Light-regulated Translation of Chloroplast Proteins. I. Transcripts of PsaA–PsaB, PsbA, and RbcL Are Associated with Polysomes in Dark-grown and Illuminated Barley Seedlings

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Abstract. We have previously observed (Klein, R. R., and J. E. Mullet, 1986, *J. Biol. Chem.* 261:11138–11145) that translation of two 65–70-kD chlorophyll *a*–apoproteins of Photosystem I (gene products of psaA and psaB) and a 32-kD quinone-binding protein of Photosystem II (gene product of psbA) was not detected in plastids of dark-grown barley seedlings even though transcripts for these proteins were present. In the present study it was found that nearly all of the psaA–psaB transcripts in plastids of dark-grown plants were associated with membrane-bound polysomes. Membrane-associated polysomes from plastids of dark-grown plants synthesized the 65–70-kD chlorophyll *a*–apoproteins at low levels when added to a homologous in vitro translation extract capable of translation elongation. However, when etioplast membranes were disrupted with detergent, in vitro synthesis of the 65–70-kD chlorophyll *a*–apoproteins increased to levels observed with polysomes of plastids from illuminated plants. These results suggest that synthesis of the chlorophyll *a*–apoproteins of Photosystem I is arrested on membrane-bound polysomes at the level of polypeptide chain elongation. In addition to the selective activation of chlorophyll *a*–apoprotein translation, illumination also caused an increase in chloroplast polysomes (membrane-associated and stromal) and induced a recruitment of psbA and rbcL transcripts into chloroplast polysomes. These results indicate that in conjunction with the selective activation of chlorophyll *a*–apoprotein elongation, illumination also caused a general stimulation of chloroplast translation initiation.

THE inner membrane of the chloroplast contains four major protein complexes: Photosystem I, Photosystem II, a cytochrome complex, and an ATP synthetase. Each of these complexes is composed of 5–12 polypeptides and associated cofactors. Some of the polypeptides of the chloroplast membrane are encoded on nuclear genes whereas others are encoded on chloroplast genes (21). The expression of chloroplast and nuclear genes is coordinated with leaf development and controlled in part by environmental signals including light (47). The synthesis and assembly of chloroplast protein complexes is highly regulated. For example, accumulation of nuclear-encoded chloroplast polypeptides is controlled at the level of transcription (18, 20, 49), translation (50), and protein stability (5, 9). The regulation of chloroplast-encoded protein accumulation is similarly complex (11, 40, 43).

The synthesis of chlorophyll and the accumulation of chlorophyll apoproteins are coupled in higher plant chloroplasts (5, 9, 29, 50, 55). Coordination of chlorophyll biosynthesis and chlorophyll–apoprotein accumulation prevents the accumulation of free chlorophyll and the resulting photooxidation of chloroplast membranes in the light (8, 30). In higher plants, light is required to convert protochlorophyllide to chlorophyllide which is then esterified with geranylgeranyl pyrophosphate to yield chlorophyll (12). In 5-d-old dark-grown barley seedlings, the formation of chlorophyll from protochlorophyllide was 90% complete after 15 min of illumination (55). Several studies have shown that the 65–70-kD chlorophyll *a*–apoproteins of Photosystem I were undetectable (26, 27, 29, 51, 55) in dark-grown barley plants. Net synthesis of the chlorophyll *a*–apoproteins was detected only after seedlings were illuminated (27, 28, 55). However, the chloroplast genes that encode the chlorophyll *a*–apoproteins are actively transcribed in plastids of dark-grown plants (43), and RNA which hybridizes to these genes accumulates (24, 27, 28, 31). These results indicate that the accumulation of chlorophyll *a*–apoproteins in dark-grown plants is regulated posttranscriptionally.

Klein and Mullet (27) showed that the chlorophyll *a*–apoproteins of Photosystems I and II were not labeled when plastids isolated from dark-grown plants were pulse-labeled with [^{35}S]methionine. The deficiency of chlorophyll *a*–apoproteins in dark-grown plants could be due to (*a*) rapid apoprotein degradation during translation so that no full-length products are detected, or (*b*) the arrest of chlorophyll *a*–apoprotein translation. Similarly, the accumulation and labeling

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of the chlorophyll *a*–apoproteins in illuminated plants could be explained by (*a*) chlorophyll stabilizing nascent chlorophyll *a*–apoproteins, or (*b*) chlorophyll activating apoprotein translation.

Klein and Mullet (28) have also shown that the synthesis of a 32-kD membrane polypeptide is rapidly induced upon illumination of dark-grown barley seedlings. Based on electrophoretic mobility and its high rate of synthesis in mature illuminated plants, the 32-kD polypeptide has been identified as the psbA gene product (28). Transcripts for psbA were detected in plastids of dark-grown plants but synthesis of the psbA gene product was not observed (27, 28). The time course of induction of psbA gene product synthesis was similar to the induction of chlorophyll *a*–apoprotein translation and the light-dependent formation of chlorophyll (29). It has recently been suggested that the psbA and psbD gene products are Photosystem II reaction center proteins that bind chlorophyll (7, 16, 45, 46, 53). Therefore, the regulation of psbA gene product synthesis may be similar to the control of Photosystem I chlorophyll *a*–apoprotein synthesis.

In the present study, the regulation of Photosystem I chlorophyll *a*–apoprotein and psbA gene product synthesis is examined. Conditions were optimized for the isolation of stromal and membrane-bound polysomes from etioplasts and chloroplasts. Using this system, the effect of light on the distribution of transcripts for rbcL (encodes the large subunit of ribulose 1,5-bisphosphate carboxylase [RUBISCO]),[1] psaA-psaB (encodes the chlorophyll *a*–apoproteins of Photosystem I), and psbA (encodes a 32-kD quinone-binding protein of Photosystem II) among membrane-bound and stromal polysomes was determined. Furthermore, conditions were optimized for the in vitro translation of isolated membrane-bound polysomes. Evidence is presented which may indicate a novel control mechanism, namely, the control of chlorophyll *a*–apoprotein gene product synthesis at the level of polypeptide chain elongation during light-induced plastid development.

Materials and Methods

Plant Growth

Barley (*Hordeum vulgare* L. var Morex) seedlings were grown as previously described (27, 28). Seeds were germinated and grown for 4.5 d in a dark chamber located in a light-tight room. At this stage of development seedlings were 7–8 cm tall. After 4.5 d in the dark, seedlings were either kept in the dark or were transferred to an illuminated chamber for 1 h before harvest (light intensity of 12 W $m^{-2}s^{-1}$, fluorescent plus incandescent bulbs).

Plastid Isolation and Plastid Number

Approximately 30 g of barley leaves (upper 4–5 cm) were cut and immediately immersed in iced water. After ∼10 min the tissue was surface-sterilized (27). Leaves were ground in 500 ml of chilled grinding media containing 0.33 M sorbitol, 50 mM Hepes-KOH (pH 8.0), 1 mM EDTA (pH 7.5 with NaOH), 1 mM dithiothreitol, and 100 μg/ml chloramphenicol. Homogenates were filtered through Miracloth and centrifuged at 4,000 *g* for 1 min. The pellet was gently resuspended in 6 ml of grinding media, and intact plastids were isolated by Percoll gradient centrifugation (41), except 5 mM dithiothreitol and 100 μg/ml chloramphenicol were added to the gradients and $MgCl_2$ and $MnCl_2$ were eliminated. Intact plastids from Percoll gradients were washed once with sorbitol-Hepes (0.33 M, and 50 mM [pH 8.0 with KOH]), centrifuged at 2,500 *g* for 5 min, and gently resuspended in chilled sorbitol-Hepes. All manipulations were performed at 2–4°C. For quantitation of plastid number (plastids per microliter of suspension volume), aliquots of isolated plastids were diluted and plastids counted in a hemocytometer with a 20× phase contrast lens.

Fractionation of Intact Plastids

For the fractionation of chloroplasts into membrane-bound and free (stromal) polysomes, intact plastids (1.25–1.75 × 10^8 plastids) were centrifuged in a microfuge (model 5413; Brinkmann Instruments Co., Westbury, NY) for 1 min at 4°C. After discarding the supernatant, the pellet of intact plastids was gently resuspended in 500 μl of cold lysis buffer (20 mM Tris-HCl [pH 8.5], 20 mM potassium acetate, 10 mM $MgCl_2$, 0.5 mg/ml heparin, 5 mM dithiothreitol) and microfuged for 5 min. The supernatant was saved and the membrane pellet was resuspended in 200 μl of lysis buffer and microfuged for 10 min. The supernatants from the first and second centrifugations were combined and centrifuged at 27,000 *g* in a JS13 rotor (Beckman Instruments Inc., Fullerton, CA) for 15 min to remove membrane fragments. The supernatant (soluble fraction) was removed and the pelleted membrane fragments were combined with the membrane pellet from the second centrifugation (membrane fraction). After fractionation, membrane and soluble samples were either electrophoresed and silver stained (28) or prepared for polysome isolation.

