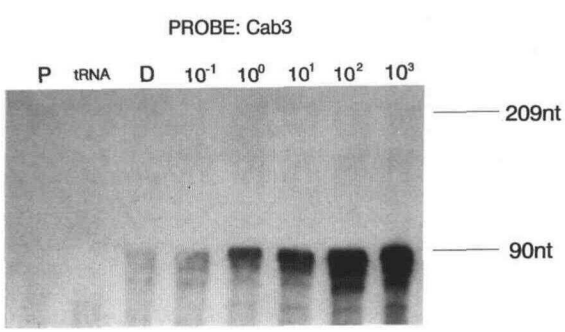

Figure 6. Fluence response analysis of the steady-state levels of the *Cab* 2 and *Cab* 3 genes. A radiolabeled *Cab*3 antisense probe was used in an RNase protection assay to define the level of RNA derived from the *Cab*2 and *Cab*3 genes. Lane designations and experimental procedures are described in Figure 5. Full protection for the *Cab*3 transcripts occurs at 209 nucleotides (nt). Partial protection for the *Cab*1 transcript occurs at 90 nucleotides.

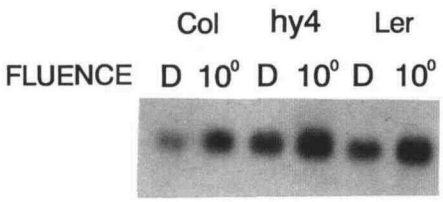

Figure 7. Blue-light-induced *Cab* RNA accumulation in *hy*4. *hy*4 and wild-type Columbia and Landsberg seedlings were planted and maintained in the dark at 4°C for 16 h, transferred to dim red light (0.2 μmol m^{-2} s^{-1}) at 21°C for 4 h, and then kept at 21°C in the dark for an additional 5 d. Seedlings received a mock (D) or real (10^{0}) 10-s pulse of blue light (total fluence 10^{0} μmol m^{-2}). Untreated and irradiated seedlings were examined for the steady-state level of total *Cab* RNA. Col, Wild-type *Arabidopsis* (Columbia). Ler, Wild-type *Arabidopsis* (Landsberg).

The *Arabidopsis Cab*1 gene has been reported to be phytochrome regulated (Karlin-Neumann et al., 1988). Thus, this specific member of the *Arabidopsis Cab* gene family is affected by both blue-light system and phytochrome excitation when a single, short pulse of light is used to irradiate etiolated seedlings.

Comparison of the *Cab*1 RNase protection data with those of the total gene family suggests that the accumulation of *Cab*1 transcript accounts for at least a portion of *Cab* gene family response to LF blue light (<10^{1} μmol m^{-2}). Experiments with *Cab*3-specific antisense RNA probes indicate that the *Cab*2 and *Cab*3 are not blue-light responsive under our assay conditions. Previous work has demonstrated a lack of phytochrome regulation for these two genes (Karlin-Neumann et al., 1988). The two remaining cloned members of the *Arabidopsis Cab* gene family, *Cab*4 (also known as Lhb1B1 or Lhcb1*4) and *Cab*5 (also known as Lhb1B2 or Lhcb1*5) (McGrath et al., 1992), were not examined in this study (or in the prior phytochrome study). It has been estimated that as many as 30 *Cab*-type genes are present in the *Arabidopsis* genome (McGrath et al., 1992).

These data suggest that the *Cab*1 member is regulated very early in leaf development and has the potential to respond to the very first light impinging on the young etiolated seedlings. The *Cab*2 and *Cab*3 members probably require continuous light or a specific developmental state (e.g. chloroplast development) prior to becoming activated.

Specific genes have been identified as responsive to either long-term blue- or red-light irradiation. For example, transcripts derived from the single-copy chalcone synthase gene in *Arabidopsis* will accumulate in red-light-grown seedlings after a 15-min irradiation with blue light at a fluence rate of 55 μmol m^{-2} s^{-1} (Feinbaum et al., 1991). Similarly, the single-copy chalcone synthase gene in parsley responds to long-term excitation of the phytochrome, blue-light, and UV-B systems (Frohnmeyer et al., 1992).

