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Transcriptional Regulation of a Gene Encoding the Small Subunit of Ribulose-1,5-Bisphosphate Carboxylase in Soybean Tissue Is Linked to the Phytochrome Response

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The effects of white light, far-red light, and darkness on the transcription of a soybean ribulose-1,5-biphosphate carboxylase small subunit gene, SRS1, were investigated. RNA was labeled with [α-^{32}P]UTP in nuclei isolated from plants grown under different conditions of light and darkness and used to probe Southern blots and dot blots. The levels of small subunit mRNA synthesis were normalized to ribosomal RNA synthesis. We demonstrate that the SRS1 gene is transcribed at a rate 16- to 32-fold higher in plants grown in the light than in those grown in darkness. Transcription of the small subunit increased dramatically when plants grown in darkness were given 30 min to 6 h of light and then leveled off after 24 to 48 h of exposure. When light-grown seedlings were exposed to greater than 2 h of darkness, a gradual decrease in transcription was detected. This decrease in transcription reached basal dark-grown levels after 48 h of exposure to darkness. The increase in transcription in etiolated seedlings treated with white light for 15 min could be reduced to basal levels if the treatment was followed by treatment with far-red light for 15 min. In addition, transcription in light-grown seedlings was reduced to basal levels when plants were exposed to far-red light for 15 min. The transcription of this ribulose-1,5-biphosphate carboxylase small subunit gene is strongly positively regulated by white light, is negatively regulated by far-red light, and exhibits a classic phytochrome-linked response.

Ribulose-1,5-bisphosphate carboxylase (RuBPC) (EC 4.1.1.39) is the most abundant soluble protein in plant leaves (19). The enzyme is composed of eight identical small subunits encoded by the nuclear genome and eight identical large subunits encoded by the chloroplast genome. The small subunit of RuBPC is synthesized as a precursor protein and then transported into the chloroplast, where it is cleaved into its mature form; there, it functions in conjunction with the large subunit in the fixation of CO_2.

Many enzymes, including the small subunit polypeptide of RuBPC, can be induced by light to high levels compared with the very low levels present in dark-grown tissue (14, 33, 36). The exposure of *Lemna gibba*, pea plants, and *Euglena gracilis* to light increases the level of the small subunit message (1, 4, 7, 21, 26, 28). In addition, light increases the transcription of the small subunit mRNA in isolated nuclei of cells from pea plants (10) and *L. gibba* (25). These data indicate that a primary mode of regulation of small subunit accumulation in response to light occurs at the level of transcription (10, 25). Posttranslational control of the level of small subunit peptide accumulation also has been demonstrated in *Chlamydomonas reinhardtii* (18, 24), however, suggesting that the regulation is complex.

Light regulation of these processes has been linked to the reversibly photoactivated chromoprotein phytochrome in *L. gibba* (34) and pea plants (23). The levels of translatable RuBPC small subunit mRNA in *L. gibba* increase after exposure to light for short periods. If light treatment is followed immediately by far-red treatment, this increase in the level of mRNA is greatly diminished, implying a phytochrome-linked response. Phytochrome itself may affect the process of transcription or it may activate intermediate regulatory molecules which act at the levels of the genes involved (28, 30, 33).

We have previously isolated a gene, SRS1, that encodes the small subunit of RuBPC in soybean tissue (4). SRS1 is one member of a multigene family containing 6 to 10 genes. The polypeptide-coding region and flanking sequences of SRS1 have been sequenced and examined in some detail. The steady-state mRNA levels produced from this gene in light-grown plants increase 50- to 100-fold over dark-grown levels. We have also shown that the location of the start of transcription is identical in light-grown and dark-grown tissues.

In this paper, we describe experiments in which we isolated nuclei from soybean plants grown under different light regimes to examine the transcriptional regulation of this RuBPC small subunit gene. Nuclear RNA transcribed in vitro was labeled with [α-^{32}P]UTP, isolated, and used to probe Southern blots and dot blots containing various DNA samples. We determined the length of illumination required to increase the transcription of the small subunit maximally and the duration of darkness necessary to return transcription to basal levels. We also demonstrate phytochrome control of the activation of transcription and that phytochrome may be actively involved in turning off transcription of the SRS1 gene.

MATERIALS AND METHODS

Light and growth conditions. Seeds of *Glycine max* var. Wayne, obtained from Ohio Seed Co., Columbus, Ohio, were imbibed for several hours and then planted in vermiculite at 26°C. Tissue was harvested after 10 to 15 days of growth.

White light was supplied by one cool white fluorescent tube (40 W; GTE Sylvania, Consumer Lighting, Atlanta, Ga.) and one Gro & Sho tube (40 W; General Electric Co.,

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Schenectady, N.Y.). Red light, provided by one sheet of Roscolene no. 823 gel (Atlanta Costume and Theatre Supply, Atlanta, Ga.) (34) over the white light source, gave results similar to those obtained with white light in initial transcriptional studies. Consequently, white light was used in the following experiments. A far-red light source was constructed as follows (29). Twenty Sylvania (30-W) floodlights were mounted five centimeters above a 0.6 sheet (0.6 m^2) of red FRF700 Plexiglas (0.125 in. [0.33 cm] thick; Westlake Plastics, Lenni, Pa.). A shallow Plexiglas tray of water between the lights and the filter cooled the light source. The plants were placed 15 cm below the filter in a closed chamber. The effects of different light intensities on transcriptional induction of small subunit were not examined.

