Structure and Blue-Light-Responsive Transcription of a Chloroplast \textit{psbD} Promoter from \textit{Arabidopsis thaliana}\(^1\)

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We characterized the effects of light on \textit{psbD} transcription and mRNA levels during chloroplast development in \textit{Arabidopsis thaliana}. After 6 to 12 hours of illumination of dark-grown seedlings, two \textit{psbD} mRNAs were detected and their 5' ends were mapped to positions -550 and -190 bp upstream from the \textit{psbD} translational start codon. Their kinetics of accumulation resembled the accumulation of chloroplast \textit{psbA} and \textit{rbcL} mRNAs but differed from the accumulation of the nuclear-encoded \textit{Lhcb} and \textit{Chs} mRNAs. A third \textit{psbD} mRNA with its 5' end at position -950 accumulated after illumination of >180 h. The 5' ends of this transcript were mapped to a nucleotide sequence that is highly conserved with functional sequences in the barley (\textit{Hordeum vulgare}) blue-light-responsive promoter (BLRP). Transcription from the \textit{Arabidopsis psbD} promoter was 3-fold higher in blue relative to red light, whereas red and blue light affected total chloroplast, \textit{rbcL}, and 16S rDNA transcription similarly. This study shows that transcription of \textit{Arabidopsis psbD} is mediated by a BLRP and suggests that \textit{psbD} genes in other land plants are regulated by a common blue-light-signaling pathway. Isolating the BLRP from \textit{Arabidopsis} will allow molecular genetic studies aimed at identifying the pertinent photoreceptor and components of this phototransduction pathway.

The chloroplast genomes of higher plants exist as multiple copies of circular DNA that range in size from 120 to 160 kb (for review, see Palmer, 1990; Sugiyura, 1992). The variability in chloroplast DNA size is due to differences in the size of an inverted repeat and small differences in gene content among plant species (Palmer, 1990). In general, chloroplast genomes encode a similar group of approximately 100 protein genes, 30 to 31 rRNA genes, and a complete set of tRNA genes, which function in photosynthesis, transcription, and translation (Shinozaki et al., 1986; Hiratsuka et al., 1989; Wakasugi et al., 1994; Maier et al., 1995). The genes are tightly spaced along the cDNA, and several are organized into polycistronic operons that resemble those of \textit{Escherichia coli} and cyanobacteria (Lindahl and Zengel, 1986; Tanaka et al., 1986; Zhou et al., 1989; Bergsland and Haselkorn, 1991). Many of these operons encode different subunits that function in the same multimerin protein complex.

The operonal organization of genes encoding related functions allows for unique regulatory mechanisms that control gene expression during chloroplast development. For example, the \textit{psbD-psbC} genes encode the D2 and CP43 subunits of the PSI reaction center (Vermaas and Ikeuchi, 1991). As chloroplasts mature, \textit{psbD-psbC} transcription rates and mRNA abundance are maintained at higher levels relative to most other chloroplast genes (Sexton et al., 1990a; Baumgartner et al., 1993; Christopher and Mullet, 1994). The differential expression of \textit{psbD-psbC} during chloroplast development involves the action of at least four promoters that drive overlapping transcription units (Sexton et al., 1990a). As barley (\textit{Hordeum vulgare}) chloroplasts mature, there is a light-induced switch in the use of the promoters. A BLRP that is differentially activated by high-fluence blue and UV-A light, but not by red or far-red light, becomes the predominant promoter driving transcription (Gamble and Mullet, 1989; Christopher and Mullet, 1994; Christopher, 1996). The blue-light-induced mRNAs make up most of the translatable \textit{psbD} mRNA in mature chloroplasts (Mullet et al., 1990; Christopher and Mullet, 1994). Blue-light-activated \textit{psbD} transcription assists in maintaining the synthesis of D2, which is photodamaged in plants and algae exposed to high-intensity light (Schuster et al., 1988; Melis et al., 1992; Christopher and Mullet, 1994).

