Maize Plastid Photogenes: Mapping and Photoregulation of Transcript Levels during Light-induced Development

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ABSTRACT Positively photoregulated regions that show increased transcript levels upon illumination of dark-grown seedlings are scattered over ~19% of the maize plastid chromosome. Some photogenes, i.e., genes within these regions, are transcribed individually, whereas others that are transcribed as polycistronic mRNAs appear to be functionally organized into operons. Multiple light-induced transcripts are complementary to most photogenes; these mRNAs are not present in equimolar amounts during plastid photomorphogenesis, but particular transcripts predominate at specific stages of development. Most, but not all, photogene RNA pools reach a maximum size (after either 10, 20, or 44 h of illumination) and then fall to approximately preillumination levels. These data and other considerations argue that photogene expression control is fundamentally transcriptional and that there is more than one expression class. Transcripts of the maize plastid gene for the large subunit of ribulose bisphosphate carboxylase reach a maximum by 20 h of illumination; transcripts of the nuclear gene for the small subunit of this enzyme continue to accumulate and fall considerably later. These data suggest that the level of transcription of the latter gene in the nucleus may be regulated by events in the chloroplast.

Pale-yellow etioplasts develop into green, photosynthetically competent chloroplasts upon illumination of dark-grown angiosperm seedlings (13, 32). Qualitative and quantitative changes in the complement of nuclear- and plastid-encoded stromal and thylakoid membrane polypeptides during light-induced plastid development (24, 28, 43) have been correlated with corresponding alterations in the abundance of specific transcripts, leading to the hypothesis that light-dependent transcriptional regulation may play a central role in the greening process (9).

In the case of nuclear-encoded plastid polypeptides, a direct correlation has been demonstrated between light-induced changes in the levels of transcripts for the precursors of the light-harvesting chlorophyll *a/b* protein (3, 21, 25, 44, 58, 62), the small subunit of ribulose bisphosphate carboxylase (10, 25, 52, 55, 58, 61, 64), and the reduced nicotinamide adenine dinucleotide phosphate/protochlorophyllide oxidoreductase (2, 51) and altered amounts of these polypeptides during early greening. The light-dependent changes in the relative abundance of all three of these transcripts appear to be under the control of the phytochrome photoreceptor (1, 2, 26, 54, 58, 63), although accumulation of the light-harvesting chlorophyll *a/b* protein in the thylakoid membrane requires the presence of chlorophyll *a*, and thus the activity of the protochlorophyll(ide) photoreceptor as well (4, 11, 21).

DNA/RNA hybridization techniques have allowed the localization of regions within the plastid genomes of maize (9), barley (47), mustard (37), *Chlamydomonas* (42), and *Euglena* (18, 22, 48) that contain photogenes, i.e., genes whose transcripts increase appreciably in abundance during light-induced plastid development. For only two plastid genes, however, have altered mRNA levels been directly correlated with altered polypeptide levels during greening—that is, for *ps2B* (or *psbA*, or photogene 32 ["*PG32*"], encoding the 32,000-mol-wt herbicide-binding B protein of photosystem II [46, 57]) and for *rcL* (the gene encoding the large subunit of ribulose bisphosphate carboxylase [RuBPCase]). A direct linkage between increased transcript levels and increased protein amounts has been demonstrated rigorously for *rcL* only in pea (53, 55) and for *ps2B* only in maize (9, 27, 28), though such a linkage is suggested for *rcL* in mung bean (45) and for *ps2B* in *Spirodela* (23, 49, 50), mung bean (45), mustard (38), and pea (60). Increases in levels of the *ps2B* transcript in greening mustard (38) and pea (60) and of the *rcL* transcript in greening pea (60) appear to be mediated by phytochrome.

The objectives of the present study were, first, to identify

regions of the maize plastid genome (other than *ps2B*) that contain photogenes, and second, to determine whether all photogenes are of a single class with regard to the times at which changes in the size of their transcript pools occur. Photogenes have been mapped to several regions of the maize plastid genome. Genes within some of these regions are transcribed individually but others appear to be functionally organized into photoregulated operons that are transcribed as polycistronic mRNAs. However, the latter regions are also represented by numerous shorter transcripts of various sizes. The levels of transcripts of all maize plastid photogenes rise noticeably within the first 2 h of illumination of dark-grown maize seedlings, but photogenes cannot be distinguished from one another on this basis in our assay. Surprisingly, even under continuous illumination, the levels of transcripts of most photogenes reach a maximum and fall to about preillumination levels—these patterns set maize plastid photogenes apart from one another. Transcript pools of the nuclear gene for the small subunit of RuBPCase were also found to rise and fall; transcripts complementary to this gene continue to accumulate long after the size of the RNA pool complementary to the plastid gene for the large subunit of this enzyme has begun to fall.

MATERIALS AND METHODS

Growth of Seedlings: Zea mays was grown as described (15). Seedlings used as sources of plastid RNA were grown for 7 d in a darkroom at 30°C and harvested either immediately (etiolated seedlings) or after illumination for variable amounts of time at room temperature with fluorescent lamps of ~1,400 lx (greening seedlings). Chlorophyll content was used as an index of the greening process, and was determined by the procedure of Arnon (5).

DNA Clones: The recombinant plasmids pZmc37, pZmc427, pZmc461, and pSSU160 have been described (10, 16, 20, 31). pZmc527, pZmc556, pZmc569, pZmc404, pZmc415, and pZmc518 contain maize plastid DNA BamHI restriction fragments 3, 17, 20, 21', 24, and 13 plus 17' (6, 36), respectively, cloned into pBR322 (17); pZr12 and pZr48 contain EcoRI fragments "b" and "e," respectively, cloned into pBR322.

Isolation of Nucleic Acids: Plastid DNA, plasmid DNA, plastid RNA, and total maize cell RNA were isolated as described (15). For the extraction of RNA from etiolated seedlings, filtered homogenates were prepared in the dark under a green safe light; as many manipulations as possible were performed at 0–4°C. An "enriched plastid RNA" fraction was prepared by a modification of the procedure for the isolation of plastid RNA (15): after homogenization of maize leaves, as described, the homogenate was filtered through four layers of Miracloth (Calbiochem-Behring Corp., San Diego, CA), and the filtrate was centrifuged at 1,500 g for 20 min at 4°C. Hot lysis buffer (15) and an equal volume of 0.1 M Tris-HCl (pH 8.0)-saturated phenol were then added immediately to each of the pellets, and the suspensions were thoroughly mixed. After centrifugation at 10,000 rpm for 10 min, the aqueous phases were collected and reextracted twice with phenol, precipitated with ethanol, and extracted twice with 2 M LiCl, as described (15).

Restriction Endonuclease Analysis and Preparation of DNA Fragments: Procedures used for restriction endonuclease digestion and mapping of plastid DNA, and for agarose gel electrophoresis have been described (15). DNA fragments were isolated from agarose gels by electroelution (65) and cleaned by spermine precipitation (30) before radioactive labeling.

Radioactive Labeling of Nucleic Acids: DNA was radioactively labeled in vitro by nick-translation, as described (15). Partially hydrolyzed RNA was radiolabeled in vitro by polynucleotide kinase, as described (15). After separation of the labeled nucleic acids from unincorporated ^{32}P-labeled nucleotides by passage through a Bio-Gel P-60 column (Bio-Rad Laboratories, Richmond, CA) (15), the specific activity of each labeled nucleic acid sample was determined after precipitation of an aliquot of the column eluate in cold 10% trichloroacetic acid.