Plants grown in darkness were kept in a light-tight 26°C chamber with continuous air flow. All manipulations involving dark-grown plants, including harvesting, were carried out in complete darkness or under green light. Green light was supplied by one warm white fluorescent tube (4 W; Sylvania) covered with one sheet each of Roscolene no. 874 and 877 gel (5). Experiments with nuclei harvested under green light or in complete darkness gave the same results.

Isolation and labeling of nuclei. Nuclei were isolated by the method of Luthe and Quatrano (16), except that tissue was collected in liquid nitrogen and ground in a Waring coffee grinder. The tissue was then suspended in buffer and homogenized with a Brinkman Polytron at low speed. Routinely, between 1 and 5 g of tissue were harvested from each set of light-treated plants, with about 5×10^5 nuclei isolated per g of tissue. This yield represents a recovery of approximately 10% of the nuclei from the original homogenized tissue sample. Nuclei were counted by examining dilutions of 4′.6-diamidino-2-phenylindole-stained homogenate (37) spread on a hemacytometer under a fluorescence microscope. Nuclei were suspended in 50% glycerol storage buffer; aliquots of nuclei could be frozen in this buffer at −70°C for labeling at a later date without adverse effects. RNA was labeled with 60 to 250 μCi of [α-^{32}P]UTP (>3,000 Ci/mmol; New England Nuclear Corp., Boston, Mass.) in vitro for 20 min at 30°C by the method of Walling and Goldberg (personal communication). Approximately 2×10^6 to 4×10^6 cpm were incorporated into RNA when 10^6 nuclei were labeled with 250 μCi of [α-^{32}P]UTP for 20 min. The transcription mix contained the following: 100 mM $(NH_4)_2SO_4$, 4 mM $MgCl_2$, 0.3 μM phosphocreatinine, 6.25 μg of bovine heart creatine phosphokinase, 0.5 mM ATP, 0.5 mM CTP, and 0.5 mM GTP in a final volume of 250 μl. (All chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.)

Labeled RNA was isolated by lysing nuclei in 5 M guanidinium thiocyanate (Fluka, Chemical Corp., Hauppange, N.Y.)–50 mM Tris-hydrochloride (pH 8.0)–50 mM EDTA (pH 8.0)–2% Sarkosyl–5 mM β-2-mercaptoethanol, heating the solution to 68°C for 1 min, briefly vortexing, and then layering the mix on 3 ml of 5.7 M CsCl adjusted to pH 8.0 with 100 mM EDTA (8, 11). The gradients were spun overnight at 125,000 × *g* in a 50 Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.). Pellets were suspended in 10 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA–500 mM NaCl with 100 μg of carrier yeast tRNA and ethanol precipitated. Phenol extraction at this point decreased the background on blots (see figures). Hybridizations were carried out at 56°C in hybridization buffer (50% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 5× Denhardt solution, 50 mM sodium phosphate buffer [pH 7.0], and 0.2% sodium dodecyl sulfate). The washes are described in the figure legends. Filters were exposed to Kodak XAR-5 film between two Du Pont Cronex intensifying screens at −70°C.

Quantitation of transcription based on rRNA transcription. We examined the effects of light on rRNA synthesis in soybean plants, since exposure to light increases rRNA synthesis above dark-grown levels in some plant systems (10, 20, 25, 31, 32). In nuclei from plants grown in both light and darkness, approximately 60 to 80% of the transcription was resistant to an alpha-amanitin concentration of 1 μg/ml. At alpha-amanitin concentrations of 5, 10, and 20 μg/ml, only 44 to 60% of the transcription was resistant for both types of nuclei. Little noticeable difference in inhibition was observed with these three higher levels of alpha-amanitin; an average of 51% of the transcription was resistant. At these concentrations of alpha-amanitin to which RNA polymerase I is resistant (13), the total level of rRNA transcription in soybean nuclei was nearly identical in light and darkness.

In approximately one-third of our experiments, we observed slightly more total labeling of RNA in nuclei from light-grown plants (up to twofold) than in nuclei from dark-grown plants. As a result, filters were probed with an equal number of radioactive counts regardless of the source of RNA.

We were also concerned that the large amount of rRNA in the probe might saturate the rDNA on the filter, making comparisons between filters based on rDNA hybridization inaccurate. The intensity of the hybridization signal to several different dilutions of this rDNA clone was directly proportional to the amount of labeled RNA probe used in the hybridization (data not shown). Therefore, hybridization to the rDNA control used in these experiments had not reached saturation.

Filter preparations. Plasmid DNA was digested for Southern blots with *Eco*RI restriction endonuclease, electrophoresed through a 1% agarose gel, and blotted to nitrocellulose by the method of Southern (27). For dot blots, DNA was linearized by restriction endonuclease digestion, extracted with phenol, and precipitated in ethanol. DNAs were suspended in water (>2 μg/μl) and denatured in 50% formamide–6% formaldehyde–10 mM sodium phosphate buffer for 10 min at 68°C. Tubes were chilled on ice and mixed with an equal volume of 20× SSC. Twofold serial dilutions of each sample were made in 10× SSC. A minifold apparatus (Schleicher & Schuell, Inc., Keene, N.H.) was used to form replicas of the dot dilution series on nitrocellulose. Wells were washed twice with 10× SSC, and filters were air dried, clamped between fiberboards, and baked for 2 h under vacuum at 80°C.

