Regulation of Chloroplast-encoded Chlorophyll-binding Protein Translation during Higher Plant Chloroplast Biogenesis*

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Etioplasts of 5-day-old dark-grown barley seedlings synthesize most of the soluble and membrane proteins found in chloroplasts of illuminated plants. Prominent among these proteins are the large subunit of ribulose bisphosphate carboxylase and the \( \alpha \)- and \( \beta \)-subunits of the chloroplast ATPase. However, etioplasts do not synthesize four chloroplast-encoded proteins which are major constituents of the chloroplast thylakoid membrane: two chlorophyll apoproteins of photosystem I (68 and 65 kDa) and two chlorophyll apoproteins of photosystem II (47 and 43 kDa). Pulse-labeling experiments show that the lack of radiolabel accumulation in the chlorophyll apoproteins in etioplasts is due to inhibition of synthesis rather than apoprotein instability. Illumination of 5-day-old dark-grown barley selectively induces synthesis of the plastid-encoded chlorophyll apoproteins and proteins of 32, 23, and 21 kDa. Synthesis of the chlorophyll apoproteins was significant in plants illuminated for 15 min and was near maximum by 1 h. The induction of photosystem I chlorophyll apoprotein synthesis was not accompanied by an increase in mRNA for these proteins. These results demonstrate that the synthesis of the plastid-encoded photosystem I chlorophyll apoproteins is blocked at the translational level in dark-grown barley. Translation of the chlorophyll apoproteins is induced rapidly by light with a time course which is similar to the light-dependent formation of chlorophyll from protochlorophyllide.

During leaf formation chloroplasts develop from small undifferentiated organelles known as proplastids (1–3). In monocotyledons such as barley, leaf development and many steps in chloroplast maturation occur in the absence of light. These include increases in plastid number/cell (1, 4), increases in volume/plastid (1, 2, 5), synthesis of most stromal proteins (4, 6–9), and accumulation of thylakoid membrane proteins such as the ATPase subunits (7, 8, 10, 11), cytochrome \( f \) and cytochrome \( b_{5} \) (8, 11, 12). When dark-grown plants are illuminated, protochlorophyllide is reduced to chlorophyll, chlorophyll-protein complexes are synthesized and assembled, and photosynthetic electron transport is activated. The events involved in the transformation of etioplasts (plastids in dark-grown plants) to chloroplasts are controlled by at least two photoreceptors, protochlorophyllide and phytochrome (13).

Notably absent from etioplast membranes are the nuclear-encoded chlorophyll \( a/b \)-binding proteins \( 14 \). RNA coding for the chlorophyll \( a/b \)-binding proteins is low in dark-grown tissue, and transcript accumulation can be induced by activating the phytochrome system (15–18). Accumulation of the chlorophyll \( a/b \)-binding proteins may also involve protein stabilization by chlorophyll since in the absence of chlorophyll the apoproteins do not accumulate (15, 16).

In oats, bean, and spinach the plastid-encoded photosystem I (PSI) chlorophyll apoproteins were reported to accumulate in dark-grown plants (8). In contrast, these proteins did not accumulate in dark-grown barley (7). When dark-grown barley seedlings were illuminated, synthesis of PSI chlorophyll apoproteins was observed within 15 min. It was hypothesized that, as with the chlorophyll \( a/b \)-binding proteins, light-induced transcription of the genes coding for the PSI chlorophyll apoproteins and association with chlorophyll may be required for stabilization of the apoproteins in the thylakoid membrane (7). In maize, mRNA from the gene coding for the PSI chlorophyll apoproteins increased 2–4-fold upon illumination of dark-grown plants (19) consistent with the transcription activation hypothesis (20). However, in spinach a lack of correspondence between changes in mRNA levels and PSI chlorophyll apoprotein accumulation was reported (11).

In this paper we have examined the effect of light on the synthesis of the plastid-encoded chlorophyll-binding proteins during light-induced development. Our results demonstrate that etioplasts are translationally competent and synthesize most of the soluble and membrane proteins found in mature chloroplasts. Our results further indicate that light rapidly and specifically increased the synthesis of PSI chlorophyll apoproteins while transcript levels for the apoproteins did not increase.