Hybridization Analyses: A competitive hybridization procedure for the detection of plastid DNA sequences complementary to plastid RNA from etiolated and greening seedlings has been described (15). Details of the procedure used here are given in Results.

For the detection of plastid RNAs complementary to plastid DNA (Northern

analysis), RNA was denatured by glyoxylation (41) and electrophoresed on horizontal 1.4% agarose gels with Tris-Borate-EDTA, pH 8.3 (15), as the running buffer. After transfer of the RNA from the agarose to Gene Screen filters (New England Nuclear, Boston, MA), filters were baked for 2 h under reduced pressure at 80°C, presoaked for 4–6 h at 37°C in 50% formamide, 3 × sodium saline citrate (0.15 M NaCl; 0.015 M sodium citrate)[1] (SSC), Denhart's solution, 0.1 mg/ml sonicated calf thymus DNA, and 0.1% SDS (15), and hybridized in the same (though fresh) solution with 10^5–10^6 cpm of denatured nick-translated DNA for 16–24 h at 47–52°C. The filters were then washed twice (once for 60 min at 70°C in 2 × SSC, 0.3% SDS, and once for 60 min at 70°C in 1 × SSC, 0.1% SDS), air-dried, and exposed to X-ray film (either with or without an intensifying screen). Autoradiographs were scanned with a Zeineh Soft Laser Scanning Densitometer, and the relative intensity of a given band determined by integration of the area under the peak. Tests were conducted to ensure that saturating amounts of labeled probe were used in the hybridization. To be sure that the photographic film was not overexposed, several exposures of different duration were made and scanned.

In some experiments ("dot-blot" analyses, see reference 59), equal amounts of unfractionated samples of RNA from greening seedlings were spotted onto a wet (equilibrated with 2 × SSC) Gene Screen filter. The filter was then baked and subsequent manipulations carried out as described above (for homologous hybridizations). For heterologous hybridizations, filters were treated as described above, with the following exceptions: (a) hybridizations were performed at 37°C; (b) the hybridization solution contained 10% (rather than 50%) formamide; and (c) after hybridization, filters were washed twice for 60 min each at 30°C in a solution containing 5 × SSC, 0.2% SDS.

RESULTS

Photoregulated Plastid Genes Are Scattered on the Genome

Competitive hybridization (15) was used in this study to identify regions of the plastid genome of *Zea mays* that contain photogenes. Filter-bound, electrophoretically separated fragments of plastid DNA were hybridized with unlabeled RNA from etiolated seedlings before their hybridization with radiolabeled RNA from greening seedlings. In this procedure, sites complementary to RNA species present in plastids from both developmental states become much less available for hybridization with the labeled RNA. It is thus expected that in titration experiments involving the prehybridization of identical filters with increasing amounts of unlabeled etioplast RNA (before the addition of constant amounts of in vitro-labeled RNA from greening seedlings to hybridization solutions for each filter), the hybridization signal from regions of DNA fragments containing genes whose transcripts are common to the two types of plastids will be progressively masked, whereas the hybridization signal from regions of those fragments containing photogenes will become increasingly apparent.

Fig. 1 illustrates the results of a typical titration experiment. Six identical nitrocellulose filters (56) containing BamHI restriction fragments of the plastid DNA were prepared (lane *a*). Four of the filters were prehybridized with increasing amounts of unlabeled enriched etioplast RNA (0-h RNA) and then hybridized with equal amounts (counts per minute) of ^{32}P-labeled enriched plastid RNA from dark-grown seedlings illuminated for 16 h (16-h RNA). (RNA extracted from leaves was end-labeled with ^{32}P in vitro because the level of labeling in vivo is too low for the hybridization assay.) Lanes *c, d, e,* and *f* show, respectively, the results of prehybridization with

[1] *Abbreviations used in this paper:* CF_0 and CF_1, the intrinsic and extrinsic protein complexes, respectively, of the plastid coupling factor for photophosphorylation; CPI, chlorophyll–protein complex of photosystem I; kb, kilobase; kbp, kilobase-pair; RuBPCase, ribulose bisphosphate carboxylase; SSC, sodium saline citrate (0.15 M NaCl, 0.015 M sodium citrate).

FIGURE 1 Identification of photogene-containing regions of the maize plastid genome by competitive hybridization. Plastid DNA was digested with BamHI and electrophoresed in 1-μg aliquots in adjoining lanes of a 0.9% Tris-Borate-EDTA agarose gel. (Lane *a*) Fluorescence of DNA after ethidium bromide staining of the agarose gel. Bands are numbered sequentially from the largest (from the origin of migration), to the smallest; those bands of interest containing two or more co-migrating fragments are also indicated. (Lanes *b–g*) Autoradiographs (1-d exposure with an intensifying screen) of individual strips cut out of a nitrocellulose filter after transfer of the plastid DNA from agarose and subsequent manipulations. (Lane *b*) Prehybridized with 50 μg of unlabeled enriched RNA from etiolated seedlings (0-h RNA) and hybridized with 1.5 × 10^6 cpm of partially hydrolyzed, kinased 0-h RNA. (Lanes *c–f*) Prehybridized with 10, 25, 50, and 100 μg, respectively, of 0-h RNA, and hybridized with 1.5 × 10^6 cpm of partially hydrolyzed, kinased, enriched RNA from 16-h-greened seedlings (16-h RNA). (Lane *g*) No prehybridization, hybridized with 1.5 × 10^6 cpm of partially hydrolyzed, kinased 16-h RNA.
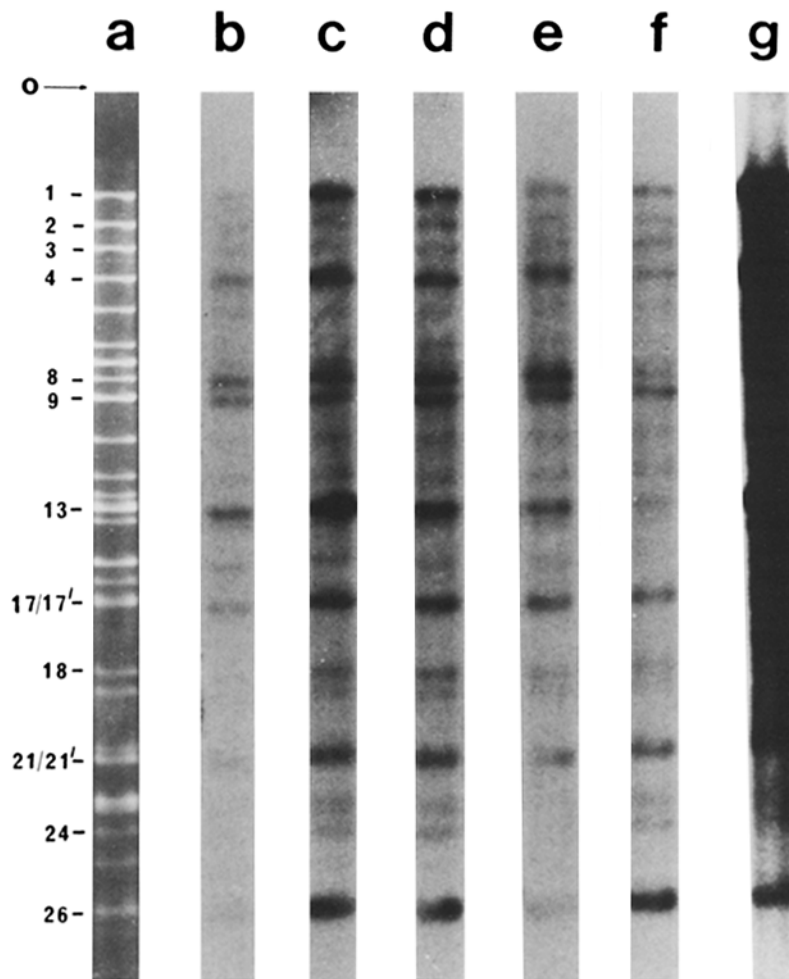
10, 25, 50, and 100 μg of unlabeled RNA from unilluminated (i.e., 0-h) plants and the subsequent hybridization with labeled 16-h RNA. To estimate the amount of hybridization masked by prehybridization with unlabeled 0-h RNA, a fifth filter (lane *g*, the nonprehybridization control) was hybridized directly with the same number of counts per minute of labeled 16-h RNA as was used for the hybridization of the first four filters. To estimate the efficiency of prehybridization with the unlabeled 0-h RNA, a sixth filter (lane *b*, background hybridization control) was prehybridized with 50 μg of unlabeled 0-h RNA, and subsequently hybridized with the same number of counts per minute of ^{32}P-labeled 0-h RNA as the labeled 16-h RNA used to hybridize the first five filters. The titration experiment demonstrates that hybridization of labeled 16-h RNA to fragments containing rRNA genes—BamHI fragments 1, 4, and 13 (8)—is decreased after prehybridization with as little as 10 μg of 0-h RNA (compare lanes *c* and *g*), and that as the concentration of unlabeled 0-h RNA in the prehybridization mixture is increased, the intensity of hybridization to these bands decreases progressively (compare lanes *c, d, e,* and *f*), whereas the intensity of hybridization to other bands—namely, to BamHI fragments 2, 3, 8, 9, 17/17′, 21/21′, and 26—becomes more prominent. (The decreased hybridization to several of these latter fragments in lane *f* has been observed consistently in titration experiments at high concentrations of prehybridized, unlabeled RNA; the cause of this decrease is not understood.) Because rRNA constitutes >90% of the total RNA pool in etioplasts and greening