Calculations. Small subunit transcription was measured as a fraction of rRNA transcription by visual comparison of the intensities of autoradiographic dots of the entire dot blot dilution series. The validity of these comparisons was confirmed by densitometric scanning of dots of similar intensity on both the rDNA and RuBPC small subunit dilution series (Zeineh Soft Laser Scanner; Biomed Instruments, Inc., Chicago, Ill.). When two or more dots of similar or equal intensity could be aligned in the rDNA and RuBPC small subunit DNA lanes, an estimate of the difference in total dilution between these dots was made. The fraction of rRNA transcription was calculated by correcting this difference in dilution for the fraction of total 18S and 25S transcripts represented by the rDNA clone, the fraction of total transcription which is rRNA, the fraction of the small subunit gene transcript represented by the various small subunit clones, and the number of micrograms of each DNA applied

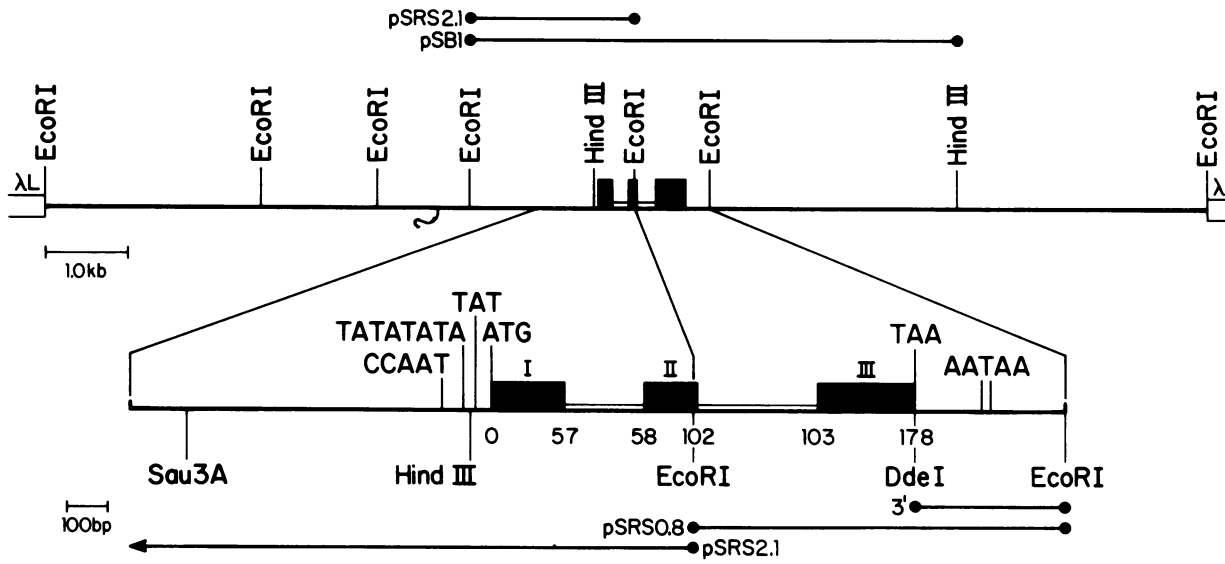

FIG. 1. Physical map of the soybean RuBPC small subunit gene, SRS1, and flanking sequences. The upper map shows all *Eco*RI and *Hin*dIII sites in the clone λSRS1. The expanded map below shows the presumptive 5′ and 3′ regulatory sequences in relation to the SRS1 gene. The three exons in both figures are shown as dark boxes and are denoted by Roman numerals in the expanded figure. Introns are shown as open rectangles. Amino acid codon numbers are shown below the exons. Only relevant *Dde*I and *Sau*3A sites are shown. The different SRS1 DNA fragments used in the following transcriptional study are aligned relative to the SRS1 restriction map. The original lambda clone, λSRS1, the two pBR325 recombinant plasmids, pSRS2.1 and pSRS0.8, and a gene-specific fragment, labeled 3′, have been described (4). The 2.0-kilobase fragment in pSRS2.1 contains about 1.5 kilobases of 5′ upstream flanking sequence and approximately 340 base pairs of encoding mature mRNA. The 0.8-kilobase fragment in pSRS0.8 contains approximately 430 base pairs of sequence encoding mature mRNA and 150 base pairs of 3′ flanking sequence. The fragment labeled 3′ is a purified 296-base-pair *Dde*I-*Eco*RI fragment containing only 3′ nontranslated sequences. The *Dde*I site is situated in the stop codon. Approximately 155 to 188 base pairs of this fragment encode mature mRNAs. This fragment was shown to be specific for the SRS1 gene by genomic Southern blots (4). The clone pSB1 contains the entire RuBPC small subunit gene with extensive 5′ and 3′ sequences on a 6-kilobase *Eco*RI-*Hin*dIII fragment, cloned into the small *Eco*RI-*Hin*dIII replacement region of pBR325.

to the filter. No attempt was made to correct for the amount of rRNA-transcribed spacer. The rDNA clone, pSR1.2B3, contains a 1.05-kilobase-pair 18S fragment in the *Bst*I-*Eco*RI replacement region of pBR325 (V. K. Eckenrode, J. Arnold, and R. B. Meagher, J. Mol. Evol., in press). Differences in the level of small subunit transcription relative to rRNA transcription were observed between independent replicate experiments (see below). Therefore, no attempt was made to interpolate the small differences in the intensities of dots that were detected with a densitometer.

RESULTS

Structure of the soybean small subunit gene SRS1. We previously presented the complete sequence of one member of the soybean RuBPC small subunit gene family designated SRS1 (4). A physical map of the λSRS1 gene and surrounding sequences is shown in Fig. 1. The peptide-coding region is composed of three exons and two introns. The 5′ transcribed leader sequence located upstream of the ATG start codon is 45 base pairs long, beginning at the A of the TAT. The sequence TATATATA, resembling a Goldberg-Hogness box (3), and the sequence CCAAT, typical of a modulator (2, 9), are found 32 and 85 base pairs, respectively, upstream from the transcriptional start. Two 3′ polyadenylate addition sites only 27 base pairs from one another have been identified, and their approximate positions are indicated in the figure (S. B. Berry-Lowe, M. A. Grandbastien, and R. B. Meagher, manuscript in preparation).