Preparation of Polysomes

For the isolation of free (soluble fraction) or membrane-bound polysomes, intact plastids (1.25–1.75 × 10^8) were fractionated into membrane and soluble phases as described above. The membrane fraction was solubilized in 700 μl of solubilization buffer (200 mM Tris-HCl [pH 8.5], 10 mM $MgCl_2$, 20 mM potassium acetate, 5 mM dithiothreitol, 0.5 mg/ml heparin, 2% polyoxyethylene 10-tridecylether (PTE), and 1.5 μg/ml proteinase K. Proteinase K was subsequently added to the soluble fraction to a final concentration of 6 μg/ml. Samples were kept on ice for 10 min then microfuged for 5 min (4°C). Samples were layered over linear gradients of 15–60% (wt/vol) RNase-free sucrose in buffer containing 50 mM Tris-HCl (pH 8.5), 25 mM KCl, and 10 mM $MgCl_2$. Sucrose gradients were centrifuged at 260,000 *g* for 2 h in a type SW41 rotor (Beckman Instruments Inc.). All operations were conducted at 4°C.

In some instances, total chloroplast polysomes (soluble and membrane-bound) were isolated from intact plastids that were not fractionated into soluble and membrane phases. For preparation of total chloroplast polysomes, intact plastids (∼1.5 × 10^8 plastids) were microfuged for 1 min and the supernatant discarded. The pellet of intact plastids was resuspended in 700 μl of solubilization buffer. Samples were kept on ice for 10 min, microfuged for 5 min, layered over 15–60% (wt/vol) sucrose gradients and centrifuged as just described. Variations from these methods are noted where appropriate in the figure legends.

For the isolation of polysomes from intact leaf tissue (70S plus 80S ribosomes), the upper 4–5 cm of 20 barley seedlings were excised with a razor blade and frozen in liquid N_2 within 3 s after excision. Frozen leaves were ground under liquid N_2 in a chilled mortar. All subsequent operations were conducted at 0–4°C. The powder was homogenized in 6–8 ml of a solution containing 200 mM Tris-HCl (pH 8.5), 200 mM KCl, 30 mM $MgCl_2$, 10 mM EGTA, 200 mM RNase-free sucrose, 2.5 mM dithiothreitol, 0.5 mg/ml heparin, 5 μg/ml proteinase K, 2% PTE, 100 μg/ml chloramphenicol, and 20 μg/ml cycloheximide. Samples were kept on ice for 10 min, filtered through 70-μm mesh filters and clarified by microfuging for 10 min. 700-μl aliquots of samples were layered over 15–60% (wt/vol) sucrose gradients and centrifuged as just described.

Analysis of Polysomes on Sucrose Density Gradients

After centrifugation, sucrose gradients of polysomes were analyzed by pumping through a UV analyzer (model UA-5; Isco, Inc., Lincoln, NE) with continuous monitoring of absorbance at 254 nm. The areas in different regions of the polysomal profiles were determined from the average of two separate profiles as described by Mason and Matsuda (36). Baselines were determined by monitoring sucrose gradient profiles of postribosomal supernatants. Postribosomal supernatants were obtained by centrifuging polysomal preparations at 390,000 *g* for 5 h in a Beckman type SW50.1 rotor. Postribosomal supernatants were then layered over 15–60% (wt/vol) sucrose gradients and centrifuged for 2 h at 260,000 *g*. It was found that the baseline from various polysomal preparations differed (i.e., stromal vs. membrane-bound polysomes). Therefore, postribosomal supernatants were prepared for each polysome fraction and baselines determined. Baselines are re-

1. *Abbreviations used in this paper*: P/T, polysome to total ribosome ratio; PTE, polyoxyethylene 10-tridecyl ether; RUBISCO, ribulose 1,5-bisphosphate carboxylase.

ported for each polysomal profile and the area below the baseline was excluded from the area calculations. The proportion of ribosomes in polysomal aggregates (P/T) was calculated as follows: (area of polysomes)/(area of polysomes + ribosomal subunits + monosomes).

Protein Synthesis by Membrane-bound Polysomes

For preparation of membrane-bound polysomes to be assayed for in vitro protein synthesis, intact plastids (3×10^7 plastids) from 4.5-d-old dark-grown seedlings and plastids from dark-grown seedlings illuminated for 1 h were gently lysed in 50 µl of buffer A (46 mM Hepes-KOH [pH 7.6], 118 mM potassium acetate, 7 mM magnesium acetate, 5 mM dithiothreitol, 10 µg/ml heparin) and microfuged for 5 min. The supernatant was discarded and the membrane pellet was resuspended in 100 µl of buffer A and microfuged for 10 min. The supernatant was discarded and the membrane pellet (with bound polysomes) was assayed for protein synthesis as described below. All manipulations were conducted at 4°C in complete darkness or in the presence of a dim green safelight (27).

For the preparation of a chloroplast S-100 fraction, chloroplasts ($\sim 3 \times 10^8$ plastids) from illuminated barley seedlings were gently lysed in 200 µl of buffer A (containing 0.2 µg/µl bovine serum albumin) and microfuged for 10 min. The supernatant was saved and the membrane pellet was resuspended in 100 µl of buffer A (containing 0.2 µg/µl bovine serum albumin) and microfuged for 10 min. The supernatants from the first and second centrifugation were combined and centrifuged at 160,000 *g* in a Beckman type SW50.1 rotor for 2 h at 4°C. The upper two-thirds of the supernatant (S-100) was frozen in small aliquots and stored at −80°C.

To find optimum translation conditions for protein synthesis by membrane-bound polysomes, we optimized some parameters of the translation medium. [^{35}S]Methionine incorporation was greatest at 2 mM ATP, 0.2 mM GTP, 7 mM magnesium acetate, and 118 mM potassium acetate. A broad pH optimum for [^{35}S]methionine incorporation was found between pH 7.6 and 8.0 (Hepes-KOH buffer, 46 mM). The presence of 2-10 µg/ml heparin were found to stimulate [^{35}S]methionine slightly (<30%) whereas higher concentrations of heparin (0.1 mg/ml) caused a twofold reduction in [^{35}S]methionine incorporation. The optimized protein synthesis mixture (50 µl final volume) for membrane-bound polysomes contained 46 mM Hepes-KOH (pH 7.6), 100 µM of each amino acid (minus methionine), 20 µCi of [^{35}S]methionine (sp act, 1,098 Ci/mmol), 2 mM ATP, 0.2 mM GTP, 7 mM magnesium acetate, 5 mM dithiothreitol, and 10 µg/ml heparin. Membrane-bound polysomes (from 3×10^7 plastids) were prepared as described and were added to the translation mixture. Assays were incubated at 23°C in the dark (membrane-bound polysomes from etioplasts) or in the light (membrane-bound polysomes from chloroplasts). After incubating for a selected time, protein synthesis was arrested by placing samples on ice. In one experiment the detergent PTE was added to the translation mixture at a final concentration of 2% (vol/vol). In a second series of experiments the protein synthesis mixture contained an S-100 fraction (A_{280} units of 0.045) prepared from isolated chloroplasts as described above.

After the labeling period, measurements of trichloroacetic acid-insoluble radioactivity were obtained (27) and samples were subsequently electrophoresed and autoradiographed (27).

Isolation and Quantitation of Plastid mRNA

Messenger RNA from intact plastids and from sucrose gradient fractions was isolated by phenol extraction as previously described (44). The phenol phase of each extract was extracted twice. To enhance the precipitation of nucleic acids by ethanol, we added phenol-extracted tRNAs (Sigma, type XXI from *E. coli*) to each extract at a final concentration of 4 µg/ml.

Northern blot analyses were performed by separating RNA on 0.8% agarose gels containing 6% formaldehyde according to the GeneScreen Instruction Manual (New England Nuclear, Boston, MA, catalogue No. NEF-972). RNA was transferred to GeneScreen membranes by capillary blot procedure. Blots were prehybridized and hybridized to nick-translated DNA probes at 42°C in the presence of 10% dextran sulfate. After hybridizing for 48 h, blots were washed for 10 min at 23°C in 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate) plus 0.5% SDS then 60 min at 50-55°C in 0.2× SSC plus 0.5% SDS. Blots were analyzed by autoradiography. Methods used for DNA labeling have been described (44). The Northern probe for rbcL mRNA was a 1.1-kbp Hind III-Pst I DNA fragment from barley (60). The Northern probe for psaA-psaB was a 1.9-kbp Bam HI-Bam HI DNA fragment from barley (10). The Northern probe for psbA was a 1.0-kbp Bgl II-Xba I DNA fragment from barley (S. K. Boyer and J. E. Mullet, unpublished data).

Preparation of Solutions and Use of RNase-free Glassware and Plasticware

To minimize exogenous RNase and bacterial contamination, all glassware was baked at 200°C for a minimum of 2 h. When possible, sterile disposable plasticware was used. All reused plasticware was rinsed with boiling SDS (2% wt/vol) and rinsed extensively with sterile distilled H_2O. All solutions were sterilized by autoclaving or filtration.

