Light-Induced Increase in the Number and Activity of Ribosomes Bound to Pea Chloroplast Thylakoids in Vivo

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ABSTRACT
Within 8 to 10 minutes of illumination, chloroplast thylakoids of pea (Pisum sativum) became enriched 30 to 100% in ribosomes bound by nascent chains. Following (or, in some experiments, coincident with) this apparent redistribution was a 25 to 65% increase in the total bound ribosome population, which was then maintained at this higher level during the normal light period. On transfer of plants to darkness, the bound ribosome population decreased to the lower dark level. White, blue (400 to 520 nanometers), and orange (545 to 690 nanometers) light were all effective in producing an increase in the bound ribosome population. The level of bound ribosomes in the oldest leaves of 16-day-old plants was 15-fold less than in the still-maturing leaf but was still increased by illumination.

In vivo experiments with chloramphenicol and lincomycin indicated a requirement for protein synthesis by the 70S ribosomes both for the light-induced shift to the population bound by nascent chains and for the increase in the total thylakoid-bound population. When thylakoids from plants in darkness or exposed to light for increasing periods were incubated in an Escherichia coli cell-free protein synthesizing system, 15 minutes of prior illumination in vivo produced a 60% increase in [3H]leucine incorporation. This stimulation preceded the increase in total bound ribosomes but corresponded in time to observed increases in the ribosomes bound by nascent chains.

A light intensity of 100 micromoles per meter$^2$ per second, but not 25 micromoles per meter$^2$ per second, caused a significant increase in bound ribosomes over a 30-minute period. Strong inhibition in vivo by 3',4'-dichlorophenyl-1,1-dimethyleurea suggests that noncyclic electron flow is essential for light-induced ribosome redistribution.

Some of the chloroplast 70S ribosomes are seen in electron micrographs to be associated with the thylakoid membranes (5, 9, 19, 28). At least some of these ribosomes must be associated with mRNA, since washed thylakoids are capable of synthesizing proteins when supplemented with soluble factors (1, 2, 8, 20). Retention in the thylakoids of the newly synthesized peptides provided evidence that the proteins synthesized were discharged into the membranes (2, 20). From this, it seemed likely that the ribosomes active in vitro were tightly bound to the thylakoids by a nascent peptide chain. The presence of this type of linkage was demonstrated more directly for Chlamydomonas (5) and for pea (28), using puromycin-induced release of the ribosomes in the presence of high salt concentrations (0.5 to 1.0 M KCl) as a criterion.

The nascent peptide linkages between ribosomes and the thylakoids of Chlamydomonas are transitory in vivo under conditions—slow cooling or the presence of a specific inhibitor of initiation (6, 19)—adverse for initiation of new chains. In addition, few or no ribosomes were found bound to the thylakoids of synchronized Chlamydomonas cells during the dark period of a diurnal cycle (6). This loss of ribosomes in darkness was attributed to lower levels of ATP and certain amino acids under the phototrophic growth conditions in use (6).

In chloroplasts of higher plants, energy requirements do not provide a compelling reason for protein synthesis to be directly dependent on light. Substrates for ATP and amino acid synthesis are available to young plants from reserves in the cotyledons and elsewhere, and, indeed, ATP levels of chloroplasts from dark plants were observed to be 40 to 50% of those in illuminated plants (25). Alscher-Herman et al. (2) found that both darkness and anoxia in vivo were needed to effect a major loss of ribosomes from pea thylakoids. In those experiments, the ribosomes reassociated during a subsequent period of illumination in the air.

Similarly, some proteins synthesized entirely or partially by chloroplast ribosomes are present in etioplasts of dark-grown plants and, therefore, do not require light for their synthesis. These include RuBPCase, CF$_1$, Cyt$f$, and Cyt $b_6$ (17, 21, 22). Thus, chloroplast ribosomes, in general, do not need specific activation by light-dependent mechanisms.

However, total protein synthesis by intact plastids in vitro was stimulated by light-driven, cyclic electron flow (23). Specific and absolute requirements for light during development were found for some thylakoid membrane polypeptides in green etioplasts (13). At least one of these polypeptides, the apoprotein of CPI, is known to be synthesized by chloroplast ribosomes (4). Finally, even without an absolute dependence on light, quantitative increases in the vigor of plastid synthesis due to light are very likely.

In the present work, we report a light-dependent increase in the number and activity of ribosomes bound to pea chloroplast thylakoid membranes, which is probably related to other aspects of the control over chloroplast protein synthesis by light. It has been possible to evaluate to some extent the kinetics of the light-induced changes and the effect of light quality and of leaf age on these changes. The involvement of protein synthesis and photosynthetic electron flow have also been assessed.

MATERIALS AND METHODS

Sources. Ascorbic acid, alumina, BSA, chloramphenicol, cycloheximide, DNA (salmon sperm, Na salt), EDTA, EGTA, DTE, DTI, GSH, orange G, puromycin, RNases A and T$_1$, sucrose (low RNase, grade 1), trypsin, and Tween 80 were purchased from

1 Abbreviation: RuBPCase, ribulose bisphosphate carboxylase; CF$_1$, chloroplast coupling factor; CPI, Chl/protein complex I; EGTA, ethylene-bis-(amino ethyl ether)-$N,N'-$tetraacetic acid; CAP, chloramphenicol; DTE, dithioerythritol.

1 Supported in part by Grant 5901-0410-9-0327-0 from the United States Department of Agriculture, Science and Education Administration, Competitive Research Grants Office program in photosynthesis.
earlier differential centrifugation and sucrose step gradients in crushed ice. Cheesecloth was used as H₂O before grinding.

Plant Material. Peas (Pisum sativum) were grown for 9 to 16 d in vermiculite watered daily with tap water in a model CU37-14 Sherrer controlled environment chamber on a 16-h light/8-h dark or a 12-h light/12-h dark schedule. Growth temperature was maintained within 1°C for any one flat of plants but ranged from 21 to 24°C for different experiments. Humidity varied with the weather. Quantum flux, provided during growth by both fluorescent and incandescent lamps, was typically 260 to 300 µmol m⁻² s⁻¹ at leaf level.