The nucleotide sequence of the barley BLRP is highly conserved with light-responsive promoters from eight different plant genera (Christopher et al., 1992; Wada et al., 1994). The light-responsive promoter and \textit{psbD-psbC} genes are linked together in all land-plant chloroplast genomes studied to date except the parasite \textit{Epifagus virginiana}, which lacks these genes (Wolfe et al., 1992), and the liverwort \textit{Marchantia polymorpha} (Ohyama et al., 1986), which lacks the light-responsive promoter.

Despite the widespread occurrence of the conserved promoter sequences in land plants, it is not known whether the blue-light-responsive transcription observed in barley (Christopher, 1996) is a conserved mechanism of gene activation for \textit{psbD} in the other plants. In barley the genetic mechanism involves a unique high-fluence blue light/UV-A phototransduction pathway (Christopher and Mullet, 1994). This pathway is modulated by phytochrome and leaf development and an extraplastidic Ser/Thr protein

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Abbreviation: BLRP, blue-light-responsive promoter.
phosphatase, which affect total chloroplast transcription activity (Christopher, 1996; Christopher et al., 1997). The biochemistry and molecular details of this blue-light signal transduction pathway are poorly understood.

An understanding of the psbD regulation by high-fluence blue light requires a comprehensive study of the relevant photoreceptor, intracellular signaling pathway, and genes for components of the pathway. *Arabidopsis thaliana* represents a genetically tractable system with which to elucidate the molecular mechanisms regulating psbD expression. However, the psbD gene to our knowledge has not been studied in Arabidopsis. As a prerequisite to using the well-developed genetics of Arabidopsis to study psbD regulation, we cloned the Arabidopsis psbD gene and identified its cognate light-responsive promoter. We mapped the psbD transcripts and studied the effect of light on psbD transcription and mRNA levels in comparison with other light-regulated chloroplast-encoded and nuclear-encoded genes. This study revealed that the Arabidopsis psbD gene is regulated by blue-light-responsive transcription from a conserved BLRP. In addition, we show that the BLRP is differentially regulated relative to other photoresponsive nuclear and chloroplast genes.

# MATERIALS AND METHODS

## Plant Material, Growth Conditions, and Light Treatments

Wild-type seeds of *Arabidopsis thaliana* ecotype Columbia were purchased from Lehle Seeds (Round Rock, TX). Seeds were surface-sterilized with 25% bleach and 0.2% Tween 20 freezing in liquid N2. and white light were measured using a quantum plant in flats containing water-saturated potting mixture. Seeds were planted on 0.8% agar containing Murashige-Skoog medium and 2% Suc. Plated seeds were refrigerated (4°C) for 15 h and then exposed to white light (90–100 μmol m−2 s−1) for 24 h to promote uniform germination. The germinating seeds were kept in white light for 9 d (216 h) or blue or red light for 6 d or were placed in the dark in light-tight, controlled environment chambers at 21 to 23°C in a dark room for 3.5 d. After the dark treatments seedlings were exposed to the light sources described below for 6, 12, or 24 h. Tissue was harvested by quick freezing in liquid N2.

To grow plants for chloroplast isolation, seeds were plated on jiffy pots containing the coding region of the nuclear gene *Lhcb1* (formerly termed *cab1*) was described previously (Christopher, 1996). Gene-specific antisense RNA probes were synthesized and radiolabeled with [α-32P]dATP (>800 Ci/mM, ICN) using T3 and T7 RNA polymerases. A plasmid containing the coding region of the *chs* gene from Arabidopsis (Feinbaum and Ausubel, 1988) was a generous gift from J. Chory (Salk Institute, San Diego, CA). The 1.75-kb *PsI* restriction fragment containing part of exon I and all of exon II of the *chs* gene was gel-purified and labeled with [α-32P]dATP using a random primer labeling kit (Promega). Radiolabeled RNA and DNA probes were hybridized to RNA gel blots as described previously (Christopher and Mullet, 1994).

The deoxyoligonucleotide 5'-GTCATAGTGATCCTC-CTATTTG spans the conserved nucleotides −16 to +5 of the psbD mRNA leader in the antisense direction (Christopher et al., 1992). The deoxyoligonucleotide 5'-AGAGATATCGACGGATCCCTA resides 60 nucleotides downstream from the 5' ends of the light-induced mRNAs of Arabidopsis and is antisense to the mRNA-like strand. The deoxyoligonucleotides were labeled at the 5' terminus using T4 polynucleotide kinase and [γ-32P]ATP (ICN).

