Regulation of Accumulation of the Major Thylakoid Polypeptides in *Chlamydomonas reinhardtii* y-1 at 25°C and 38°C

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ABSTRACT The amount of messenger RNA (mRNA) for polypeptides of the chlorophyll a/b-protein complex of thylakoid membranes in etiolated and greening cells of *Chlamydomonas reinhardtii* y-1 was examined by immunoprecipitation and electrophoresis of products of in vitro translation to determine at which stage production of these polypeptides is regulated. Cells grown 4 d in the dark at 25°C contained small amounts of translatable mRNA for the major membrane polypeptides. Exposure of these etiolated cells to light, under conditions in which the membrane polypeptides accumulated, resulted in a significant increase in the quantity of the mRNA. In contrast, when etiolated cells were incubated for 1–2 h in the dark at 38°C, translation assays indicated that mRNA for the membrane polypeptides became abundant. Moreover, the quantity of the mRNA did not increase when these cells subsequently were exposed to light. Therefore, at 38°C the cellular level of the polypeptides is not regulated by synthesis of mRNA. The in vitro synthesized polypeptides, which were precipitated with antibodies prepared against the purified thylakoid polypeptides, had apparent molecular weights of 31,500 and 30,000. The corresponding immunoprecipitated polypeptides made in vivo had apparent molecular weights of 29,500 and 26,000. Thus, the membrane polypeptides are made as precursors.

No net accumulation of the polypeptides occurred in cells in the dark at 38°C, but immunoreactive polypeptides the size of the mature membrane components were labeled during incubation of cells with [14C]acetate in the dark. These results indicated that the mRNA was translated in the dark, but since the polypeptides did not accumulate, the products of translation were probably degraded. We conclude from our experiments that at 25°C production of the polypeptides is regulated by the level of translatable mRNA in the cells. At 38°C, however, the accumulation of the polypeptides is controlled by posttranslational processes.

Thylakoid membranes of *Chlamydomonas reinhardtii* contain three major polypeptide components, separable by electrophoresis, that are structurally related (1, 2). They were designated 11, 16, and 17 by Chua and Bennoun (3). In our hands, the apparent Mr of polypeptide 11 is 29,500. Polypeptides 16 and 17 have an apparent Mr of 26,000, and resolution of these two is dependent upon the conditions of electrophoresis. The three polypeptides are synthesized on cytoplasmic ribosomes (4–6) as precursors to the mature forms (7). They serve as components of the light-harvesting chlorophyll a/b-protein complex within the chloroplast membranes (8, 9) and accumulate normally only under conditions that permit synthesis of chlorophyll.

In wild-type *Chlamydomonas* cells, synthesis of chlorophyll and of the polypeptides occurs in the dark (5). In contrast, no net synthesis of either of these membrane components can be detected in dark-grown cells of the y-1 mutant strain (5, 10). When etiolated y-1 cells are transferred to the light at 25°C, there is a lag of 1–2 h before significant rates of production of chlorophyll and the polypeptides are achieved (11, 12). This process of greening is sensitive to the addition of inhibitors of RNA synthesis (13), which suggests that in cells grown in the...
dark at 25°C the initial rate of greening is limited by the level of mRNA.

The initial lag in greening of *Chlamydomonas* cells can be eliminated by increasing the temperature to 38°C before illumination. After a 1–2 h preincubation in the dark at 38°C, accumulation of both chlorophyll and the membrane polypeptides begins immediately when cells are exposed to light (5). Greening of the cells at 38°C is relatively insensitive to inhibition of RNA synthesis by actinomycin D, when the drug is added just before exposure of the cells to light (5). Therefore, accumulation of the mRNA for the membrane polypeptides apparently occurs in the dark at the higher temperature, and consequently the rate of greening may not be limited by the amount of mRNA under these latter conditions.

