**Arabidopsis rbcS Genes Are Differentially Regulated by Light**

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Individual members of the *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (*rbcS*) gene family are differentially regulated by light of different qualities. In 10-d-old etiolated seedlings, the expression of only three of the four genes is under inductive phytochrome control. *rbcS* mRNA levels reach a maximum (3- to 5-fold higher than the dark level) about 6 h after a red light pulse, but the rate of decay differs among the genes. Moreover, *rbcS* 2B requires a higher fluence for induction. At early stages of development, *rbcS* 1A, 2B, and 3B are highly expressed in the dark and cannot be further induced by red light, indicating a developmental component in the overall regulatory mechanism. Continuous light experiments indicate that high-irradiance responses may play a role in the induction of at least three of the four *rbcS* genes. Under conditions of phytochrome saturation, *rbcS* 1A is insensitive to blue light pulses, whereas among the three B locus genes, at least *rbcS* 3B appears to respond to a blue-light photoreceptor. These results add to the data suggesting that individual members of *rbcS* gene families in higher plants may be subject to a variety of differing regulatory mechanisms.

Rubisco is a multimeric enzyme consisting of eight SSUs and eight large subunits of 14 and 55 kD, respectively. The enzyme is located in the stroma of the chloroplast, the genome of which encodes the large subunit (Coen et al., 1977). The SSU polypeptides are encoded on nuclear genes (*rbcS*) and translated on cytoplasmic ribosomes as precursors that are posttranslationally imported and processed by the chloroplast (Highfield and Ellis, 1978).

Extensive work has shown that, in most higher plants, the SSU is encoded by a gene family ranging from four to 10 or more genes (reviewed by Dean et al., 1989). Because Rubisco is the most abundant protein in leaves, this is not surprising. However, further work in a number of species has shown that individual members of a gene family may be differently regulated, suggesting that the multigene families do not serve solely to amplify the product. For example, in the leaves of tomato plants all five *rbcS* genes are expressed but at different levels. Transfer to darkness has different effects on different genes. Three of the five tomato genes are expressed in cotyledons of dark-grown plants, and other differences in organ-specific gene expression exist (Sugita et al., 1987; Wanner and Gruissem, 1991). These variations are based on differ-ences at both the transcriptional and posttranscriptional levels; variations in both DNA-protein interactions (Manzara et al., 1991) and in mRNA stability (Wanner and Gruissem, 1991) have been observed among different genes. Transcripts from the different members of the potato *rbcS* family are differentially degraded when the plants are placed in the dark (Fritz et al., 1991). In pea, differences in the relative levels of expression in different organs were found (Fluur and Chua, 1986), whereas in petunia, the differences are less pronounced (Dean et al., 1989).

The objective of this work was to determine whether individual *rbcS* genes within a species respond differently to light of different qualities. It has been known for some time that *rbcS* genes are regulated by light and that this process is at least in part mediated at the level of transcription, e.g. in pea (Gallagher et al., 1985), *Lemna* (Silverthorne and Tobin, 1984), soybean (Berry-Lowe and Meagher, 1985), and rye (Ernst et al., 1987). Light can also affect gene expression at the posttranscriptional level (Jenkins, 1991), and some data suggest that photoregulation of *rbcS* gene expression can also be exercised at the level of transcript stability (Ernst et al., 1987; Wanner and Gruissem, 1991). The role of phytochrome as the photoreceptor mediating the expression of many light-responsive genes, including *rbcS*, has been demonstrated in a variety of species (reviewed by Tobin and Silverthorne, 1985). In addition to phytochrome, blue/UV-A photoreceptor(s) and UV-B photoreceptor(s) may be involved (Horwitz and Gressel, 1986), and an interaction between phytochrome and a blue-light receptor has been proposed by several authors (for review, see Thompson and White, 1991). The interactions between these photoreceptors is thought to be complex; in pea, photoregulation of the *rbcS* genes depends on the developmental stage of the plant. In young, etiolated pea seedlings, the light-induced changes in *rbcS* gene expression are phytochrome mediated, and there is no evidence for action of other photoreceptors. However, in mature leaves, a blue-light receptor acts in concert with phytochrome (Fluur and Chua, 1986; Sasaki et al., 1988; Clugston et al., 1990). Blue-light receptors have been implicated in other species as well (Wehmeyer et al., 1990). Thus, it is clear that developmental and light cues must interact. Finally, inhibitor and other studies show that developmental state of the chloroplast influences *rbcS* gene expression, and a "plastid factor"

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Abbreviations: HIR, high irradiance response; LF, low fluence; *rbcS*, small subunit of Rubisco; SSC, standard sodium citrate; SSU, small subunit; VLF, very low fluence.
has been postulated (Simpson et al., 1986; reviewed by Thompson and White, 1991).