A relatively large number of the gene families identified as light regulated will respond to excitation of either a blue-light or phytochrome system, e.g. the *Fed* (Fd) gene family in pea (Kaufman et al., 1985; Marrs and Kaufman, 1989). The data presented in this paper in combination with that from other laboratories (Dedonder et al., 1993) establish that,

although the *RbcS* gene family in *Arabidopsis* is regulated by phytochrome, it is not regulated by a blue-light system under the conditions described herein.

The threshold fluence for the blue-light-affected change in the steady-state level of *Cab*1 RNA is at or just below 10^0 μmol m^{-2} (Fig. 1). This is similar to the threshold observed for blue LF system activation of the *Cab* gene family in etiolated peas. Activation of the blue LF system in peas results in an increase in the rate of transcription for the *Cab* gene family. This response is also immediate and occurs in the absence of cytoplasmic protein synthesis. We do not know whether the response in *Arabidopsis* is transcriptional and/or the result of altered stability of the *Cab* RNA.

Run-on transcription assays in isolated nuclei, similar to those used in the pea system, have proven almost impossible to perform in etiolated *Arabidopsis*. *Arabidopsis* strains harboring chimeric genes consisting of the upstream regulatory regions of the *Cab*1 gene spliced to an appropriate reporter gene should offer the means to answer the transcription/turnover question.

The outcome of the experiments with *RbcS* RNA indicates that the steady-state level of this transcript in *Arabidopsis* is affected by the VLF phytochrome response (Fig. 3). This contrasts with pea, in which the *RbcS* gene family is not regulated by a VLF response (Kaufman et al., 1984). We observed no effects of LF blue light on the steady-state level of *RbcS* RNA. Dedonder et al. (1993) reported *Arabidopsis RbcS* RNA accumulation in response to 10^3 μmol m^{-2} of blue light. When assayed as described herein, blue light at a fluence of 10^3 μmol m^{-2} delivered in 100 s is enough to elicit a phytochrome effect as defined by far-red prevention of the response (not shown).

The time course in response to irradiation with saturating red light reported herein (Fig. 4) differs from that reported by Karlin-Neumann et al. (1988). In the latter case, expression was transient, and *Cab* transcript levels returned to the dark level within 8 h. Our data do not identify a return to control levels but do suggest a rhythmicity in the level of *Cab* RNA. The contrasting results are likely due to the very plastic nature of *Cab* gene expression in *Arabidopsis* coupled to different growth conditions (Brusslan and Tobin, 1992).

The dual regulation of the *Cab*1 gene invites the question of whether regulation by the blue-light system and phytochrome occur by biochemically distinct pathways or whether they intersect at some point. The data presented herein suggest, but do not confirm, that the blue-light response does not depend on prior or concomitant activation of the phytochrome system. This is also the case for blue-light regulation of the transcription of the pea *Cab* gene family (Warpeha and Kaufman, 1990). However, blue-light-regulated transcription of the *Cab* gene family in tomatoes appears to require phytochrome excitation (Oelmuller et al., 1989).

The time course for blue-light regulation of the *Arabidopsis Cab* gene differs from that of phytochrome regulation. This would suggest, but cannot confirm, that the biochemistry governing blue-light and phytochrome *Cab* regulation are distinct and, if they do intersect, the intersection point is removed from the point of receptor excitation. Clearly, more work will need to be done to determine the relationship between the blue-light system and the phytochrome system regulation of this single member of the *Arabidopsis Cab* gene family.

The *Arabidopsis* hypocotyl elongation mutant *hy*4 does not exhibit suppression of hypocotyl elongation when grown in continuous blue-light conditions. The mutant exhibits suppression of growth in red and/or far-red conditions, confirming that the phytochrome system is functional and that the lesion is in a blue-light system (Koornneef et al., 1980). Furthermore, the presence of red-/far-red-induced suppression indicates that *hy*4 is normal for the elongation press, suggesting that the mutation affects the ability to capture or process the blue-light signal.

The *hy*4 locus has the potential to encode a blue-light receptor active in suppression of epicotyl elongation (Ahmed and Cashmore, 1993). Our results suggest that the *hy*4 mutant is unaffected in blue-light regulation of the steady-state level of *Cab* RNA. Combined with previous reports indicating that *hy*4 exhibits normal blue-light effects on stomates, chloroplast development, and phototropism (reviewed by Chory, 1992), the data suggest the existence of several independent blue-light receptors and/or blue-light-mediated signal transduction pathways.

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