We examined the levels of mRNA for polypeptides 11, 16, and 17 of the light-harvesting chlorophyll a/b-protein complex of thylakoid membranes in *Chlamydomonas* cells to obtain definitive evidence regarding this proposal by which light and temperature affect the rate of greening. Our results indicate that the accumulation of these polypeptides at 25°C is limited by translatable mRNA levels, while at 38°C it is determined by posttranslational processes.

**MATERIALS AND METHODS**

**Growth and Greening of Cells**

Etiolated cells of *Chlorella reinhardtii* were obtained as previously described by transfer of green cells to the dark and subsequent growth at 25°C for 4 d (5, 14). The level of chlorophyll of the dark-grown cells began to increase after a 24-h incubation at 25°C, whereas the green cells grown at 25°C provided ~1.7 mg total RNA/10^11 cells. The green cells were harvested at centrifugation and suspended in fresh medium to a density of ~1 × 10^11 cells/ml and incubated at 25 or 38°C. For experiments conducted at 25°C, cell suspensions were incubated with shaking for 3 h in the dark or light (15 W/m² from cool-white fluorescent lamps). Since the doubling time for these cells in the dark is 18–20 h at 25°C (14), and cell division is suppressed during greening (15), there was no significant increase in cell number during the 3-h incubation period. For experiments at 38°C, equal volumes of cell suspensions were incubated with shaking in a water bath (5) for 2 h in the dark. Cells from one flask were harvested at this time, while the remainder were exposed to incandescent light (~20 W/m²) by passing through a glass 4602 IR filter, Coming Glass Co., Cortland, NY.

Stock cultures of the algal cells on agar medium (14) were checked periodically for revertants to wild-type by extended growth in the dark. No green colonies, indicative of reversion, were observed over the course of this work.

**Labeling of Proteins In Vivo**

Cellular proteins were pulse-labeled, after incubating suspensions of dark-grown cells in growth medium lacking acetate for 1.5 h in the dark at 38°C, by adding [35S]methionine (85 Ci/mM; Amersham, Arlington Heights, IL) to 10 or 20 μCi/ml of the suspension. The cells were kept in the dark during the labeling period or immediately exposed to light. Aliquots were withdrawn at prescribed times and mixed with trichloroacetic acid (final concentration, 10% wt/vol). Cells were collected by centrifugation.

**Preparation of Poly(A)-rich RNA**

Poly(A)-rich RNA was separated from bulk RNA with columns of oligo(dT) cellulose (P-L Biochemicals Inc., Milwaukee, WI) (17). The buffer used for adsorption contained 0.5 M NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 0.2% SDS. NaCl was omitted from the buffer used for elution of poly(A)-rich RNA. Fractions containing this RNA were adjusted to 0.2 M ammonium acetate and the RNA was precipitated with 2 vol of 95% ethanol at -20°C overnight. The precipitate was dried by lyophilization, dissolved in distilled water and stored at -70°C. For the preparation of mRNA, all glassware was autoclaved and all solutions were either autoclaved or passed through a 0.45-μm pore filter (Millipore Corp., Bedford, MA).

**Translation of Poly(A)-rich RNA**

RNA (0.2 μg) was translated essentially according to the method of Pelham and Jackson (18). Each assay mixture (30 μl) contained 25 mM HEPES-KOH (pH 7.2), 1.2 mM MgCl₂, 135 mM K⁺ (as acetate and chloride salts), 23 mM NaCl, 0.2 mM dithiothreitol, 10 mM creatine phosphate, 16 μg/ml creatine kinase, 8 μg hemin, 50 μM of each of 19 amino acids (except methionine), 30 μCi [35S]methionine (800–1,000 Ci/mM; Amersham, Arlington Heights, IL), and 10-μl micrococcal nuclease-treated rabbit reticulocyte lysate (Bethesda Research Laboratories, Gaithersburg, MD). The mixtures were incubated 60 min at 30°C. The incorporation of 35S into protein was measured by transferring 2 μl of the mixture to 1-cm squares of filter paper, which then were treated as described by Mans and Novelli (19). Total incorporation routinely ranged between 2 × 10^5 and 3 × 10^6 dpm/0.2 μg RNA in each assay tube. The amount of protein synthesized was linearly related to the quantity of poly(A)-rich RNA up to at least 1 μg per assay tube. The remaining translation mixture was treated with 2 vol of a saturated solution of (NH₄)₂SO₄, chilled in ice for 30 min and centrifuged 3 min at 12,000 g in a Model 5412 Eppendorf centrifuge. The pellets contained essentially all of the protein translated from the algal RNA, whereas major proteins endogenous to reticulocytes were recovered mostly in the supernatant fraction (Fig. 1).