Results

Various investigators have isolated polysomes from chloroplasts of higher plants (3, 4, 13-15, 19, 22, 23, 31, 35, 59) and have succeeded in establishing the site of synthesis (stromal or membrane fraction) of several membrane polypeptides (22, 23, 39) and the large subunit of RUBISCO (22, 39). Using previously reported protocols for isolating chloroplast polysomes, an attempt was made to isolate polysomes from plastids of 4.5-d-old dark-grown barley seedlings. Significant yields of undegraded polysomes could not be obtained from isolated barley plastids using existing methods (data not shown). Therefore, in this report a procedure was developed for obtaining undegraded polysomes from isolated barley plastids. This protocol was used to examine the point at which the synthesis and/or accumulation of several light-induced chloroplast membrane proteins is regulated. Also, the distribution of transcripts for the large subunit of RUBISCO, a stromal phase enzyme which is synthesized at high rates in etiolated tissues (28), was examined.

Effect of RNase Inhibitors on Transcript Stability

A major technical problem in isolating polysomes from plant tissue is the rapid degradation of the mRNA backbone of polysomes by RNases after homogenization of the tissue (1, 17). The degradation of polysomes by RNase can be retarded by minimizing exogenous RNase contamination, and retarding the activity of endogenous RNases. Davis et al. (17) showed that buffers containing 200 mM Tris-HCl of pH 8.5 can retard RNase activity. However, these conditions alone are no guarantee for sufficient preservation of the in vivo distribution of ribosomal material (32). Therefore, before attempting a quantitative investigation of the distribution of ribosomes and mRNA, it was first necessary to check the stability of mRNA under the present experimental conditions.

Fig. 1 shows the stability of mRNA coding for the gene products of psaA-psaB, rbcL, and psbA in lysed plastids in the presence of reported inhibitors of RNase action. Of the RNase inhibitors examined, heparin (0.5 mg/ml) was the most effective in inhibiting RNase activity (lane *3*). The human placenta RNase inhibitor, RNasin (Promega Biotec, Madison, WI), was less effective (lane *2*), and proteinase K (lane *4*) was the least effective inhibitor examined. Vanadyl-ribonucleoside complexes (VRC) inhibited RNase activity but its high UV absorbance precluded its use (data not shown). Other experimental conditions that were found to stabilize mRNA in polysomes included the addition of dithiothreitol, the use of Tris-HCl buffers of pH 8.5 and a ratio of 4 µl solubilization buffer per 1×10^6 plastids. Therefore, to improve the yield of polysomes, we lysed barley plastids and extracted polysomes in Tris-HCl buffers of pH 8.5 containing 0.5 mg/ml heparin and 5 mM dithiothreitol. Furthermore, the protocol was modified so that the ratio of solubilization buffer to plastid number exceeded 4:1.

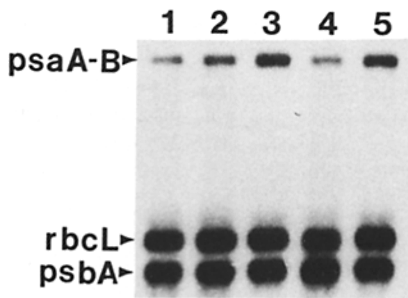

Figure 1. The effect of RNase inhibitors on the stability of rbcL, psaA-psaB, and psbA mRNA. Plastids (1.0 × 10^8) isolated from 4.5-d-old dark-grown seedlings were lysed in 200 μl of buffer (25 mM Tris-HCl [pH 8.5], 15 mM potassium acetate, 10 mM $MgCl_2$, and 10 mM dithiothreitol) containing various RNase inhibitors. Lysed plastids were incubated for 2 h at 4°C and nucleic acids isolated by phenol extractions. Nucleic acid from an equal number of plastids was loaded on 6% formaldehyde gels, transferred to nylon membranes and hybridized simultaneously with nick-translated probes of rbcL, psbA, and psaA-psaB. Lane *1*, control; lane *2*, RNasin ribonuclease inhibitor, 1 U/μl; lane *3*, heparin, 0.5 mg/ml; lane *4*, proteinase K, 5.0 μg/ml; lane *5*, heparin plus proteinase K, 0.5 mg/ml and 5.0 μg/ml, respectively.

Sucrose Gradient Profiles of Plastid Polysomes

Sucrose gradient profiles of polysomes from intact plastids are shown in Fig. 2. After 1 h of illumination a 41% increase in the amount of ribosomal material in polysomes was observed compared with etiolated samples (compare Fig. 2, *A* and *B*). This light-induced increase in large polysomes (4-mers or larger) is consistent with a general stimulation of translation initiation (54). A light-induced stimulation of translation initiation has been observed in pea (19) and *Chlamydomonas reinhardtii* (14, 15) chloroplasts. The P/T ratios (and the shape of the profiles) also indicate that a considerable portion of 70S ribosomes of etioplasts and 1-h-illuminated plastids were in polysomes.

When polysomes of etioplasts and 1-h-illuminated plastids were treated with RNase A, polysomal material was completely shifted to monosomes and ribosomal subunits (Fig. 2, *C* and *D*) indicating that the polysomal fractions represented in Fig. 2, *A* and *B* were not contaminated with ribosomal material attached to membrane fragments (22, 35). Ribosomes attached to membrane fragments are often evident in polysome profiles as a characteristic shoulder of material which comigrates with large polysomes (1). This artifact, and the artifactual aggregation of polysomes via their nascent chains, was eliminated in the present study by solubilizing membranes with the detergent PTE and by degrading nascent chains with proteinase K (1).

Fractionation of Plastids into Stromal and Membrane Phases

To estimate the degree of cross-contamination between stromal and membrane fractions, we electrophoresed, silver stained, and compared polypeptides from these compartments (Fig. 3). The distribution of the small subunit and large subunit of RUBISCO indicates that the membrane phase is largely free of these stromal polypeptides (compare lanes *1* and *2*, *3* and *4*). Further, the stromal fraction is essentially free of membrane polypeptides including the chlorophyll *a*-apoproteins of Photosystem I and the chlorophyll *a*/*b*-binding proteins of Photosystem II. This indicates that the fractionation of plastids into stromal and membrane phases results in only a slight amount of cross-contamination.

Distribution of Transcripts between Membrane and Stromal Fractions

Because stromal and membrane fractions can be prepared nearly free of cross-contamination, the distribution of transcripts for psaA–psaB, psbA, and rbcL between the membrane and stroma was examined by Northern blot analyses (Fig. 4 *A*). Transcripts for psaA–psaB and psbA would be restricted to the stroma of dark-grown plastids if synthesis of these polypeptides was blocked at the level of translation initiation. Alternatively, if translation were arrested early in chain elongation, transcripts of psaA–psaB and psbA would

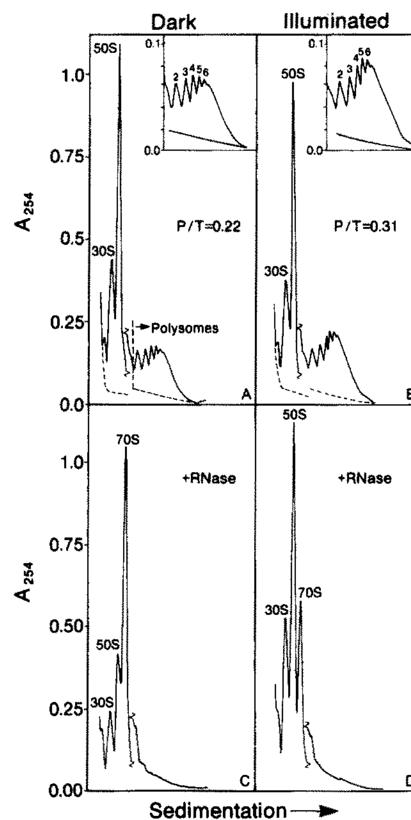

Figure 2. Sucrose density gradient profiles of ribosomal material of isolated plastids before and after RNase treatment. Intact plastids were isolated from 4.5-d-old dark-grown (*Dark*) seedlings and from seedlings illuminated (*Illuminated*) for 1 h before harvest. Plastids (1.5 × 10^8) were solubilized with 700 μl solubilization buffer in the absence (*A* and *B*) or presence (*C* and *D*) of 0.25 μg/ml RNase A (heparin omitted from buffer for RNase A treatment). Samples were incubated for 20 min at 4°C followed by separation on 15–60% (wt/vol) sucrose gradients as described in Materials and Methods. Gradients were scanned at 254 nm and P/T ratios calculated. Insets in *A* and *B* represent the polysomal region of each profile, with numbers indicating number of ribosomes associated with a given mRNA. A 2.5-fold decrease in full scale absorbance is indicated by a break in the absorbance tracing of each profile. Dashed lines represent baselines. The top of gradients is to the left.