In this work we examined the response of the members of the Arabidopsis thaliana rbcS family to different light regimens to determine whether they are similarly or differently regulated by phytochrome or a blue-light photoreceptor. In Arabidopsis, the rbcS gene family consists of four members that have been divided into two subfamilies on the basis of linkage and sequence similarities (Krebbers et al., 1988). Three genes (rbcS 1B, 2B, and 3B) are tightly linked at a single locus, and the fourth gene (rbcS 1A) is at least 10 kb removed from, or completely unlinked to, the B subfamily. Using gene-specific probes derived from the 3'-untranslated regions of the different genes, we have compared the effect of different light quantities and qualities on the mRNA levels of all members of the gene family in both etiolated and green seedlings. The results indicate that the four genes are differentially regulated by light. We have determined that one of the four does not appear to be under inductive control of either phytochrome or a blue-light receptor, whereas the other three genes differ in the kinetics of their response and the fluence level required for expression. The genes are differentially expressed in the dark, and there are also complex variations in their response to continuous irradiation at different wavelengths.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of Arabidopsis thaliana (L. Heynh.) (a derivative of the Columbia ecotype) were obtained from plants grown in a plant growth chamber at 21°C and 60% humidity with a 16-h photoperiod (white fluorescent light Philips TL D 33 lamps, average fluence rate 80 pmol m~2 s~1). After harvest, seeds were dried for 1 week at 27°C and stored at 4°C for at least 1 month before use. Seeds were surface sterilized by treatment with ethanol for 2 min, followed by 5% NaOCl containing 0.02% Tween for 15 min. After four washes with sterile water, the seeds were suspended in 0.2% agar and sown at about 100 seeds/Petri dish on medium containing 2.15 g/L of Murashige and Skoog salts and 15 g/L of Suc (pH 5.8). The medium was solidified with 7 g/L of agar. The dishes were wrapped in aluminum foil and kept in darkness at 20°C and 90% RH. Additional treatments to induce germination were not necessary.

Light Treatments

Light pulses (0.1–1000 s, depending on the desired fluence) were given after 9 or 10 d of etiolated growth. After a dark period (length indicated in the legend of the figures), seedlings were harvested under dim green light (fluence rate at a distance of 1 m: 20 μmol m~2 s~1) and frozen in liquid nitrogen. The green light (λmax: 515 nm) was obtained by wrapping a Philips TL 40-W/17 fluorescent tube with two layers of green and two layers of blue cellophane (Sidac, Brussels, Belgium). For continuous irradiation, Petri dishes with seeds were placed in the light fields immediately after sowing for a period of 10 d. Twenty-four-hour irradiations always started on day 9 after sowing. After continuous or 24-h irradiation, seedlings were harvested immediately and frozen in liquid nitrogen. Red light (λmax: 660 nm) was supplied by red fluorescent lamps (TL 15, Philips) combined with a red filter (Röhm and Haas No. 501). In the fluence-response experiment (Fig. 4), a narrow-band red-light source combined with a Compur (Germany) electronic m-1 shutter was used as described earlier (De Petter et al., 1985). Far-red light (λmax: 730 nm) was obtained by filtering the light of Osram Linestra lamps through a 10-cm water layer and an FRF-700 filter (Westlake Plastics Co.). Blue light (λmax: 450 nm) was obtained by filtering the light of Philips TL 18 fluorescent lamps through a blue plexiglass sheet and an 8-cm-thick CuSO4 solution (100 g of CuSO4/L containing 0.2% H2SO4). White light was provided by Philips TL D 33 fluorescent lamps.

Different fluence rates were obtained by varying the distance between the light source and the seedlings and were measured with a spectroradiometer system (IL 600; International Light, Newburyport, MA).

