Plant Physiol. (1991) 97, 335–342
0032-0889/91/97/0335/08/$01.00/0

Received for publication February 13, 1991
Accepted May 8, 1991

Regulation of Light-Induced Chloroplast Transcription and Translation in Eight-Day-Old Dark-Grown Barley Seedlings[1]

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ABSTRACT

Plastid transcription and translation are light-activated in 8-day-old dark-grown barley (*Hordeum vulgare* L.) seedlings. Pretreatment of dark-grown seedlings with cycloheximide (inhibitor of cytoplasmic protein synthesis) abolished the activation of rbcL, psbA, and psaA-B transcription by light. In contrast, inhibition of plastid protein synthesis by chloramphenicol stimulated light-activated transcription of rbcL, psbA, and psaA-B. Light-induced transcription of the plastid genome occurred normally in the chlorophyll-deficient mutant xan-J^{64}. These results suggest that although the light-induced activation of plastid transcription is modulated by cytoplasmic and organellar protein synthesis, transcriptional activation is not dependent on the absorption of light by protochlorophyllide or the attainment of photosynthetic competence. In addition, plastid translation increased dramatically when 8-day-old dark-grown seedlings were illuminated and activation was dependent on cytoplasmic protein synthesis. Blockage of light-activated plastid transcription by Tagetin treatment (inhibitor of plastid RNA polymerase) did not attenuate the activation of plastid translation by light. These results suggest that while light simultaneously activates plastid transcription and translation, the rapid burst in plastid protein synthesis is due mainly to cytoplasmic-derived changes that regulate the rate of translation of pre-existing mRNAs.

The development of the chloroplast and the expression of its genome are coordinated with leaf formation and controlled, in part, by environmental signals including light (for reviews, see refs. 22 and 32). During barley leaf formation, the number of plastids in developing mesophyll cells increases from 10 to 65, and plastid DNA copy number increases five- to 10-fold/cell (22). Plastid transcription activity also increases during the early stages of mesophyll cell development (22). The induction of plastid gene expression is coordinated with the expression of a set of nuclear genes that leads to the rapid accumulation of the photosynthetic apparatus and, in illuminated plants, to the formation of mature chloroplasts.

Although expression of the nuclear and plastid genomes is coordinated, the mechanisms controlling gene expression of nuclear and plastid-encoded subunits appear quite different. The expression of nuclear genes encoding plastid proteins appears to be regulated primarily, but not exclusively, at the transcriptional level (for reviews, see refs. 6, 14, and 32). Expression of Cab and rbcS is regulated by light and tissue-specific factors largely at the level of transcription initiation (6, 14). Both Cab and rbcS have been shown to be regulated in a positive manner by light acting through phytochrome and a second blue-light photoreceptor (14). In contrast to the expression of Cab and rbcS, recent models have emphasized post-transcriptional mechanisms of light-dependent expression of chloroplast genes (7, 8, 22, 23). However, the mechanisms by which light regulates chloroplast genes and the extent of light control depends on the plant species, leaf type (cotyledon *versus* primary leaf), and the developmental stage of the tissue. For example, in young developing barley leaves, the accumulation of the plastid-encoded Chl *a*-apoproteins is strictly light-dependent and is controlled at a post-transcriptional level (23). Overall plastid transcriptional activity is near maximum and largely light-independent in these tissues (13). In older dark-grown barley, additional levels of regulation are superimposed on the post-translational stabilization of the Chl apoproteins. As barley plastids mature in the dark, chloroplast transcription and translation decline precipitously (12, 13). At this stage of plastid development, transcription becomes light-dependent exhibiting a four- to fivefold stimulation upon illumination. This increase in transcriptional activity of chloroplast genes is associated with the differential accumulation of plastid mRNA (13) and a concomitant burst in translational activity (4). At present, the molecular mechanisms governing the activation of plastid transcription are not clear, though a light-dependent change in the protein composition of the plastid RNA polymerase has been proposed (19). Furthermore, it has not been clearly demonstrated that the reactivation of plastid protein synthesis in older dark-grown seedlings is dependent on *de novo* transcription of plastid photogenes or whether the reactivation of protein synthesis is controlled at a post-transcriptional level.

In the present study, I examined the molecular mechanisms controlling the activation of plastid transcription in 8-d-old barley seedlings upon illumination. It was observed that the light-dependent transcription of the plastid genome was dependent on *de novo* cytoplasmic protein synthesis. Evidence was also obtained with inhibitors of plastid translation implicating possible negative regulation of light-activated plastid transcription by a plastid-encoded factor. Chl-deficient mutants were also analyzed to determine whether the signal for light-activated plastid transcription requires photosynthesis or the biosynthesis of Chl. The reactivation of plastid protein

[1] The investigation reported in this paper (No. 90–3–184) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

synthesis in 8-d-old barley seedlings was also examined and a role for cytoplasmic protein synthesis in this process was indicated. Finally, although light simultaneously reactivates plastid transcription and translation, the present results demonstrate that the reactivation of plastid protein synthesis does not depend on *de novo* plastid transcription, but rather utilizes mRNA transcribed before illumination.