MATERIALS AND METHODS

Plant Growth—Barley (Hordeum vulgare L. var. Morex) seedlings were grown and maintained for all experiments in controlled environment chambers at 23 °C. Seeds were planted in vermiculite and watered with full-strength Hoagland's nutrient solution. For developmental studies seeds were germinated and grown for 5 days in a dark chamber located in a light-tight room. At this stage of development seedlings were ~9–10 cm tall. After 5 days, seedlings were transferred to an illuminated chamber with a light intensity of 10,000 lux (fluorescent plus incandescent bulbs). All manipulations of dark-grown plants were performed when possible in complete darkness. However, when required, light was provided by a dim green safelight which was unable to photoconvert measureable amounts of protochlorophyll.

Plastid Isolation—Approximately 50 g of barley primary leaves were cut and immediately placed in iced water. After approximately 10 min, water was drained from the tissue and replaced with a solution

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1The abbreviations used are: PSI, photosystem I; PSII, photosystem II; LS, large subunit of ribulose bisphosphate carboxylase; SS, small subunit of ribulose bisphosphate carboxylase; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NaN_3SO_4, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
containing 2.1% (v/v) sodium hypochlorite, 0.25% (v/v) Tween 80, and 1.0% (w/v) NaCl. After 5 min, the sterilization fluid was drained and leaves thoroughly rinsed with 5–10 changes of ice-cold water. Leaves were ground in 300 ml of chilled grinding media containing 0.33 M sorbitol, 50 mM Hepes-KOH (pH 8.0), and 2 mM EDTA (pH 7.5 with NaOH). The homogenate was filtered through Miracloth and centrifuged at 2500 × g for 5 min, and gently resuspended in chilled sorbitol-Hepes (0.33 M and 50 mM, pH 8.0, with KOH). Generally, the preparation yielded 90–95% intact plastids. All manipulations were performed in a cold room (2–4 °C) in the presence of a dim safelight with wavelength emission of 515–580 nm.

**Protein Synthesis in Isolated Intact Etioplasts and Plastids from Illuminated Tissue**—Conditions for ATP-dependent protein synthesis by isolated intact chloroplasts have been previously reported (22). To find optimum incubation conditions for protein synthesis by isolated intact etioplasts, some parameters of the incubation media were investigated. Total [35S]methionine incorporation (membrane plus soluble) was greatest in 250 μM ATP, 5 mM MgCl₂ for etioplasts and 10 mM ATP, 10 mM MgCl₂, for chloroplast translation. Optimum translation conditions for other parameters (amino acid concentration, pH, K⁺ concentration) were similar for intact etioplasts and intact chloroplasts. The optimized protein synthesis mixture (75 μl, final volume) contained 0.33 M sorbitol, 50 mM Hepes-KOH (pH 8.0), 40 μM of each amino acid (minus methionine), 10 mM dithiothreitol, 25 μCi of [35S]methionine (specific activity, 1098 Ci/mmol), and 5 mM ATP, 5 mM MgCl₂. Intact plastids were added at a final concentration of approximately 1.25 × 10⁶ plastids/translational assay. The suspension was warmed to room temperature (23 °C) and incubated with gentle shaking in the light (10,000 lux, for plastids isolated from illuminated tissue) or in the dark (etioplasts from dark-grown tissue). Plastids were incubated for 30 min at which time unlabeled methionine (8.5 mM final concentration) was added to block further incorporation of [35S]methionine and to allow complete elongation of complete nascent polypeptides (the chase period was 30 min). Sorbitol-Hepes was added to terminate the reaction. In one experiment, plastids were pulse labeled for 5 min in the dark, and then sorbitol-Hepes was added to terminate the reaction.

Following the labeling period, plastids were fractionated into membrane and soluble polypeptides as previously described (22). Measurements of trichloroacetic acid-insoluble radioactivity were obtained (23), and samples were subsequently electrophoresed and autoradiographed (22).

**In Vivo Labeling**—Excised barley seedlings were labeled with [35S]methionine in the absence or presence of cycloheximide as previously described (22) but with several modifications. Briefly, barley leaves of 5-day-old dark-grown seedlings or leaves of 5-day-old dark-grown seedlings illuminated for 8 h were cut under water at the base of their stems and quickly placed in ice-cold water. Leaves were surface sterilized as described above but under water (the base of the leaves, and quickly immersed in 300 μl of 40 mM Hepes-KOH (pH 8.0) in shortened nitricelulose ivals (10 leaves/vial). When appropriate, cycloheximide was added to a final concentration of 20 μg/ml. Seedlings were preincubated for 20 min at 23 °C with the evaporative demand increased by cool air from a hair drier. After 20 min, 300 μCi of [35S]methionine was added to each vial, and leaves were allowed to incorporate radioactivity under the same conditions for an additional 3 h. When solutions became low during this period, 40 mM Hepes-KOH (plus cycloheximide when appropriate) was added to each vial. Following incubation, leaves were placed in chilled water and subsequently macerated in 6 ml of grinding media with a razor blade. The suspension was filtered through a 20-μm nylon mesh filter, layered directly onto Percoll gradients, and intact plastids were isolated and fractionated as described above.