Light control of transcription: (i) transcriptional assays. The conditions for pulse-labeling of transcripts were determined in preliminary experiments. Transcriptionally active nuclei were isolated from light-grown and dark-grown plants, and the RNAs were labeled in vitro with [α-^{32}P]UTP for 5 to 90 min. We found that incorporation into trichloroacetic acid-precipitable counts proceeded linearly for at least 30 min in nuclei obtained from plants grown under either light regime.Transcription had declined considerably by 90 min. All subsequent nuclei preparations were labeled for 20 min.

To examine the integrity of our labeled RNA preparations, the size of the RNA probe was examined on a morpholinepropanesulfonic acid-formaldehyde denaturing agarose gel (15) and compared with the sizes of 18S and 25S soybean rRNAs. The sizes of labeled soybean RNAs ranged from 5 kilobase pairs to smaller, with a mean of 1.5 kilobase pairs. This size range is comparable to that reported for labeled transcripts of *L. gibba*, *Drosophila melanogaster*, and adenovirus (12, 17, 25).

(ii) Qualitative studies. Initial qualitative studies were performed to determine whether transcription was specific for the SRS1 gene. We also wanted to know whether we could detect differences between small subunit transcriptions in light and darkness. RNA isolated from nuclei of plants grown in light or darkness was used as a probe to hybridize to *Eco*RI restriction digests of the plasmid clone pSB1. The *Eco*RI fragments within pSB1 hybridized specifically to pulse-labeled RNA from light-grown plants and not to the plasmid vehicle (Fig. 2A). No hybridization was detected between labeled RNA from plants grown in darkness and pSB1 fragments. In a separate experiment, pulse-labeled RNA was hybridized to *Eco*RI fragments of the entire lambda clone, λSRS1 (Fig. 1). Of the six *Eco*RI soybean fragments contained in the clone, only the 2.0- and 0.8-kilobase *Eco*RI fragments hybridized to RNA from light-

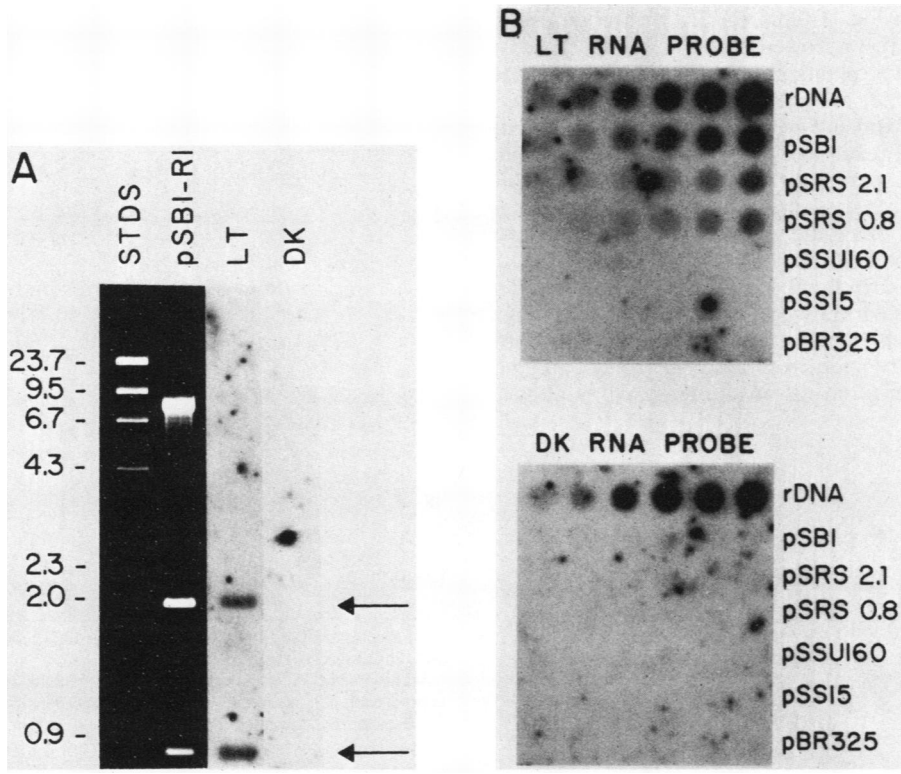

FIG. 2. Induction of RuBPC small subunit transcription by light. (A) Fragments from *Eco*RI-digested pSB1, containing the RuBPC small subunit gene SRS1, were electrophoresed through an agarose gel and blotted to nitrocellulose. The stained gel containing the standards (STDS) and pSB1 digest (pSB1-RI) is shown on the left. The autoradiogram of this filter hybridized to RNA labeled with [α-^{32}P]UTP in isolated nuclei from light-grown (LT) or dark-grown (DK) soybean plants is shown on the right. Arrows indicate gene containing fragments of 0.8 and 2.0 kilobases. Filters were washed at 56°C in 0.5× SSC. (B) Dot blots of plasmid DNAs probed with RNA labeled with [α-^{32}P]UTP in isolated nuclei from light-grown (LT) or dark-grown (DK) soybean plants. Plasmid DNAs, pSB1, pSRS2.1, pSRS0.8 (see Fig. 1), the pea cDNAs pSSU160 and pSS15, and pBR325 and a soybean 18S rDNA clone were prepared as described in the text and were loaded in a twofold dilution series. The first dot in the series contains 1 μg of each plasmid. Blots were hybridized at 56°C and washed with 0.2× SSC at 40°C.

grown nuclei (data not shown). This finding suggests that no other highly transcribed genes are closely linked to the SRS1 gene.