plastids (9), it can be assumed that at concentrations of unlabeled 0-h RNA sufficiently high to mask the hybridization of labeled rRNA, any increase noted in the hybridization of labeled 16-h vs. labeled 0-h RNA to a given fragment is due to the presence of transcripts from genes whose expression is photoregulated. This is seen most clearly by comparing lanes *b* and *e*; both of these filters were hybridized with 50 μg of unlabeled 0-h RNA before their hybridization with identical amounts (counts per minute) of either labeled 0-h (lane *b*) or labeled 16-h RNA (lane *e*). The intensity of hybridization to BamHI fragments 1, 4, and 13 that contain rDNA is approximately the same in both lanes, but in lane *e* increased hybridization is particularly evident to BamHI fragments 8, 9, 17/17′, and 21/21′. Also evident, though not as pronounced in this particular experiment, is increased hybridization to BamHI fragments 2, 3, and 26. In other titration experiments performed with RNA prepared from sucrose gradient-purified etioplasts and plastids from seedlings illuminated for 16 h (rather than with enriched RNA samples), hybridization to BamHI fragments 1, 4, and 13 was masked completely (data not shown); hybridization was seen only to BamHI fragments 2, 3, 8, 9, 17/17′, 21/21′, and 26, thus confirming the results presented in Fig. 1 and ruling out the possibility that the rDNA-containing fragments contain strongly regulated photogenes (i.e., genes showing much increased levels of hybridization with labeled 16-h RNA).

It is concluded from the titration experiments that BamHI fragments 2, 3, 8, 9, 17 and/or 17′, 21 and/or 21′, and 26
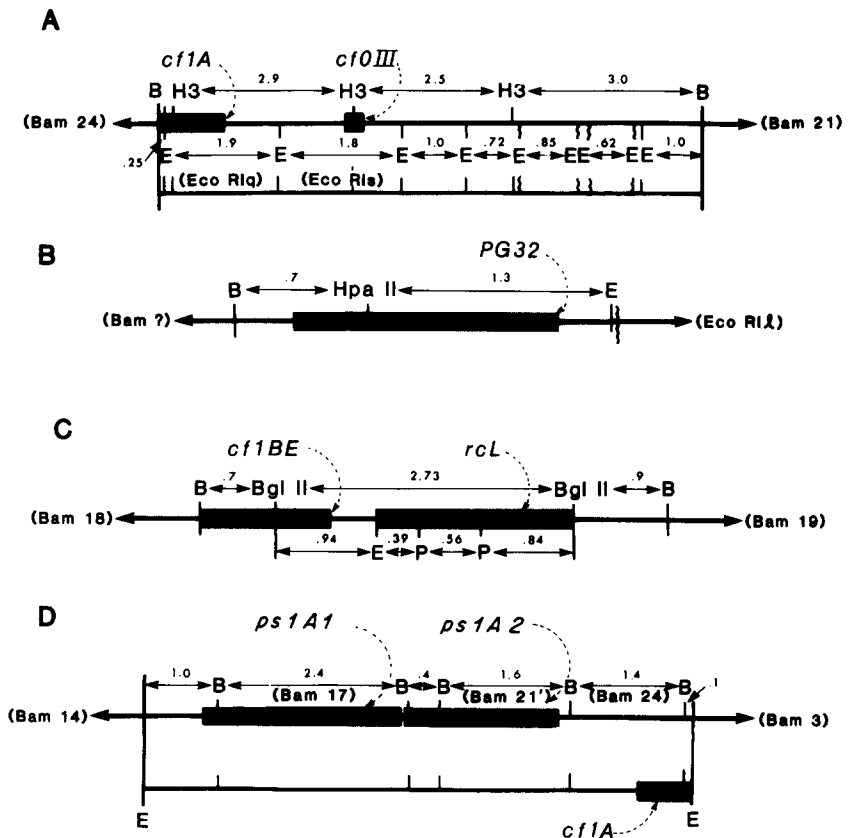
A

FIGURE 2 Restriction endonuclease maps of cloned photogene-containing regions of the maize plastid genome. Sizes of subfragments are given in kilobase pairs, and the positions of sequenced genes are indicated by thick lines on the appropriate coding strand. (A) BamHI fragment 3 (pZmc527); (B) a 2.0-kbp portion of BamHI fragment 8 (pZmc427); (C) BamHI fragment 9 (pZmc37); and (D) EcoRI fragment b (pZr12), containing BamHI fragments 17 (2.4 kbp), 21' (1.6 kbp), and 24 (1.4 kbp). (B, BamHI, E, EcoRI, H3, HindIII, P, PstI). The photogene-containing sequences in BamHI fragments 2 and 20 are not shown.

contain photogenes. Of these, BamHI fragments 2 and 26 will not be discussed further in this paper: studies concerning BamHI fragment 2 will be presented in a separate publication (Krebbers, E. T., K. M. T. Muskavitch, and L. Bogorad, manuscript in preparation); BamHI 26 is located within the 16S–23S rDNA spacer region (see Fig. 4) and contains portions of the coding regions of the tRNA^{Ile} and tRNA^{Ala} split genes (33). The behavior of BamHI 26 in titration experiments is thus consistent with the data of Haff and Bogorad (29) that show an increase in the level of these plastid-encoded tRNAs during greening.