Controlled Illumination. For experiments involving colored light, or a light intensity different from that in the controlled environment chamber, a plywood box with a Plexiglas top was constructed; it was large enough to accommodate a 30.5×45.7-cm flat. The top was fitted with a Plexiglas tank holding a 10-cm-deep layer of H₂O or of CuSO₄ solution. Illumination was from 12 flood lamps of 300 w each, placed above the tank, or from the fluorescent lamps of the controlled environment chamber. Temperature in the light box was controlled at 24.5 ± 0.5°C with cooled air. RH in the light box was measured during experiments and ranged from 35 to 50%.

Quantum flux received by the plants was measured with a Lambda Instrument Corp. Model LI-185A quantum/radiometer/photonmeter and a model LI-190S quantum sensor with cutoff limits of 400 and 700 nm.

Different colors of light were isolated using 36–49-cm gelatin filters containing dyes (27), either Victoria pure blue BO plus 8-hydroxyquinoline sulfate or tartrazine plus orange G. A deep orange cineoid filter (No. 5A from Kliegl Bros., Long Island City, NY) was also satisfactory for orange light. These secondary filters were placed beneath the primary filter consisting of 10 cm of either 10 or 50 g CuSO₄·5H₂O per L in 0.5% H₂SO₄ (for blue light) or 10 cm of H₂O for orange light. The resulting transmission bands had cut-off limits of 400 and 500 nm (blue) and 545 and 690 nm (orange).

Sampling Procedure. Because of some variability in the level of thylakoid-bound ribosomes in plants from different parts of a single flat, shoots were sampled in a predetermined pattern from a grid of 16, 32, or 48 equal portions of the area of the flat. In time-course experiments, plants from three to eight sections of the flat were sampled at each time point. When many samples had to be assayed, leaves or shoots were cut with scissors and immediately immersed in liquid N₂. The tissue was crushed, then stored in plastic vials under liquid N₂ until thylakoid membranes were isolated. In most experiments, the samples were divided into two to four portions, and thylakoids were isolated separately from each portion to generate replicate data for statistical analysis.

When intact chloroplasts were isolated from fresh tissue, the shoots were cut and then immediately immersed in H₂O containing crushed ice. Cheesecloth was used as a sling to remove excess H₂O before grinding.

Thylakoid Isolation. Preparation of washed thylakoids by differential centrifugation and sucrose step gradients was described earlier (28). These are referred to as 'sucrose thylakoids.' Chl was measured by the procedure of Arnon (3). 'Percoll thylakoids' were prepared from intact chloroplasts isolated on gradients of Percoll, as described in detail elsewhere (L. E. Fish and A. T. Jagendorf, in preparation), using 330 mm sorbitol and pH 7.5 in both the grinding medium and Percoll gradients. After centrifugation of the Percoll gradients, the supernatant and the upper green band of naked thylakoids were aspirated off. The lower green band was removed; diluted with 20 ml of resuspension buffer containing 330 mm sorbitol, 50 mm Hepes-KOH (pH 7.5 at 4°C), 2 mm EDTA, 1 mm MgCl₂, and 1 mm MnCl₂; then sedimented for 4 min at 1,480 g in an HB-4 rotor. The resulting pellet of intact chloroplasts (92 to 96% refractile in the phase contrast microscope) was resuspended in 20 ml of a hypotonic buffer containing 20 mm Tris-Cl (pH 8.5 at 4°C), 20 mm KCl, and 15 mm Mg-acetate and passed four times through a 15- or 18-gauge canula and syringe, and the thylakoids were sedimented at 27,000 g for 4 min in a Sorvall SS-34 rotor. Resuspension, syringing, and centrifugation were repeated two more times. The final thylakoid pellet was drained briefly at 4°C and resuspended in a buffer appropriate for further treatments (see individual experiments).

Measurement of total thylakoid-bound RNA was described earlier (10).

Release of Bound Ribosomes by High Salt and Puromycin. For experiments involving measurement of high salt- and puromycin-sensitive ribosomes, washed Percoll thylakoids (30 to 150 µg Chl) were resuspended in 3.0 ml 50 mm Hepes-KOH (pH 7.5 at 22°C), 1.0 mm KCl, 15 mm Mg-acetate with or without 50 µm puromycin and incubated for 30 min at either 25 or 30°C (see specific experiments). Thylakoids were removed by centrifugation for 15 min at 21,000 g at 4°C, washed once with the same incubation medium lacking puromycin, and assayed for residual bound RNA.

Preparation of Leaf Polysomes and Stroma Extract. Total pea leaf polysomes were isolated by the method of Davies et al. (7) with some modifications. Stroma extract, for use in isolation of 70S ribosomes and their 30S and 50S subunits, was prepared from intact Percoll chloroplasts. The intact chloroplast pellet was resuspended in 50 mm Tris-Cl (pH 7.6 at 4°C), 20 mm KCl, and 15 mm Mg-acetate at 1.6 mg Chl per ml and sonicated for 15 s at maximum power with a Fisher model BP2 ultrasonic probe. The membranes were removed by centrifugation for 25 min at 23,700 g in the Sorvall HB-4 rotor at 0°C. The supernatant (crude stroma extract), which contained 2.1 mg RNA per ml, was stored in liquid N₂.

Sucrose Gradient Analysis of Ribosomes. Washed thylakoids were resuspended in 50 mm Tris-Cl (pH 7.6 at 4°C), 20 mm KCl, 15 mm Mg-acetate and incubated with 50 µg/ml tryps in 0.6 to 1.9 mg Chl per ml for 1 h at 0°C to release the thylakoid-associated ribosomes (18). Aliquots of 100 to 150 µl were loaded directly onto a 4.5-m1 gradient of 10 to 34% (w/v) sucrose in the same buffer, prepared according to the method of Stone (26). The gradients were centrifuged at 50,000 rpm (300,000g max) in a Beckman SW 50.1 rotor for 60 to 70 min at 0 to 1°C. Ribosome profiles were displayed using ISCO fractionating equipment, with UV absorbance measured with an ISCO model A5 monitor and type 6 optical unit. Gradients were displaced by pumping 60% glycerol through the bottom of the tube at 0.95 ml/min.