(iii) Quantitative studies. RNAs labeled in nuclei from light-grown and dark-grown seedlings were hybridized to nitrocellulose dot blots to determine the extent of light-induced transcription. A soybean ribosomal clone, pSR1.2B3 (rDNA), was used as an internal control to verify that hybridization between the two filters was equivalent. The dot blot shown in Fig. 2B indicates that transcription of the small subunit occurs at approximately a 32-fold higher rate in the light than in darkness. The calculated transcription rates based on hybridization to the plasmid containing the intact RuBPC small subunit gene, pSB1, and the 5′ and 3′ halves of the gene, pSRS2.1 and pSRS0.8, are approximately equivalent. Two pea RuBPC small subunit cDNA clones, pSS15 (6) and pSSU160 (1), and the plasmid vehicle, pBR325, did not hybridize to the labeled RNA, confirming that the moderate stringency used during hybridization was sufficient to permit hybridization of only very closely related small subunit sequences. We have evidence that one other small subunit gene, SRS4, which is very closely related to SRS1, is present in the soybean genome and is also transcriptionally active in the light (Berry-Lowe et al., in preparation). Therefore, to be certain that we were examining SRS1 gene-specific transcription, we needed to use conditions more stringent than those used in this experiment.

Levels of light-induced transcription of SRS1 similar to those shown in Fig. 2B were obtained when labeled RNAs were hybridized to nitrocellulose dot blots under conditions of higher stringency. The hybridization of RNA labeled in nuclei from light-induced plants to the SRS1 gene on pSB1 is shown in Fig. 3G, lane 2, and that from plants grown in darkness is shown in Fig. 3A, lane 2. Through normalization of small subunit transcription against rRNA transcription measured by hybridization to the rDNA clone pSR1.2B3, the levels of SRS1 transcription can be compared. The level of transcription of SRS1 in continuous light represents 0.8 to 1.5% of the total transcription, whereas that in darkness is approximately 0.04% (see above for calculations). As stated above, this represents a 16- to 32-fold increase in transcription in the light. Hybridization to the 3′ gene-specific probe is shown in Fig. 3G, lane 4 (dilutions were not carried out). Lane 3 of Fig. 3G contains pBR322 DNA.

Time course for transcriptional induction in the light. The length of light treatment required to initiate transcription of the small subunit was determined. Seedlings were germinated in darkness for 11 days, exposed to light for periods ranging from 30 min to 48 h, and then harvested for in vitro labeling of nuclei. Transcription was compared with that from nuclei of seedlings grown continuously in light or continuously in darkness. The transcription levels were normalized to ribosomal RNA transcription rates. The results of two experiments (Fig. 3) typify the data from several experiments. A summary of the quantitative analysis of the

dot blots presented in Fig. 3 (dark circles on the graph) is given in Table 1. There was often a detectable increase in transcription in etiolated plants exposed to 15 or 30 min of light, but a reproducible, measurable increase in transcription required 2 h of exposure to light. SRS1 small subunit gene transcription had reached 80% of maximum levels in 6 h but continued to increase with longer or continuous light treatment. The smooth curve in the figure serves only as a guide in interpreting the data and not as a quantitative summary of these experiments.

Time course for transcriptional decrease in the darkness. To explore the effects of darkness on transcription, seedlings were grown in the light for 11 days and then returned to darkness for periods ranging from 2 to 48 h before being harvested. The transcription rate was measured as above.