The first radiolabeled primer was used to assay equal amounts of total cell RNA in primer extension analysis experiments, as described previously (Christopher et al., 1992). To map the 5' ends of the light-induced mRNAs, a DNA-sequencing reaction was conducted using the second primer and the plasmid pAtEH100 (described below), and this reaction was electrophoresed in parallel with the primer extension reactions. Radioactivity on the blots and in bands on polyacrylamide gels was quantitated with a liquid scintillation counter (series LS1801, Beckman) or a gel/blot analysis system (model 4000, Ambis, San Diego, California).
Chloroplast Isolation and Chloroplast Transcription Assays

Leaves were homogenized (Powergen homogenizer, Fisher Scientific) at a dial setting between 2 and 3. Plastids were isolated by centrifugation (4100g) of cell lysates on Percoll gradients (40–80%) essentially as described previously (Christopher et al., 1992), except that plastids were pelleted at 2600g. Intact plastids were counted in a hemacytometer using a phase-contrast microscope. Chloroplast transcription activity was assayed using [α-32P]UTP and 5 × 10⁷ purified plastids at a final concentration of 4.55 × 10⁸ plastids mL⁻¹, as described previously (Christopher, 1996).

The tagetitoxin experiment utilized 5 × 10⁹ plastids at a final concentration of 9.6 × 10⁹ plastids mL⁻¹. Chloroplast transcription activities from duplicate experiments were expressed as pmol [32P]UMP incorporated (5 × 10⁸ plastids)⁻¹ (10 min)⁻¹, whereas the units for the tagetitoxin experiment were pmol [32P]UMP incorporated (5 × 10⁹ plastids)⁻¹ (10 min)⁻¹. Radiolabeled run-on transcripts were hybridized as described previously (Christopher, 1996) to nonradiolabeled, gene-specific probes rbcL, 16S rRNA, and rbcL mRNA levels were more abundant after 24 h of light, whereas the units for the tagetitoxin experiment were pmol [32P]UMP incorporated (5 × 10⁹ plastids)⁻¹ (10 min)⁻¹. Radiolabeled run-on transcripts were hybridized as described previously (Christopher, 1996) to nonradiolabeled, gene-specific probes rbcL, 16S rRNA, and rbcL mRNA levels were more abundant after 24 h of light.

RESULTS

We used primer extension assays to examine the expression of the Arabidopsis psbD gene and to map the 5' ends of psbD mRNAs during greening of young seedlings. As shown in Figure 1, no psbD mRNAs were detected in dark-grown seedlings. Three major mRNA 5' ends, located 190, 550, and 950 nucleotides upstream from the psbD translational start codon, were detected in illuminated seedlings using a primer specific to the psbD gene (Fig. 1). The 190- and 550-nucleotide fragments increased in abundance from 6 to 24 h of continuous light and by 216 h of light, these fragments were highly abundant. However, after 216 h of light a third primer extension product of 950 nucleotides was detected. This 950-nucleotide product was detected after 180 to 200 h of light (data not shown), with significant levels being detected by 216 h of light. Therefore, during greening, the accumulation of mRNA corresponding to the 950-nucleotide fragment was different from the accumulation of the mRNAs corresponding to the 190- and 550-nucleotide fragments. The appearance of the 950-nucleotide band was affected by seedling development, with lower levels present in cotyledons compared with leaves of light-grown seedlings (D.A. Christopher and P.H. Hoffer, unpublished data).