**Preparation of Antibodies to Polypeptide 11**

The polypeptide was prepared from thylakoid membranes, purified as described previously (4, 22), by preparative electrophoresis on polyacrylamide slab gels (24 × 19 × 0.3 cm) containing a 7.5 to 15% linear acrylamide gradient (24). Gels were stained with 0.25% Coomassie Brilliant Blue in methanol:14% acetic acid (1:1, vol/vol), destained in methanol:10% acetic acid (3:7, vol/vol) and then washed with water for 0.5 h. The polypeptide 11 band was cut out, ground in a mortar and pestle, lyophilized and then reground to a fine powder. The powder was swollen with 9.2 ml of resolving gel buffer (24) per gram of powder and embedded in an equal volume of fresh 7.5% polyacrylamide gel in the same buffer in 1-cm diameter tubes to a height of 10 cm. The protein was eluted electrophoretically at 10 mA per tube at room temperature for 2 h into a bag of dialysis tubing. These samples were lyophilized, taken up in 1–2 ml water and dialyzed against three changes of 20 mM Tris-HCl (pH 9.2) containing 0.1% SDS. The protein then was precipitated with 10 vol of acetone to remove dye, and the pellet was washed once with acetone and air-dried. The protein was prepared for injection into rabbits by solubilization in 4% SDS by heating 1 min in boiling water and then dilution with water to make the final concentration of SDS 0.1 to 0.2%.

Preimmune serum was obtained from each rabbit before injection. Rabbits were injected each time in the thickest muscle of all four limbs using a total of 1 ml emulsion consisting of 1 vol of antigen and 3 vol of Freund's complete adjuvant. The first injection contained a total of 100 μg of antigen and two subsequent injections of 50 μg per rabbit were given at 2 and 5 wk after the first injection. Rabbits were bled 1 wk later. Booster injections of 50 μg of antigen per rabbit were given 1 wk after each additional bleeding. IgG fractions were prepared by (NH₄)₂SO₄ fractionation and DEAE-Sephadex chromatography (25).

**Immunoprecipitation**

Pellets obtained from the translation mixtures by precipitation with (NH₄)₂SO₄, were dissolved in 20 μl of 50 mM Tris-HCl (pH 7.5) containing 2% SDS by heating 1 min in boiling water and then mixed with 380 μl of buffer B (50 mM Tris-HCl, pH 7.5, containing 300 mM NaCl, 5 mM EDTA, and 1% Triton X-100 [26]). Each sample was mixed with 20 μg of a nonimmune IgG fraction and, after incubation at room temperature for 15 min, with 50 μl of formalin-fixed Staphylococcus aureus cells (10% suspension, IgG sorb. The Enzyme Center, Boston, MA), which were prewashed with buffer B and suspended in buffer B containing 1 mg/ml ovalbumin (27). The sample was incubated at room temperature for 10
RESULTS