For preparation of 30S, 50S, and 60S subunit peaks to be used in RNA extractions, sucrose gradients (10 to 25%) containing 50 mm Tris-Cl (pH 7.5 at 4°C), 25 mm MgCl₂, 0.5 mm KCl, and 5 mm DTE were used. Stroma extract or pea leaf polysomes were treated with 0.5 mm KCl, 5 mm DTE; or 0.5 mm KCl, 5 mm DTE, 1 mm puromycin; respectively; for 15 min at 0°C, then separated on the 10 to 25% gradients by 2-h of centrifugation at 50,000 rpm.

RNA Extraction and Electrophoresis. Intact RNA was extracted from thylakoids and ribosome preparations, using the method of Leaver and Ingle (15) with some modifications. When extracting thylakoids, 5 ml of a solution at 0°C containing 10% Triton X-100, 10 mm Tris-Cl (pH 7.4 at 4°C), 30 mm KCl, and 10 mm MgCl₂ was added to a thylakoid pellet containing up to 5 mg Chl. This mixture was homogenized briefly with a chilled Polytron homogenizer; then 5 ml of 2% triisopropylphosphoranesulfonate,
12% p-aminosalicylate, 70 mM KCl, 10 mM Tris-Cl (pH 7.6 at 4°C), 10 mM MgCl₂, and 12% isobutanol at 0°C were added, and the mixture was homogenized for 10 to 15 s with the Polytron. Five ml of 10% m-cresol, 0.1% 8-hydroxyquinoline, in liquid phenol saturated with 10 mM Tris-HCl (pH 7.4), were added at room temperature, and the mixture was homogenized for about 10 s. After standing at room temperature for 10 min, the mixture was homogenized again for 10 s, then extracted two to four times with chloroform as described previously (15). RNA was precipitated from the aqueous phase by incubating overnight at -20°C with two volumes of ethanol, then collected by centrifugation. The RNA content was estimated in a diluted, redissolved aliquot by measuring the A₂₆₀, using an extinction coefficient of 22 ml mg⁻¹ cm⁻¹.

For extraction of intact RNA from sucrose gradient ribosome peaks, the procedure for washed thylakoids was followed, except that the initial homogenization with Triton medium was omitted. Intact RNA was fractionated on 2.4% (w/v) acrylamide, 0.12% (w/v) bisacrylamide tube gels (0.6 x 8 cm) at room temperature in a buffer containing 20 mM Tris base, 16 mM HCl, and 0.1% (w/v) SDS (15). Other conditions are given in the figures. The gels were scanned in a Gilford model 240 spectrophotometer with a model 2410-S linear transport accessory. The relative areas of peaks in scans of polyacrylamide gels and of sucrose gradients were determined from copies of the traces by cutting and weighing the peaks.

DNA Analysis. Possible DNA contamination in the RNA analyses was estimated by the modified diphenylamine method of Giles and Myers (11). The DNA concentration was calculated by comparison to a standard curve using 2 to 40 μg of highly polymerized salmon sperm DNA. The concentration of the standard DNA solution was estimated from A₂₆₀ measurements, using an extinction coefficient of 22.3 ml mg⁻¹ cm⁻¹.

Measurement of Photosynthesis. O₂ evolution by 3.5-mm leaf discs cut with a No. 1 cork borer was measured using a Clark type O₂ electrode. Twenty leaf discs were stirred rapidly in a 1.8 ml electrode chamber filled with distilled H₂O at 25°C. Measurements were taken of respiration rates for 4 min in the dark, both before and after a 20-min illumination period. A saturating light intensity of 1,200 μmol m⁻² s⁻¹ (at 400 to 700 nm), measured at the outer surface of the water jacket, was provided by a rheostat-regulated Sylvania Sun Gun.

CO₂ fixation as a function of light intensity was measured for single leaves on intact plants using a water-cooled Plexiglas chamber and a Beckman 315A FLO-TRON reference IR gas analyzer. Leaf temperature, continuously monitored with a thermocouple, was maintained at 23.5 ± 0.5°C. Illumination was provided by a General Electric lucofex lamp. Light intensity was decreased by raising the lamp and shading the chamber with layers of clear polyethylene.

Preparation of E. coli Extract and Measurement of Protein Synthesis. Soluble factors needed for protein synthesis by thylakoid-associated ribosomes were isolated as the S-30 fraction of E. coli strain K-12 mid-log harvest cells, as described earlier (1). Incubation conditions for in vitro protein synthesis and measurement of incorporated radioactivity were also the same as those described (1).

RESULTS

Estimation of Contamination by 80S Ribosomal Material in Washed Thylakoids. The level of thylakoid-bound 70S ribosomes can be estimated by measuring total thylakoid-associated RNA only if contamination by 80S ribosomes is insignificant. This was, indeed, the case for thylakoids extracted from previously purified intact Percoll gradient chloroplasts (Fig. 1B). However, intact chloroplasts could only be used in experiments in which fresh tissue was used for analysis. Freezing the tissue in liquid N₂ precluded isolation of intact chloroplasts but made possible the collection of many samples over a short period of time. The samples were then stored until they could be used for isolation of sucrose thylakoids. Analyses of the RNAs associated with these sucrose thylakoids by polyacrylamide gel electrophoresis showed that substantial amount of RNA from 80S ribosomes were present. Thylakoids prepared from fresh tissue, using the same procedure, were found to contain a similar level of contamination, indicating that neither freezing nor subsequent thawing during homogenization is the cause of the contamination (Table I). A shorter homogenization time was found to reduce the amount of 80S ribosome RNAs sedimenting with the thylakoids, but, even with a short homogenization time, the contamination was still high. The period of the first centrifugation, within a useful range of times, did not have a significant effect (Table I).