MATERIALS AND METHODS

Plant Growth and Mutant Identification

Barley (*Hordeum vulgare* L. var Morex) seedlings were planted and grown as described previously (10, 13). Seedlings were grown and maintained in a dark chamber in a light-tight room. After 7.5 to 8 d in complete darkness, seedlings were either maintained in complete darkness or were illuminated as described below.

Seedlings of the barley mutant xan-J^{64} were planted as above and were maintained in complete darkness for 8.5 d and then transferred to an illuminated chamber (300 $\mu E \cdot m^{-2} \cdot s^{-1}$, fluorescent plus incandescent bulbs). After 14 h of illumination, mutants were identified (Chl-deficient, yellow) and harvested, and transcription activity of isolated plastids was examined (see below).

All manipulations of seedlings before light treatments were conducted in complete darkness or with light provided by a dim green safelight as described previously (10).

In Vivo Inhibitor Treatment

Conditions for *in vivo* uptake of inhibitors of protein synthesis were as described previously (11, 24). Approximately 100 leaves from dark-grown seedlings (upper 3 cm) were cut and immediately immersed in water and recut at the base of the leaf. Leaves were quickly placed in small vials containing either 40 mM Hepes-KOH (pH 8.0, control), or Hepes buffer plus CHI[2] (20 μg/mL) or chloramphenicol (100 μg/mL). Seedlings were incubated in complete darkness for 40 min with the evaporative demand increased by blowing cool air from a hair drier. After 40 min, seedlings were either maintained in complete darkness (dark control) or transferred to an illuminated chamber (300 $\mu E \cdot m^{-2} \cdot s^{-1}$ white light). After incubation, intact plastids were isolated and transcriptional or translational activity examined as described below.

In one experiment, CHI was added after seedlings were illuminated. In this case, seedlings were transferred to light for 5 h, then excised and placed in vials containing Hepes buffer or buffer plus CHI as described above. After various times of incubation, intact plastids were isolated and transcriptional activity examined. In an additional experiment, seedlings were treated with Tagetin[3] RNA polymerase inhibitor (Epicentre Technologies, Madison WI) before illumination. In this experiment, leaves from 8-d-old dark-grown seedlings were excised and placed in a solution of buffer plus Tagetin (800 units/mL). After approximately 7 h of dark incubation, leaves were transferred to the light for 7 h. Plastids were then isolated and translational activity examined as described below. Further details of inhibitor treatments are given in individual figure and table legends.

Plastid Isolation and Plastid Number

Plastids were isolated from the apical 3-cm section of barley leaves by Percoll gradient purification (24). Plastids were quantitated using a hemocytometer and a microscope equipped with a 20× phase-contrast lens.

Transcription in Lysed Plastids and Filter Hybridization of α^{32}P-Labeled Transcripts

Transcription in lysed plastids of barley was assayed as described (13). Standard transcription assays were carried out for 5 to 10 min. After this labeling period, the incorporation of [α^{32}P]UTP was determined, and α^{32}P-labeled transcripts were phenol-extracted (13). Unincorporated [α^{32}P]UTP was removed by passing phenol-extracted samples over a spun column of Sephadex G-50–80 resin. Transcripts were precipitated with ethanol overnight (−85°C).

Hybridization of α^{32}P-labeled transcripts to filter-bound DNA specific probes was as described (13). Duplicate aliquots of linearized Bluescript plasmid (Stratagene, La Jolla, CA) containing inserts of rbcL (1 pmol/aliquot), psbA (1 pmol/aliquot), and psaA-B (1 pmol/aliquot) were filter-bound to GeneScreen Plus nylon membranes (DuPont). After hybridization, blots were washed (13), dried, and exposed to x-ray film. Subsequently, individual dots were excised from the blot and counted by liquid scintillation. Estimates of the percent hybridization of ^{32}P-labeled transcripts of rbcL, psbA, and psaA-B were obtained (13). Incorporation of UTP was then corrected for the percent hybridization of radiolabeled transcripts and normalized per unit length of the DNA probe. Transcription activities of the individual genes are thus reported on a pmol of UTP incorporated per kilobase DNA basis.

In one experiment, Tagetin RNA polymerase inhibitor was added to lysed transcription assays. Further details of this experiment are found in the text.

Protein Synthesis by Isolated Intact Plastids

Conditions for ATP-driven protein synthesis by isolated intact plastids were as described previously (10, 11, 24). After the labeling period, plastids were fractionated into membrane and soluble polypeptides. Measurements of TCA insoluble radioactivity were obtained and samples were subsequently electrophoresed and autoradiographed (11).

Chloroplast DNA Probes and Plasmid Vectors

Portions of the open reading frames of the rbcL, psbA, and psaA-psaB genes of barley were cloned into Bluescript plasmid cloning vectors (Stratagene). The cloned barley chloroplast DNA fragments for rbcL, psbA, and psaA-B were as described previously (1, 13).

[2] Abbreviations: CHI, cycloheximide; CP1, chl-binding proteins of PSI; CP43 and CP47, 43 and 47 kD Chl-binding proteins of PSII; D1 and D2, 30–32 kD reaction center proteins of PSII.