**Determination of Plastid Number and Volume**—For quantitation of plastid number per μl of suspension volume (μl) aliquots of isolated plastids were diluted and plasts counted in a hemocytometer with a × 20 phase contrast lens on a Nikon photomicroscope. Plastid dimensions (length and width) of approximately 50 plastids/treatment were measured on photographic prints of negatives enlarged approximately 3000 times magnification on a Sony 4000. Plastid sections with the largest values for length and width were recorded since the longest value for each dimension should represent the median plane section of the organelle and thus give an accurate approximation of the dimensions of the plastid (5). Measurements of plastid dimensions obtained in this manner are in close agreement with previously reported estimates of length and width of barley plastids (1). Based on the observation that plastids of dark-grown barley (5–7 day-old) are ellipsoidal (6) and that plastids do not change shape during the early phase of illumination, we have estimated the volume of each plastid by the volume of rotation equation for an ellipse:

\[
\text{volume} = \frac{\pi}{3} L W
\]

where \(L\) is the long axis of ellipsoid and \(W\) is the short axis of ellipsoid.

**Isolation and Quantitation of Plastid rRNA and mRNA**—Pea chloroplast RNA was isolated by phenol extraction as previously described (24). To ensure high yield and reproducibility the phenol phase of each extract was re-extracted twice. Variation of nucleic acid recovery/plastid volume was less than 5% (based on 2 extractions done in triplicate). Detection of RNA was done by separating total nucleic acid isolated from a known plastid volume (approximately 0.5 μg/lane) on methyl mercury gels (25). Separated RNA was detected by ethidium bromide staining. A portion of the open reading frames of psaA and psbA (28). The Northern probe for psbA was a 0.55-kbp EcoRI-PstI internal DNA fragment of pea psbA (29).

**RESULTS**

**Tissue Selection**—The growth of the barley primary leaf is only marginally different for the first 5 days when dark-grown plants are compared to light-grown plants. Since the leaves of grasses have basal meristems, the various cells of the leaf represent an ontogenetic sequence with the most mature cells situated at the apex and the youngest cells at the base at any point in time (3, 4). In a 5-day-old dark-grown barley seedling, plastids in the basal part of the primary leaf are typical proplastids and contain almost no membranes (1). Plastids in more mature regions (5th cm from the base to the leaf tip) are larger, more numerous per cell, and have a well defined prolamellar body (1). Mature fully developed etioplasts require a relatively brief period of illumination to be transformed into mature functional chloroplasts while the undifferentiated proplastids (basal leaf sections) require a much longer period of illumination to develop into a mature chloroplast (1). This is because proplastids have not already synthesized the membranes, pigments, and enzymes that are present in mature etioplasts. Based on these facts we decided to concentrate investigations on the 4-cm apical section of 5-day-old dark-grown barley leaves whose plastids represent near fully developed etioplasts.