Competitive hybridization experiments were also used to localize photogene-containing regions within the larger BamHI fragments identified in the titration experiments as containing photoregulated genes (i.e., 3, 8, and 9) and to determine which one (or both) of the BamHI fragments that electrophorese at the same position in an agarose gel (i.e., 17 and/or 17', 21 and/or 21') contains photogene sequences. DNA for these detailed analyses was obtained from clones of each of the fragments of interest and a restriction map of each cloned fragment was determined (when necessary). The plasmid DNA was electrophoresed on an agarose gel after digestion with a specific restriction enyzme(s) and the DNA was transferred to nitrocellulose; competitive hybridization analyses were conducted as outlined above.

Restriction endonuclease maps of the plastid DNA fragments in the following clones (described in Materials and Methods) are shown in Fig. 2, A–D: (A) pZmc527 (containing BamHI fragment 3); (B) pZmc427 (containing a 2.0-kilobase-pair (kbp) portion of BamHI fragment 8); (C) pZmc37 (containing BamHI 9); and (D) pZr12 (containing EcoRI fragment "b," which includes BamHI fragments 17, 21', and 24). Sequenced genes within these fragments include: the proteo-

lipid subunit of the intrinsic membrane portion of the plastid coupling factor for photophosphorylation, CF$_0$ (cf0III), within BamHI fragment 3 (Rodermel, S. R., and L. Bogorad, unpublished observation); the α-subunit of the extrinsic membrane portion of the plastid coupling factor for photophosphorylation, CF$_1$ (cf1A), within BamHI fragments 3 and 24 (Rodermel, S. R., and L. Bogorad, unpublished observation); ps2B (or "PG32") within BamHI fragment 8 (McIntosh, L., and L. Bogorad, unpublished observation); rcL within BamHI fragment 9 (40); the fused gene for the β- and ε-subunits of CF$_1$ (cf1BE) within BamHI fragments 9 and 18 (34); and the duplicated genes for the apoprotein(s) of the chlorophyll–protein complex of photosystem I (CPI)[1] (ps1A1 and ps1A2) within BamHI fragments 17 and 21', respectively (Fish, L., U. Kück, and L. Bogorad, article in press).

Fig. 3 illustrates the results of a typical competitive hybridization experiment designed to localize photogene-containing regions within a cloned BamHI fragment. pZmc527 (Fig. 2A) was digested with EcoRI and co-electrophoresed in the same lane of an agarose gel with a BamHI digest of pZmc518, a plasmid containing BamHI fragments 13 and 17'. Lane a shows the pattern of restriction fragments generated after ethidium bromide staining of the gel; lane b is the "background" hybridization control filter; lane c is the filter prehybridized with 0-h RNA and subsequently hybridized with ^{32}P-labeled 16-h RNA; and lane d is the "nonprehybridization" control filter. The data indicate that: (a) in the control, hybridization of labeled 16-h RNA to BamHI fragment 13 (3.1 kbp), containing r16 (the plastid gene for 16S rRNA), is effectively masked at the concentration of unlabeled 0-h RNA utilized in the prehybridization (lanes d vs. b and c); (b) the 2.4-kbp fragment (BamHI 17') contains a photogene(s) (the slight increase in hybridization intensity, lanes c versus b);

pZmc518·pZmc527

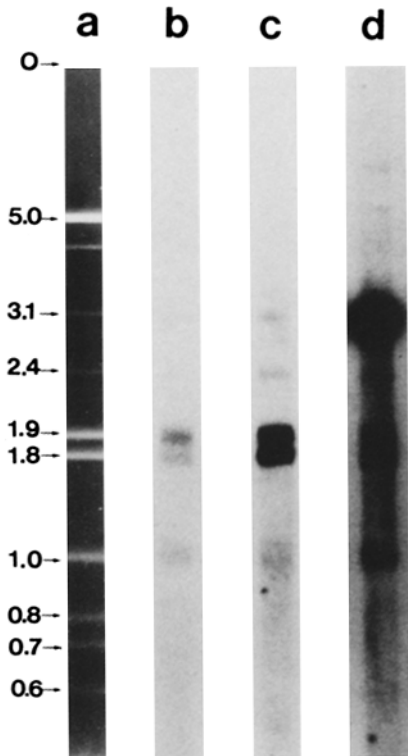a b c d

0—
5.0—
3.1—
2.4—
1.9—
1.8—
1.0—
0.8—
0.7—
0.6—

Figure 3   Localization of photogene-containing regions within pZmc518 and pZmc527 by competitive hybridization. A restriction digest containing 1 μg of pZmc527 (digested with EcoRI) and 0.1 μg of pZmc518 (digested with BamHI) was loaded onto each of four adjoining lanes in a 0.9% Tris-Borate-EDTA agarose gel. (Lane a) Fluorescence of DNA after electrophoresis and ethidium bromide staining of the gel. Numerals indicate the sizes (in kilobase pairs) of plastid DNA-containing fragments: the 1.9- (EcoRI "q"), 1.8- (EcoRI "s"), 1.0-, 0.8-, 0.7-, and 0.6-kbp fragments represent plastid DNA sequences derived from pZmc527; the 5.0-kbp fragment is a chimeric fragment containing 1.0 kbp of BamHI 3 and 4.0 kbp of pBR322; the 3.1- and 2.4-kbp plastid DNA fragments represent maize plastid BamHI fragments 13 and 17', respectively, from pZmc518. (Lanes b–d) Autoradiographs of individual strips cut out of a nitrocellulose filter after transfer of the DNA from agarose and subsequent manipulations. (Lane b) Prehybridized with 650 μg of unlabeled total maize cell RNA from etiolated seedlings and hybridized with 1 × 10⁷ cpm of partially hydrolyzed, kinased 0-h RNA. (Lane c) Prehybridized with 650 μg of unlabeled total maize cell RNA from etiolated seedlings and hybridized with 1 × 10⁷ cpm of partially hydrolyzed, kinased 16-h RNA. (Lane d) No prehybridization, hybridized with 5 × 10⁶ cpm of partially hydrolyzed, kinased 16-h RNA.

and (c) the 1.9- (EcoRI "q"), 1.8- (EcoRI "s"), and 1.0-kbp EcoRI fragments (located adjacent to one another within BamHI fragment 3 [see Fig. 2A]) contain photogene sequences (lanes c vs. b).

In competitive hybridization experiments similar to those shown in Fig. 3, the following regions of the maize plastid genome were found to contain "strongly regulated" photogenes: (a) the 1.3-kbp HpaII–EcoRI and the 0.7-kbp BamHI–HpaII subfragments of BamHI fragment 8 (see Fig. 2B); (b) the 2.7-kbp BglII–BglII and the 0.7-kbp BamHI–BglII subfragments of BamHI fragment 9 (see Fig. 2C); and (c) BamHI fragments 17 and 21' (see Fig. 2D). In addition, BamHI

fragments 18, 20, and 24 were identified as photogene-containing regions of the plastid genome in competitive hybridization experiments (such as in Fig. 1, or such as that shown in Fig. 3); these fragments showed only slight to moderate increases in hybridization intensity with labeled 16-h (vs. labeled 0-h) RNA.

Photogene-containing regions of the plastid genome of Zea mays identified and localized by competitive hybridization, and confirmed in experiments described below, are shown in Fig. 4.