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table I. *Transcriptional Activity of Plastids Isolated from Inhibitor-Treated Seedlings*

Dark-grown seedlings were excised and incubated with inhibitors or with buffer alone (control) for 40 min. Excised leaves were then maintained in the dark or were irradiated with white light. After leaves were illuminated for either 7 or 14 h, plastids were isolated and transcriptional activities of lysed plastids determined as described in "Materials and Methods." Data represent the mean of four experiments ± SE.

Treatment	UTP Incorporation
	pmol·5 × 10⁶ plastids⁻¹·5 min⁻¹ × 10¹
8-d dark (control)	3.5 ± 0.8
8-d dark, 7-h light (control)	15.1 ± 1.1
8-d dark, 7-h light plus CHI	2.9 ± 0.3
8-d dark, 7-h light plus chloramphenicol	17.3 ± 1.4
8-d dark, 14-h light (control)	12.5 ± 0.7
8-d dark, 14-h light plus chloramphenicol	17.6 ± 0.1

RESULTS

Specificity of Protein Synthesis Inhibitors

A major aim of the present study was to examine the light-dependent processes governing the activation of plastid transcription in 8-d-old dark-grown barley. In part, the effect of the protein synthesis inhibitors, CHI (inhibitor of 80S ribosomes) and chloramphenicol (inhibitor of 70S ribosomes) on the activation of plastid transcription by light was examined. The specificity of CHI and chloramphenicol as protein synthesis inhibitors has been previously determined in barley (10, 11) and pea (24). *In vivo* treatment of seedlings with CHI has been found to selectively block the translation of plastid proteins encoded in the nucleus (translated on 80S ribosomes), whereas chloramphenicol selectively blocks the incorporation of radiolabel into proteins translated within the plastid. To determine whether chloramphenicol or CHI exhibit secondary effects on chloroplast transcription, [^{32}P]UTP incorporation by isolated plastids was quantified in the presence of CHI and chloramphenicol. The addition of CHI or chloramphenicol to plastid transcription assays did not inhibit the incorporation of [^{32}P]UTP into plastid RNA (data not shown). Under the same conditions, Tagetin, an inhibitor of the chloroplast RNA polymerase (20, 21), reduced UTP incorporation by lysed plastids by more than 95%.

Effect of Protein Synthesis Inhibitors on Light-Activated Plastid Transcription

The role of *de novo* protein synthesis in light-activated plastid transcription was examined and the results are shown in Table I. Plastid transcriptional activity of dark-grown seedlings was stimulated approximately fourfold after 7 h of illumination. When seedlings were pretreated with CHI before illumination, the activation of plastid transcription by light was abolished. In contrast, chloramphenicol did not inhibit, but rather, stimulated the transcriptional activity of illuminated plastids. The stimulatory effect of chloramphenicol on plastid transcriptional activity was observed after 7 and 14 h of illumination.

Having examined the effect of protein synthesis inhibitors on the overall transcriptional activity of illuminated barley plastids, the next objective was to quantify the effect of these inhibitors on the transcription of specific chloroplast genes. A typical dot blot analysis of the transcriptional activity of rbcL (encodes the large subunit of the enzyme rubisco), psbA (encodes a 32-kD reaction center polypeptide of PSII) and psaA-B (encodes 70–80 kD reaction center polypeptides of PSI) is shown in Figure 1 (upper panel). Quantitation of the transcriptional activity of these genes showed a low level of rbcL, psbA, and psaA-B expression in plastids isolated from 8-d dark-grown seedlings (Fig. 1, lower panel). Illumination of dark-grown seedlings for 7 h resulted in a 19-fold enhancement of psbA transcription, whereas rbcL and psaA-B transcription was stimulated eight- and 14-fold, respectively. Illumination of CHI-treated seedlings did not enhance psbA, rbcL, or psaA-B transcription. Furthermore, the transcription