**Protein Synthesis in Vivo in Plastids of Dark-grown and Illuminated Plants**—It has been suggested by Siddell and Ellis (6) that plastids at different stages of development may synthesize a different spectrum of proteins than those from mature chloroplasts. Based on this and the observation that chlorophyll-binding proteins of PSI did not accumulate in dark-grown barley (7), we characterized the patterns of protein synthesis of plastids from dark-grown and illuminated plants. To do this [35S]methionine was used to label plastid proteins in vivo in the presence or absence of cycloheximide, an inhibitor of cytoplasmic protein synthesis. After the labeling period, plastids were isolated from the apical 4 cm of barley leaves, and radiolabeled polypeptides were examined.
by autoradiography of NaDodSO₄ gels (Fig. 1). This experiment indicated that major light-induced changes in the synthesis of plastid polypeptides occur in the membrane fraction. Both the large subunit (LS) and small subunit (SS) of ribulose bisphosphate carboxylase were synthesized in mature etioplasts and in plastids from illuminated seedlings (Fig. 1, lanes 5–6). As expected, cycloheximide inhibited synthesis of SS and most of the soluble plastid polypeptides but did not inhibit synthesis of LS and two proteins which comigrated with the α- and β-subunits of the chloroplast ATPase (Fig. 1, lanes 7–8). It was noted that illumination of dark-grown barley altered the synthesis of only a few nuclear-encoded soluble proteins (Fig. 1, lanes 5 versus 6). In contrast, numerous differences were observed when radiolabeled membrane proteins from dark-grown plants were compared to illuminated plants (Fig. 1, lane 1 versus 2 or 3 versus 4). Of the nuclear-encoded membrane polypeptides (synthesis inhibited by cycloheximide), light induced the synthesis of chlorophyll a/b-binding proteins (i.e., LHCII) and inhibited the synthesis of several unidentified high molecular weight (>70 kDa) polypeptides of etioplast membranes (Fig. 1, lane 1 versus 2). Of the plastid-encoded membrane polypeptides (synthesized in the presence of cycloheximide), etioplasts synthesize the ω- and β-subunits of the ATPase, a low level of a 32-kDa polypeptide (possibly the gene product of psbA), and several lower molecular weight polypeptides (Fig. 1, lane 3). Etioplasts, in general, synthesized a nearly complete set of chloroplast-encoded polypeptides with the exception of the chlorophyll-binding proteins of PSI and PSII (Fig. 1, lane 3 versus 4). Net synthesis of the chlorophyll apoproteins of PSI and PSII was not obtained until seedlings were illuminated. In addition, the relative accumulation of label in a 32-kDa protein increased significantly in illuminated plants (Fig. 1, lane 3 versus 4). These results indicated that while etioplasts are translationally active and are capable of synthesizing a nearly complete set of plastid-encoded polypeptides, light selectively stimulated the synthesis of a limited number of membrane polypeptides which included chlorophyll apoproteins of PSI and PSII.

The results in Fig. 1 indicate that the expression of plastid-encoded chlorophyll-binding proteins was tightly coupled to early events of light-induced plastid development. However, it was unclear whether expression was regulated at the transcriptional or post-transcriptional level. Post-transcriptional regulation could involve apoprotein or mRNA stability or inhibition of apoprotein mRNA translation. Unfortunately, complications associated with in vivo labeling methods concerning the secondary effects of plant excision, inhibitor application, and plant-to-plant variation in label uptake make quantitative studies of plastid gene expression difficult. Therefore, to examine the regulation of chlorophyll apoprotein synthesis in a more quantitative way, we examined protein synthesis, protein turnover, and RNA levels in plastids isolated from dark-grown and illuminated seedlings.

**Changes in Plastid Size**—Light-induced changes in the size of plastids from the primary leaf of barley were determined during the first 16 h of illumination (Table I). A 31% increase in plastid volume occurred when 5-day-old dark-grown barley seedlings were illuminated for 16 h. Other workers have also noted significant increases in plastid size and plastid number/cell when dark-grown tissue was illuminated (1, 30–32). Therefore, to account for the observed increase in plastid volume during light-induced development, data obtained with isolated plastids (protein synthesis, RNA content, protein composition and turnover) was expressed on an equal plastid volume basis. Data expressed in this way separate plastid events from other cellular changes yet allow expression on a per cell basis using published values for plastid number/cell (3, 4, 33, 34).

**Polypeptide Composition, rRNA Content, and Protein Synthesis of Plastids Isolated during Light-induced Development**—The time course of polypeptide accumulation during light-induced development is represented in Fig. 2. When expressed on an equal plastid volume basis, light-induced changes in

<p>| Table I |
|------------------------|------------------------|------------------------|------------------------|
| <strong>Average plastid length, width, and estimated plastid volume from 5-day-old dark-grown and illuminated seedlings</strong> |</p>
<table>
<thead>
<tr>
<th>Hours of illumination</th>
<th>Plastid length (µm)</th>
<th>Plastid width (µm)</th>
<th>Plastid volume (µl x 10^-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.5 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td>1</td>
<td>6.4 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>10.3 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>6.8 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>12.8 ± 0.4</td>
</tr>
<tr>
<td>16</td>
<td>7.1 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>14.0 ± 0.5</td>
</tr>
</tbody>
</table>

Five-day-old dark-grown barley seedlings were transferred to a lighted chamber and plastids isolated after 0, 1, 4, or 16 h. Dimensions (length and width) of approximately 50 plastids/treatment were measured on photographic prints of plastids at a final magnification of 10,000 times. Results are expressed as mean ± S.E.
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11141

Membrane

Soluble

1 2 3 4 5 6 7 8

200. 974. 68. 43. 25.7 184. 143.