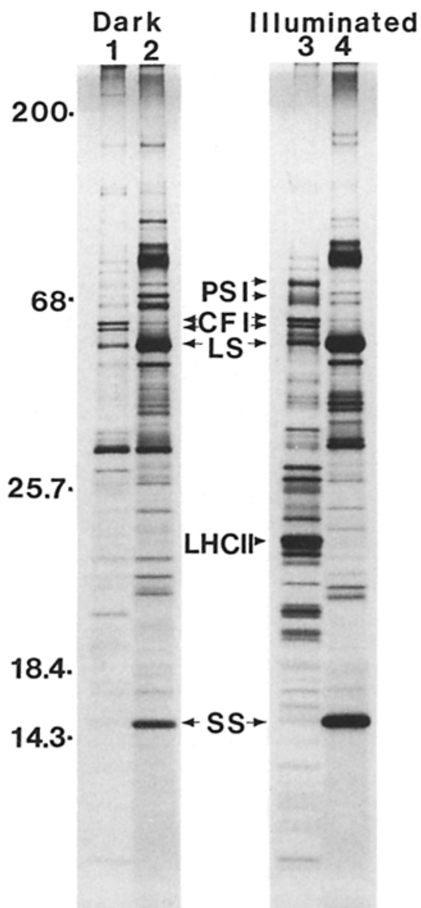

Figure 3. Separation of membrane-associated and stromal polypeptides of dark-grown and illuminated plastids. Intact plastids were isolated from 4.5-d-old dark-grown seedlings (*Dark*) and from seedlings illuminated for 16 h before harvest (*Illuminated*). Intact plastids were fractionated into membrane (lanes *1* and *3*) or stromal (lanes *2* and *4*) polypeptides and samples were loaded on NaDod-SO_4-PAGE gels on an equal plastid number basis. Polyacrylamide gels were fixed and silver stained. Numbers to the left indicate mobility of molecular weight standards (kD). Plastid polypeptides identified include the α- and β-subunits of the chloroplast ATPase (*CF 1*), chlorophyll *a*-apoproteins of Photosystem I (*PSI*), chlorophyll *a/b*-binding antennae protein(s) of Photosystem II (*LHCII*), and the large (*LS*) and small subunits (*SS*) of RUBISCO.

be found associated with small polysomes (monomers, dimers) possibly in the stroma of dark-grown plastids. In either case, if light stimulated the synthesis of these proteins by activating translation initiation or by bypassing a block early in polypeptide elongation, a shift in mRNA distribution to the membrane fraction would be expected upon illumination. As shown in Fig. 4 *A*, psaA–psaB transcripts were largely confined to the membrane fraction of both etioplasts and plastids from 1-h-illuminated seedlings. In contrast, transcripts for rbcL and psbA were found in both stromal and membrane compartments. Whereas the proportion of rbcL and psbA transcripts associated with membranes increased upon illumination, a majority of the transcripts remained in the stroma. Further, when expressed on a per-plastid basis, illumination of seedlings for 1 h did not increase the level of psbA, rbcL, or psaA–psaB transcripts (compare lanes *1* and *4*). During this same period, however, light rapidly induced the synthesis of the psaA–psaB and psbA gene products, whereas rbcL gene product synthesis was slightly stimulated (27).

The Northern blot in Fig. 4 *A* was hybridized simultaneously with probes for rbcL, psbA, and psaA–psaB. To demonstrate that transcripts for psbA, psaA–psaB, and rbcL can be simultaneously quantitated, we hybridized Northern blots with individual probes for rbcL, psbA, or psaA–psaB or simultaneously with all three probes (Fig. 4 *B*). Nick-translated DNA probes hybridized to a similar extent when probes were hybridized separately or when hybridized together (compare Fig. 4 *B*, lanes *1–3* vs. *4*). Furthermore, transcripts for psaA–psaB, psbA, and rbcL did not comigrate, indicating that plastid RNA can be probed simultaneously with the three DNA probes and the transcript levels quantitated.

Analysis of Plastid RNA in Polysome Profiles

The preceding results establish the subchloroplast distribution of transcripts for psbA, psaA–psaB, and rbcL but do not distinguish between transcripts that are associated with polysomes or those that sediment as free RNA or as mRNPs. Therefore, sucrose gradient profiles of stromal and membrane-associated polysomes were obtained and the distribution of psaA–psaB, psbA, and rbcL transcripts among non-

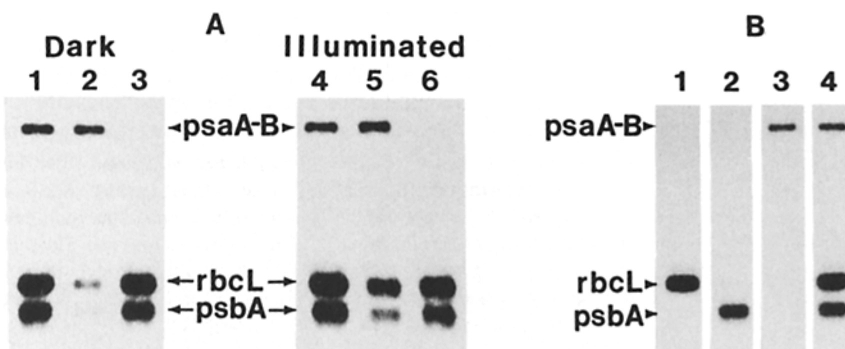

Figure 4. (*A*) Distribution of transcripts for psbA, psaA–psaB, and rbcL between the stromal and membrane fractions of etiolated and illuminated plastids. Intact plastids were isolated from 4.5-d-old dark-grown (*Dark*) seedlings and from seedlings illuminated for 1 h before harvest (*Illuminated*). Plastids (0.5×10^8) were fractionated into membrane and stromal fractions. RNA was isolated from membrane and soluble fractions and from intact plastids (0.5×10^8) by phenol extractions. RNA from intact plastids (lanes *1* and *4*) and from membrane (lanes *2* and *5*) and stromal (lanes *3* and *6*) fractions was loaded on 6% formaldehyde gels on an equal plastid number basis. Nucleic acid was subsequently transferred to nylon membranes and hybridized simultaneously with nick-translated probes of rbcL, psbA, and psaA–psaB. Blots were subsequently washed and exposed to x-ray film for 1 h. (*B*) Quantitation by Northern blot analysis of transcripts for rbcL, psaA–psaB, and psbA. Nucleic acid, isolated from an equal number of plastids, was separated on 6% formaldehyde gels and transferred to nylon membranes. Nylon membranes were cut into strips with each strip containing an identical sample of transferred nucleic acid. Nylon strips were then hybridized with nick-translated DNA from rbcL (lane *1*), psbA (lane *2*), psaA–psaB (lane *3*), or hybridized simultaneously with probes for psaA–psaB, psbA, and rbcL (lane *4*). Blots were subsequently washed and autoradiographed for 1 h.