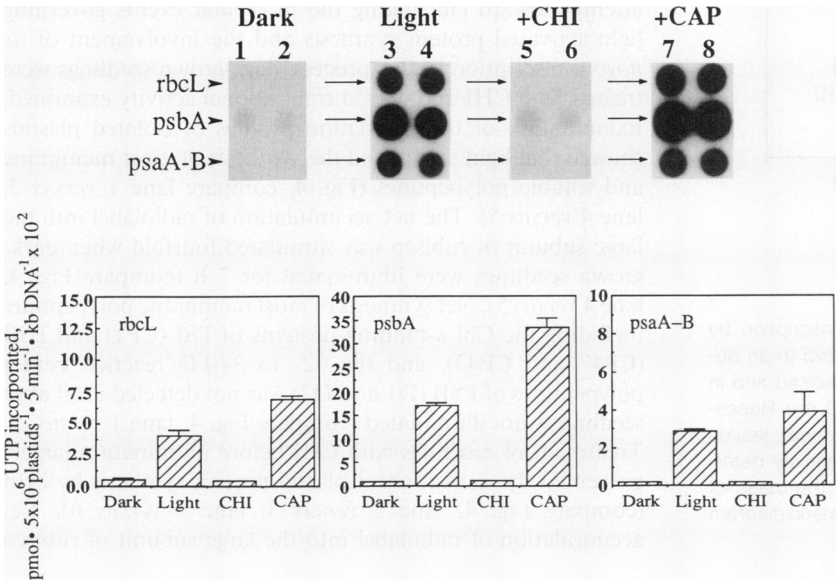

Figure 1. Effect of inhibitor treatment on transcriptional activity of plastid genes. Run-on transcripts were synthesized by plastids isolated from 8-d-old dark-grown seedling (upper panel, lanes 1 and 2) and from leaves treated with inhibitors in the light for 7 h. Run-on transcripts from light-grown control (lanes 3 and 4) and inhibitor-treated plastids (lanes 5–8) were hybridized to strips of nylon membrane containing DNA probes for rbcL, psbA, and psaA-B (upper panel). The radioactivity associated with each dot was counted by liquid scintillation. Incorporation was subsequently corrected for percent hybridization of radiolabeled transcripts and normalized per kb length of each DNA probe (lower panel). Transcription rates are expressed as the pmol of UTP incorporated by 5 × 10^6 plastids/kilobase of DNA during a standard 5-min transcription assay. CAP, chloramphenicol. Each data point represents the mean of two observations ± SD.

rates of the genes were nearly identical to dark-grown seedlings, indicating that CHI abolishes light-activated transcription but has little effect on the basal level of expression. Treatment of seedlings with chloramphenicol stimulated the light-induced transcription of all three genes examined, indicating that the effect was not specific for a given chloroplast gene. Transcriptional activity of rbcL, psbA, and psaA-B in chloramphenicol-treated seedlings was approximately 1.4-, 1.9-, and 1.7-fold greater than the activity observed in control illuminated seedlings, respectively.

The present study demonstrated that inhibition of cytoplasmic protein synthesis before illumination blocked the activation of plastid transcription. To determine whether concomitant cytoplasmic protein synthesis is necessary to maintain light-induced plastid transcriptional activity, seedlings were illuminated for 5 h and then treated with CHI. At various times after CHI treatment, plastids were isolated and transcriptional activity examined (Fig. 2). Treatment of illuminated seedlings with CHI caused a 20% decline in plastid transcriptional activity after 1 h of CHI treatment and by 4 h, transcription activity had declined by 60%. The transcription activity of plastids from control seedlings increased slightly between 5 and 7 h of illumination and declined slightly by 9 h. Therefore, *de novo* cytoplasmic protein synthesis is necessary for the activation of plastid transcription, and continued cytoplasmic protein synthesis is necessary to maintain light-activated plastid transcription.

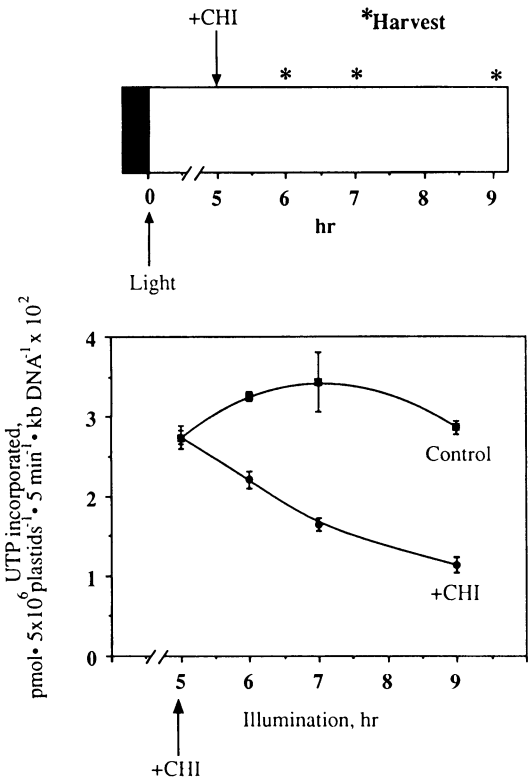

Figure 2. Rapid reversal of light-activated plastid transcription by CHI. Eight-day-old dark-grown seedlings were transferred to an illuminated chamber. After 5 h of light, seedlings were excised and in either placed in a solution of CHI (20 μg/mL) or in 40 mM Hepes-KOH, pH 8.0 (control). At various times after CHI treatment, leaves were harvested, plastids isolated, and transcriptional activity determined. Each data point in the lower panel represents the mean of four observations ± SD. The upper panel shows a schematic diagram depicting the experimental protocol.

Analysis of Chl-deficient Mutant, xan-J^{64}

The results presented thus far demonstrate that light differentially activates the transcription of rbcL, psbA, and psaA-B, and the activation is governed by both cytoplasmic and organellar protein synthesis. During this period of plastid transcription activation, light also induces the synthesis of Chl and activates photosynthetic electron transport. To determine whether the activation of plastid transcription is dependent on the light-dependent synthesis of Chl and the attainment of photosynthetic competence, the transcriptional activity of plastids of the Chl-deficient mutant, xan-J^{64}, was examined. Mutants of the xan-J loci are blocked in the biosynthesis of Chl *a* at the conversion of Chlide to Chl *a* (10 and references therein) and are therefore photosynthetically incompetent. The transcription activity of illuminated wild-type seedlings was fivefold greater than activities of dark-grown seedlings (data not shown). By comparison, overall transcription activity of plastids of illuminated xan-J^{64} mutant seedlings was nearly identical to wild-type illuminated seedlings. Dot blot analyses showed that the transcription of rbcL, psbA, and psaA-B in mutant and wild-type illuminated plastids was noticeably greater than the activities in dark-grown plastids (Fig. 3, upper panel). Quantitation of the activities of the individual genes indicated that the transcription of psbA and psaA-B in Chl-deficient mutant plastids was similar to (or slightly exceeded) the activity in wild-type illuminated plastids (Fig. 3, lower panel). Furthermore, transcription activity of rbcL in mutant plastids was sevenfold greater than the activity of dark-grown plastids.