To corroborate the estimations of contamination by 80S ribosomes using a different method, sucrose gradient profiles were generated of ribosome material released from thylakoids by 50 μg/ml of trypsin. The simpler pattern from the Percoll thylakoids (Fig. 1B) indicates greater purity than that found in the complex
Twelve-d-old plants were illuminated 1 h before harvesting the leaves. In experiment 1, pea shoots were frozen in liquid N₂, crushed, and divided into four portions of 20 g each. Sucrose thylakoids were isolated as described, except that the length of the first centrifugation of the filtered homogenate was varied as shown. In experiment 2, cut shoots were mixed and divided into three uniform portions of 15 g each. One portion was homogenized without freezing. The other two were frozen, then homogenized in the blender for either 7 or 20 s at 100 v. In both experiments, washed sucrose thylakoids were prepared, and intact RNA was isolated and analyzed to estimate the extent of contamination with 80S ribosomes.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Condition</th>
<th>80S r RNA % of total</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Length of 1st centrifugation</td>
<td>2 min</td>
</tr>
<tr>
<td>2</td>
<td>Fresh tissue</td>
<td>4 min</td>
</tr>
<tr>
<td></td>
<td>Frozen tissue</td>
<td>6 min</td>
</tr>
<tr>
<td></td>
<td>7-s homogenization</td>
<td>8 min</td>
</tr>
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</table>

pattern from sucrose thylakoids (Fig. 1C). Identification of the 70S ribosomal peak was made by comparison with stroma ribosomes (Fig. 1A); that of the 80S ribosomes was made by comparison with the pattern of total leaf ribosomes, which contain more cytoplasmic than chloroplast ribosomes (Fig. 1D). In addition, subunits generated from stroma ribosomes and total leaf poly- somes were separated on sucrose density gradients (data not shown), and their RNA was analyzed by gel electrophoresis; its size was estimated by comparison to RNA extracted from E. coli and from yeast cells. These procedures permitted positive identifications of the peaks shown in Figure 1, A to D. The pattern of the ribosomes taken from sucrose thylakoids, thus, clearly demonstrates the existence of major contamination (20 to 40%) with 80S ribosomes.

Lack of Interference by DNA. The specificity of the method used for measuring RNA was further checked by analyzing the final extract for DNA (see "Materials and Methods"). In samples containing 32 to 42 μg of RNA from sucrose thylakoids, no DNA could be detected by the analytical method (11), which would have shown as little as 1 to 2 μg. Thus, the chloroplast DNA did not interfere with analysis of RNA.

Effect of Light on the Amount of Thylakoid-Bound Ribosomes. Samples from 17-d-old pea plants were frozen in liquid N₂, starting as shown in Figure 2. Total thylakoid-associated RNA reached a maximum within about 50 min after illumination, then remained constant up to the last sampling at 8.5 h into the light period. The low level of thylakoid-associated RNA in this experiment is related to the advanced age of the plants (see below and "Discussion"). When 11-d-old plants were sampled through a 24-h light/dark cycle, a similar pattern was obtained. Following the onset of the dark period, the thylakoid-associated RNA had dropped to its lowest level by the time of the first dark sampling at 30 min. Within the resolution of the experiment, the level of RNA remained constant through the remaining 7.5 h of darkness (data not shown).

Inasmuch as a significant fraction of the measured RNA represented contaminating 80S ribosomes (see above), it was important to know if the extent of their binding followed the same pattern as that of the 70S ribosomes. Accordingly, sucrose thylakoids were isolated from plants in the dark or after 1 h of light, and separate portions were used to measure total RNA for sucrose gradient fractionation of ribosomes released by trypsin and for electrophoretic analysis of intact RNA extracted by phenol. For the calculations of percentage of 70S and 80S rRNA, it was assumed that other species of RNA present were quantitatively negligible and that the two varieties of ribosomal RNA could be estimated from the areas under the appropriate peaks on the RNA electrophorogram as on the sucrose gradient profile. The results from the two kinds of analysis agree closely (Table II) and indicate that light causes little or no change in the level of contaminating 80S ribosomes and that 76 to 100% (overall mean for both methods, 86%) of the light-induced increase in the thylakoid-
associated RNA is attributable to an increase in 70S ribosomes.

In control experiments, we found that 67 to 76% of the thylakoid-bound RNA was released by trypsin. The extent of ribosome release did not differ significantly between chloroplasts isolated from plants in the light or in the dark, and increasing the trypsin level from 30 to 200 μg/ml did not change the amount released. The close agreement of the estimates of 80S ribosomal contamination by sucrose gradient profiles with those from electrophoretic analysis of phenol-extracted RNA indicates that the 24 to 33% of the ribosomal material not removed by trypsin was not selectively enriched in one or the other type of ribosome.

**Effect of Leaf Age on the Amount of Bound RNA.** Experiments with plants of different ages have yielded a wide range of values for thylakoid-associated RNA. The variation was troublesome, since it was essential, for experiments in which several samples were being compared, that the plants in each sample were truly representative of the whole population. The most likely source of variability was the relative proportion of young and old leaves on each sample. To test this idea, leaves of different ages on 16-d-old plants were sampled in the dark and then in the light. Relative leaf sizes and their positions on the plants are illustrated in Figure 3. The level of RNA in the young, folded leaves was dramatically higher than it was in the older leaves, but in all leaves the level of thylakoid-associated RNA increased in response to light (Fig. 4). The increase in RNA in the lower leaves is not evident in Figure 4, because of the scale of the graph. The absolute and relative increases are tabulated in Table III. The youngest leaves contain chloroplasts at early stages of development and might be expected to have a greater level of thylakoid-associated ribosomes. However, young chloroplasts have less Chl also, so that expression of the thylakoid-associated RNA in terms of Chl is not a direct measure of the number of thylakoid-associated ribosomes per chloroplast. To clarify this point, the amount of Chl per chloroplast for leaves of different ages was determined for 12-d-old plants. Using the values in Table III for thylakoid-bound RNA, the amount of RNA and the number of ribosomes bound to the thylakoids were calculated per chloroplast. After taking into consideration the difference in the amount of Chl per chloroplast for the different leaves, the number of thylakoid-associated ribosomes per chloroplast was calculated to be 30-fold higher for the youngest leaf than for the oldest leaf (Table III).