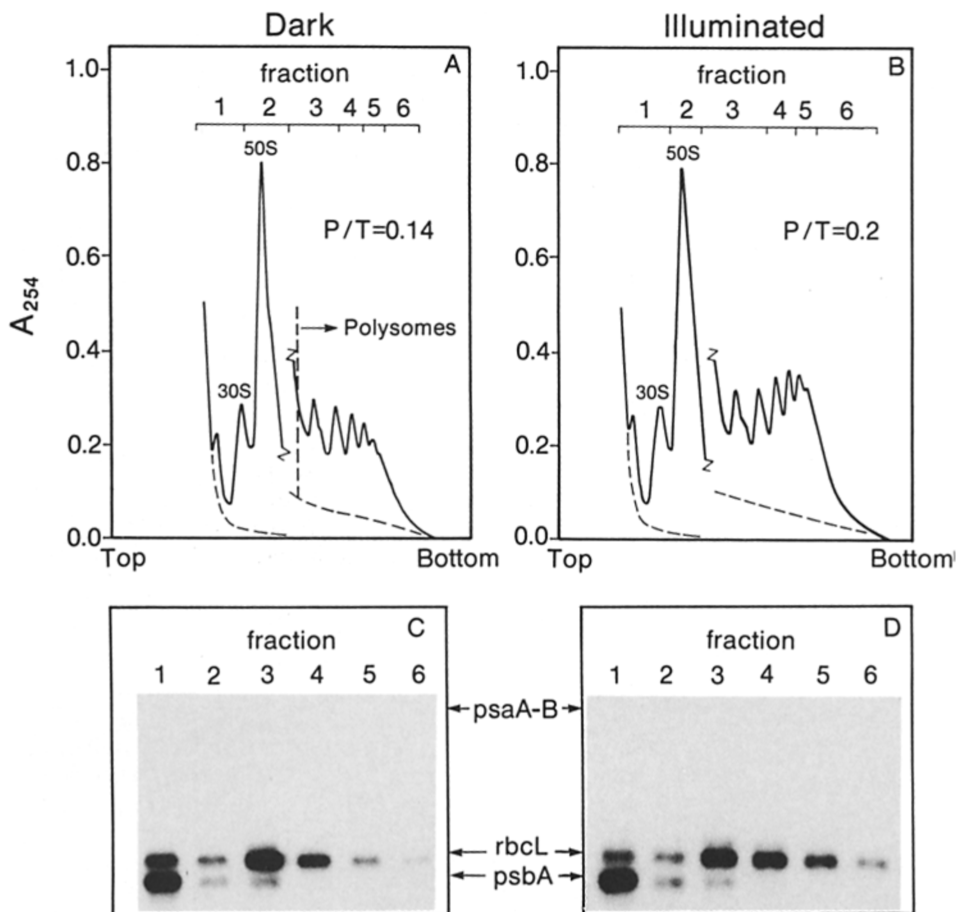

Figure 5. (*A* and *B*) Sucrose density gradient profiles of stromal ribosomal material of dark-grown and illuminated plastids. Intact plastids (1.5 × 10^8) were isolated from 4.5-d-old dark-grown seedlings (*Dark*) and from seedlings illuminated for 1 h before harvest (*Illuminated*). Plastids were fractionated into membrane and stromal phases, and stromal samples were layered over sucrose density gradients and centrifuged as described in Materials and Methods. Sucrose density gradients of stromal ribosomal material was scanned at 254 nm and simultaneously fractionated into six fractions as indicated. A fivefold decrease in full scale absorbance is indicated by a break in the absorbance tracing of each profile. Dashed lines represent base lines. The top of each gradient is to the left. (*C* and *D*) Northern blot analyses of sucrose density gradient profiles of stromal ribosomal material from dark-grown and illuminated plastids. Nucleic acid was isolated from sucrose density gradient fractions of stromal ribosomal material by phenol extractions. Nucleic acid of each fraction was loaded on 6% formaldehyde gels on an equal plastid number basis and subsequently electrophoresed, transferred to nylon membranes and hybridized with nick-translated DNA probes by rbcL, psbA, and psaA–psaB. Nylon membranes were washed and exposed to Kodak x-ray film (type XAR) for ~3 h. Northern blot analysis of etiolated and illuminated plastids are shown in *C* and *D*, respectively.

polysomal and polysomal fractions determined (Figs. 5 and 6).

The dominant ribosomal fractions of the stroma of etioplasts were ribosomal subunits (30S, 50S) representing nearly 86% of the stromal ribosome material (Fig. 5, *A*). Illuminating tissue for 1 h caused a 38% increase in the proportion of polysomes (fractions 4–6) but a majority of stromal ribosomes remained as free subunits (fractions 1–2). The low recovery of stromal ribosomes as polysomes should not have resulted from RNase action or polysome runoff because conditions were optimized to prevent these events. Margulies and Michaels (35) also showed that only 10% of the stromal ribosomes of *Chlamydomonas reinhardtii* chloroplasts were recovered as polysomes even when chloramphenicol was used to prevent runoff and heparin was used to prevent RNase action. These results suggest that the majority of stromal ribosomes of barley plastids are not involved in protein synthesis at this stage of development.

The relative distribution of rbcL, psbA, and psaA–psaB transcripts across stromal polysome gradient profiles is shown in Fig. 5, *C* and *D*. RbcL transcripts were found distributed throughout the polysome gradients with the largest portion being associated with small polysomes (fraction 3, dimers and trimers). Illumination of seedlings for 1 h increased the proportion of rbcL transcripts in large polysomes (fractions 4–6) and decreased the amount of transcripts in nonpolysome fractions (fractions 1–2). The recruitment of rbcL transcripts into large stromal polysomes is consistent with the slight increase in large subunit synthesis that has been observed upon illumination of etiolated barley seedlings (27). In contrast to rbcL, psbA transcripts in the stroma were not associated with polysomes but were found in nonpolysomal fractions in both etioplasts and plastids from illuminated tissues (Fig. 5, *C* and *D*, lane *1*). Therefore, while a majority of psbA transcripts were found in the stromal phase, these transcripts appear to be translationally inactive and may exist as mRNPs. Finally, only a small percentage (<10% of total) of psaA–psaB transcripts were detected in fractions 2–3 of the stromal phase of etioplasts and illuminated plastids (observable with longer exposures of Northern blots), indicating that the synthesis of these polypeptides is largely restricted to membrane-associated polysomes.

Sucrose density gradient profiles and mRNA distribution of membrane-associated polysomes is shown in Fig. 6. In contrast to the distribution of stromal ribosomal material, the majority of the membrane-associated ribosomes were in polysomes (P/T > 0.5) with illumination causing an increase in large membrane-associated polysomes (compare Fig. 6, *A*

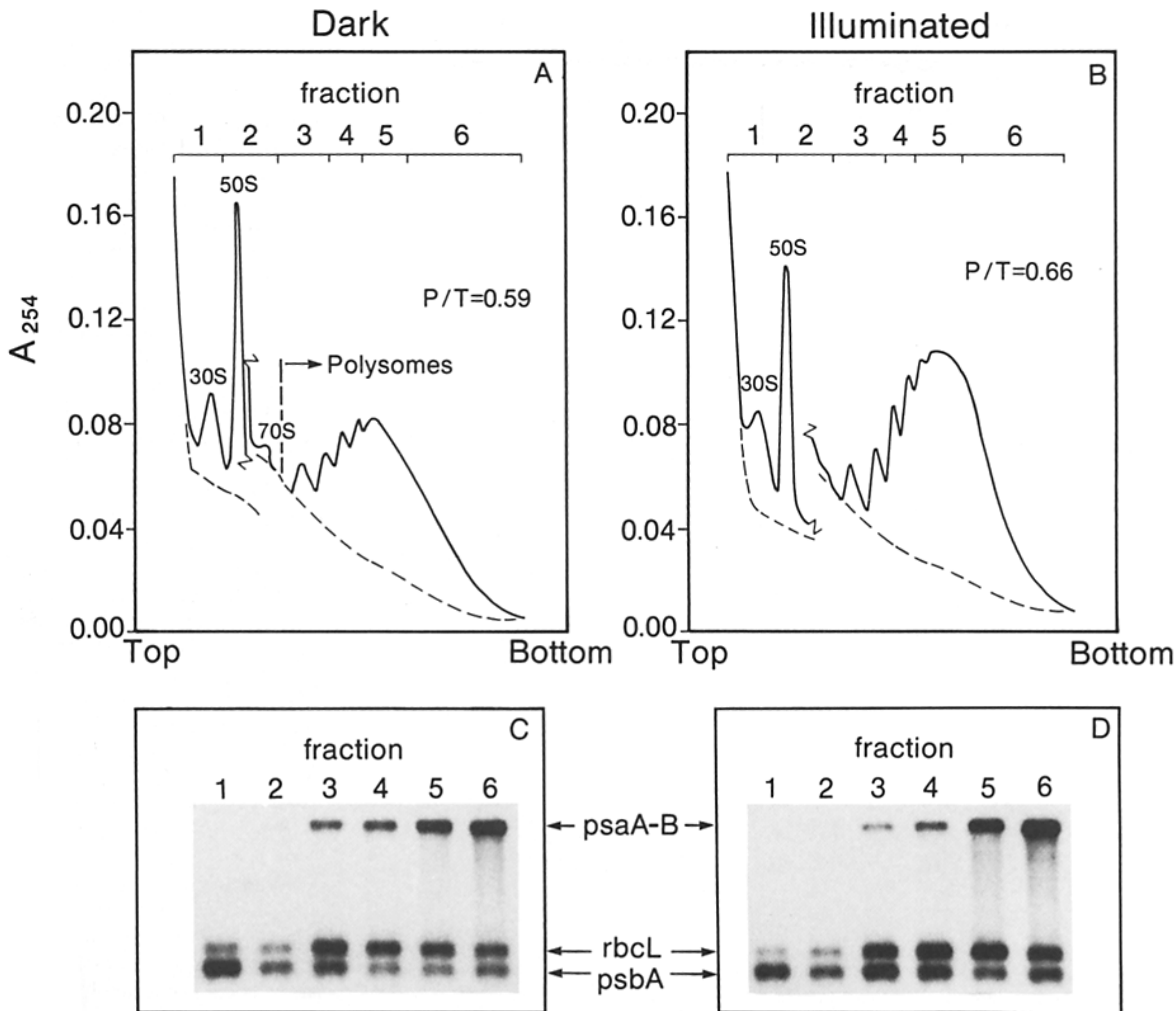

Figure 6. (*A* and *B*) Sucrose density gradient profiles of membrane-associated ribosomal material of dark-grown and illuminated plastids. Intact plastids (1.5 × 10^8) were isolated from 4.5-d-old dark-grown seedlings (*Dark*) and from seedlings illuminated for 1 h before harvest (*Illuminated*). Plastids were fractionated into membrane and stromal phases, and membranes were solubilized as described in Materials and Methods. Ribosomal material of membrane fractions were layered over sucrose density gradients, centrifuged, and scanned at 254 nm. Gradients of ribosomal material were fractionated into six fractions as indicated. A 2.5-fold decrease in full scale absorbance is indicated by a break in the absorbance tracing of each profile. Dashed lines represent base lines. The top of each gradient is to the left. (*C* and *D*) Northern blot analysis of sucrose density gradient profiles of membrane-associated ribosomal material from dark-grown and illuminated plastids. Nucleic acid from sucrose gradient fractions was isolated and subsequent Northern blot analyses were conducted as described for stromal ribosomal material (see Fig. 5 legend). Northern blots were exposed to Kodak x-ray film (type XAR) for ~12 h. Northern blot analyses of etiolated and illuminated plastids are shown in *C* and *D*, respectively.

and *B*, fractions 4–6). The polysomes found associated with the thylakoid membranes are likely attached via nascent chains because dissociation required 0.5 M KCl plus puromycin (data not shown). The fact that 0.5 M KCl alone did not remove the polysomes from membranes argues against a nonspecific association of ribosomes with the membrane (14, 15, 22, 59).