Oligonucleotide Probes

The Arabidopsis rbcS gene-specific probes were: 5'-TTTTGAGGTTTACACAAAAG-3' (rbcS 1A), 5'-CGGATTAGTCAACATGGATAT-3' (rbcS 1B), 5'-AGATAATTCAACGCTGAATAT-3' (rbcS 2B), and 5'-AGATAATTCATAGAATGTTCAT-3' (rbcS 3B). Synthetic oligonucleotides were prepared using the β-cyanophosphoramidite method on an Applied BioSystems 394 DNA synthesizer. Probes were labeled with [γ-32P]ATP using T4 polynucleotide kinase (Maniatis et al., 1982). The specificity of the probes, designed on the basis of computer comparisons of the 3' ends of the mRNA-encoding sequences, was confirmed using Southern blots in which each probe was tested against plasmids carrying each of the four rbcS genes (data not shown).

Preparation of RNA

Total RNA was prepared from about 2 g of seedling material using an extraction buffer containing 0.1 M NaCl, 0.01 M Tris-HCl (pH 7.5), 1 mM EDTA, and 1% SDS. After two phenol:chloroform:isoamyl alcohol (25:24:1) extractions, the nucleic acids were precipitated with ethanol. After the pellet was resuspended in extraction buffer containing only 0.1% SDS, DNA contamination was removed by pelleting the RNA in 4 M LiCl. After solubilization, the RNA was again precipitated with ethanol, washed with 70% ethanol, and finally resuspended in water at a concentration of about 1 mg/mL.

Northern Analysis

RNA (10 μg) was denatured in 50% formamide, 5.9% formaldehyde, 1× Mops buffer (40 mM Mops [pH 7.0], 10 mM NaCl, 1 mM EDTA) at 65°C for 5 min and separated on an 1.5% agarose gel containing 2.2 M formaldehyde in 1× Mops buffer. A gel loaded with 5 μg of RNA was stained with acridine orange (2 μg/mL) both to check RNA integrity and to ensure that equal amounts of RNA were loaded in each slot (data not shown). The RNA was transferred to Hybond-N (Amersham) in 20× SSC (3 M NaCl, 0.3 M sodium citrate). The filter-bound RNA was hybridized with one of
the $^{32}$P-labeled oligonucleotides after purification of the probe over a Nensorb-20 column (New England Nuclear).

After a 6-h prehybridization period, hybridization with the end-labeled oligonucleotide probe (30 pmol) was carried out for 15 h at 40°C in a buffer containing 5X SSC, 10X Denhardt's solution, 0.1% SDS, 0.05% Na$_4$P$_2$O$_7$, and 100 µg/mL of herring sperm DNA. The washes were in 6X SSC, twice at 40°C for 15 min and twice at 45°C for 15 min. Filters were exposed to x-ray film at —70°C with intensifying screens. The films were scanned densitometrically to determine the amounts of rbcS mRNA relative to the dark-grown control level. The autoradiogram in Figure 1 shows a representative result of three independent experiments. Other data are averages of two to three independent experiments.

**RESULTS**

Only Three of the Arabidopsis rbcS Genes Show a Phytochrome Response

As reported previously (Krebbers et al., 1988), all four Arabidopsis rbcS genes are expressed in leaves of light-grown plants. To determine whether the expression of the rbcS genes in Arabidopsis is controlled by light through phytochrome action, etiolated seedlings were irradiated with white light or were given a short irradiation before transfer to darkness. Figure 1 shows the accumulation of rbcS mRNA after 24 h of white light in comparison with the mRNA levels measured 24 h after pulses of red light (5 min, fluence 5000 µmol m$^{-2}$), far-red light (10 min, fluence 5000 µmol m$^{-2}$), red light (5 min) followed by far-red light (10 min), or blue light (10 min, fluence 1000 µmol m$^{-2}$). The LF red pulse enhanced the levels of the rbcS 1A, 2B, and 3B mRNAs 3- to 4-fold above the dark-grown control level, but a far-red pulse also led to an increase of the same transcripts, albeit to a lower level. If a red pulse was followed by a far-red pulse, the amount of mRNA was reduced to that observed with a far-red pulse alone. The effect of a blue pulse was similar to the effect of a short far-red irradiation. These results indicate that phytochrome is involved in the regulation of these genes but suggest that other factors may also play a role. Short light pulses, whatever the wavelength, could not induce a significant increase of the rbcS 1B transcript level above the dark-grown control, although a 3-fold increase was measured after 24 h of white light (Fig. 1).