Photogene Transcripts Increase in Abundance Differently during Light-induced Plastid Development

The competitive hybridization experiments showed that several regions of the maize plastid genome contain genes whose transcripts increase in abundance during light-induced plastid development. The next question was whether all the photogenes are regulated on the same schedule, i.e., do the sizes of transcript pools for all photogenes change at the same time? To address this question, each photogene probe was hybridized to a filter containing equal amounts of total maize cell RNA from etiolated and greening seedlings (dot-blot assay). Because rRNA comprises >90% of the plastid RNA pool in etioplasts and greening plastids (9), the amounts of RNA applied to each filter were adjusted (normalized) to equal amounts of 16S rRNA when necessary (to compensate for DNA contamination and/or cytoplasmic RNA excess in one sample vs. another). 16S rRNA pool sizes were estimated for each sample from densitometric scans of autoradiographs, after the hybridization of nick-translated pZmc518 (containing r16) to a Gene Screen filter containing approximately equal amounts of the RNAs of interest. For example, densitometric analysis of the "r16" filter in Fig. 5 shows that the 16S rRNA pool sizes are very nearly the same in these samples of total maize cell RNA; as a consequence, the amounts of these samples applied to each filter were not adjusted.

Fig. 5 shows the results of a typical "dot-blot" experiment in which each photogene probe was hybridized to a filter containing equal amounts of RNA from dark-grown seedlings ("0") illuminated either continuously for 2, 10, 20, 44, or 68 h, or continuously for 20 h followed by 24 (44D) or 48 h ("68D") of darkness. (The chlorophyll content of seedlings sampled from each experimental condition was used as an "index" of the greening process, and the pattern of increase is shown in Fig. 6.) Three general patterns of change in the size of the RNA pools complementary to each photogene probe emerge from densitometric analyses of the data, using several different autoradiographic exposures of each filter (Fig. 7, a–f): (a) the sizes of the RNA pools complementary to the contiguous BamHI fragments 3 and 24 (containing cf0III and cf1A) and to a portion of BamHI fragment 9 within the coding region of the rcL gene increase slightly during very early greening (0–2 h) then increase at an elevated rate until pool sizes reach maxima by ~20 h; the pool sizes then decline to about their preillumination levels, regardless of whether illumination is continued or not. Although the differences are small, the decrease in the sizes of RNA pools in seedlings returned to darkness is consistently more rapid than in seedlings maintained in the light. (b) RNA pools complementary to the contiguous BamHI fragments 17 and 21' (containing ps1A1 and ps1A2, respectively) show a pattern of increase and

FIGURE 4 Positions on the circular *Zea mays* plastid genome of photogene-containing regions identified by competitive hybridization. The restriction endonuclease map is after Bedbrook and Bogorad (6) and Larrinua et al. (36). On the outer circle, recognition sites for BamHI are indicated by black triangles, and individual BamHI fragments by numerals. The locations of the two large inverted repeats, and the map positions of known genes are also shown; the direction of transcription of each sequenced gene or unidentified open reading frame (*UORF*) is indicated by its placement on either the innermost (clockwise) or middle circle (counterclockwise). Sequenced genes whose direction of transcription is unknown are indicated by open boxes, and photogene-containing regions by stippled boxes.
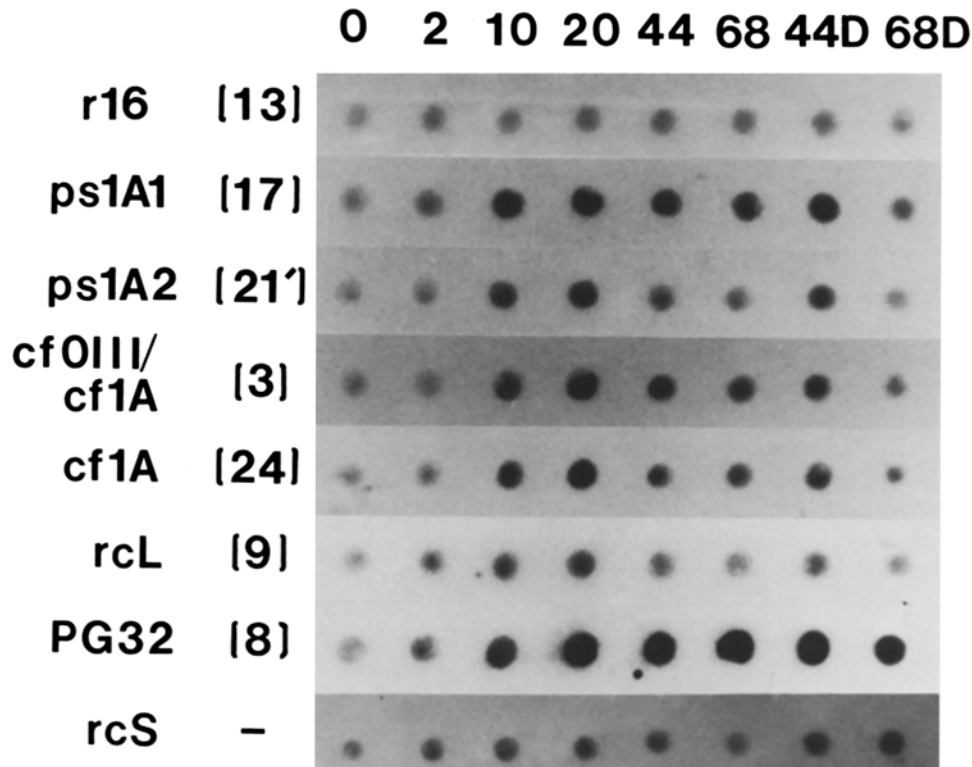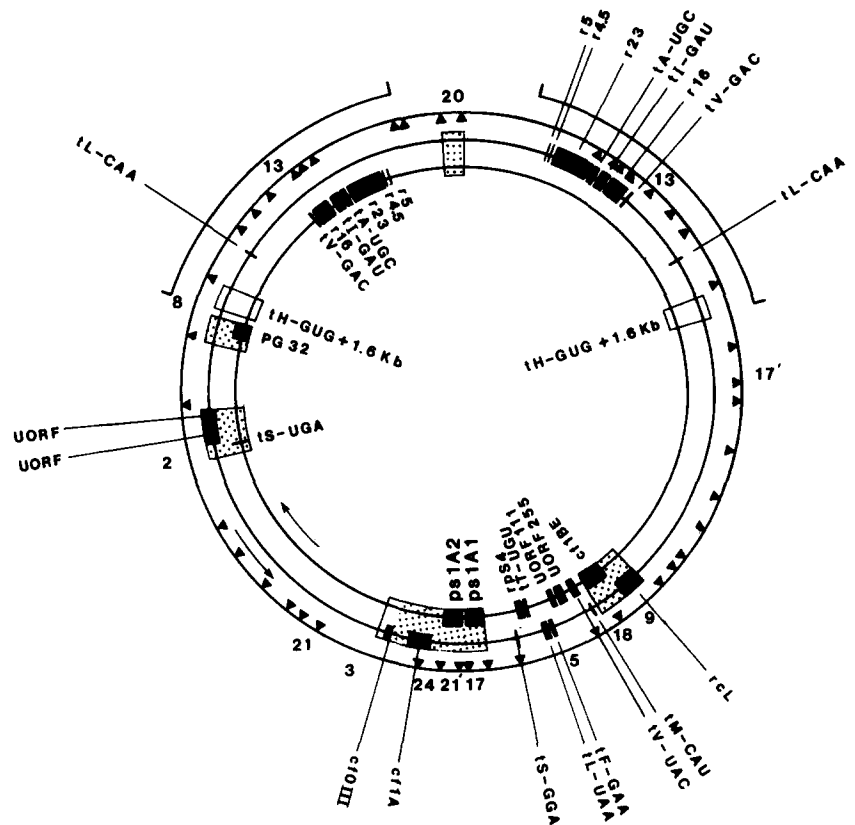