We examined as controls the expression of two other plastid genes, psbA (encodes the D1 subunit of the PSI reaction center) and rbcL (encodes the large subunit of Ribisco), and two light-regulated nuclear genes, Lhcb (encodes the chlorophyll α/β-binding protein) and Chs (encodes chalcone synthase) (Fig. 2). The analysis of the 18S and 16S rRNAs are also presented in Figure 2. Low levels of 16S rRNA and psbA and rbcL, mRNAs were detected in 3.5-d-old dark-grown seedlings. The levels of psbA and rbcL increased during the greening process (Fig. 2). Both psbA and rbcL mRNA levels were more abundant after 24 h of continuous light. The increase in abundance of psbA and rbcL mRNAs resembled the increase in the psbD 190- and 550-nucleotide fragments (Figs. 1 and 2). This increase could be due to a general increase in total plastid transcription and in the stability of psbA and rbcL mRNAs that accompanies light-induced chloroplast and leaf develop-
The abundance of Chs mRNA increased by 6 h of light, decreased by 24 h of light, and was undetected by 216 h of light (Fig. 2), which is similar to the previously observed pattern of Chs expression (Kubasek et al., 1992). The psbD 950-nucleotide fragment (Figs. 1 and 2B) accumulated later during seedling development relative to Chs mRNAs but moderately resembled Lhcb mRNA accumulation. By analyzing the expression of the four other genes, we show that the low level of the psbD 950-nucleotide fragment detected after 6 to 24 h of illumination was not an artifact caused by inadequate light treatments, since the light-responsive expression of Chs, Lhcb, and rbcL and the psbD 190- and 550-nucleotide mRNAs was detected. Rather, the accumulation of the psbD 950-nucleotide fragment followed a dif-

Figure 1. Transcript mapping and analysis of psbD expression in greening Arabidopsis chloroplasts. A, 3.5-d-old dark-grown seedlings (3.5 DD) were exposed to 0, 6, 12, 24, or 216 h of continuous light (HL). psbD mRNAs were analyzed by primer extension assays. Three bands corresponding to three different mRNA 5' ends are labeled by approximate size (in nucleotides) as 190, 550, and 950. The arrow denotes the bands for mRNA 5' ends appearing in the continuous light-grown sample (216 h). B, The psbD gene (black bar), putative light-responsive promoter (LRP, striped box), and position of transcripts (open arrows) are indicated as derived from the experiments in A. The black half-arrow refers to the location of the primer used in the primer extension assays.

Figure 2. Analysis of chloroplast and nuclear gene expression during Arabidopsis chloroplast development. A, Treatments were the same as described in Figure 1. Transcript levels were analyzed for the chloroplast psbA and rbcL genes and the nuclear Lhcb and Chs genes using RNA hybridization experiments. The ethidium bromide-stained nuclear 18S and chloroplast 16S rRNAs are included as references. B, The relative transcript levels for Lhcb, Chs, psbA, and the 950-nucleotide psbD band (from Fig. 1) were quantitated. The averages ± so for two experiments done in duplicate are shown.
High-resolution primer extension mapping of the 5' ends of the light-induced psbD mRNAs from Arabidopsis. Lanes labeled cDNA (A, T, C, and G) refer to the dideoxynucleotide sequencing reactions conducted on plasmid pATEH100 using the same primer as for primer extension of Arabidopsis RNA (lane R) from light-grown cDNA (A, T, C, and G) refer to the dideoxynucleotide sequencing reactions conducted on plasmid pATEH100 using the same primer as for primer extension of Arabidopsis RNA (lane R) from light-grown plants. The cDNA sequence read from the panels is printed to the right of the panel. The arrows point to the nucleotides mapping to the 5' ends of the psbD mRNAs that correspond to the 950-nucleotide mRNA ends of the light-induced psbD gene. Information about the nucleotide sequence was used to map the 5' end of the 950-nucleotide mRNA and to analyze the promoter structure and function. As shown in Figure 3, the 5' ends of the mRNAs were mapped to the nucleotides TTGA in the cDNA sequence 5'-GAATTT-GAATATCAGA. The mRNAs for the psbD light-responsive promoters from eight other plants also have multiple 5' ends that map to the same T residues in this conserved sequence (Christopher et al., 1992). The 5' ends of the psbD mRNAs reside exactly 949 to 952 bp upstream from the psbD translational start codon.