Light-Activated Plastid Protein Synthesis

Illumination simultaneously activates plastid transcription and translation in 8-d-old barley seedlings (4, 13). As an initial attempt toward elucidating the molecular events governing light-activated protein synthesis and the involvement of *de novo* transcription in this process, dark-grown seedlings were treated with CHI and plastid translational activity examined. Examination of the translation profiles of isolated plastids showed that light stimulated the synthesis of most membrane and soluble polypeptides (Fig. 4, compare lane 1 *versus* 2; lane 4 *versus* 5). The net accumulation of radiolabel into the large subunit of rubisco was stimulated fourfold when dark-grown seedlings were illuminated for 7 h (compare Fig. 4, lane 4 *versus* 5). Net synthesis of most membrane polypeptides including the Chl *a*-binding proteins of PSI (CP1) and PSII (CP47 and CP43), and the 32- to 34-kD reaction center polypeptides of PSII (D1 and D2) was not detected until after seedlings were illuminated (compare Fig. 4, lane 1 *versus* 2). Treatment of seedlings with CHI before illumination largely prevented the activation of plastid protein synthesis by light (compare Fig. 4, lane 2 *versus* 3; lane 5 *versus* 6). Net accumulation of radiolabel into the large subunit of rubisco

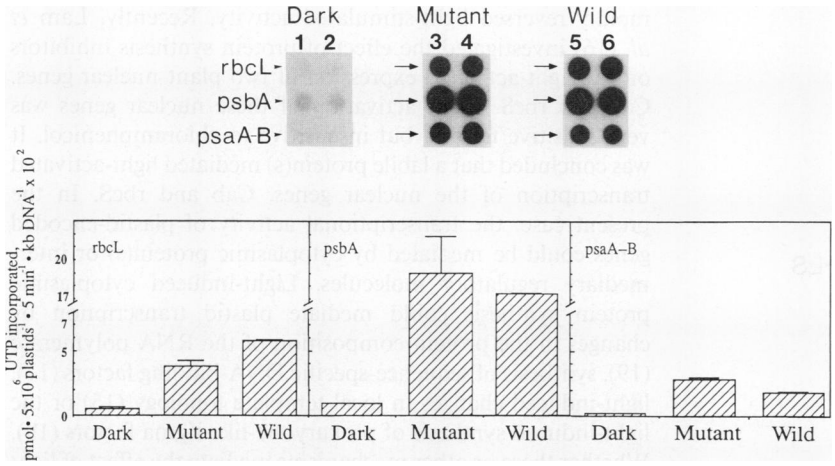

Figure 3. Transcriptional activity of plastids from Chl-deficient mutant seedlings. Seedlings of the Chl-deficient mutant, xan-J[64], were grown for 8.5 d in complete darkness and then transfered to an illuminated chamber. After 14 h of illumination, mutants were identified (yellow, Chl deficient) and wild-type and mutant seedlings were harvested. Run-on transcripts from dark-grown (lanes 1–2), illuminated mutant (lanes 3–4), and illuminated wild-type plastids (lanes 5–6) were hybridized to strips of nylon membranes containing DNA probes for rbcL, psbA, and psaA-B (upper panel). Lower panel shows the quantitation of transcriptional activity of individual genes in illuminated mutant and wild-type plastids. Each data point represent the mean of two observations ± SD.

and the Chl *a*-binding proteins of PSI and PSII was similar in dark-grown seedlings and illuminated seedlings treated with CHI. CHI treatment, however, did not completely block the light-induced synthesis of D1, but net incorporation of label into D1 was reduced approximately sixfold when compared with control illuminated seedlings (compare Fig. 4, lanes 2 *versus* 3).

It is apparent that the activation of plastid transcription and translation by light is dependent on cytoplasmic protein synthesis. What is not apparant is whether the rapid burst in plastid protein synthesis is due mainly to changes that regulate the rate of translation of pre-existing mRNAs or whether light-induced changes in plastid transcription are sufficient to account for the burst in plastid translation. To address this question, seedlings were treated with the plastid RNA polymerase inhibitor Tagetin before illumination. Subsequently, plastids were isolated and plastid translational activity and mRNA levels examined. The results are presented in Figures 5 and 6. The illumination of dark-grown seedlings for 7 h increased the steady state level of rbcL, psbA, and psaA-B mRNA (compare Fig. 5, lane 1 *versus* 2). Treatment of seedlings with Tagetin before illumination prevented the light-dependent increase in plastid transcripts (compare Fig. 5, lane 2 *versus* 3). The levels of psbA and rbcL mRNAs were similar in dark-grown seedlings and in illuminated seedlings treated with Tagetin (compare Fig. 5, lane 1 *versus* 3). The level of psaA-B mRNA in Tagetin-treated seedlings had declined by 7 h of light. The decline in psaA-B mRNA in illuminated tissue treated with Tagetin may reflect the blockage of psaA-B transcription by Tagetin and accelerated psaA-B mRNA turnover in illuminated plastids (12).