FIGURE 5 Dot-blot analysis of RNA from greening maize seedlings. Gene Screen filters were spotted with 5 μg of each total maize cell RNA sample described in the text. The filters were hybridized with the following nick-translated photogene-containing probes: pZmc556 (BamHI 17), pZmc404 (BamHI 21'), pZmc527 (BamHI 3), pZmc415 (BamHI 24), pZmc461 (containing a 560-bp PstI subfragment of BamHI 9 from within the coding region of the *rcL* gene [see Fig. 2C]), pZmc427 (a portion of BamHI 8 containing the *ps2B* (*PG32*) gene sequence), and pSSU160 (containing the gene for the pea small subunit of RuBPCase [*rcS*]). In addition, one filter was hybridized with nick-translated pZmc518 (containing a copy of the 16S rRNA gene) as a control. The second column gives the cloned plastid BamHI fragment (or portion thereof) used as a probe, and the first column the sequenced gene(s) on each fragment.

FIGURE 6 Total chlorophyll content of the greening seedlings used in Fig. 5 (in micrograms of pigment per gram of fresh weight of tissue).
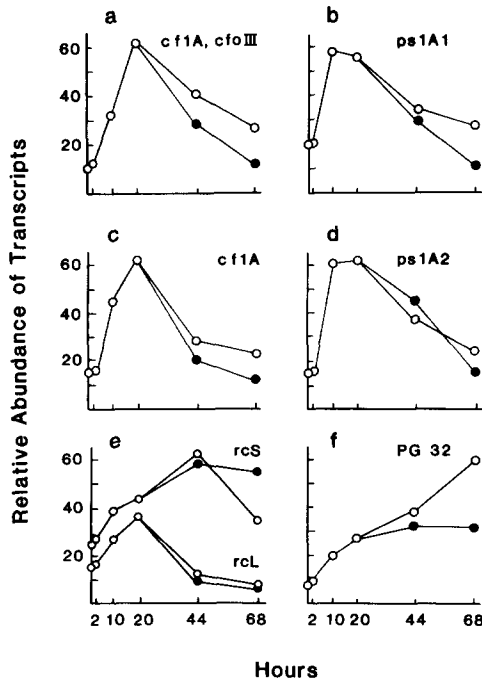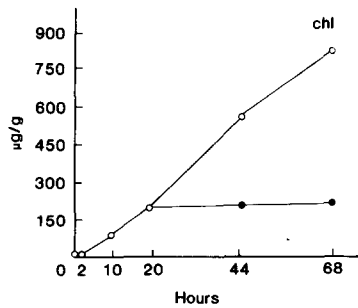

FIGURE 7 The relative abundance of transcripts complementary to photogene-containing regions of the maize plastid genome. Densitometric scans were performed upon several different autoradiographic exposures of each of the dot-blot filters in Fig. 5 to determine the relative abundance of transcripts complementary to a given photogene probe at each time point. (a) pZmc527 (containing cf0III and a portion of cf1A), (b) pZmc556 (containing ps1A1), (c) pZmc415 (containing a portion of cf1A), (d) pZmc404 (containing ps1A2), (e) pZmc461 (containing an internal portion of rcL) and pSSU160 (containing the pea rcS), and (f) pZmc427 (containing ps2B ["PG32"]). Seedlings were maintained in the light (O) or returned to darkness (●) after 20 h illumination. Densitometric analysis of several different autoradiographic exposures of the r16 filter shows that the amounts of 16S rRNA are approximately the same in all samples. Northern hybridization data (Fig. 13) confirm the pattern of change observed for pZmc461 (rcL) and also show that the pattern of change in the cf1BE transcript resembles the pattern of change in transcript pools complementary to the polycistronic genes cf1A and cf0III (within BamHI fragments 3 and 24—see Fig. 7); pools of all transcripts of all three regions reach a maximum size by 20 h illumination and then fall to approximately preillumination levels.

decrease similar to pools complementary to BamHI fragments 3 and 24 and to the rcL gene, except that maximum pool sizes are reached earlier (between 10 and 20 h); the pattern of change in the pool size of transcripts complementary to BamHI 20 resembles that of BamHI 17 and 21′ (data not shown). (c) Unlike the transcript pools of other photogenes, the RNA pool complementary to the plastid DNA fragment cloned in pZmc427 (consisting mainly of the ps2B [PG32] transcript, see Fig. 12) increased approximately linearly (or perhaps biphasically) throughout the 68-h experimental period of continuous illumination. Furthermore, the pool of transcripts remained constant at the 20-h level in seedlings returned to darkness for 24 or 48 h.

These three general patterns of change have been confirmed by other dot-blot experiments using samples of RNA from seedlings greened for 0, 2, 4, 6, and 10 h, or using samples of RNA from seedlings exposed to continuous illumination for 0, 3, 6, 12, 18, 24, and 36 h, or continuous illumination for 12 h followed by a return to darkness for 6, 12, or 24 h (data not shown). These results have also been confirmed by dot-blot experiments that used samples of RNA from seedlings greened under conditions of continuous illumination but at a higher temperature than that used in the present studies (Zhu, Y. S., and L. Bogorad, unpublished); in these experiments, the same patterns of increase and decrease were seen (except for the RNA pool complementary to ps2B, which showed a continual increase), with the exception that the maxima occurred later during greening. These three general patterns of change have also been confirmed by Northern experiments (described below), in which it has been observed that the vast majority of transcripts complementary to all photogene probes show noticable increases by 2 h of illumination, and all photogene transcripts (with the exception of the ps2B mRNA) show a decrease to approximately preillumination levels after 10–20 h of illumination.

Because it was of interest to determine whether or not the transcript pools for the plastid-encoded rcL gene and the nuclear-encoded small subunit gene (rcS) are coordinately regulated during greening, pSSU160 (containing the sequence of the pea rcS gene) was hybridized under low-stringency conditions to a Gene Screen filter containing 5 μg each of the same samples of RNA used in Fig. 5. Densitometric analysis of the rcS filter shown in Fig. 5 (as well as of several other similar filters) reveals that the pool size of transcripts complementary to the rcS gene increases by 2 h of illumination, reaches a maximum after 44 h of illumination, and then declines; the RNA pools in those seedlings returned to darkness after 20 h of illumination continue to increase in size for 24 h, then decline moderately. The data thus indicate that the rcS RNA pool continues to increase long after the rcL pool reaches a maximum and falls much later than the rcL pool (44 h vs. 20 h). The rcS RNA pool also appears to be more dark-stable than the rcL transcript pool.

Based upon the patterns of change observed in the dot blot (and Northern) analyses, photogenes whose RNA pool sizes reach a maximum after either ~10 h, 20 h, or 44 h of illumination before falling to approximately preillumination levels have been classified as either "early-max," "mid-max," or "late-max" photogenes, and those photogenes whose RNA pools show no maximum size (but rather a continual increase) have been designated as "no-max" photogenes. The data thus indicate that the apoprotein(s) of CPI are encoded by early-max photogenes; the large subunit of RuBPCase and the α-, β-ε- (see later, Fig. 13 and 14), and proteolipid subunits of the photosynthetic coupling factor are encoded by mid-max photogenes; the small subunit of RuBPCase by a late-max photogene; and the B protein of photosystem II by a no-max photogene.