**Effect of Protein Synthesis Inhibitors on the Light-Induced Changes.** Our working hypothesis is that the light-dependent increase in the level of thylakoid-associated ribosomes reflects changes in translational and, perhaps, transcriptional activity in the chloroplast. If translational activity by the chloroplast ribo-

**FIG. 4.** Effect of leaf age on the level of thylakoid-bound ribosomes. Two flats of 16-d-old plants were divided with markers into 24 sections each and sampled in darkness and in light at 300 μmol m⁻² s⁻¹. At each time point, plants were cut from sections of each flat, rapidly divided into the three parts illustrated in Figure 3, and frozen in liquid N₂. Average sampling time was 15 min for points in the light and 23 min for points in the dark. The midpoint of each sampling period was used for the graphs. Sucrose thylakoids were isolated and assayed for bound RNA.

som is required for either the light-induced increase or the decrease in darkness of thylakoid-associated ribosomes, then inhibitors of protein synthesis by 70S ribosomes should modify these responses. Lincomycin and CAP were applied to plants prior to illumination, either by feeding the inhibitor through cut shoots for 3 h or by spraying the leaves with a solution of the inhibitor in 0.05% (v/v) Tween-80. The light-induced increase in thylakoid-associated RNA in two separate experiments was inhibited 71% by CAP and 51% by lincomycin (Table IV). In another experiment, CAP inhibited the dark-induced decrease by 52%.

No effect of cycloheximide, with uptake of 70 μg/g fresh weight during a 3-h period, could be found on these in vivo responses to light. However, the significance of this result is not entirely clear, because, at the levels used, incorporation of added radioactive amino acids into total protein of the shoot was inhibited only 37% (data not shown).

**Effect of Light and of Brief Preillumination on the Increase in Bound Ribosomes and on the High Salt-Sensitive and Puromycin/Salt-Sensitive Subpopulations.** Puromycin, in the presence of a high concentration of salt, has been used to remove membrane-bound ribosomes that are held to the membrane by a nascent peptide chain (5, 6, 18, 28). The puromycin causes release of the nascent peptide from the ribosome and high salt functions to disrupt electrostatic bonds which also hold the ribosome to the membrane. Conditions for this release were recently described for pea thylakoids (28). These criteria—release by high salt or by high salt plus puromycin—were applied to ribosomes of thylakoids from plants harvested in the light or in the dark (Table V). It is clear in the two experiments shown that the number of ribosomes bound by a nascent chain (defined by the need for puromycin as well as high salt for release) did increase substantially in the light. The increase was 100% in experiment 1 and 60% in experiment 2 of Table V, due to only 8 to 10 min of illumination (compare lines 2 and 3 with line 1 in experiment 1, lines 3 and 4 with line 1 in experiment 2). During this time, the total bound RNA increased only one-half as much (Table V, experiment 1) or a negligible amount (Table V, experiment 2).

Table V also includes data generated in an attempt to see if a brief period of illumination (5 min in experiment 1, 4 min in experiment 2) would cause an inductive set of changes in bound ribosomes during a subsequent 60-min dark period. The experi-
Table III. Bound Ribosomes in Chloroplasts from Leaves of Different Ages

For estimation of the Chl content of chloroplasts, leaves of 12-d-old plants were separated according to morphological stage of development: youngest, immature leaf; almost fully expanded, but still folded leaf; youngest, mature, and expanded leaf; oldest leaf (see Fig. 3). Tissue samples were homogenized with a Polytron homogenizer in the grinding medium used for isolating sucrose thylakoids. After filtering through cheesecloth, aliquots were used directly for measuring Chl and for counting chloroplasts using a Levy corpuscle counting chamber.

For calculation of the number of ribosomes bound per chloroplast, typical values for thylakoid-bound RNA for leaves at these stages of development (e.g. see below) were used (column 4). The mass of RNA per ribosome was assumed to be 1.69 × 10^6 daltons (2.81 × 10^18 g).

<table>
<thead>
<tr>
<th>Leaf</th>
<th>No. of Chloroplasts Counted</th>
<th>Chl per Chloroplast</th>
<th>Thylakoid-Bound RNA</th>
<th>Ribosomes per Chloroplast</th>
<th>Bound RNA on Thylakoids* Harvested In</th>
<th>Increase in Light</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>µg</td>
<td>µg/mg Chl</td>
<td>µg RNA/mg Chl</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Youngest</td>
<td>985</td>
<td>1.0</td>
<td>1,500&lt;sup&gt;a&lt;/sup&gt;</td>
<td>530,000</td>
<td>320</td>
<td>487</td>
</tr>
<tr>
<td>Folded</td>
<td>434</td>
<td>1.5</td>
<td>450&lt;sup&gt;a&lt;/sup&gt;</td>
<td>230,000</td>
<td>46</td>
<td>85</td>
</tr>
<tr>
<td>Youngest mature</td>
<td>706</td>
<td>1.4</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50,000</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Oldest</td>
<td>772</td>
<td>1.5</td>
<td>30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16,000</td>
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<sup>a</sup> Data taken from experiment shown in Figure 7. The values for dark-harvested leaves are the mean of two time points; for light-harvested leaves, the mean of the last three points in the light (see Fig. 7).

<sup>b</sup> Typical values for leaves harvested in the light. Dark values would be somewhat lower.

Table IV. Effect of Chloramphenicol and Lincomycin on In Vivo Changes in Thylakoid-Bound Ribosomes

In the three experiments shown, plants were treated as follows. In experiment 1, 15-d-old plants were cut above the cotyledons, and the stems were placed in 2.5-ml vials containing either water or 1 mg/ml CAP. After 3 h of incubation in darkness, a sample consisting of the youngest mature leaf and the upper part of the shoot was collected from one-half of the plants. The remaining plants were illuminated for 1 h at 275 µmol m<sup>−2</sup> sec<sup>−1</sup>, then sampled in the same manner. In experiment 2, 15-d-old plants were sprayed with 1 mg/ml CAP in 0.1% Tween-80 or just with Tween-80. These were incubated in the light for 2 h, then one-half were sampled as in experiment 1; the remainder were placed in darkness for 60 min, then sampled. In experiment 3, 13-d-old plants were sprayed with 200 µg/ml lincomycin in 0.1% Tween-80 or just with Tween-80, then incubated for 2 h in darkness. One-half of the plants were collected, and the remaining plants were incubated in the light for 1 h before harvesting. All samples were frozen in liquid N<sub>2</sub> at the specified times, then divided into three or four portions for replicate determinations. High-Tris thylakoids were isolated individually from each portion; RNA assays from each portion were made in triplicate.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Type of Transition</th>
<th>Inhibitor</th>
<th>Bound RNA</th>
<th>Change in RNA</th>
<th>Inhibi-tion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dark</td>
<td>Light</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>µg RNA/mg Chl</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Dark to light</td>
<td>CAP</td>
<td>255 ± 46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>424 ± 19</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>299 ± 17</td>
<td>342 ± 31</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>Light to dark</td>
<td>CAP</td>
<td>184 ± 9</td>
<td>309 ± 26</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>221 ± 12</td>
<td>281 ± 10</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>Dark to light</td>
<td>Lincomycin</td>
<td>97 ± 2</td>
<td>152 ± 12</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>94 ± 7</td>
<td>121 ± 4</td>
<td>27</td>
</tr>
</tbody>
</table>