Given that Tagetin treatment prevented the light-induced increase in plastid mRNAs, the translation profiles of plastids from Tagetin-treated seedlings were examined. In agreement with the results of Figure 4, illumination stimulated the net accumulation of radiolabel into most membrane and soluble polypeptides (Fig. 6, compare lane 1 *versus* 2; lane 4 *versus* 5). Examination of the translation profiles from Tagetin-treated seedlings showed that Tagetin did not prevent the activation of plastid translation by light. In particular, the translation of the large subunit of rubisco was stimulated fivefold in control illuminated seedlings and fourfold in illuminated seedlings treated with Tagetin. Net accumulation of radiolabel by Tagetin-treated plastids into D1 polypeptide equaled (or slightly exceeded) that by control illuminated plastids (Fig. 6B, compare lane 2 *versus* 3). Furthermore, despite a significant decline in psaA-B mRNA in illuminated seedlings treated with Tagetin, net accumulation of radiolabel into CP1 was stimulated when Tagetin-treated seedlings were illuminated (compare Fig. 6B, lane 2 *versus* 3).

DISCUSSION

Photosynthesis, together with other plastid functions, requires the products of several hundred genes, of which about 120 are located on the chloroplast genome (22). Recent reviews of chloroplast gene expression emphasize post-transcriptional and translational mechanisms of control (7, 8, 22). However, numerous observations of light-induced fluctuations in plastid mRNA populations have been reported (for review, see ref. 19). The extent to which light-induced fluctuations in plastid mRNA pools are determined by changes in transcription rates has only recently been addressed. Klein and Mullet (13) have shown the light-induced increases in plastid mRNAs in 8-d-old dark-grown barley seedlings is due, in part, to light-activated transcription of chloroplast genes. Furthermore, associated with the light-dependent increase in plastid transcriptional activity in 8-d-old barley seedlings is renewed translation of plastid-encoded polypeptides. Gamble *et al.* (4) concluded that the increase in plastid protein synthesis was controlled at the level of translation initiation. However, it was not directly determined whether protein synthesis was activated by the translation of pre-existing mRNAs (*i.e.* translational control) or whether the increase in translational activity could be accounted for by an increase in transcription rate of previously silent plastid genes. In the present study, I investigated the light-dependent processes controlling the activation of plastid transcription and determined whether *de novo* transcription of plastid photogenes accounts for the rapid burst in plastid protein synthesis.

Light-Induced Plastid Transcription

As a first step toward understanding the light-dependent processes controlling the activation of plastid transcription

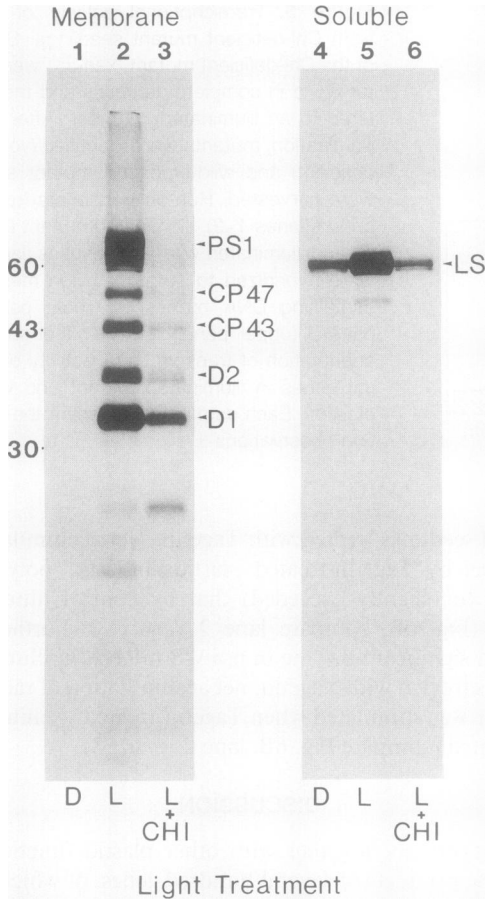

Figure 4. Synthesis of polypeptides by plastids isolated from CHI-treated leaves. Dark-grown barley leaves were excised and incubated with or without 20 μg/mL CHI for 40 min before irradiation with white light. After illumination for 7 h, intact plastids were isolated and incubated with [^{35}S]methionine for 20 min and the chased for 20 min with unlabeled methionine as described in "Materials and Methods." Following the labeling period, plastids were fractionated into membrane and soluble polypeptides and samples were loaded on SDS-PAGE gels containing 8 M urea. Gels were fixed, fluorographed, and exposed to x-ray film for 72 h. Lanes 1 and 4, membrane and soluble polypeptides, respectively, synthesized by dark-grown plastids; lanes 2 and 5, membrane and soluble polypeptides, respectively, labeled in plastids isolated from control leaves (minus cycloheximide); lanes 3 and 6, membrane and soluble polypeptides, respectively, labeled in plastids from CHI-treated leaves. Numbers to the left indicate mobility of standards (kD). LS, large subunit of rubisco; CPI, Chl *a*-apoproteins of PSI; D1, 32-kD psbA gene product; D, dark; L, light.