As shown in Figure 4, the nucleotide sequence flanking the Arabidopsis psbD region at position 950 nucleotides is highly conserved with the barley BLRP and the light-responsive promoters of three dicots, five monocots, and similar sequences from two other evolutionarily distant plants, black pine and broad bean. Equally important is the presence in Arabidopsis of conserved nucleotide sequences centered at positions 10 (AAGTAAGT), 30 (TTGAAT), and 5' coding regions of the psbD gene. Information about the nucleotide sequence was used to map the 5' end of the 950-nucleotide mRNA and to analyze the promoter structure and function. As shown in Figure 3, the 5' ends of the mRNAs were mapped to the nucleotides TTGA in the cDNA sequence 5'-GAATTT-GAATATCAGA. The mRNAs for the psbD light-responsive promoters from eight other plants also have multiple 5' ends that map to the same T residues in this conserved sequence (Christopher et al., 1992). The 5' ends of the psbD mRNAs reside exactly 949 to 952 bp upstream from the psbD translational start codon.

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To obtain more information concerning the DNA region in Arabidopsis from which the psbD mRNA at position 950 arises, we cloned the psbD gene and flanking regions from an Arabidopsis chloroplast genomic library and determined the nucleotide sequence of the upstream transcribed, untranscribed, and 5' coding regions of the psbD gene. Information about the nucleotide sequence was used to map the 5' end of the 950-nucleotide mRNA and to analyze the promoter structure and function. As shown in Figure 3, the 5' ends of the mRNAs were mapped to the nucleotides TTGA in the cDNA sequence 5'-GAATTT-GAATATCAGA. The mRNAs for the psbD light-responsive promoters from eight other plants also have multiple 5' ends that map to the same T residues in this conserved sequence (Christopher et al., 1992). The 5' ends of the psbD mRNAs reside exactly 949 to 952 bp upstream from the psbD translational start codon.

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light, chloroplast transcription assays were conducted using chloroplasts from dark-adapted plants treated with red, blue, or white light. Measurements of the total transcription activity of intact Arabidopsis chloroplasts are shown in Figure 5. Transcription rates from 28-d-old dark-adapted plants exposed to red and blue light were similar and were 2-fold higher relative to transcription rates of plants maintained in darkness (Fig. 5A). Total chloroplast transcription rates from plants exposed to white light were on average 4-fold higher than dark-adapted controls.

The addition of tagetitoxin, an inhibitor of chloroplast transcription (Mathews and Durbin, 1990), reduced transcription by 65%. This degree of inhibition falls within the previously determined range obtained by titration of tagetitoxin in transcription assays using pea chloroplasts (Mathews and Durbin, 1990). Radiolabeled transcripts synthesized by the Arabidopsis chloroplasts hybridized to ctDNA restriction fragments in a manner proportional to their transcription rates (Fig. 5C). These data confirm that chloroplast transcription activities were being measured in our run-on assays.

We measured the transcription activities of the psbD light-responsive promoter region in comparison with rbcL and 16S rRNA transcription from the same plants. As shown in Table I, transcription from the psbD promoter was not detected in the dark-adapted controls. Transcription increased slightly in red light and was highest in blue and white light (Table I). In contrast, total chloroplast transcription was similar in red and blue light (Fig. 5), and rbcL transcription did not differ markedly in red, blue, or white light. In the dark controls transcription of the 16S RNA was highest among all genes. The transcription of the 16S RNA gene was stimulated 3- and 2-fold in red and blue light, respectively, and more than 20-fold in white light. When transcription of the psbD promoter was normalized relative to rbcL and 16S rRNA transcription, only psbD transcription was significantly higher in blue light relative to red light (Table I). This indicates that blue light specifically stimulated transcription from the psbD promoter relative to other chloroplast genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Dark</th>
<th>Red Light</th>
<th>Blue Light</th>
<th>White Light</th>
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<tbody>
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<td>psbD-LRP</td>
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<td>0.38 ± 0.04</td>
<td>1.11 ± 0.16</td>
<td>1.98 ± 0.18</td>
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<tr>
<td>rbcL</td>
<td>0.04 ± 0.01</td>
<td>0.15 ± 0.04</td>
<td>0.13 ± 0.01</td>
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</tr>
<tr>
<td>16S rRNA</td>
<td>0.44 ± 0.05</td>
<td>1.36 ± 0.20</td>
<td>0.99 ± 0.04</td>
<td>9.34 ± 0.20</td>
</tr>
<tr>
<td>LRP: rbcL</td>
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<td>2.5</td>
<td>8.5</td>
<td>15.0</td>
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<td>LRP: 16S rRNA</td>
<td>0.0</td>
<td>0.2</td>
<td>1.1</td>
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</tr>
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**Table I. Quantitation of the effect of light quality on transcription activities of the psbD-light-responsive promoter (LRP) and rbcL and 16S rRNA genes**

Gene- and promoter-specific transcription activities (mean ± so) were measured on an equal chloroplast basis from dark-adapted plants exposed to 7 h of additional darkness, red, blue, or white light as described in “Materials and Methods.” In the ratios the transcription of the psbD-LRP was normalized to rbcL and 16S rRNA transcription.