Characterization of Antibody Preparations

The antigen used to raise antibodies consisted of a single polypeptide, as evaluated by electrophoresis through a polyacrylamide gradient gel (Fig. 2A), which corresponded in size to polypeptide 11 (1, 3). Additional peaks occurred at positions corresponding to polypeptides 16 and 17, even though the preparation of antigen used to raise the antibodies was free of detectable cross-reactivity with polypeptide 11. No precipitation bands were observed with preimmune IgG (not shown), but a rather complex immunoprecipitation pattern was obtained with immune IgG (Fig. 2B). The heaviest and sharpest peak of immunoprecipitation occurred in the position corresponding to polypeptide 11. Additional peaks occurred at positions corresponding to polypeptides 16 and 17, even though the preparation of antigen used to raise the antibodies was free of detectable amounts of polypeptides 16 and 17 (Fig. 2A). Cross-reactivity of polypeptides 11, 16, and 17 was observed previously by Chua and Blomberg (1) with a different preparation.

Effect of Treatment of Cells on mRNA Levels

Poly(A)-rich RNA was isolated from dark-grown cells and from cells exposed to light for 3 h at 25°C and translated in vitro. The electrophoretic patterns for the total translation products are shown in Fig. 3. Polypeptides in the M, 25,000–35,000 range were more abundant after translation of RNA from illuminated cells (Fig. 3, lane 3) than from dark-grown cells (Fig. 3, lane 1). As shown in Fig. 3 (Fig. 3, lanes 2 and 4), when the translation products were treated with antibodies produced against mature membrane polypeptide 11, two polypeptides, apparent M, 31,500 and 30,000, were the major products that were immunoprecipitated. Considerably more of these polypeptides were synthesized from RNA from greening cells (Fig. 3, lane 4) than from dark-grown cells (Fig. 3, lane 3). This suggestion was confirmed by in vitro translation of poly(A)-rich RNA extracted from etiolated cells incubated 2 h in the dark at 38°C. As shown in Fig. 4 (lanes 1–3), a given amount of RNA produced the same pattern of trans-

min with frequent mixing and then centrifuged to pellet the cells. The supernatant fluid was mixed with 20 μg of the IgG immune fraction. After incubation at 37°C for 30 min and 4°C for 2 h, 50 μl of the S. aureus cell suspension were added. The suspension was incubated at room temperature for 15 min with frequent mixing. The pellet obtained by centrifugation was washed three times with buffer B containing 0.1% SDS, suspended in buffer B with 0.1% SDS, transferred to a clean tube and pelleted again by centrifugation (26).

Electrophoresis

Electrophoresis of translation products and immunoprecipitates on 10 to 20% polyacrylamide gradient gels was performed as described previously (2). In some cases, the buffer system described by Chua (24) was also used. Autoradiography of dried gels was performed as described before (28).

FIGURE 1 Fractionation of translation mixtures. After translation of algal mRNA, 0.5, 1, or 2 vol of a saturated solution of (NH₄)₂SO₄ (prepared at room temperature) was added to separate mixtures. The samples were chilled on ice 30 min and then centrifuged at 12,000 g for 3 min. Protein in the supernatant fractions was precipitated by adding trichloroacetic acid to 10% (wt/vol). Each pellet was dissolved in 0.1 ml of 0.1 M Tris base containing 2% SDS, 0.3 M sucrose, and 4% 2-mercaptoethanol, and 10 μl of each sample was applied to a 10–20% polyacrylamide gradient gel (2). Lanes 1–7 show autoradiograms of ³⁵S-labeled polypeptides recovered in: a total (T), unfractionated sample precipitated with 10% trichloroacetic acid (lane 1), supernatant fractions (S) after adding 0.5 (lane 2), 1 (lane 3) or 2 vol (lane 4) of (NH₄)₂SO₄ and the corresponding pellet fractions (P) after adding 0.5 (lane 5), 1 (lane 6) or 2 vol (lane 7) (NH₄)₂SO₄. Lanes 8–11 show the patterns of protein stain for pellet fractions (P) from 0.5 (lane 8), 1 (lane 9), and 2 vol (lane 10) (NH₄)₂SO₄ and a total protein sample (T) obtained with 10% trichloroacetic acid (lane 11). 2 vol of (NH₄)₂SO₄ provided the maximal recovery of labeled translated polypeptides in the insoluble fraction while precipitating relatively little of the major endogenous polypeptides. This fractionation scheme was based on the solubility of polypeptide 11, which corresponded in size to polypeptides 16 and 17, even though the preparation of antigen used to raise the antibodies was free of detectable amounts of polypeptides 16 and 17 (Fig. 2A). Cross-reactivity of polypeptides 11, 16, and 17 was observed previously by Chua and Blomberg (1) with a different preparation.