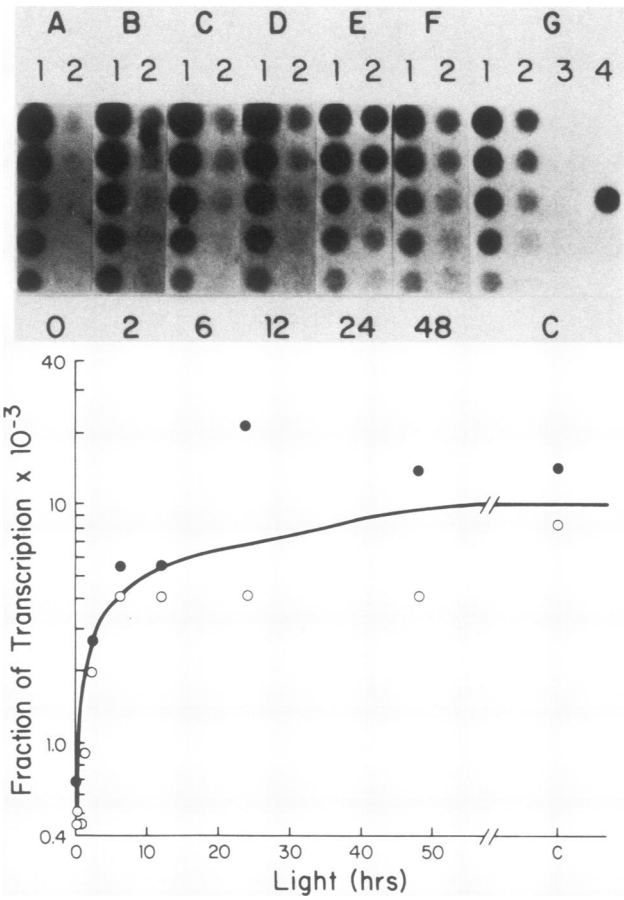

FIG. 3. Time course for transcriptional induction in light. In dot blots (A through G), rDNA transcription (lanes 1) is compared with SRS1 transcription (lanes 2). In panel G, lane 1 contains rDNA (1 μg) from the soybean ribosomal clone pSR1.2B3, lane 2 contains pSB1 RuBPC small subunit DNA (5 μg), lane 3 contains DNA from pBR325 (1 μg), and lane 4 contains one dot (0.6 μg) of the SRS1 gene-specific 3′ fragment. Only the first five of eight twofold dilutions are shown. Filters were probed with RNA labeled with [α-^{32}P]UTP in isolated nuclei from etiolated seedlings exposed to light for 0 (A), 2 (B), 6 (C), 12 (D), 24 (E), and 48 (F) h. RNA labeled in isolated nuclei from plants grown in continuous light (C) was used for comparison (G). Filters were washed with 0.1× SSC at 56°C. In the graph below, two such experiments are summarized. Dark circles in the graph correspond to the dot blots shown. Open circles represent times from an independent experiment. The fraction of transcription was calculated as described in the text (see also Table 1) and is plotted against hours of exposure to light. The probes used in this experiment were phenol extracted.

TABLE 1. Quantitation of SRS1 transcription

Time (h) in light[a]	rDNA[b]		RuBPC small subunit[b]		Difference in dilution	Fraction of transcription (10^{-3})[c]
	Dilution	Peak area	Dilution	Peak area		
0	128	4.0	1	6.5	128	0.68
2	32	29.7	1	26	32	2.7
	64	12.8	2	14		
6	16	22	1	25.5	16	5.4
	32	7.2	2	10.2		
12	16	32	1	25	16	5.4
	32	13	2	14		
24	4	22	1	23	4	22
	8	6.5	2	16		
48	16	10.2	2	11	8	10.8
	32	4	4	3		
Continuous	8	23.3	1	21.1	8	10.8
	16	6.3	2	10.7		

[a] Samples are the same as those described in the legend to Fig. 3 (dot blots and closed circles on graph).
[b] The integrated densities of dots of similar intensity are shown for comparison. The laser scanner measures the density of the entire dot.
[c] See the text for calculations.

Results from these experiments are shown in Fig. 4. A decrease in the transcription rate was detectable within 2 to 6 h of exposure to darkness and reached dark-grown levels within 48 h of darkness.

Phytochrome response. The induction of small subunit transcription by light can be reversed by far-red light in *L. gibba* and pea plants (14, 23, 25, 28, 34). We wanted to determine whether the light induction of soybean small subunit transcription exhibited far-red reversibility. Ten-day-old etiolated soybean seedlings were exposed for various times to white light and then to far-red light followed by periods of darkness before harvest for transcriptional assays. An example of the reproducible and quantitatively significant results obtained from a variety of experiments is presented in Fig. 5. When dark-grown plants received a 15-min white light treatment followed by 9 h of exposure to darkness, a sixfold induction of small subunit transcription was detected (Fig. 5C). When etiolated plants were exposed to white light for 15 min followed by a 15-min exposure to far-red light and harvested after 9 h in the darkness, no increase in transcription was observed (Fig. 5D). The induction of soybean RuBPC small subunit transcription caused by a pulse of white light was reproducibly reversed by a 15-min treatment with far-red light, thereby exhibiting a classical phytochrome-linked response.

It has been shown in some plants that the light treatment must be followed immediately by far-red light treatment to effect reversal of the induction of steady-state mRNA levels (34). We have shown that far-red light treatment immediately after red light treatment does cause a significant reversal of transcriptional induction in soybean. We have not examined the effects of various times between the red or white light and the far-red light treatments, but we have studied the effect of far-red light on transcriptionally active light-grown plants. Seedlings were germinated and grown in white light for 10 days, treated with far-red light for 15 min, and then placed in darkness for 15 min or 2 h (Fig. 6B) before being