<sup>a</sup> All values shown ±SD.
Table V. Effects of Light and Preillumination on the Puromycin-Sensitive Bound Ribosomes

Twelve-d-old pea plants were kept in darkness for 20 h (experiment 1) or for 16 h (experiment 2) prior to preillumination for 5 min (experiment 1) or for 4 min (experiment 2) where indicated. All plants were kept for another 60 min in darkness. Some were harvested rapidly and chilled to 0°C, while the remainder were illuminated at 275 μmol m⁻² sec⁻¹ for 10 min (experiment 1) or 8 min (experiment 2), then harvested and chilled. All plants were ground as soon as possible without freezing, and Percoll chloroplasts were prepared. Thylakoid membranes were isolated and washed, and total bound RNA and sensitivity to high salt or high salt plus puromycin were determined in triplicate, as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Light Regime</th>
<th>Time Sequence</th>
<th>Thylakoid-Bound RNA</th>
<th>Change Caused by Illumination*</th>
<th>Change Caused by Illuminationb</th>
<th>Change Caused by Illuminationc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Released by 1 M KCl Released by Puromycin¹</td>
<td>Total Puromycin-Sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>μg RNA/mg Chl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5 min</td>
<td>60 min</td>
<td>10 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>Dark</td>
<td>Dark</td>
<td>338 ± 12</td>
<td>164 ± 12</td>
<td>79 ± 7</td>
<td>+48</td>
</tr>
<tr>
<td>Dark</td>
<td>Dark</td>
<td>Light</td>
<td>501 ± 14</td>
<td>198 ± 15</td>
<td>155 ± 6</td>
<td>+61</td>
</tr>
<tr>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>544 ± 23</td>
<td>230 ± 26</td>
<td>160 ± 12</td>
<td>+61</td>
</tr>
<tr>
<td>2</td>
<td>4 min</td>
<td>60 min</td>
<td>8 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>Dark</td>
<td>Dark</td>
<td>607 ± 22</td>
<td>322 ± 51</td>
<td>138 ± 34</td>
<td>-3</td>
</tr>
<tr>
<td>Light</td>
<td>Dark</td>
<td>Dark</td>
<td>589 ± 3</td>
<td>281 ± 21</td>
<td>161 ± 23</td>
<td>-3</td>
</tr>
<tr>
<td>Dark</td>
<td>Dark</td>
<td>Light</td>
<td>631 ± 18</td>
<td>247 ± 33</td>
<td>220 ± 33</td>
<td>+5</td>
</tr>
<tr>
<td>Light</td>
<td>Dark</td>
<td>Light</td>
<td>677 ± 16</td>
<td>253 ± 30</td>
<td>216 ± 28</td>
<td>+12</td>
</tr>
</tbody>
</table>

* Difference between RNA released by puromycin plus 1 M KCl and that released by 1 M KCl alone.

b Light-induced change compared to 'Dark, Dark, Dark.'

c So for the difference calculated by the compounding formula, $\text{d}_A = (\text{d}_B + \text{d}_C)^{1/2}$.

Photosynthesis, measured with an IR gas analyzer, saturated at about 2,000 μmol m⁻² sec⁻¹ for our plants and half-saturated at about 475 μmol m⁻² sec⁻¹ (determined from a double reciprocal plot of the data shown in Fig. 6). The light-induced increase in ribosome binding was found to occur at much lower light intensities. A level of 100 μmol m⁻² sec⁻¹, but not 25 μmol m⁻² sec⁻¹, was adequate to produce the maximum increase of bound ribosomes within 30 min of illumination (Fig. 6).

Blue and orange light, provided by broad band-pass filters, were tested for inducing ribosome binding. The light transmitted by the blue filter (410-510 nm) was in a region of minimal effectiveness in the photoconversion of phytochrome. The orange filter (545-690 nm) eliminated the active wavelengths absorbed by blue-light-activated photoreceptors. Flats of peas that had been in darkness were sampled at two or three time points, then illuminated with filtered light and sampled again at three or four time points in the light. The results, summarized in Table VI, indicate that both colors of light were effective in this function. The intensities used (about 55 μmol m⁻² sec⁻¹ for blue light and 110-140 for orange light) are close to threshold values for white light. A blue light intensity of 16 μmol m⁻² sec⁻¹ was entirely ineffective, in line with the lack of effect of an intensity of 25 μmol m⁻² sec⁻¹ for white light (Fig. 6).

Inhibition by DCMU. The results shown in Figure 6 indicate a fairly low light saturation point and, thus, a low requirement for electron flow. These results do not distinguish between a requirement for ATP and a requirement for reducing electrons. To clarify this point, we examined the effect of DCMU.

The time course of DCMU entry into the chloroplasts of intact plants in light was assessed by measuring O₂ evolution from leaf discs cut from plants sprayed with DCMU and 0.4% (v/v) Tween-80 or with just 0.4% (v/v) Tween-80 (Fig. 7). Complete inhibition of O₂ evolution occurred within 2 h after spraying with DCMU. To insure that the observed inhibition was not a result of entry of DCMU into the cells after cutting of the leaf discs, the leaves were rinsed repeatedly in ice water before the discs were cut. In addition,

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**Fig. 5.** Effect of in vivo illumination on the total activity and specific activity in protein synthesis of thylakoid-bound ribosomes. Plants 14 d old were sampled in darkness (using a dim safelight) and then illuminated with 138 μmol m⁻² sec⁻¹ of orange light. Sucrose thylakoids were isolated from each sample and divided up for triplicate measurements of Chl, thylakoid-bound RNA, and protein synthesis when supplemented with a soluble extract from *E. coli*. The protein synthesis reaction mixtures contained, in 250 μl, 100 μg Chl and 40 μl *E. coli* S-30 fraction. Incubations were for 60 min at 30°C. Protein synthesis was measured in duplicate; the mean percentage difference between replicates was 5.2%, and an incorporation of 220,000 cpmp/mg Chl represents 23 pmol of [³H]Leu incorporated per mg Chl.