DIscussion

The Arabidopsis psbD Gene Is Regulated by a BLRP

Several lines of evidence support the idea that the Arabidopsis psbD gene is regulated by a BLRP that is common to most plant cDNAs. The 5' ends of the light-induced psbD mRNAs arise from a region residing 950 bp upstream from the psbD translational start codon. This -950 position is the same location of the light-responsive promoters of the psbD genes from tobacco and spinach chloroplasts (Yao et al., 1989; Christopher et al., 1992). The nucleotide sequences of the light-responsive promoters from dicots are highly conserved with the sequences in nucleotide sequences of the light-responsive promoters (Christopher et al., 1992). These conserved DNA sequences from barley and tobacco have been identified as functional promoter elements using transcription assays, RNA-capping experiments, transcription inhibitor studies, gel mobility shift experiments, and promoter assays in transgenic chloroplasts (Yao et al., 1989; Sexton et al., 1990a; Christopher et al., 1992; Wada et al., 1994; Allison and Maliga, 1995; Kim and Mullet, 1995; Christopher, 1996).

DNA sequence analysis of the 100-bp region flanking the -950-bp transcription initiation site of Arabidopsis (Fig. 4) indicated that it also contains the conserved promoter elements (-10, -35, AAGTAAGT, and transcription initiation motifs) necessary for transcription (Christopher et al., 1992; Wada et al., 1994; Allison and Maliga, 1995; Kim and Mullet, 1995). We demonstrated that transcription from this region is light-dependent and is highest in blue relative to red light. The increase in abundance of transcripts in response to light correlated closely with the increase in psbD transcription. Therefore, we conclude that a conserved BLRP resides 950 to 985 bp upstream from the Arabidopsis psbD gene. In addition, because blue light activates this promoter in the dicot Arabidopsis and the monocot barley, we suggest that the blue light signal transduction pathway is common to other land plants.

In addition to blue light, developmental signals could modulate promoter activity. For example, in young seedlings grown in continuous light, the -950-nucleotide mRNA that arises from the BLRP was detected at significant levels only after extended periods (>10 h) of illumination, whereas psbD transcription was readily activated in older seedlings exposed to a relatively short (7 h) period of illumination. Recently, we found that the level of the mRNA from the BLRP is higher in primary leaves, whereas low levels were observed in light-grown cotyledons (D.A. Christopher and P.H. Hoffer, unpublished data). Similarly, the expression of the Arabidopsis Lhcb gene is strongly influenced by developmental signals (Brusslan and Tobin, 1992) in addition to light (Kaufman, 1993; Gao and Kaufman, 1994).

Two other psbD mRNA 5' ends were detected in our experiments (190- and 550-nucleotide bands). Analogous psbD RNAs are also found in all other plant species studied (Yao et al., 1989; Sexton et al., 1990a; Christopher et al., 1992; Wada et al., 1994). Multiple 5' ends for psbD transcripts are generated by multiple promoters and 5' RNA-processing events. We suggest that the 190- and 550-nucleotide bands correspond to mRNAs that arise from additional promoters and/or RNA processing within the Arabidopsis psbD operon. Transcription and RNA-processing assays (Sexton et al., 1990a) will further define the origin of the mRNAs arising from the -550 and -190 positions.