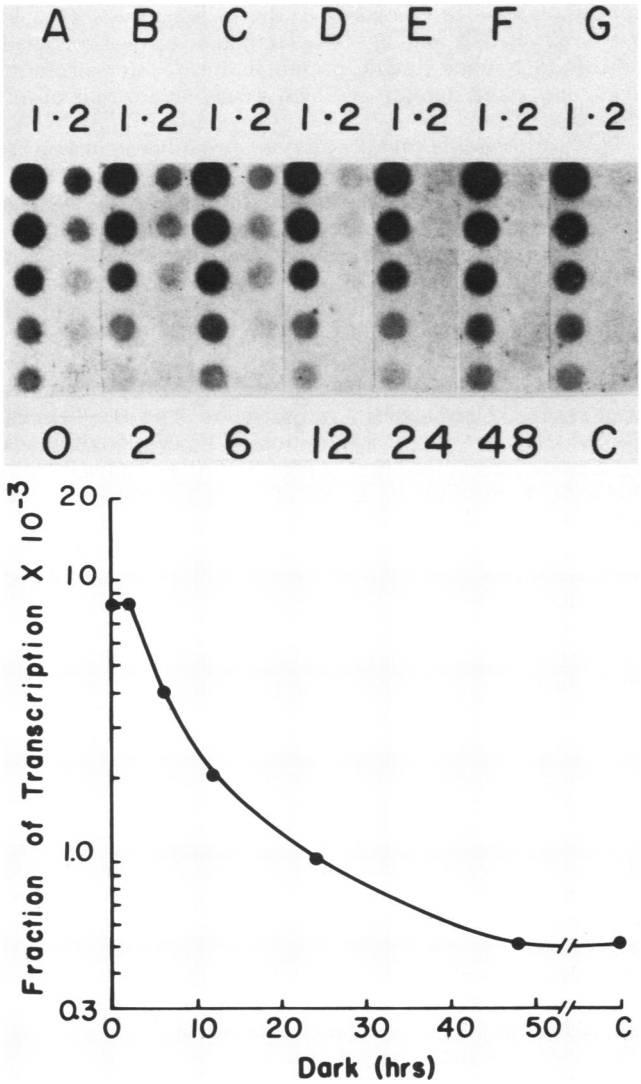

FIG. 4. Time course for transcriptional decrease in darkness. Dot blots showing rDNA transcription from a soybean ribosomal clone (lanes 1) and SRS1 gene transcription from clone pSB1 (lanes 2) were prepared as described in the legend to Fig. 3. RNAs labeled in isolated nuclei from seedlings grown in the light and exposed to darkness for 0, 2, 6, 12, 24, or 48 h or to continuous darkness were used to probe the filters. The fraction of transcription was calculated as described in the text. This value is plotted against hours of exposure to darkness. The graph illustrates the results from one experiment. The probes used in this experiment were phenol extracted and washed at 56°C in 0.1× SSC.

harvested. In both experiments, transcription was reduced to levels near the basal levels of dark-grown plants by the far-red treatment. In most of our experiments (like the one shown), far-red treatment resulted in a 20- to 40-fold decrease in transcription (compare Fig. 6A to B). When light-grown plants were treated with 2 h of darkness alone without the far-red treatment, transcription was reduced by a factor of zero to two (at most) over controls (Fig. 4). Therefore, far-red treatment reversed the effects of continuous white light treatment.

DISCUSSION

Light-regulated transcription has been shown to be a major mechanism controlling small subunit mRNA and

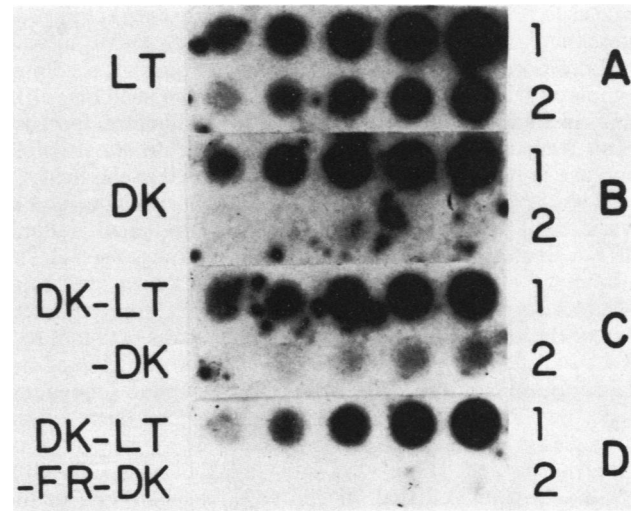

FIG. 5. Phytochrome regulation of SRS1 transcription. Dot blots in which rDNA transcription from a soybean ribosomal clone (lanes 1) was compared with RuBPC small subunit transcription from clone pSB1 (lanes 2) were probed with RNA transcribed and labeled in isolated nuclei from plants grown in continuous light (A), from plants grown in total darkness (B), from plants grown in darkness and exposed to 15 min of white light (C), and from plants grown in the dark and exposed to white light for 15 min and then immediately exposed to far-red light for 15 min (D). Plants from treatments C and D were placed in darkness for 9 h before harvest. Blots were washed at 50°C with 0.25× SSC.

protein levels in pea plant cells (10). The effects of white, red, and far-red light on RuBPC small subunit transcription have been shown for the aquatic monocot, *L. gibba* (25). We have demonstrated that the transcription of the SRS1 gene encoding the small subunit of RuBPC in soybean is under strong positive regulation by white light and strong negative regulation by far-red light. Our data establish that the chromoprotein phytochrome is involved in the control of transcription of the small subunit gene in soybean.

We observed a 16- to 32-fold increase in soybean RuBPC small subunit transcription in seedlings germinated in white light compared with the level found in seedlings germinated in darkness. Changes in small subunit transcription were

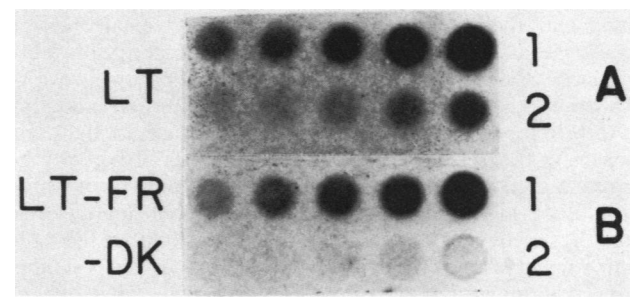

FIG. 6. Effect of far-red light on transcription of SRS1 in light-grown soybean. In dot blots rDNA transcription from a soybean ribosomal clone (lanes 1) was compared with RuBPC small subunit transcription from clone pSB1 (lanes 2). RNA probe was transcribed and labeled in isolated nuclei from seedlings grown in continuous light (A) or in continuous light but exposed to far-red light for 15 min and placed in the dark for 2 h before harvest in the dark (B). Blots were washed with 0.1× SSC at 56°. The probes used in this experiment were phenol extracted. See Fig. 4, 2-h dark-treated sample for comparison.

measured relative to rRNA synthesis. These data are in good agreement with the 18-fold increase in pea small subunit transcription observed in nuclei from light-grown seedlings compared with the level found in dark-grown seedlings (10). This 18-fold increase translated into a ninefold increase when transcription was measured relative to the twofold increase in pea rRNA transcription observed in the light. In *L. gibba*, plants treated with intermittent red light (2 min every 8 h) transcribed significantly more small subunit mRNA than did plants shifted to total darkness for 7 days.