**Effect of Light Intensity and Quality.** A thorough evaluation of the effect of light intensity would involve measurements of the rate of change in the ribosomes bound at different intensities. The small relative changes over short time intervals and the logistics of these experiments precluded such a study. Instead, we chose one time point (30 min) and attempted to determine the minimum light intensity that would produce a significant increase in bound ribosomes.

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for more or nim; orange values for in plants four immediately in measurement. umol m-2 sec-1, was group, and accomplished youngest fully open leaves and the shoot above were frozen in liquid N2. Sucrose thylakoids were isolated separately from four replicates in each group, and RNA was assayed in triplicate for each replicate. Photosynthesis was measured as CO2 fixation by the youngest fully opened leaf of an intact plant, monitored with an IR gas analyzer at five light intensities.

Table VI. Stimulation of Ribosome Binding by White, Blue, and Orange Light

Plants 10 to 13 d old that had been in darkness for 12 h or more were sampled at three time points in the dark, illuminated, and then sampled at four or more time points in the light. Shoot or leaf samples were frozen immediately in liquid N2, then used later for isolation of sucrose thylakoids and measurement of the bound RNA in triplicate. To summarize the data, values for bound RNA in dark samples were averaged, as were those for plants in the light (Fig. 2). The blue-light wavelength range was 400 to 520 nm; orange light was 545 to 690 nm.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Light Color</th>
<th>Light Intensity ( \mu \text{mol m}^{-2} \text{s}^{-1} )</th>
<th>Bound RNA ( \mu \text{g RNA/mg Chl} )</th>
<th>Light-Induced Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>White</td>
<td>275</td>
<td>133 ± 10*</td>
<td>175 ± 10</td>
</tr>
<tr>
<td>2</td>
<td>White</td>
<td>400</td>
<td>303 ± 7</td>
<td>478 ± 16</td>
</tr>
<tr>
<td>3</td>
<td>Blue</td>
<td>52</td>
<td>153 ± 15</td>
<td>207 ± 17</td>
</tr>
<tr>
<td>4</td>
<td>Blue</td>
<td>55</td>
<td>139 ± 6</td>
<td>180 ± 5</td>
</tr>
<tr>
<td>5( ^{b} )</td>
<td>Blue</td>
<td>16</td>
<td>815 ± 20</td>
<td>832 ± 34</td>
</tr>
<tr>
<td>6</td>
<td>Orange</td>
<td>110</td>
<td>143 ± 5</td>
<td>187 ± 27</td>
</tr>
<tr>
<td>7</td>
<td>Orange</td>
<td>140</td>
<td>158 ± 14</td>
<td>199 ± 8</td>
</tr>
</tbody>
</table>

* Values are shown with sd of the mean for thylakoids isolated at three or more time points. Each number, thus, represents the average of nine or more RNA determinations.

\( ^{b} \) Only the youngest folded leaves were used; two points were averaged for ‘Dark,’ so that the variability shown is range + 2.

spraying the 150-min control plants with DCMU just before harvesting and washing had no effect on O2 evolution. For thylakoid-bound ribosome measurements, plants were sprayed with DCMU or control solution (0.4% [v/v] ethanol/0.4% [v/v] Tween 80) at least 3 h before harvest. Shoot samples were collected before illumination, and also after 10 to 15 min of light, and used for isolation of washed thylakoids. DCMU inhibited the light-induced increase in puromycin/high salt-sensitive ribosomes by 72 to 100%, and in total RNA by 44 to 100%, in the four experiments (Table VII).

Inhibition of O2 evolution from leaf discs by DCMU was determined in experiment 3 (Table VII). No O2 evolution was detected at the time of harvest.

DISCUSSION

We used the convenient and rapid enzymic method for measuring total RNA (10) to estimate the number of ribosomes bound to thylakoids, because there is no simple, quantitative method to obtain this estimate more directly. For this procedure to be valid, however, the total RNA has to consist very largely of the rRNA of 70S ribosomes. Nonribosomal RNAs were found to be an insignificant fraction of the total RNA bound to thylakoids (10).

Contamination by 80S ribosomes is essentially absent from intact chloroplasts prepared on Percoll gradients (24) and from the thylakoids extracted from them (Fig. 1). However, in time-course or other experiments that required assay of many samples, this procedure was not used, since time limitations necessitated that some samples be stored for an extended period while the first ones were being processed. We felt that this storage period might introduce complications that would be difficult to assess. For this reason, we used immersion in liquid N2 to stop physiological
changes within seconds after harvesting, and we stored the frozen samples until time permitted processing. Washed sucrose thylakoids, but not intact chloroplasts, could be obtained from these frozen shoots.

Whereas our early results (10) (Fig. 4B) and the report of Reardon et al. (24) seemed to indicate that 80S ribosomes were contributing 5% or less to the RNA associated with these washed sucrose thylakoids, we discovered later that this was an incorrect result caused by insertion of a step involving dissolution of the thylakoids in Triton X-100 and centrifugation to remove undissolved material prior to assay of the RNA present (as in Ref. 15). For reasons that are still not clear, most of the 80S ribosomal material present initially was removed in that centrifugation, leaving almost pure 70S ribosomes in the supernatant. Without the Triton dissolution step, 15 to 40% of the RNA present in the sucrose thylakoids, despite extensive washing, was due to adhering 80S ribosomal RNA. Several modifications of the thylakoid isolation procedure (Table I) failed to diminish this problem.