and the involvement of *de novo* protein synthesis in this process, the effect of protein synthesis inhibitors on transcription activity of 8-d-old dark-grown barley was examined. The present results indicate that the light-induced synthesis of a cytoplasmic factor(s) may act as a positive regulator of light-activated plastid transcription. This conclusion is based on the observation that inhibition of cytoplasmic protein synthesis abolished the light-induced transcription of chloroplast genes. Furthermore, the cytoplasmic signal controlling plastid transcription appears labile in nature since inhibition of cytoplasmic protein synthesis after transcription was reactivated rapidly reversed light-stimulated activity. Recently, Lam *et al.* (16) investigated the effect of protein synthesis inhibitors on the light-activated expression of two plant nuclear genes, Cab and rbcS. Light activation of these nuclear genes was very sensitive to CHI but insensitive to chloramphenicol. It was concluded that a labile protein(s) mediated light-activated transcription of the nuclear genes, Cab and rbcS. In the present case, the transcriptional activity of plastid-encoded genes could be mediated by cytoplasmic protein(s) or intermediary regulatory molecules. Light-induced cytoplasmic protein synthesis could mediate plastid transcription by changes in the protein composition of the RNA polymerase (19), synthesis of sequence-specific DNA-binding factors (17), light-induced changes in local template topology (15) or the light-induced synthesis of prokaryotic-like sigma factors (19). Whether these or other mechanisms mediate the effect of light on chloroplast transcription is presently under investigation.

Unlike the inhibitory effect of CHI on light-activated chloroplast transcription, inhibition of plastid protein synthesis with chloramphenicol did not abolish the activation of plastid transcription. Since several of the subunits of the RNA polymerase are encoded by the chloroplast genome (22, 25, 29), induction of plastid transcription does not require *de novo* synthesis of plastid-encoded subunits of the RNA polymerase. The results obtained with chloramphenicol also implicate possible negative regulation of light-activated plastid transcription. Inhibition of organelle protein synthesis stimulated the transcriptional activity of rbcL, psbA, and psaA-B when compared with activities in control illuminated seedlings. The stimulatory effect of chloramphenicol might be explained on

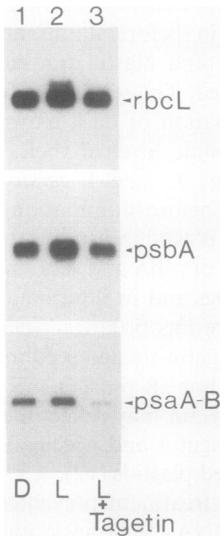

Figure 5. Effect of Tagetin RNA polymerase inhibitor on accumulation of plastid mRNA. Dark-grown barley leaves were excised and incubated with or without Tagetin RNA polymerase inhibitor (800 U/mL) for 7 h in the dark. Subsequently, seedlings were transferred to light and after 7 h intact plastids were isolated. Nucleic acid was isolated from an equal number of plastids from dark-grown seedlings (lane 1) and from illuminated seedlings incubated without (lane 2, control) or with Tagetin (lane 3). Northern blots were conducted as described in "Materials and Methods."

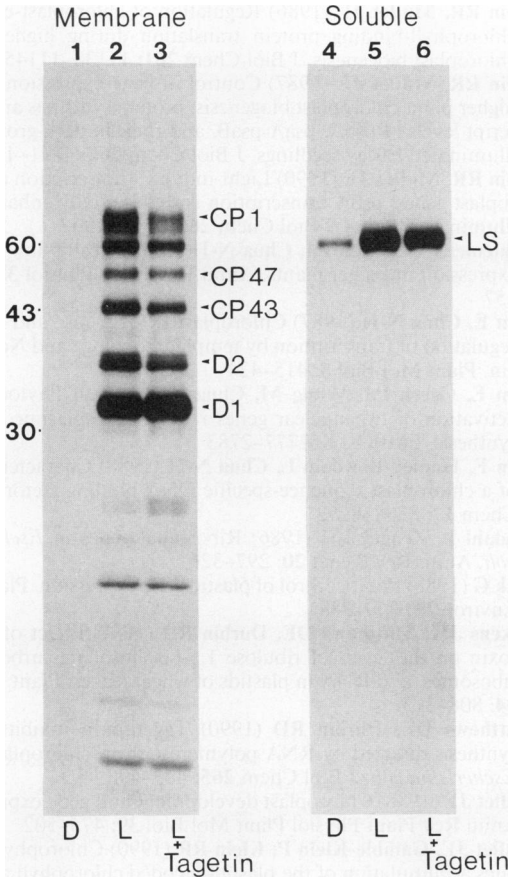

Figure 6. Effect of Tagetin RNA polymerase inhibitor on light-activated plastid translation. Barley leaves were treated with Tagetin as described for Figure 5. Intact plastids were isolated and incubated with [^{35}S]methionine as described for Figure 4. After labeling, plastids from dark-grown seedlings (lanes 1 and 4) and from illuminated seedlings incubated without (lanes 2 and 5, control) or with Tagetin (lanes 3 and 6) were fractionated, electrophoresed, and autoradiographed as described in Figure 4. Legends same as Figure 4 plus rbcL, encodes the large subunit of rubisco (LS); psaA-B, encodes 70 to 80-kD PSI reaction center polypeptides (CPI); psbA, encodes the 32-kD PSII reaction center polypeptide (D1).