The distribution of transcripts across sucrose gradient profiles of membrane-associated ribosomes is shown in Fig. 6, *C* and *D*. RbcL transcripts were found in polysome and nonpolysome association with membranes of both etioplasts and illuminated plastids (Fig. 6, *C* and *D*). Transcripts observed in nonpolysome fractions may represent trapped or sticky mRNA (22) or mRNA released upon disassociation of monosomes into ribosomal subunits by PTE and proteinase K (1). PsbA transcripts were distributed among nonpolysomal and polysomal fractions of etioplasts even though synthesis of the psbA gene product was not detected in dark-grown plants (28). Illumination caused a slight recruitment of psbA transcripts into membrane-associated polysomes when compared with etiolated tissues (compare Fig. 6, *C* and *D*, lanes *3–6*). PsaA–psaB transcripts were found in membrane-associated polysomes of etioplasts with little or no psaA–psaB message in nonpolysome fractions (fractions *1–2*). Illumination of seedlings for 1 h caused only a slight alteration in the distribution of psaA–psaB transcripts among polysome fractions (compare Fig. 6, *C* and *D*). As with the psbA gene product, the synthesis of psaA–psaB gene products was not detected in dark-grown plants but translation of the polypeptide was observed after only 15 min of illumina-

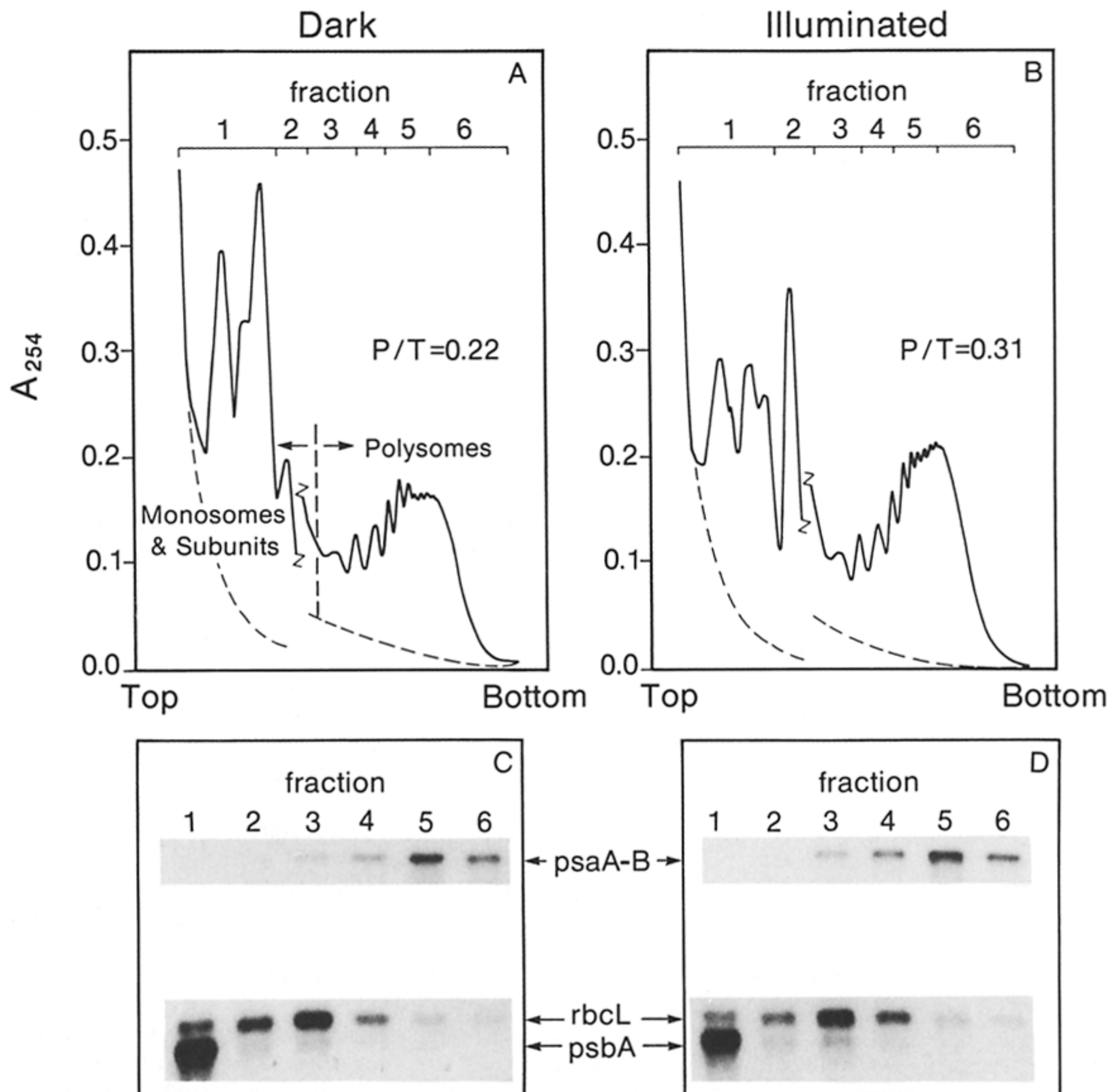

Figure 7. (*A* and *B*) Sucrose density gradient profiles of total leaf ribosomal material of dark-grown and illuminated seedlings. Total ribosomal material (70S and 80S) was extracted from leaves of 4.5-d-old dark-grown seedlings and seedlings illuminated for 1 h before harvest as described in Materials and Methods. Ribosomal material was layered over sucrose gradients, centrifuged, and scanned at 254 nm. Gradients of total leaf ribosomal material was fractionated into six fractions as indicated. A 2.5-fold decrease in full scale absorbance is indicated by a break in the absorbance tracing of each profile. Dashed lines represent base lines. The top of each gradient is to the left. (*C* and *D*) Northern blot analyses of sucrose density gradients of total leaf ribosomal material of dark-grown and illuminated seedlings. Nucleic acid from each sucrose gradient fraction was isolated and Northern blot analyses were conducted as described in the legend of Fig. 4. Nucleic acid samples were loaded on 6% formaldehyde gels as a constant percentage of each fraction. Northern blots were exposed to Kodak x-ray film (type XAR) for ~6 h. Northern blots of nucleic acid of etiolated and illuminated seedlings are shown in *C* and *D*, respectively.

tion (27). The association of psaA–psaB and psbA transcripts with membrane-bound polysomes in etioplasts indicates that light controls the synthesis (or accumulation) of chlorophyll *a*-apoproteins and the psbA gene product during polypeptide chain elongation or at the posttranslational level.

Transcript Distribution in Polysomes from Rapidly Frozen Leaf Tissue

To verify the association of psaA–psaB and psbA transcripts with polysomes of dark-grown plastids, total polysomes (70S and 80S) were isolated from barley leaves rapidly frozen in liquid N_2 (Fig. 7, *A–D*). Translation is rapidly arrested by liquid N_2 temperatures and the distribution of ribosomes should reflect that in vivo at the time of harvest. Further, the polysome extraction buffer contained 200 mM Tris buffer, 5 µg/ml proteinase K, and 100 µg/ml chloramphenicol, which should prevent translation during polysome isolation.

In agreement with the polysome profiles obtained from isolated plastids, illumination of etiolated seedlings resulted in a greater proportion of ribosomal material in polysomes as indicated by an increased P/T ratio (Fig. 7, *A* and *B*). The ratios of polysomes to total ribosomal material (P/T) ob-

tained here were similar to ratios observed for mustard cotyledons during phytochrome-mediated photomorphogenesis (42). Higher reported P/T values have commonly been obtained by purifying crude ribosomal preparations via short-term centrifugation through a sucrose cushion. This procedure, however, causes the selective exclusion of ribosomal subunits from the polysome pellet (32, 33).

The distribution of transcripts for psbA, rbcL, and psaA–psaB in rapidly frozen leaf tissue parallels the distribution of transcripts obtained with isolated plastids (compare Figs. 5 and 6 to Fig. 7). RbcL transcripts were predominately found in small polysomes (fraction 3) with a shift of rbcL mRNA to larger polysomes upon illumination. The majority of psbA transcripts were found in nonpolysomal fractions similar to what was observed in isolated plastids (compare Figs. 5 and 7). However, difficulties in completely solubilizing membranes were encountered when polysomes were extracted from rapidly frozen leaf tissue (data not shown). Incomplete solubilization would prevent the complete release of membrane-bound polysomes and therefore underestimate the proportion of psbA mRNA in polysomal fractions. Despite the incomplete release of membrane-associated polysomes, psaA–psaB mRNA was found largely in polysomal fractions of etioplasts and plastids from 1-h-illuminated tissues (Fig. 7, *C* and *D*). These results agree with the polysome profiles and mRNA distribution of isolated plastids.