The kinetics of the response to a red-light pulse were not identical among rbcS 1A, 2B, and 3B (Fig. 2). Expression of rbcS 1A and rbcS 3B reached a maximum 6 h after the light pulse. The response of rbcS 2B differed; the level of mRNA accumulated 6 h after the light pulse was lower, but it was maintained for a longer time. No significant expression of the rbcS 1B gene was measured during the 48-h course, and at the end of that period, the mRNA levels of all four genes had decreased to the initial value.

Arabidopsis rbcS Genes Are Expressed in the Dark and Are Differently Regulated at Different Developmental Stages

To determine whether the four rbcS genes are expressed in the dark, gene-specific probes were used in an RNA gel blot against RNA isolated from 10-d-old dark-grown Arabidopsis seedlings. The results presented in Figure 1 (lane DC) show...
a weak but detectable dark expression of the $rbcS$ 1A, 2B, and 3B genes. Expression of the $rbcS$ 1B gene in the dark was almost undetectable after normal exposure times.

When RNA was prepared from dark-grown seedlings of different ages, 2- to 4-fold more mRNA from the $rbcS$ 1A, 2B, and 3B genes was present at day 5 than at later stages (Fig. 3). When red light pulses were administered to 5-d-old dark-grown seedlings, no significant induction over the dark-grown controls could be observed at 5 d, whereas at 10 d or later, $rbcS$ 1A, 2B, and 3B all showed significant inductions of mRNA levels (Fig. 3). These data suggest that these genes are under different regulatory controls at different stages of development.

$rbcS$ Genes Do Not Respond Identically to Different Fluences of Red Light

Fluence-response data for accumulation of the $rbcS$ mRNAs after a pulse of red light are presented in Figure 4. Induction of the $rbcS$ 1A, 2B, and 3B genes started at fluences between 1 and 10 $\mu$mol m$^{-2}$. However, as fluence levels increased, the corresponding increase in the $rbcS$ 2B mRNA level did not equal that of $rbcS$ 1A and 3B. $rbcS$ 2B responded relatively weakly at LFs, attaining levels similar to the other two when the fluence of the red-light pulse reached 10,000 $\mu$mol m$^{-2}$ (10 min at a fluence rate of 16.6 $\mu$mol m$^{-2}$ s$^{-1}$). Induction of the three genes by a blue pulse required a fluence of at least 1000 $\mu$mol m$^{-2}$ (2 min at a fluence rate of 8.33 $\mu$mol m$^{-2}$ s$^{-1}$, data not shown).

In summary, the four Arabidopsis genes respond differently to red light pulses, $rbcS$ 1B shows essentially no response, whereas $rbcS$ 2B differs from $rbcS$ 1A and 3B in both the kinetics of its response and the fluence level required for significant induction, $rbcS$ 2B being much less responsive than $rbcS$ 1A and 3B.

Arabidopsis $rbcS$ Genes Also Differ in Their Responses to 24-h Continuous Light

In addition to inductive phytochrome responses, photomorphogenic responses to continuous irradiation have been described. These HIRs are induced by red light but also show strong responsiveness to far-red and blue light (Mancinelli, 1980; Kronenberg and Kendrick, 1986). Although red/far-red reversibility of HIR responses is not possible, it has been shown that far-red-induced HIRs are indubitably mediated by phytochrome (Hartmann, 1966). We have compared the effect of 24 h of continuous white light irradiation on $rbcS$ mRNA levels with the response of the $rbcS$ genes to 24 h of continuous irradiation with red, far-red, and blue light. As shown in Figures 1 and 5A, when 9-d-old etiolated seedlings were exposed to 24 h of white light, the $rbcS$ 1A and 3B genes...
Differential Light Regulation of Arabidopsis rbcS Genes