Time illuminated, hr

Fig. 2. Accumulation of plastid polypeptides during the first 16-h illumination of barley seedlings. Five-day-old dark-grown barley seedlings were transferred to a lighted chamber and plastids isolated after 0, 1, 4, or 16 h. Plastid concentrations (plastids/μl of solution) and plastid volumes (μl/plastid) were estimated as described under "Materials and Methods." Plastids were fractionated into membrane and soluble polypeptides and loaded onto NaDodSO4-PAGE gels on an equal plastid volume basis. Polyacrylamide gels were fixed and silver stained. Numbers to the left indicate mobility of Mr standards (kDa). PchIrd marks the position of the protochlorophyllide reductase protein. CF1 and LHCII are defined in the legend to Fig. 1.

stainable levels of polypeptides were restricted to membrane proteins (Fig. 2, lanes 1–4). Little change in stainable levels of soluble polypeptides (including LS and SS of ribulose bisphosphate carboxylase) were observed during greening (Fig. 2, lanes 5–8). This is in agreement with reports that light is not required for the accumulation of ribulose bisphosphate carboxylase in barley (4, 7, 35). In contrast, after 16 h of illumination marked accumulation of nuclear encoded chlorophyll a/b-binding proteins (LHCII) and polypeptides in the region of the chlorophyll-binding proteins of PSI and PSII was observed (Fig. 2, lanes 1–4). A decrease in the level of a 36-kDa polypeptide, tentatively identified as protochlorophyllide reductase, was also observed. Little change was observed in the stainable levels of the α- and β-subunits of the ATPase. This experiment established the time frame during which major light-induced changes in the protein composition of the thylakoid membrane occur.

The protein synthesis activity of intact plastids isolated from plants illuminated for 0–16 h is shown in Fig. 3. A slight increase in the activity of soluble polypeptide synthesis by isolated plastids was observed during the first hour of illumination followed by a decrease in synthesis during the next 3 hours of illumination. In agreement with previous reports (6, 22), the LS of ribulose bisphosphate carboxylase was the major soluble product synthesized by isolated etioplasts and illuminated plastids. In contrast, a 3–4-fold increase in synthesis of membrane polypeptides was observed within 1 hour after onset of illumination (Fig. 3). Thereafter, a gradual decline in synthesis of membrane polypeptides was observed.

The increase in membrane protein synthesis activity during light-induced plastid development could be due to an increase in chloroplast ribosome number. To examine this possibility nucleic acid was isolated from plastids of dark-grown and illuminated seedlings. The nucleic acid was treated with DNase I, and the RNA content/plastid was determined after phenol extraction and precipitation (Table II). This analysis

Fig. 3. Quantitation of [35S]methionine incorporation into soluble and membrane polypeptides by isolated plastids. Plasts were isolated from seedlings after 0, 1, 4, or 16 h of illumination and incubated with [35S]methionine in optimized protein synthesis mixtures for 30 min. After 30 min, excess unlabeled methionine (8.5 mM) was added to each mixture and incubated for 30 min. Plasts were subsequently fractionated into membrane and soluble polypeptides, and 1-μl aliquots were processed for measurement of trichloroacetic acid-insoluble radioactivity. Plastid concentrations (plastids/μl of solution) and plastid volumes (μl/plastid) were estimated and incorporation of label expressed on a equal plastid volume basis.
Regulation of Chlorophyll-binding Protein Synthesis

Table II
RNA content of plastids from 5-day-old dark-grown and illuminated barley seedlings

<table>
<thead>
<tr>
<th>Hours of illumination</th>
<th>RNA/plastid</th>
<th>RNA/plastid volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1266</td>
<td>5.9</td>
</tr>
<tr>
<td>0.25</td>
<td>1224</td>
<td>5.7</td>
</tr>
<tr>
<td>0.5</td>
<td>1200</td>
<td>5.6</td>
</tr>
<tr>
<td>1.0</td>
<td>1224</td>
<td>5.7</td>
</tr>
<tr>
<td>16.0</td>
<td>1158</td>
<td>4.1</td>
</tr>
</tbody>
</table>

showed that the total RNA content/plastid remained constant during the first hour of seedling illumination with a small decrease in RNA content apparent after 16 hours of illumination. Examination of plastid nucleic acid on methyl mercury agarose gels revealed that rRNA (marked by arrows) in mature seedlings were transferred to a lighted chamber, and plastids isolated after 0, 1, 4, 13, and 24 h. Plastid concentrations (plastids/µl) and plastid volumes (µl/plastid) were determined, and plastid RNA was isolated by phenol extractions. Ribosomal RNA isolated from an equal volume of plastids was loaded on methyl mercury gels (approximately 0.5 µg/lane), and separated RNA was detected by ethidium bromide staining. Arrows mark ribosomal RNA.