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A stained gel is shown after electrophoresis of 10 μg of polypeptide 11 (lane 7) and 100 μg of thylakoid membrane polypeptides (lane 2) on a 7.5 to 15% polyacrylamide gradient gel (24). (B) Characterization of anti-11 IgG. Membrane samples were electrophoresed in the first dimension as in A and an unstained lane was electrophoresed at right angles into a 1.0% agarose gel (1), containing 0.5 mg/ml anti-11 IgG, which was then stained. A stained lane from the first dimension gel is shown in position for reference.
with the immunoprecipitated in vitro products of poly(A)-rich RNA translation (Fig. 5). The in vitro products (Fig. 5, lane 5) were of higher $M_r$ than the in vivo products (Fig. 5, lane 4). We could not detect the precursor forms among the immunoprecipitated polypeptides labeled in vivo (Fig. 5, lane 4). Thus processing of the precursors, which is thought to accompany transport into the chloroplast (7, 29-31), occurs rapidly after synthesis.

To confirm that the polypeptides, immunoprecipitated after in vitro translation, were precursors of the major polypeptides of thylakoid membranes, the experiment as described in Fig. 5 was repeated with antibodies prepared against polypeptide 11 (generously supplied by Dr. N.-H. Chua, The Rockefeller University). This antibody preparation provided the same results as antibodies against polypeptide 11 (results not shown).

Although three major polypeptides were resolved from Chlamydomonas thylakoid membranes (1-3), application of the same high resolution systems for electrophoresis of immunoprecipitated in vitro translation products succeeded only in separating two major components. The ratio of the radioactivity in these two in vitro synthesized polypeptides was similar to the ratio of the amounts of the $M_r$ 29,500 and 26,000 polypep-

**Figure 3** Immunological analysis of the products of in vitro translation of poly(A)-rich RNA prepared from etiolated and greening cells incubated at 25°C. RNA was translated in the presence of $^{35}$S]methionine, the products were precipitated with (NH$_4$)$_2$SO$_4$ and redissolved material was treated with anti-11 IgG as described in Materials and Methods. The figure presents a radioautogram after electrophoresis on a 10 to 20% polyacrylamide gradient gel (2) of the total (lane 1) and the corresponding immunoreactive polypeptides (lane 2) produced by translation of 0.2 μg of RNA from etiolated cells. Lanes 3 and 4 show the total translation pattern and immunoreactive polypeptides, respectively, obtained after translation of 0.2 μg of RNA from cells exposed to light for 3 h. Total $^{35}$S incorporated into protein was 1.93 × 10$^6$ cpm with RNA from etiolated cells and 1.96 × 10$^6$ cpm with RNA from greening cells. Equal portions of the samples were placed on the gel. The numbers ($\times 10^3$) on the left side of radioautograms in this and subsequent figures present the scale of apparent $M_r$ determined with a series of standard proteins (bovine serum albumin, $M_r$ 67,000; ovalbumin, $M_r$ 43,000; carbonic anhydrase, $M_r$ 30,000; soybean trypsin inhibitor, $M_r$ 21,000; $\beta$-lactoglobulin, $M_r$ 18,400, and globin, $M_r$ 16,000). The numbers ($\times 10^3$) on the right side of the figure indicate the apparent $M_r$ of the major immunoreactive polypeptides.