The chloroplast psbA and rbcL genes, the other psbD mRNAs (−190 and −550) and the photoresponsive nuclear genes (Lhcb and Chs) differed from the expression of the psbD BLRP. These different genetic responses of nuclear and chloroplast genes during greening could be triggered by the same signaling system that has unique downstream components. Alternatively, the blue light photoreceptor pathway activating the BLRP could be different from, and could interact with, the signaling pathways regulating the other photoresponsive genes. Along this line, psbD and 16S transcription rates in white light were much greater than the sum of the individual transcription rates in red and blue light (Table I). This suggests a synergistic effect of white light on the transcription of these genes. Pretreatment of dark-adapted plants with 30 min of red light followed by 7 h of blue light yielded transcription rates for these genes similar to those for blue light alone (data not shown). This suggests that the synergistic effect requires continuous white light and cannot be mimicked by short exposure to red followed by blue light.

In barley a synergistic effect of continuous blue plus far-red light on psbD transcription was observed, whereas far-red light alone had no effect (Christopher, 1996). Phytochrome A has been shown to mediate the high irradiance response to far-red light (Smith, 1995). It was proposed that phytochrome A modulates the activity of the psbD-BLRP by activating total chloroplast transcription, whereas blue light induces a BLRP-specific transcription factor (Christopher, 1996). The capacity of the factor to activate transcription could be increased by the higher total plastid transcription levels. Therefore, the synergistic effect of white light is interpreted to be due to these two separate influences of light quality on psbD transcription (Christopher, 1996). Alternatively, the higher fluences of white light used here could have resulted in a greater stimulation of transcription. Further investigation of the effect of phytochrome and fluence rate on the regulation of the psbD BLRP is needed. These investigations will be enhanced by using Arabidopsis because of the ease of generating mutants and

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<th>Nucleotide Position</th>
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<tr>
<td></td>
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</tr>
<tr>
<td>25</td>
<td>C</td>
</tr>
<tr>
<td>32</td>
<td>G</td>
</tr>
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<td>C</td>
</tr>
<tr>
<td>58</td>
<td>A</td>
</tr>
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<td>70</td>
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the large variety of photomorphogenetic mutants already available.

DNA Sequence Similarity Is Retained among Evolutionarily Related Plants

The sequence comparison of the Arabidopsis psbD BLRP with the promoters from 11 plants (Fig. 4) revealed some interesting differences and similarities among the monocot cereals, dicots, and gymnosperms, which are summarized in Table II. At positions 25, 32, 35, 39, 58, and 70, the monocot cereals use a different base than that used by the dicots and the one gymnosperm, black pine. At positions 25, 35, and 39, the monocots use C instead of A, which is used by both dicots and black pine. At positions 32, 58, and 70, the monocots use G, A, and T compared with pyrimidine (C,T), T, and G in dicots and black pine, respectively. Therefore, the DNA sequence in the black pine promoter is more similar to the dicot sequences than to the monocot cereal sequences. The sequence similarity suggests that the gymnosperm promoter is more closely related evolutionarily to the dicots than the monocots. This is interesting because the dicots are hypothesized to have originated from gymnosperms, whereas the monocots came later in evolution from the dicots (Doyle et al., 1994). The retention of these evolutionary similarities in a small, non-protein-coding, functional DNA sequence is noteworthy.

Role of Blue Light in Plant Adaptation to High-Light Environments

The evolutionary conservation of the BLRP in land plants raises an interesting question about its physiological significance. What is the selection pressure maintaining the association of the BLRP with the promoters from 11 plants (Fig. 4) revealed some interesting differences and similarities among the monocot cereals, dicots, and gymnosperms, which are summarized in Table II. At positions 25, 32, 35, 39, 58, and 70, the monocot cereals use a different base than that used by the dicots and the one gymnosperm, black pine. At positions 25, 35, and 39, the monocots use C instead of A, which is used by both dicots and black pine. At positions 32, 58, and 70, the monocots use G, A, and T compared with pyrimidine (C,T), T, and G in dicots and black pine, respectively. Therefore, the DNA sequence in the black pine promoter is more identical to the dicot sequences than to the monocot cereal sequences. The sequence similarity suggests that the gymnosperm promoter is more closely related evolutionarily to the dicots than the monocots. This is interesting because the dicots are hypothesized to have originated from gymnosperms, whereas the monocots came later in evolution from the dicots (Doyle et al., 1994). The retention of these evolutionary similarities in a small, non-protein-coding, functional DNA sequence is noteworthy.

LITERATURE CITED


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