To determine whether the increase in synthesis of membrane polypeptides during illumination results from a general increase in membrane polypeptide synthesis or from increased synthesis of selected light-induced polypeptides, the pattern of proteins synthesized during the first 16 h of illumination was examined (Fig. 5). The profile of membrane polypeptides synthesized by isolated intact etioplasts was very similar to that obtained in vivo plus cycloheximide (Fig. 1, lane 3 versus Fig. 5, lane 1). No net synthesis of either PSI or PSII chlorophyll apoproteins was observed in plastids isolated from dark-grown plants. After 15 min of illumination, however, synthesis of either PSI and PSII apoproteins was observed, and synthesis increased to an apparent maximum at 1 hour. No further qualitative changes in polypeptide synthesis were observed out to 16 h of tissue illumination. These results demonstrate that while etioplasts do not synthesize the chlorophyll apoproteins of PSI and PSII, the light-regulated induction of apoprotein synthesis occurs within 15 min of illumination. It was also noted that induction of chlorophyll apoprotein synthesis was selective. Light did not induce a general increase in the synthesis of all membrane polypeptides. Rather, except for the chlorophyll apoproteins and proteins of 32, 23, and 21 kDa, light did not alter the synthesis of membrane polypeptides.

The results in Fig. 5 verified the changes in protein synthesis observed in vivo and provided a more quantitative analysis of the time course and magnitude of the changes in protein synthesis which occur when dark-grown plants are illuminated. However, it was not clear whether the lack of detectable label in chlorophyll apoproteins is due to the inability of etioplasts to synthesize the chlorophyll apoproteins or to rapid turnover of newly synthesized apoproteins. To determine which of these mechanisms accounts for the lack of detectable label in chlorophyll apoproteins, isolated plastids from dark-grown plants were pulse-labeled to detect transient polypeptides synthesized within etioplasts (Fig. 6, lanes 1 and 4). During a 5-min pulse label etioplasts synthesized measurable levels of full length polypeptides along with incomplete nascent polypeptides (marked by open arrows). No PSI or PSII chlorophyll apoproteins were detected in either the membrane or soluble phase of pulse-labeled etioplasts (radiolabeled polypeptides of isolated chloroplasts are shown in lanes 3 and 6 for comparison). When pulse-labeled etioplasts were chased with unlabeled methionine, incomplete nascent polypeptides (marked by open arrows) read out to mature full length polypeptides (22), yet again the chlorophyll apoproteins were not radiolabeled (Fig. 6, lanes 2 and 5). These results, therefore, demonstrate that the lack of radiolabel in chlorophyll
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FIG. 6. Pulse-chase labeling of etioplast polypeptides. Five-day-old dark-grown barley seedlings were transferred to illuminated chambers and plastids isolated after 0 or 1 h. Etioplast polypeptides were pulse-labeled with [35S]methionine for 5 min and then chased for 0 (lanes 1 and 4) or 30 min (lanes 2 and 5) with excess unlabeled methionine. Plastids from 1-h illuminated seedlings were also pulse-labeled for 5 min and chased for 30 min (lanes 3 and 6) with unlabeled methionine. Reactions were terminated by the addition of 0.375 ml of chilled sorbitol-Hepes to reaction vessels. Plastids were fractionated and protein samples loaded on NaDodSO4-PAGE gels on an equal cpnm basis. Gels were fixed, fluorographed, and exposed to x-ray film for 1 week. CFI and D are defined in the legend to Fig. 1.

apoproteins is not due to chlorophyll apoprotein turnover but rather to their lack of synthesis in etioplasts.