Sizes of Transcripts of Photogenes

The competitive hybridization and dot blot analyses have been confirmed and extended by determining the sizes of the transcripts complementary to photogene sequences, mapping transcripts on cloned plastid DNA sequences, and estimating the changes in abundance (in etiolated versus greening seedlings) of each RNA type by Northern analyses. Cloned photogene-containing probes were hybridized to filters containing either the same RNA samples as were used in the dot-blot experiments or plastid RNA enriched samples from etiolated and 16 h-greened seedlings (0-h and 16-h RNA, as in the competitive hybridization experiments). As in the dot-blot experiments, the rough equivalence of the plastid RNA concentrations in the 0- and 16-h enriched RNA samples was assessed by Northern hybridizations with nick-translated pZmc518 ($rl6$) DNA.

BamHI FRAGMENT 3: As shown in Fig. 2A, BamHI fragment 3 contains $cf0III$ and most of $cf1A$, encoding, respectively, the proton-translocating subunit of CF_0 and the α-subunit of CF_1 (Rodermel, S. R., and L. Bogorad, unpublished observations); no long, open-reading frames are found on the noncoding strands of these genes. Fig. 8 shows the hybridization of pZmc527 (containing BamHI 3) to 0-h (lane D) and 16-h RNA (lane L); at least 14 major transcripts are hybridized, ranging in size from 0.85 to 5.0 kilobases (kb). All of these transcripts are more abundant in the 16-h than 0-h RNA samples, but not all change to the same extent; some transcripts increase slightly (the 0.9-, 1.0-, 1.4-, 1.75-, and 3.25-kb mRNAs), others increase moderately (the 0.85-, 2.3-, 3.0-, and 3.15-kb mRNAs), and still others increase a good deal (the 2.55-, 2.65-, 4.0-, 4.5-, and 5.0-kb mRNAs).

In an effort to localize the region(s) of BamHI fragment 3

to which each of the 14 transcripts is complementary (Fig. 9), nick-translated HindIII subfragments of BamHI 3 (see Fig. 2A) were hybridized to filters containing 0-h (lanes a, c, e,
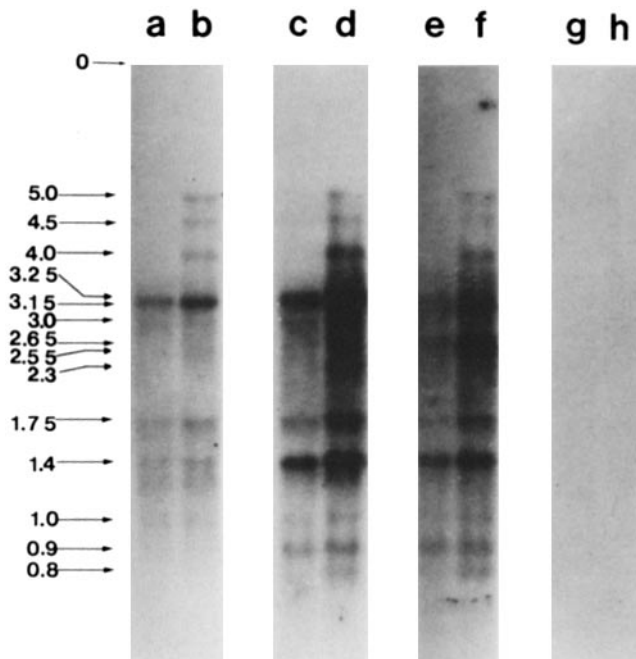

FIGURE 9 Localization of transcripts within HindIII subfragments of BamHI fragment 3. HindIII subfragments of BamHI 3 were generated by digestion of pZmc527 with BamHI and HindIII, electrophoresis, and electroelution (as described in Materials and Methods). Each fragment was then nick-translated and hybridized to a filter containing 0- and 16-h RNA. See text for further details.
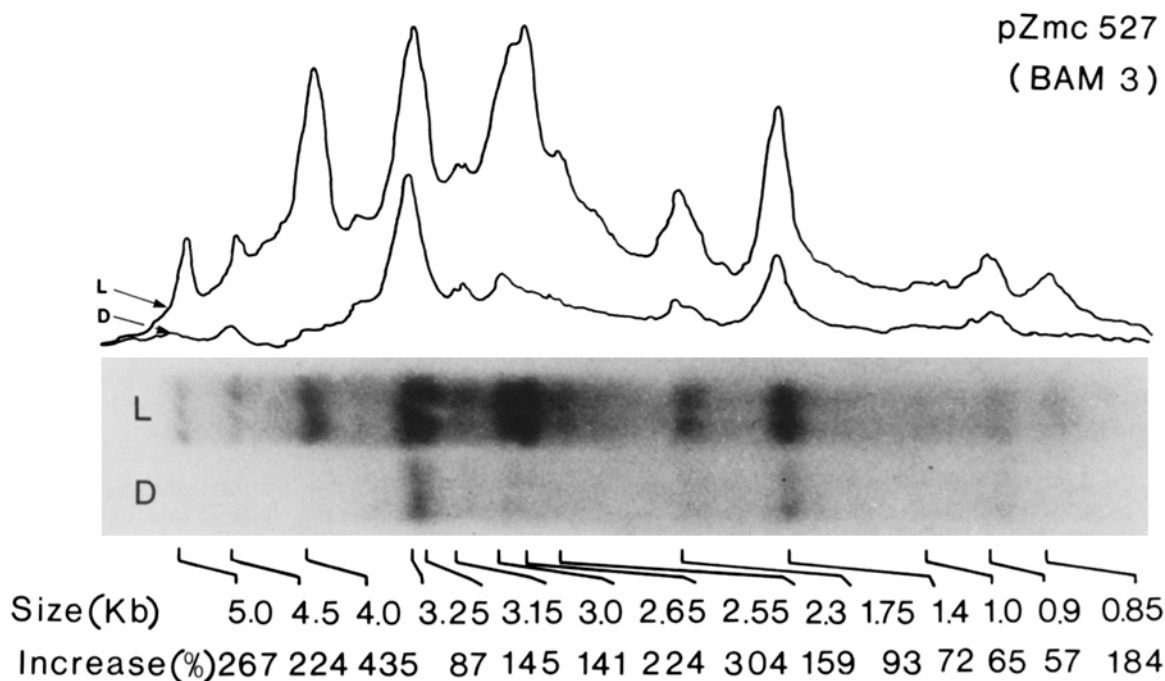

pZmc 527
(BAM 3)

Size(Kb)	5.0	4.5	4.0	3.25	3.15	3.0	2.65	2.55	2.3	1.75	1.4	1.0	0.9	0.85
Increase(%)	267	224	435	87	145	141	224	304	159	93	72	65	57	184

FIGURE 8 Hybridization of nick-translated pZmc527 to 20 μg each of "enriched" plastid RNA from etiolated (lane D) and 16-h greened (lane L) maize seedlings. The Northern analysis was performed as described in Materials and Methods. The size of each transcript (in kilobases) and the percent change in the abundance of each transcript in etiolated vs. 16-h greened seedlings is indicated. This latter number was derived from densitomeric scans of the D and L lanes of numerous autoradiographs of filters such as that shown in the figure by comparing the areas under the respective 0- and 16-h peaks of each transcript. The 0- and 16-h scans resulting from the filter shown in the figure are depicted, with the bottom scan showing the hybridization to 0-h RNA, and the upper scan the hybridization to 16-h RNA.