It was necessary, therefore, to see if the light-induced increases in thylakoid-associated RNA (Figs. 2 and 4) were due to changes in the contaminating 80S rRNA, only the 70S rRNA, or both. Polyacrylamide gels of phenol-extracted RNA and sucrose gradient profiles of trypsin-released ribosomes indicated that the changes caused by light are restricted almost completely to the 70S ribosomal population (Table II). The full extent of light-induced changes was also found when using Percoll thylakoids, thus confirming this conclusion.

The level of thylakoid-bound ribosomes changed in response to both light and dark treatment of the plants. Light typically increased the number of bound ribosomes between 25 and 65% over the dark level. This was true for both the youngest still-folded leaves and the mature expanded leaves (Table III and Fig. 4). When plants were transferred to darkness after a normal 12- or 16-h light period, the number of bound ribosomes returned to the previous dark level (Table IV, Fig. 4). The time required for completion of the transition from light to dark levels, or vice versa, varied from 15 to 50 min, though in one experiment (Table V, experiment 2), only 10 min were necessary. The thylakoid-associated ribosome level remained constant throughout the time in light or in darkness, once the transition had been made (Fig. 2 and other experiments not shown).

These results are in the same direction, but not as dramatic, as those seen with synchronized cells of *Chlamydomonas* (6), where the level in the dark was only 17% of that in the light, and the transition was rapid.

Results of *in vivo* studies permit a tentative definition of the
nature of the light reaction causing the increase in ribosome binding to thylakoids. Although the technical difficulty of obtaining an adequate number of early data points precluded any real kinetic studies of the phenomenon, it was possible to examine steady-state alterations in response to different light treatments. We feel that the fact that orange light was effective rules out a blue light-absorbing pigment as the sole photoreceptor. The similar responses to blue, orange, and white light (Table VI) argue against the participation of phytochrome. However, dose response measurements are needed to verify this.

If photosynthetic reactions are involved, they seem to require less than full light intensity, since the transition to the higher steady-state ribosome level is effected at intensities approximately 10% of those needed for saturation of photosynthesis (Fig. 6). However, a kinetic analysis would be necessary to establish the threshold intensity for the response. Nevertheless, electron transport through the PSII region seems to be required in view of the significant inhibition by DCMU (Table VII). Although this DCMU effect could have resulted from restriction of the ATP supply, cyclic electron flow would still be supporting ATP synthesis. Indeed, protein synthesis in isolated chloroplasts was supported entirely by ATP from cyclic flow (23). We think the DCMU inhibition more likely results from a need either for reducing equivalents, as in the activation of Calvin cycle enzymes, or for the regulatory function of redox poising of the electron transport chain, as in control of activation of thylakoid-bound protein kinase by the redox state of plastoquinone (14).

The concentration of DCMU in the chloroplasts in vivo cannot be measured directly; we observed that isolated chloroplasts from DCMU-treated plants had regained up to 40% of their O2-evolving capability (data not shown). However, monitoring of the gas exchange of small leaf discs with an O2 electrode permitted us to observe (Fig. 7) that inhibition by DCMU was complete within about 2 h in the light (using 400 μM DCMU—a super-saturated solution) or after 4 h in the dark (with 200 μM DCMU). Since in experiments 1 and 2 of Table VII the DCMU was applied about 18 h before harvest, it is conceivable that its internal concentration became considerably higher than that needed to inhibit linear electron flow. However, in experiment 3, monitoring of the DCMU effect on O2 evolution in parallel samples showed that leaves were harvested for RNA measurement shortly after complete inhibition had occurred. Since the concentration of DCMU needed to inhibit cyclic electron flow partially is 1,000 times higher than that needed for equivalent inhibition of PSII (12), certainly in experiment 3 of Table VII and most probably in others as well, added DCMU inhibited noncyclic electron flow only.

The proteins made by thylakoid-bound polysomes most likely include those of the thylakoid membrane (1). Thus, the higher level of bound ribosomes on thylakoid membranes from very young leaves is no doubt an indication of their higher content of rapidly growing, younger plastids. Also, the light-induced increase means that thylakoid growth is probably slower in darkness but still appreciable. This contrasts with synchronized *Chlamydomonas*, where thylakoid-bound ribosomes are largely absent in the dark phase (6), and spectinomycin hardly inhibited total cell protein synthesis in the dark.

The earliest effects of light observed in these studies were an increase in the number of bound polysomes (defined by the puromycin-released population) (Table V) and their activity in amino acid incorporation (Fig. 5). When these effects were seen in as little as 8 min, the total bound RNA showed little change. The increase in the total amount of bound RNA usually took 20 to 45 min to become apparent, although the response was occasionally more rapid (Table V, experiment 1). This sequence could be explained in one of two ways. There might be a rapid discharge of bound (high salt-sensitive) monomeric ribosomes simultaneous with the attachment of new polysomes via their nascent chains. Or the new ribosomes, attached by nascent chains, may be derived largely from the monomeric ribosomes already present in salt-sensitive bonds on the thylakoid surface. We have no data that can distinguish between these hypotheses as yet, but, in *Euglena*, the exchange of large subunits between the thylakoid-bound and unattached ribosomes may be much lower than that of the small subunits (16). This result suggested the possibility of a direct transition for the large subunit from a high salt-sensitive state to a puromycin-sensitive state, involving binding of the small subunit and subsequent chain elongation. Other evidence for the requirement of interaction between the nascent chain and the thylakoid for light-enhanced binding is found in the inhibition by CAP and lincomycin (Table IV).

Release of ribosomes from the thylakoids may also require protein synthesis (Table IV). In *Chlamydomonas*, adding chloramphenicol during harvesting of cells and isolation of thylakoids prevented chain termination and permitted retention of bound ribosomes (5, 19). Presumably, continuing protein synthesis leads to runoff of ribosomes from the mRNA, thereby leading to breakdown of the polysomes and release of the ribosomes from the membrane-associated nascent chains.

Further work is needed to see if light stimulates the synthesis of all the thylakoid peptides made by chloroplast ribosomes, or one or another more specifically than the rest. *In vitro* experiments will undoubtedly be needed to define further the mechanism of the light-induced increase in ribosome binding to thylakoid membranes.

Acknowledgments—We wish to thank Dr. P. Ludford, for help in the use of the IR gas analyzer for CO2 fixation measurements, and Drs. R. Alscher-Herman and H. Nivison, for critical reading of the manuscript.

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