Transcript Levels in Plastids of Dark-grown and Illuminated Barley Plants—It has previously been reported that two mRNAs (1.8 and 1.6 kbp), differing at their 5'-ends, are formed from barley rbcL (the gene coding for ribulose bisphosphate carboxylase LS (36)). The shorter of the rbcL transcripts predominates in etioplasts of 7-day-old dark-grown barley, and the relative amount of the larger transcript increases upon illumination of these plants (36). The shorter of the rbcL transcripts predominates in etioplasts of 7-day-old dark-grown barley, and the relative amount of the larger transcript increases upon illumination of these plants (36). Our results show that LS synthesis is active in etioplasts of 5-day-old dark-grown barley, and illumination of the plants for 1 h has little effect on LS synthesis (Fig. 5). Northern blots were done to determine if the level of rbcL mRNA or ratio of the two transcripts changed in response to illumination. Fig. 7 shows that both rbcL transcripts are present in the 5-day-old dark-grown barley plastids used in this study. In addition, illumination of plants for 1 h does not alter the concentration or the ratio of the two rbcL transcripts (Fig. 7, lanes 1 and 2).

In contrast to LS, the PSI chlorophyll apoproteins and a 32-kDa protein show a dramatic light-induced increase in synthesis (Figs. 1, 5, and 6). The light-induced increase could be a consequence of activated transcription or translation of mRNA. To address this question the mRNA levels for the PSI chlorophyll apoproteins and a 32-kDa protein (psbA gene product) were determined in etioplasts and in plastids isolated from illuminated plants (Fig. 7). The genes for the PSI chlorophyll apoproteins (psaA-psaB) and the 32-kDa protein (psbA) have not been mapped on the barley chloroplast genome. Therefore, for psbA we used an internal 5'-end probe from peas to detect barley mRNA in the two plastid populations. This probe hybridized to an RNA of a size similar to that found in peas (1.3 kbp) (Fig. 7, lanes 3 and 4). This analysis also showed that psbA transcript levels did not increase during the first hour of plant illumination. During this same period a significant increase in synthesis of a 32-kDa protein comigrating with the psbA gene product was observed. To analyze psaA and psbB mRNA levels we used an internal psaA probe from spinach (KpnI-BamHI) and a probe which contained portions of both psaA and psbB (BamHI-BamHI) (28, 37). These probes as well as a psaB-specific probe hybridized to a barley chloroplast RNA of approximately 5.8 kbp (data not shown). This suggests that psaA and psbB are cotranscribed in barley as has been found in spinach (28) and peas (37). The spinach psaA-psaB probe was used to analyze RNAs extracted from etioplasts and plastids isolated from illuminated plants. This analysis showed that barley psaA-psaB transcript levels did not increase during the first hour of illumination of dark-grown barley (Fig. 7, lanes 5 and 6). During this same period PSI chlorophyll apoprotein synthesis went from undetectable to near maximum levels.

DISCUSSION

Etioplasts in 5-day-old dark-grown barley synthesize most of the soluble and membrane-bound polypeptides found in

FIG. 7. Northern blot of rbcL, psbA, and psaA-psaB mRNA. RNA was isolated from an equal volume of plastids isolated from plants grown in the dark (lanes 1, 3, 5) or from plants illuminated for 1 h (lanes 2, 4, 6). The RNA was separated on glyoxal gels, transferred to nylon membranes, and hybridized with nick-translated DNA from rbcL (lanes 1, 2), psbA (lanes 3, 4) or psaA-psaB (lanes 5, 6). RbcL encodes LS, psbA encodes a 32-kDa quinone-binding protein, and psaA-psbB encode chlorophyll apoproteins of PSI.
chloroplasts (Figs. 1 and 5). Prominent among these proteins are the LS of ribulose bisphosphate carboxylase and the α- and β-subunits of the chloroplast ATPase. Illumination of dark-grown barley does not significantly alter the synthesis of most plastid-encoded proteins but selectively enhances the synthesis of 7 thylakoid polypeptides: two PSI proteins of 68 and 65 kDa which bind chlorophyll α (7, 38), two PSII proteins of 47 and 43 kDa which bind chlorophyll α (39–43), a 32-kDa protein which comigrates with the psbA gene product, and polypeptides of 23 and 21 kDa. It has recently been suggested that the psbA gene product is a PSI reaction center protein and also binds chlorophyll (44). The induction of the synthesis of these proteins occurred within 15 min and was near maximum by 1 hour of illumination.