**Figure 4** Immunological analysis of the products of translation of poly(A)-rich RNA prepared from cells incubated at 38°C. Lanes 1-3 contained portions of the total products translated from 0.2 μg of RNA from either etiolated cells (lane 1) or from cells exposed to light for 15 (lane 2) and 60 min (lane 3). Total $^{35}$S incorporated into protein was 2.72 × 10$^6$, 2.75 × 10$^6$, and 2.80 × 10$^6$ cpm with RNA from etiolated, 15-min illuminated or 60-min illuminated cells, respectively. Equal portions of the samples were applied to the gel. Lanes 4-6 contained polypeptides immunoprecipitated with anti-11 IgG from the total translation mixtures of RNA from etiolated cells (lane 4), and from cells exposed to light for 15 (lane 5) and 60 min (lane 6). The $^{35}$S-labeled products were detected by radioautography after electrophoresis.

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shown. Only the central portion of a radioautogram of the gel is shown. Lane 1, total proteins of cells labeled in the dark; lane 2, immunoprecipitated polypeptides of cells labeled in the dark; lane 3, total protein from cells labeled in the light; lane 4, immunoprecipitated polypeptides of cells labeled in the light; lane 5, immunoprecipitated products of in vitro translation; and lane 6, total products of in vitro translation.

polypeptides in thylakoid membranes (2). Either the precursors to polypeptides 16 and 17 are the same size or polypeptide 17 may be a form of 16 that is modified posttranslationally (see reference 2).

Synthesis of Immunoreactive Polypeptides In Vivo

Although the mRNA was present in dark-grown cells at 38°C, no accumulation of the membrane polypeptides was detected (5, 10). It is possible, however, that the mRNA was translated under these conditions but the polypeptides were subsequently degraded. To test for in vivo translation of the mRNA, cells were incubated in the dark or light at 38°C and then pulse-labeled with [14C]acetate. Since thylakoid membranes are not formed in the dark, in these experiments immunoprecipitation of in vivo labeled products was carried out on total cellular protein to insure a proper comparison between cells labeled in the dark or light. The antibodies to polypeptide 11 reacted with two major components from illuminated cells (Fig. 5, lane 4), which corresponded in size to, and comigrated with, the major membrane polypeptides. The same polypeptides were immunoprecipitated from cells labeled in the dark, but lesser amounts were recovered (Fig. 5, lane 2). Therefore, the mRNA for these polypeptides was translated in the dark even though net accumulation did not occur.

The identity of the minor, M, 29,000 polypeptide, which was immunoprecipitated from cells labeled in vivo, is not known. Since it migrates during electrophoresis only slightly faster than polypeptide 11, it may be polypeptide 12, which could have been a contaminating antigen in the preparation used to raise antibodies. Its synthesis was not sensitive to chloramphenicol and thus this polypeptide was a product of cytoplasmic protein synthesis (see also reference 6).

Evidence for Turnover of Membrane Polypeptides in the Dark

The results of Fig. 5 demonstrated that the cells translated mRNA for the major thylakoid polypeptides in the dark at 38°C. However, since the polypeptides do not accumulate in the dark, they possibly are degraded. The kinetics of accumulation of thylakoid polypeptides in the dark and light at 38°C were examined over a 15-min labeling period with [14C]acetate.

A qualitative analysis of radioautograms after electrophoresis of total protein from these cells (Fig. 6) showed that by 2 min of light the M, 29,500 and 26,000 thylakoid polypeptides (labeled 11, 16, and 17) were the most prominent products of protein synthesis (Fig. 6, lane 4). Radioautograms were scanned to determine the kinetics of synthesis of these polypeptides relative to a series of other polypeptides chosen as internal reference components (marked with symbols in Fig. 6).