Figure 5. Effect of 24-h (A) or continuous (B) irradiation on the mRNA levels of the four rbcS genes. Twenty-four-hour white light (WL), 24-h red light (R), 24-h far-red light (FR) (all fluence rates 15 μmol m⁻² s⁻¹), and 24-h blue light (BL) (fluence rate 5 μmol m⁻² s⁻¹) were given after 9 d of etiolated growth. Continuous white light (cWL) and continuous red light (cR) (both at a fluence rate of 10 μmol m⁻² s⁻¹) were given during 10 d after sowing. The blue pulse (fluence 10,000 μmol m⁻²) was given after 9 d of cR growth and was followed by 24 h of cR. Plant material given different light treatments was harvested after 10 d. The four rbcS mRNA levels are expressed relative to the dark-grown control levels of 10-d-old, rbcS 1A; □, rbcS 1B; ■, rbcS 2B; ▽, rbcS 3B. The data are means of two or three independent determinations (±SE indicated).

Figure 6. Fluence rate responses of the different rbcS mRNA levels to red (○), far-red (●), and blue (△) light. Irradiations were continuous for 24 h after 9 d of etiolated growth. The four rbcS mRNA levels are expressed relative to the dark-grown (DC) controls (x). The data are means of two independent determinations with a range of <25%.

were most highly expressed. The stimulation of rbcS 2B gene expression by 24 h of white light was less pronounced but still higher than for rbcS 1B (Fig. 1). In Figure 5A, the effect of 24 h of white light is also compared with the effect of 24 h of irradiation with red, far-red, or blue light. After 24 h of far-red or red light, the mRNA levels for the rbcS 1A, 2B, and 3B genes were comparable to those after 24 h of white light, but induction above the dark-grown control level of the rbcS 1B gene under the same conditions was weaker, only about 40% of that observed in white light. Finally, when 24 h of blue light were given, rbcS 1B, 2B, and 3B reached approximately the same mRNA levels as under other conditions, but rbcS 1A was present at slightly but significantly lower levels.

Because HIRs are often strongly fluence-rate dependent (Kronenberg and Kendrick, 1986; Smith et al., 1991), the effects of 24-h red, far-red, and blue light at different fluence rates (0.2, 2.0, 20 μmol m⁻² s⁻¹) were investigated. rbcS 1A, 2B, and 3B all responded at 2 μmol m⁻² s⁻¹; the mRNA levels relative to the dark-grown control levels after the red-light treatment were 2.8, 1.9, and 3.6 for rbcS 1A, 2B, and 3B, respectively; 2.1, 1.5, and 3.5 after far-red light; and 1.6, 1.9, and 2.2 after blue light. Thus, rbcS 3B was more sensitive at this fluence rate than the others. As shown in Figure 6, all of the genes responded more intensely at 20 μmol m⁻² s⁻¹. The relative mRNA levels at 20 μmol m⁻² s⁻¹ confirmed the results shown in Figure 5A; rbcS 1A responded equally well to 24 h of red or far-red light and showed less induction by blue light. The induction of the rbcS 1B and 2B genes was similar under all three light sources but, again, rbcS 1B was expressed weakly relative to the other three. The rbcS 3B gene was expressed at the highest level after 24 h of far-red light, and blue light was marginally less effective than red light. In summary, the effects of far-red and blue light and the fluence rate dependency indicate that an HIR is involved in the light regulation of the Arabidopsis rbcS 1A, 2B, and 3B genes.
A Blue-Light Photoreceptor Is Also Involved in Photoregulation

The weak response to blue pulses shown in Figure 1 can be due to blue-light excitation of phytochrome, but a blue-light photoreceptor can also be involved in the light response. When short blue pulses are given to seedlings germinated and grown in continuous red light, the phytochrome response is assumed to be saturated (Briggs and Iino, 1983). When seedlings grown under such conditions for 9 d were given a pulse of blue light (fluence 10,000 μmol m⁻²), a weak increase in the mRNA level of rbcS 3B was observed (compare in Fig. 5B, cR and cR + Bl). It was not possible to confirm a similar trend for rbcS 1B and 2B, but at least in the case of rbcS 3B, a blue-light photoreceptor is implicated.

In the same experiment, plants were also grown under continuous white light. The results presented in Figure 5B show somewhat similar effects of continuous white and red light on induction of all four genes. It is interesting that comparison of the effect of continuous white light with the data presented in Figure 5A about the effect of 24-h white light irradiation of 9-d-old dark-grown seedlings show much higher mRNA levels for rbcS 1B, 2B, and 3B after continuous irradiation. rbcS 1A showed no such increase.