Protein Synthesis by Membrane-bound Polysomes

We have previously reported that translation of the 65–70-kD chlorophyll *a*-apoproteins was not detectable in dark-grown tissue (28). The results of Fig. 6 indicate that this lack of apparent translation is not due to the absence of psaA–psaB transcripts in membrane-bound polysomes. However, the presence of psaA–psaB transcripts in etioplast polysomes does not necessarily indicate that these polysomes are translationally competent. To determine whether psaA–psaB transcripts associated with etioplast membrane-bound polysomes are translatable, we isolated membrane-bound polysomes and added them to a homologous in vitro translation system. For the isolation of polysomes to be assayed for in vitro protein synthesis, several modifications of our polysome isolation protocol were necessary. First, because Tris-HCl buffers can inhibit chloroplast translation, membrane-bound polysomes were isolated in a buffer of 46 mM Hepes–KOH (pH 7.6) containing 118 mM potassium acetate and 7 mM magnesium acetate. Second, the heparin concentration of the polysome isolation buffer was lowered to 10 µg/ml because higher concentrations of heparin inhibit translation by membrane-bound polysomes (data not shown). Finally, the use of proteinase K and chloramphenicol for polysome preparation was eliminated.

The characterization of translation by membrane-bound polysomes of illuminated plastids is shown in Table I. Incorporation of [^{35}S]methionine into protein was reduced >90% by RNase A and chloramphenicol which is consistent with translation by 70S ribosomes. [^{35}S]Methionine incorporation by membrane-bound polysomes was insensitive to low concentrations of aurin tricarboxylic acid (25 µM) whereas higher concentrations inhibited [^{35}S]methionine incorporation by nearly 50%. Aurin tricarboxylic acid at low concentration (20 µM) was reported to selectively inhibit initiation of translation whereas increasing concentrations of aurin

Table I. Characterization of Translation by Membrane-bound Polysomes

Treatment	[^{35}S]Methionine incorporation
	%
Control	100
10 µg/ml RNase A	7.4
100 µg/ml chloramphenicol	9.6
25 µM aurin tricarboxylic acid	99.0
100 µM aurin tricarboxylic acid	56.5
2% (vol/vol) PTE	115.3
0.045 A_{280} units S-100*	111.5
2% (vol/vol) PTE plus 0.045 A_{280} units S-100	123.5

Membrane-bound polysomes were prepared from 3 × 10^{7} chloroplasts as described in Materials and Methods. Translation by membrane-bound polysomes was conducted with additions as indicated. After 10 min, reactions were terminated by placing the translation mixtures on ice. 1-µl aliquots were processed for measurement of trichloracetic acid–insoluble radioactivity and incorporation of label expressed on an equal plastid number basis.
* [^{35}S]Methionine incorporation by S-100 fraction (minus membrane-bound polysomes) was equal to 4% the incorporation in the presence of membrane-bound polysomes.

tricarboxylic acid nonspecifically inhibit chain elongation (23). These results indicate that [^{35}S]methionine incorporation by isolated membrane-bound polysomes is largely due to chain elongation with little or no reinitiation occurring during the experimental period.

When the detergent PTE was added to solubilize chloroplast membranes, [^{35}S]methionine incorporation was slightly stimulated, suggesting that the attachment of polysomes to membranes is not required for chain elongation. Furthermore, when an S-100 fraction prepared from chloroplasts was added to the translation mixture, [^{35}S]methionine incorporation was increased ~10%. It should be stated, however, that the stimulation of [^{35}S]methionine incorporation by S-100 fractions was variable and was dependent on the concentration of membrane-bound polysomes in the translation mixture and on the preparation of S-100. In general, greater stimulation of [^{35}S]methionine incorporation by S-100 addition was observed when the translation mixture also contained the detergent PTE. In the presence of S-100 and the detergent PTE, [^{35}S]methionine incorporation by isolated polysomes were nearly linear for 10 min (data not shown). After 15 min, no further [^{35}S]methionine incorporation was observed. Under these conditions, [^{35}S]methionine incorporation by membrane-bound polysomes was nearly twice that reported for isolated intact chloroplasts (28).

The profiles of polypeptides synthesized in vitro by membrane-bound polysomes are shown in Fig. 8. Several translation products of membrane-bound polysomes comigrated with chloroplast polypeptides synthesized in vivo (Fig. 8, lanes *1* vs. *2–4*, and lanes *5* vs. *6–7*). A major translation product of membrane-bound polysomes was the large subunit of RUBISCO. These results indicate that rbcL transcripts associated with membrane-bound polysomes are translatable. Also present amongst the translation products of membrane-bound polysomes of illuminated plastids was a polypeptide that comigrated with a 65–70-kDa chlorophyll *a*-apoprotein of Photosystem I (compare lanes *5* vs. *6* and *7*). Synthesis of the chlorophyll *a*-apoprotein was observed

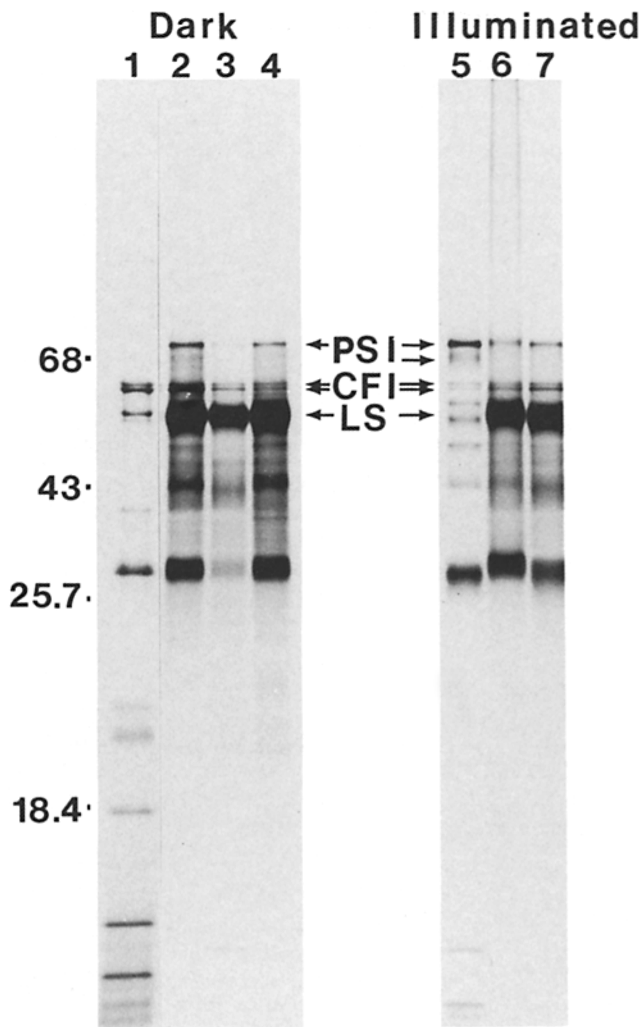

Figure 8. Protein synthesis by membrane-bound polysomes of etiolated and illuminated plastids. Membrane-bound polysomes were isolated from plastids of 4.5-d-old dark-grown seedlings (lanes *2–4*) and from seedlings illuminated for 1 h before harvest (lanes *6* and *7*). Membrane-bound polysomes were added to translation mixtures containing 0.045 A_{280} units S-100 plus 2% PTE (lanes *2* and *6*), 0.045 A_{280} units S-100 (lanes *3* and *7*), or 2% PTE (lane *4*). After 10 min, unlabeled methionine (final concentration of 5 mM) was added to block further incorporation of [^{35}S]methionine and to allow chain elongation of incomplete polypeptides. Lanes *1* and *5* represent polypeptides synthesized in vivo by dark-grown and illuminated plastids, respectively. After labeling, samples were electrophoresed, fluorographed, and exposed to Kodak x-ray film (Type BB-1) for 72 h. Numbers to the left indicate mobility of molecular weight standards. Samples were loaded on an equal plastid number basis.

when polysomes were anchored to membranes via their nascent chains (lane *7*, minus detergent) or when membranes of illuminated plastids were solubilized by the detergent PTE (lane *6*).

When membrane-bound polysomes from etioplasts were translated in vitro, synthesis of a chlorophyll *a*–apoprotein of Photosystem I was also detected (lanes *2–4*). In contrast to illuminated plastids, in vitro synthesis of the chlorophyll *a*–apoprotein by etioplast membrane-bound polysomes was greatly stimulated upon solubilization of membranes by the detergent PTE (compare lanes *3* vs. *2* and *4*). Further, while chlorophyll *a*–apoprotein synthesis by etioplast polysomes was stimulated by addition of S-100, synthesis was observed in the absence of added chloroplast S-100 (compare lanes *2* and *4*). These results indicate that psaA–psaB transcripts associated with etioplast membrane-bound polysomes are translationally competent and that association with etioplast membranes inhibits their translation.