The label in the reference polypeptides increased at a linear rate over a 15-min period both when cells were incubated in the light and in the dark (Fig. 7A and B). (Extrapolation of the lines in Fig. 7 to the time axis suggested that ~1 min was required to fully label the amino acid pools with 14C acetate under these conditions.) In contrast, the label in polypeptides 11 and 16 + 17 increased linearly in the light but ceased to accumulate in these polypeptides in the dark after 5 min (Fig. 7C). This result indicated that in the dark the
Whether the changes in amounts of translatable mRNA observed in these experiments reflect changes in transcription cannot be determined from the data. Light or an increase in temperature also may affect the amount of translatable mRNA in these cells by altering rates of degradation of the mRNA or posttranscriptional reactions involved in processing of the mRNA. There is no evidence at present to discriminate between these possibilities, particularly in cells incubated at 38°C. Nevertheless, the higher temperature appears to result in the same effect as exposure to light. The mechanisms of the two responses, however, may be different.

Our results here show that the absence of net synthesis of the membrane polypeptides in the dark at 38°C observed previously (5, 10) was not because the mRNA was not translated, since in the dark the membrane polypeptides became labeled in vivo (Fig. 5). We conclude from these results that posttranslational processes determine the intracellular amounts of the polypeptides at 38°C. Since the immunoreactive polypeptides labeled in vivo were the same size as the mature membrane polypeptides, and the precursor forms of these polypeptides were not detected, it appears that the precursor polypeptides were rapidly transported into the chloroplast and processed (7, 29–31) in the dark. As the membrane polypeptides were synthesized in the dark, yet did not increase above a small, constant level, a proteolytic system apparently is present in the chloroplast capable of degrading them.

Because of the cross-reactivity of the antibodies we cannot conclude with certainty which polypeptide immunoprecipitated after in vitro translation is related to polypeptide 11 and which to polypeptides 16 and 17. However, since the in vitro translation products were larger than these membrane polypeptides, it seems clear that the immunoprecipitated products represent precursor proteins. This conclusion is consistent with the findings that other cytoplasmically synthesized chloroplast proteins, in particular, the small subunit of ribulose-1,5-bisphosphate carboxylase (30–32) and the chlorophyll a/b protein (29, 33–35), are synthesized as larger precursors. We suggest that the Mr 31,500 and 30,000 polypeptides are precursors of the Mr 29,500 and 26,000 membrane polypeptides, respectively. This would make the additional mass (the "transit sequence" [7]) in the precursor to polypeptide 11 and polypeptides 16 and 17 ~2,000 and 4,000 daltons, respectively. It is interesting to note that the transit sequence in the precursor of the carboxylase subunit is about 5,000 daltons (32).

The y-1 mutant strain of Chlamydomonas mimics higher plants in its dependence on light for formation of functional photosynthetic membranes. Illumination causes an increase in translatable mRNA for the polypeptides of the light-harvesting complex in higher plants (33–35) by a process that appears to involve phytochrome (36). However, in higher plants, as in Chlamydomonas at 38°C, accumulation of the polypeptide was not detected in vivo except under conditions that allowed synthesis of both chlorophyll a and chlorophyll b (35–37). Bennett (38) observed turnover of the chlorophyll a/b protein in the dark in pea leaves and suggested that chlorophyll synthesis is required to protect the protein from degradation.

In conclusion, our results demonstrate that in cells of C. reinhardtii y-1 an increase in temperature promoted accumulation of translatable mRNA for the major thylakoid polypeptides in the dark. Although the polypeptides were synthesized in the dark, they did not accumulate. Illumination of these cells resulted in a linear increase in the amount of the polypeptides, in parallel with the synthesis of chlorophyll (5). Formation of complexes with chlorophyll (9) possibly protects these polypeptides from degradation.

The authors thank Mr. H. Lee Tiffany for his skilled technical assistance in the preparation and analysis of the antibodies.
This work was supported by grant PCM-8007283 from the National Science Foundation. A preliminary account of the results was presented at the Twenty-first Annual Meeting of the American Society for Cell Biology in Anaheim, California.

Received for publication 7 April 1982, and in revised form 9 July 1982.

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