Soybean small subunit transcription was measured relative to rRNA transcription. We found that approximately 51% of the transcription in soybean nuclei was resistant to 5 to 20 μg of alpha-amanitin per ml. Therefore, 51% of the transcription is presumably from ribosomal gene sequences, since only RNA polymerase I is active under these conditions. This calculation is comparable to the data of Silverthorne and Tobin (25) and Gallagher and Ellis (10), who have estimated that 40 and 64%, respectively, of the total transcription in isolated light-grown nuclei is alpha-amanitin resistant. We did not observe an increase in rRNA transcription in the light as has been seen in pea, *L. gibba*, and mustard plants (10, 25, 31, 32).

When measured against rRNA transcription, the light-induced transcription of the SRS1 RuBPC small subunit gene reached extremely high levels. We calculated in repeated experiments that SRS1 gene transcription represented between 0.8 and 1.5% of the transcription in light-induced nuclei (Fig. 3). High levels of transcription in the light could account for the significant steady-state levels of mRNA. Pulse-chase labeling of nuclear transcripts in pea and *L. gibba* tissues suggest that small subunit mRNA has a very slow turnover time, at least in light-treated tissue (10, 25). We have already reported that the steady-state levels of soybean small subunit mRNA are at least 50-fold higher in light-grown tissue than in dark-grown tissue (4). Similar differences in steady-state mRNA levels for light-grown and dark-grown seedlings have been reported in peas and wheat (7, 26). An eightfold increase was observed in steady-state mRNA levels between *L. gibba* plants placed in complete darkness for 7 days and those given intermittent light treatment (28). Increased transcription appears to be a major mechanism for increasing the steady-state levels of small subunit mRNA in the light.

The time course of transcriptional induction was examined by exposing soybean seedlings to white light for various times and then assaying them for RuBPC small subunit transcription. Continuous increases in transcription were dramatic when etiolated seedlings were given up to 6 h of exposure to light, showing approximately a 16-fold induction at this time. After this initial (6-h) exposure to light, increases in transcription were usually more gradual, with no increase after 24 to 48 h of exposure to light as compared with those in seedlings grown in continuous light. When seedlings were germinated and grown in continuous light and then subjected to darkness, a small decline in small subunit transcription was detected within 2 to 6 h. This decline continued until 48 h of exposure to darkness, at which time it reached the basal levels for dark-grown plants, resulting in a 16- to 32-fold reduction in transcription. Comparable studies on the time course of transcriptional induction of pea and *L. gibba* small subunit have not been published, although the effect of light-dark shifts on steady-state small subunit mRNA and protein levels have been examined in these and a number of other systems (22, 35). The effects of light on the steady-state levels of mRNA and polypeptide are not dramatic when compared to the increases observed in transcription. The inability to detect major changes in small subunit mRNA and RuBPC protein in these systems is most likely due to the stability and high steady-state levels of the gene products.

Phytochrome is a photoreversible chromoprotein known to be involved in the regulation of light-induced processes. We wanted to know whether phytochrome was involved in the induction of soybean small subunit transcription in the light and in the decrease in transcription rates in darkness. A 15-min white light treatment increased transcription in etiolated soybean seedlings above basal levels. This induction of transcription was reversed when the white light treatment was followed by a 15-min far-red light treatment. The decrease in transcription brought on by far-red treatment often reached basal levels of transcription. The involvement of phytochrome in the transcription of small subunit genes has also been examined in *L. gibba* (25). After 7 days in total darkness, transcriptional levels of small subunit were still detectable. When these plants were given 2 min of red light followed by 2 h of darkness, an increase in transcription was observed. This increase in transcription was eliminated when the red light treatment was followed immediately by a 10-min far-red treatment. Light-induced transcription in soybean and *L. gibba* is reversible by far-red light, typical of a phytochrome-linked response.

Soybeans germinated in total light were irradiated with far-red light and placed in darkness for 2 h before harvest. A 16- to 32-fold decrease in small subunit transcription was detected in the far-red-treated plants relative to continuous-light-grown plants. Transcription usually decreased to dark-grown levels. Differences could not be attributed to turnover of the message in darkness, since light-grown plants treated with 2 h of darkness exhibit only a zero to twofold decrease in transcription (Fig. 4). This result implicated the direct involvement of phytochrome in turning off transcription. Tobin has presented data in *L. gibba* demonstrating that a short light treatment must be followed immediately by the far-red light treatment to obtain a substantial far-red reversal effect (34). The data imply that a significant delay between the onset of the light treatment and the far-red light treatment which followed resulted in no reversal effect; i.e., transcription continued. Given these data for *L. gibba*, our results on light-grown plants treated with far-red light are somewhat surprising. We would not have expected such a complete reduction of transcription if the plants were already in a committed light-grown state for such a long period. Our data indicate that phytochrome must continuously be present in the far-red-absorbing (active) form to sustain transcription in soybean. The very slow reduction in soybean small subunit transcription during the shift from the light to darkness (Fig. 4) may parallel the dark reversion of phytochrome, that is, the slow, spontaneous conversion of phytochrome from the far-red-absorbing (active) to the red-absorbing form (14, 29). Data on the kinetics of spontaneous dark reversion of phytochrome are not available for soybean.

In summary, the light and phytochrome effects on transcription of soybean RuBPC small subunit are dramatic, far more so than the effects on steady-state levels of mRNA and protein in any system. Soybean may be a useful system in which to study phytochrome-linked regulation of transcription.

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