**DISCUSSION**

The four *Arabidopsis* rbcS genes respond to light in quite different ways. In etiolated seedlings, rbcS 1A, 2B, and 3B are influenced by light acting through inductive phytochrome action, a response that is lacking in rbcS 1B. The three genes are induced by a pulse of red light, and this induction can be reversed by a pulse of far-red light. However, the reversal does not turn off expression altogether but, instead, leads to a level that can also be induced by far-red light alone. rbcS 1B is not induced by a single pulse of far-red light. The three genes that are induced by a pulse of red light do not share the same kinetics of response, rbcS 2B remaining at or near the maximum-induced level over a much longer period than the other two (Fig. 2). rbcS 2B also differs in the fluence level required to obtain full induction with a red-light pulse, requiring 50- to 100-fold greater light levels to be induced to levels similar to those of rbcS 1A and 3B. The genes also differ in their response to 24 h of continuous light of different wavelengths. rbcS 1B and 2B respond more weakly to 24 h of light than the other two, but rbcS 1A is the only gene not to maintain the same level of induction under 24-h blue light conditions (Fig. 5A). This complex pattern of responses to different light sources among the *Arabidopsis* rbcS genes adds to the variety of data showing that members of rbcS families in various species are differentially regulated with respect to organ specificity, expression in the dark, and various other parameters (Dean et al., 1989). It should be noted that these experiments address only steady-state levels of mRNA; the relative contributions of transcription, posttranscriptional processes, and stability differences to the observed changes in the RNA levels of the individual genes are unknown.

As shown in Figure 3, 5-d-old etiolated *Arabidopsis* seedlings show high rbcS 1A, 2B, and 3B mRNA levels that decrease at later stages of development in the absence of light. This is consistent with the results of Brusslan and Tobin (1992), who reported developmental control of both the *Arabidopsis* cab genes and the *rbcS* 1A gene in young seedlings. This control mechanism is independent of light or other environmental stimuli and results in an increased expression of these genes early in germination, which is later decreased in the absence of light. It is unlikely that the message present at the 5-d stage is some form of stored message left over from seed development. Light-grown *Arabidopsis* seedlings show a complete decay to dark-grown control rbcS mRNA levels after a 4-d dark period (A. Dedonder, unpublished observations). Also, Brusslan and Tobin (1992) found no cab mRNA in imbibed seeds or in 1-d-old seedlings.

Although rbcS 1A is at a different locus than the rbcS B genes and is the most divergent of the four in terms of protein sequence (Krebbers et al., 1988), rbcS 1B deviates most strikingly from the other three genes in its response to red light. This situation is somewhat reminiscent of that in tomato, which has two unlinked genes (rbcS 1 and rbcS 2) and three linked genes (rbcS 3A-C); the coding regions of the latter are more highly conserved than the unlinked genes (Sugita et al., 1987). However, tomato rbcS 3A is different from rbcS 1, 3B, and 3C in its response upon transfer of the plant to darkness. If we assume that tandemly linked genes arise from duplications, these results indicate that there may be differences in the rates of evolution of coding sequences and regulatory sequences.

When the 5'-flanking sequences of the *Arabidopsis* rbcS genes are compared, the G box (C/A-CACCTG GCC), demonstrated by Donald and Cashmore (1990) to be necessary for expression of the *Arabidopsis* rbcS 1A promoter in transgenic tobacco plants grown under greenhouse conditions, is found to be present in its entirety in rbcS 3B as well. However, in rbcS 2B, the final GC is, instead, AT; the final A is the +4 position, which Schindler et al. (1992) reported must be a G or T for the box to bind to GBF1. The region containing the G box is missing completely in rbcS 1B. It is part of a 43-bp deletion in the 5'-flanking region of rbcS 1B relative to the other three genes in an otherwise highly conserved region (see figure 5 in Krebbers et al., 1988; the rbcS 1A G box is at position −259 relative to the initiation codon in the numbering used in that figure). A partial form of the G box can be found 49 bp downstream of the expected position in rbcS 1B (CATGTG GT), but this sequence matches none of the variants found to have binding activity by Schindler et al. (1992).