Discussion

We have previously reported that the translation of the 65–70-kD chlorophyll *a*–apoproteins of Photosystem I and the 32-kD gene product of psbA was not detectable in plastids of dark-grown barley seedlings (27, 28). The lack of apparent translation of these proteins is not due to the absence of active ribosomes or mRNA encoding these polypeptides (27, 28, 43). Furthermore, the posttranscriptional regulation of the accumulation of these proteins is selective; the translation and accumulation of other membrane proteins (i.e., α- and β-subunits of the ATPase complex) and soluble proteins (i.e., the large subunit of RUBISCO) occurs in plastids of dark-grown plants. Illumination of dark-grown plants for as little as 15 min induced the appearance of the chlorophyll *a*–apoproteins of Photosystem I and the psbA gene product in isolated plastid protein synthesis assays (27). These results could indicate that the accumulation of the gene products of psaA, psaB, and psbA is regulated at the level of protein translation.

We examined the distribution of transcripts for three light-induced proteins (psaA, psaB, and psbA gene products) and for the large subunit of RUBISCO (rbcL gene product) in polysome gradients of dark-grown and illuminated plastids. Examination of polysome profiles, in conjunction with protein synthesis studies, have been used to identify potential points of light regulated translation in mature chloroplasts of peas (4, 19), barley (31), and *Chlamydomonas reinhardtii* (14, 15, 23). To accurately assess the distribution of plastid transcripts, it is necessary to obtain undegraded polysomes. Therefore, we have made several modifications of existing protocols resulting in reproducible plastid polysome profiles.

The first modification was the use of heparin which reduced the degradation of mRNA. The presence of heparin during polysome workup increased P/T ratios and improved mRNA yields. Second, it has been noted that slow cooling of *Chlamydomonas reinhardtii* cells during harvesting preferentially blocked polypeptide chain initiation but allowed chain elongation and termination to proceed (15). To help prevent elongation and termination after tissue harvesting, excised barley leaves were immediately immersed in iced water and were maintained at 4°C for the remainder of the experimental period. In addition, chloramphenicol, an inhibitor of 70S ribosome elongation, was added to all solutions used in plastid isolation. Therefore, translation should have been arrested at the time of harvest and profiles should reflect the distribution of ribosomes that existed in vivo. The third modification was the use of the detergent PTE to solubilize membranes and proteinase K to dissociate polysome aggregates. The use of PTE and proteinase K prevented the migration of polysome aggregates or membrane fragments

(with associated ribosomal material) with large polysomes in sucrose gradients. The above modifications allowed reproducible polysome profiles to be obtained from isolated plastids thereby permitting the examination of translational regulation during light-induced chloroplast biogenesis.

Mechanism of Light-regulated Protein Synthesis

Translation of mRNA can be regulated at the point of chain initiation, elongation, or termination (for review, see reference 25). The arrest of translation at each of these points can result in a unique polysome profile that permits the site of translational regulation to be determined. If translation of psaA–psaB and psbA transcripts was selectively inhibited at the point of initiation, then transcripts would be largely restricted to nonpolysomal fractions (15, 19). Alternatively, if arrest was at an early point in chain elongation, as has been reported for secretory proteins (38, 56–58), then psaA–psaB and psbA transcripts should be largely restricted to association with small polysomes. In either case, illumination should cause a redistribution of psaA–psaB and psbA transcripts to larger polysomes. By comparison, a large light-induced redistribution of rbcL transcripts was not expected because the large subunit of RUBISCO is a major translation product of both etioplasts and illuminated chloroplasts (27, 28).

In the present study, psaA–psaB mRNA was found almost exclusively in membrane-bound polysomes in plastids of dark-grown and illuminated seedlings. Further, psbA and rbcL transcripts showed only a moderate redistribution to large membrane-associated polysomes upon illumination. The lack of a large light-induced redistribution of psbA and psaA–psaB transcripts argues that translation of these mRNAs was not inhibited in etioplasts at the level of translation initiation nor at a point early in elongation. Despite the association of psaA–psaB and psbA transcripts with large polysomes, several features suggest that the translation of chlorophyll *a*–apoproteins and the psbA gene product is arrested (or retarded) during chain elongation. First, radiolabeling of these polypeptides was not detected in dark-grown plastids even under short pulse-labeling conditions (27). Second, Photosystem I chlorophyll *a*–apoproteins were not detected in immunoassays of dark-grown barley plastids (26, 51, 55). These features are consistent with proposed models of translation controlled during chain elongation (54). Further, in vitro chlorophyll *a*–apoprotein synthesis was nearly undetectable when etioplast polysomes remained anchored to membranes. However, when released from membrane association by the detergent PTE, polysomes of dark-grown plastids synthesized a 65–70-kD polypeptide comigrating with a chlorophyll *a*–apoprotein of Photosystem I. This result, and the distribution of psaA–psaB transcripts on etioplast polysomes, indicates that translation of psaA–psaB mRNA may be blocked (retarded) at several points in chain elongation through an association with etioplast membranes.

In most systems examined so far the regulation of protein synthesis was during the initiation of translation (for review, see reference 25). However, several instances where chain elongation of a select set of mRNAs was arrested have been reported. The best known example is the translation of secretory proteins blocked at an early stage of elongation by the signal recognition particle unless the appropriate endoplasmic reticulum components are also present (38, 56–58). However, recent evidence shows that the signal recognition particle can retard the synthesis of an integral membrane protein and several secretory proteins at multiple points in elongation (34). Furthermore, translation can be arrested as late as when 67% of the nascent IgG light chain has been completed (2). When rough microsomes were added to the translationally blocked polysomes it was found that regardless of their length, nascent chain translation could be resumed and polypeptides were translocated into the rough microsomes (2, 34). These reports indicate that the arrest of membrane protein and secretory protein synthesis by the signal recognition particle is not confined to a single point early in chain elongation, but rather translation is retarded at discrete points throughout chain elongation (34). It is likely that transcripts for these translational-arrested proteins would be distributed throughout polysome profiles as was observed here for psaA–psaB and psbA transcripts of etioplasts. Several other reports showing mRNA associated with polysomes that are translationally inactive include reports of normal cellular mRNAs of *Drosophila* tissue culture (6) and HeLa cells (52), which remain associated with polysomes under stress conditions. These untranslated mRNAs are found in association with polysomes of similar size in heat-shocked and control cells. In addition, the untranslated mRNAs appear undegraded and are translatable when deproteinized and added to a reticulocyte translation system (6).

The present results suggest that there is a block in the elongation of psaA–psaB and psbA mRNA in etioplasts and the lesion is rapidly overcome upon illumination. It is interesting that the time course of induction of chlorophyll *a*–apoprotein synthesis is similar to the time course of light-dependent chlorophyll *a* conversion from protochlorophyllide (29, 55). It is possible that the binding of chlorophyll *a* molecules to the apoproteins may release the inhibition of chain elongation thereby permitting the rapid synthesis and assembly of chlorophyll–protein complexes. Alternatively, the chlorophyll *a*–apoproteins may be synthesized and, in the absence of chlorophyll, rapidly turned over such that no detectable full-length apoproteins can be detected under short (5 min) pulse-labeling conditions. The fact that full length 65–70-kDa chlorophyll *a*–apoproteins were synthesized in vitro by etioplast polysomes would indicate that nascent chains were not being proteolyzed during translation. Other plastid proteins have been reported to exhibit accelerated turnover due to the absence of chlorophyll (9), copper (37), or subunits of a common protein complex (40, 48). The half-life of these proteins ranged from 5 (48) to 90 (9) min depending on the lesion and the plant species. In all of these examples, translation of unstable proteins could be detected using pulse-labeling techniques. In the present case, proteolysis would need to be very rapid to explain the absence of detectable chlorophyll *a*–apoprotein translation in dark-grown plastids.

Finally, the distribution of rbcL transcripts is worth noting in comparison to previous studies (22, 39). The majority of rbcL transcripts were found associated with stromal polysomes as expected for mRNA coding for a soluble protein. However, a portion of rbcL transcripts were associated with membrane-bound polysomes, and a significant portion of these transcripts were translatable in vitro. This observation

is consistent with a previous report by Hattori and Margulies (22) who showed that a large fraction of rbcL mRNA was associated with membrane polysomes. Our results suggest that, in barley plastids, the majority of large subunit synthesis occurs on stromal polysomes and that a smaller portion of rbcL translation may occur on membrane polysomes. However, an apparent association of polysomes with the membrane may be due in part to stickiness of stromal polysomes (22). As was first suggested by Hattori and Margulies (22), the possibility of a membrane-associated step in large subunit synthesis or in the formation of the RUBISCO holoenzyme cannot be excluded.

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