the basis of blocking the synthesis of a labile repressor protein or other negative signals dependent on plastid protein synthesis. Stimulatory effects of protein synthesis inhibitors on gene activation are well documented in other systems (2, 9, 26). In most of these cases, the models that were presented emphasized that inhibition of protein synthesis would decrease the concentration of a labile factor, which acts to inhibit expression of select genes. However, the entire sequence of two plastid genomes has been determined, and most genes identified encode structural components of the transcriptional or translational apparatii of plastids, or are involved in photosynthesis (22, 28). Therefore, it is unlikely that many regulatory genes controlling plastid gene expression reside within the chloroplast genome. Alternatively, plastid protein synthesis may regulate transcriptional activity via negative feedback control by free or unassembled polypeptide subunits. Although this mechanism has not been shown to operate in plastids, it is involved in the transcription of the rRNA operons in *Escherichia coli* (18). rRNA molecules or other components such as mature ribosomes (or ppGpp) appear to inhibit transcription of the rRNA operons. This type of regulation may also occur in plastids.

Klein *et al.* (10) have previously demonstrated that the light-induced accumulation of plastid encoded Chl *a*-apoproteins in 4.5-d-old dark-grown barley seedlings requires the light-dependent conversion of protochlorophyllide to Chl *a*. Among the evidence for an involvement of Chl *a* accumulation was that the synthesis of Chl *a*-apoproteins was not detected in Chl-deficient barley mutants (10). To determine whether the light-dependent activation of chloroplast transcription in 8-d-old dark-grown barley is also dependent on Chl *a* accumulation and the attainment of photosynthetic activity, the transcriptional activity of the Chl-deficient barley mutant, xan-J^{64}, was examined; xan-J^{64} mutant plastids are blocked in the esterification of Chlide (see 10 and references therein) and therefore, do not accumulate Chl *a* upon illumination. Examination of the transcriptional activity of illuminated xan-J^{64} mutant seedlings indicates that the accumulation of Chl *a* is not necessary for the activation of plastid transcription. Furthermore, xan-J^{64} mutant plastids are photosynthetically incompetent, thereby indicating that the attainment of photosynthetic activity is not the primary photoevent controlling light-activated plastid transcription. Studies are presently being conducted to identify the photoreceptor and the cytoplasmic factor mediating light-activated plastid transcription. Preliminary studies have implicated phytochrome as the primary photoreceptor controlling activation of plastid transcription (JE Mullet and RR Klein, unpublished data). Therefore, light may be preceived by phytochrome and *de novo* cytoplasmic protein synthesis is required for the signal transduction between Pfr and the transcription of rbcL, psbA, and psaA-B.

Control of Plastid Translation

Ellis (3) in 1977 proposed the "cytoplasmic control principle," which states that the translation of plastid gene products is controlled by cytoplasmic translation products. Consistent with this hypothesis was the present finding that CHI caused a general inhibition of light-activated plastid translation. Evidence has accumulated with the use of specific inhibitors (5, 31), nuclear mutations (for review, see ref. 31), and transgenic plants (27) that nuclear factors govern the expression of the plastid genome. What has not been directly determined is whether cytoplasmic factors control the light-induced burst in plastid protein synthesis in older tissues by altering the rate of translation of pre-existing mRNAs (translational control) or by increasing the concentration of template mRNA via enhanced transcriptional activity. The recent commercial availability of Tagetin, an inhibitor of the chloroplast RNA polymerase, permitted an assessment of the dependence of light-activated translation on *de novo* transcription. The present results indicate that activation of plastid translation by light was not dependent on light-induced transcription or the associated accumulation of plastid "photogene" mRNA. Rather, cytoplasmic factors apparently control the burst in plastid protein synthesis by altering the rate of translation of

pre-existing mRNA. Hence, despite the concomitant increase in plastid photogene mRNA, control of protein synthesis is at the level of translation. Consistent with a translational control mechanism is an increase in plastid polysome content and a shift of nonpolysomal mRNA into large polysomes upon illumination (4). Further studies are in progress to identify the cytoplasmic translation products governing the activation of plastid protein synthesis.

Finally, the coordination of nuclear and plastid gene expression consists of a complex array of mechanisms in which transcriptional and translational activity of the plastid are controlled by cytoplasmic translation products. Emerging evidence also indicates that nuclear genes may be responsible for regulating plastid gene expression at the level of transcript stability (30) and RNA processing (5). This complex array of control mechanisms may be necessary to coordinate the assembly of multiprotein complexes in which components are encoded in two distinct genomes—one of a eukaryotic nature (nuclear) and the second of prokaryotic origin.

ACKNOWLEDGMENTS

The author thanks Mrs. Zonnie McBride, Dr. Patricia Klein, and Mr. Martin Wiglesworth for their help during the preparation of this manuscript. The author also thanks Dr. John E. Mullet for providing DNA probes and for his advice and guidance during the conception of this study. Finally, thanks go to Dr. David Simpson for providing the barley mutants used in this study.

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