The present results and those of Vierling and Alberte (7) indicate that the synthesis of PSI and PSII chlorophyll apoproteins and a 32-kDa polypeptide is tightly coupled to an early event of light-induced plastid development. One such early event is the light-dependent conversion of protochlorophyllide to chlorophyllide with subsequent conversion to chlorophyll (45). In 5-day-old dark-grown barley the formation of chlorophyll from protochlorophyllide was 90% complete after 15 min of illumination (7). This time course agrees closely with the induction of apoprotein synthesis which suggests that formation of chlorophyll α may be a key regulatory point for chlorophyll apoprotein synthesis. A tight coupling between chlorophyll formation and chlorophyll apoprotein synthesis provides several advantages to the plant. First, the build-up of a pool of chlorophyll precursors (protochlorophyllide) and chlorophyll apoprotein transcripts in dark-grown plants allows rapid synthesis and assembly of PSI complexes once plants are illuminated. This is consistent with reports of rapid activation of PSI activity upon illumination of etiolated plants (40, 46). Second, it is known that chlorophyll which is not associated with its normal protein-carotenoid complex can photooxidize membranes (47). Therefore, lack of coordination between chlorophyll synthesis and chlorophyll apoprotein synthesis could have deleterious consequences.

We also note that these early light-induced events precede the major increase in chlorophyll and chlorophyll α/β apoprotein accumulation which occurs between 2 and 16 hours of illumination (7, 48) see Fig. 2). These later light-induced events are influenced by phytochrome (13).

Investigation of transcript levels showed that transcripts for the PSI chlorophyll apoproteins were present in etioplasts and that their levels did not increase during the first hour of illumination. Therefore, light rapidly and specifically increased the synthesis of these polypeptides at a time when transcript levels did not change indicating that synthesis is regulated at the post-transcriptional level. Pulse-labeling experiments clearly show that the failure to radiolabel PSI chlorophyll apoproteins in etioplasts was not due to protein turnover but rather due to the lack of synthesis of the apoproteins. These results show that, at the stage of seedling development investigated here, a marked effect of light upon plastid protein synthesis is exerted at the translational level.

In addition to activating PSI apoprotein synthesis, light also caused increased synthesis of two PSII chlorophyll apoproteins (products of the psbB and psbC genes), a 32-kDa protein which comigrates with the psbA gene product and proteins of 23 and 21 kDa. RNA from the psbC gene is present in barley etioplasts as part of a polycistronic RNA which suggests that this PSI apoprotein is also under translational control. The same is true for psbA; however, further analysis is needed to test whether the 32-kDa polypeptide which shows light-induced synthesis is a product of the psbA gene. Finally, the 23- and 21-kDa polypeptides which show light-induced synthesis are the products of unknown genes.

Previous reports have shown that qualitative and quantitative changes of plastid-encoded polypeptides during light-induced development correlated with corresponding alterations in the abundance of specific transcripts (19). This led to the idea that light-dependent increases in transcription played a central role in light-induced chloroplast development. The genes whose transcript levels were reported to increase upon illumination included psaA-psbB, psbA, and genes for subunit III and the α-subunit of the chloroplast ATPase (19). However, Herrmann et al. (11) and Altman et al. (49) have shown in spinach and maize that plastid-encoded genes are not shut off in etiolated tissues. When expressed on a total cellular RNA basis, illumination of spinach leaves increased the steady state mRNA concentrations of all plastid-encoded genes, not a selective set of photogenes (11). Even then, there was a lack of correspondence between changes in mRNA levels and polypeptide accumulation. Our results, in contrast to those of Rodermel and Bogorad (19), show that changes in synthesis of the chlorophyll apoproteins of PSI and a 32-kDa polypeptide occur rapidly in response to light and that these changes precede large changes in steady-state mRNA levels. These conflicting results may be resolved by the fact that plants of different developmental stages had been used in these experiments. We have observed changes in transcript levels of plastid-encoded genes when older (7–10-day-old) etiolated tissue is illuminated. However, even though changes in transcript levels of plastid-encoded genes were observed in the older tissue, light-induced control of gene expression also appears to be exerted at the translational level. Further, reported observations of light-dependent regulation of transcript levels and the reported low level of plastid-encoded transcripts in etiolated tissue may, in part, be related to the manner of data expression. When expressed on a whole tissue basis (mg of fresh weight, mg of total protein, leaf area basis, or total cellular RNA basis), the influence of light on plastid gene expression is confounded by effects of light on leaf development and associated increases in plastid number/cell and increased volume/plastid.

Finally we should note that the selective regulation of chlorophyll apoprotein translation is an example of a growing list of plastid events which are regulated at the level of translation. These include regulation of gene expression during Euglena plastid biogenesis (50), regulation of rbcL translation in Amaranth cotyledons (9) and in pea buds (51), and synthesis of the psbA gene product in mature Spirodela plants (52). These examples suggest that translational regulation may be important for coordination of nuclear and plastid gene expression and the coordination of chromophore and cofactor biosynthesis during chloroplast biogenesis.

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