Thus, despite lacking a sequence motif whose mutation has been shown by Donald and Cashmore (1990) to abolish rbcS 1A expression, full expression of the rbcS 1B gene is observed in continuous white light at levels similar to those of the other rbcS B genes and surpassing those of rbcS 1A (Fig. 5B). cis elements and trans-acting factors other than the G box and factors binding specifically to it must, therefore, play a role in the light-regulated expression of rbcS 1B. For example, there is evidence that a “plastid factor,” whose presence is correlated with chloroplast development (which has occurred when rbcS 1B is expressed), plays a role in the expression of rbcS genes (Simpson et al., 1986; Dean et al., 1989), although different members of a gene family can differ in their responsiveness to such factors (Wanner and Grunse, 1991). Some of the differences in expression pattern

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*Note: The reference to “Dedonder et al.” in the text is not present in the provided image.*
observed in this work may be related to different sensitivities of the *Arabidopsis rbcS* genes to this or other factors. In etiolated *Arabidopsis* seedlings, the *rbcS* 1A, 2B, and 3B genes can be weakly stimulated by a pulse of far-red light. This has also been reported for *rbcS* genes of other species (tomato and tobacco, Wehmeyer et al., 1990; rye, Ernst et al., 1988), and similar results were obtained when the *rbcS* 1A promoter was used to drive a chimeric gene in transgenic tobacco plants (De Almeida et al., 1989). In contrast to the data showing induction of the *cab* genes by VLFs of red or far-red light in etiolated pea seedlings, no clear VLF response could be demonstrated for *rbcS* mRNA accumulation (Kaufman et al., 1984; Yoshida, 1989). This is confirmed by the results presented here; no induction was obtained with red-light pulses lower than 1 μmol m^{-2} (Fig. 4). Typical LF responses need a Pfr:total phytochrome ratio higher than 0.03 and, consequently, cannot be induced by far-red light. A small shift of the LF-response curve (see figure 2 in Kronenberg and Kendrick, 1986) to lower values would reduce the Pfr:total phytochrome threshold for induction of the LF response and could explain the effect of a 5000-μmol m^{-2} (nearly saturating) far-red pulse.

Although the action of a specific blue-light photoreceptor cannot be excluded, the weak increase of the *rbcS* 1A, 2B, and 3B mRNA levels observed in etiolated *Arabidopsis* seedlings after a pulse of blue light (Fig. 1) is most probably the result of inductive phytochrome action, because the Pfr level induced by blue light is about 40% (Hayward, 1984). The situation is different when seedlings are grown under conditions that saturate the phytochrome response (Fig. 5B); only *rbcS* 3B exhibits a clear phytochrome-independent increase in expression level. The induction required an LF of 10,000 μmol m^{-2}. When similar experiments were carried out using *Pisum sativum*, LF blue-light-induced accumulation of different transcripts, including *cab*, was observed, and the involvement of a blue-light photoreceptor in the photoregulation of four different nuclear genes was postulated (Warpeha et al., 1989). The results are consistent with regulation of at least the *rbcS* 3B gene by a blue-light photoreceptor in 10-d-old red-light-grown seedlings. Because these seedlings show Chl accumulation, the involvement of a blue-light photoreceptor in the effect of LF blue light on the expression of three *Arabidopsis rbcS* genes is probably correlated with the developmental stage of the seedlings, as was described for pea (Fluur and Chua, 1986; Clugston et al., 1990).

Blue light is also implicated in HIR (Kronenberg and Kendrick, 1986). The response of the *rbcS* 1A, 2B, and 3B genes to a 24-h irradiation with blue or far-red light (Fig. 5A) and the fluence rate dependence of that response (Fig. 6) indicate that these genes have such a response. However, it seems to be much reduced in *rbcS* 1B, for which 24 h of white light cannot be replaced by 24 h of far-red or blue light.

The different responses of the *Arabidopsis rbcS* genes to light stimulation suggest that, as in other species, multiple mechanisms exist for their regulation. It will be interesting to determine whether the *Arabidopsis* genes are as complex in their patterns of organ specificity and whether other gene families have equally complex responses to light of different types. Presumably such complexity has a selective advantage that is as yet not understood.

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