and *g*) and 16-h RNA (lanes *b*, *d*, *f*, and *h*): lanes *a* and *b* show the hybridization of the 0.25-kbp HindIII–BamHI subfragment; lanes *c* and *d* the hybridization of the 2.9-kbp HindIII subfragment; lanes *e* and *f* the hybridization of the 2.5-kbp HindIII subfragment; and lanes *g* and *h* the hybridization of the 3.0-kbp HindIII–BamHI fragment. The data indicate that some transcripts (either the same transcript or the same-sized transcript) are complementary to all three of the contiguous 0.25-, 2.9-, and 2.5-kbp subfragments (i.e., the 5.0-, 4.5-, 4.0-, 1.75-, 1.4-, and 1.0-kb mRNAs), whereas other transcripts are complementary to either the contiguous 0.25- and 2.9-kbp subfragments (the 3.25-kb mRNA) or the contiguous 2.9- and 2.5-kbp subfragments (the 3.15-, 0.9-, and 0.85-kb mRNAs). No transcripts were complementary only to the noncontiguous 2.5- and 0.25-kbp fragments. Two transcripts were found to be complementary primarily to the 2.9-kbp subfragment (the 3.0- and 2.3-kb mRNAs), and two complementary primarily to the 2.5-kbp subfragment (the 2.65- and 2.55-kb mRNAs). Hybridization to the 3.0-kbp subfragment could not be detected. This assignment of the 14 transcripts complementary to HindIII-generated subfragments of BamHI fragment 3 has been confirmed and extended by the hybridization of nick-translated EcoRI subfragments of BamHI 3 to 0-h and 16-h RNA (data not shown). EcoRI fragments "q" and "s," and the 1.0-kbp EcoRI fragment, are complementary to all, or various combinations, of the 14 transcripts, thus confirming the identification of these as photogene-containing fragments in the competitive hybridization experiments (Fig. 3).

The approximate regions of complementarity of the 14 transcripts of BamHI fragment 3 are depicted schematically

in Fig. 10. The lines representing the transcripts are proportional in length to the sizes estimated from the Northern experiments but their placement is only approximate; we have no evidence to permit precise assignment of the 5' or 3' ends of these transcripts. An extension of the 5.0-, 3.25-, 1.75-, 1.4-, and 1.0-kb mRNAs from BamHI 3 into BamHI 24 is based upon the complementarity of pZmc415 (containing BamHI fragment 24) to transcripts of these sizes in Northern analyses such as those described in Fig. 8 (data not shown). The data thus indicate that *cf1A* and *cf0III* are complementary to 9 and 11 light-stimulated transcripts, respectively, and that the levels of these mRNAs increase differently during light-induced plastid development.

BamHI FRAGMENTS 17, 21', AND 24:   BamHI fragments 17, 21', and 24 were identified as photogene-containing regions of the plastid genome in competitive hybridization experiments; as shown in Fig. 2*D*, these fragments are located adjacent to one another on the chromosome. *psIA1* and *psIA2* (the duplicated genes for the apoprotein[s] of CPI) have been sequenced on BamHI fragments 17 and 21' (24a), and the 3' end of the *cf1A* gene has been sequenced on BamHI fragment 24 (Rodermel, S. R., and L. Bogorad, unpublished observation); no long open-reading frames are found on the noncoding strands of these genes. Fig. 11 shows the hybridization of pZmc556 (containing BamHI fragment 17) to a filter containing 0-h (lane *D*) and 16-h RNA (lane *L*). At least eight major transcripts are hybridized, all of which increase in abundance in the light; some of these transcripts increase a little (the 1.9-, and 3.2-kb mRNAs), while others show moderate (the 1.0-, 1.4-, 1.75-, 3.1-, and 3.4-kb mRNAs) to high (the 4.9-kb mRNA) amounts of increase in 16-h-greened
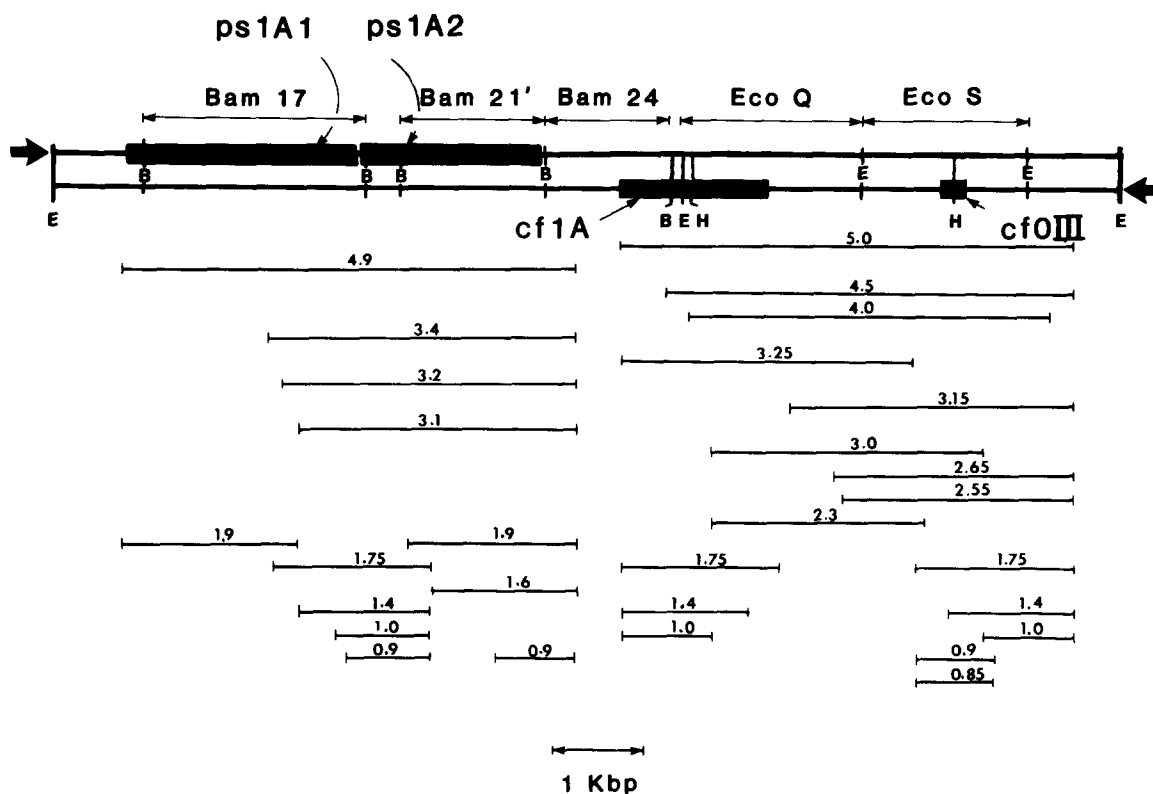

FIGURE 10   Schematic illustration of all transcripts complementary to BamHI fragments 3, 17, 21' and 24; the levels of all these transcripts were found to increase in the light (Figs. 8 and 11). Transcripts of the designated size (in kilobases) are found to be complementary to each of the fragments indicated. BamHI (B), EcoRI (E), and HindIII (H) restriction sites are shown. The directions of transcription of sequenced genes are indicated by bold arrows.
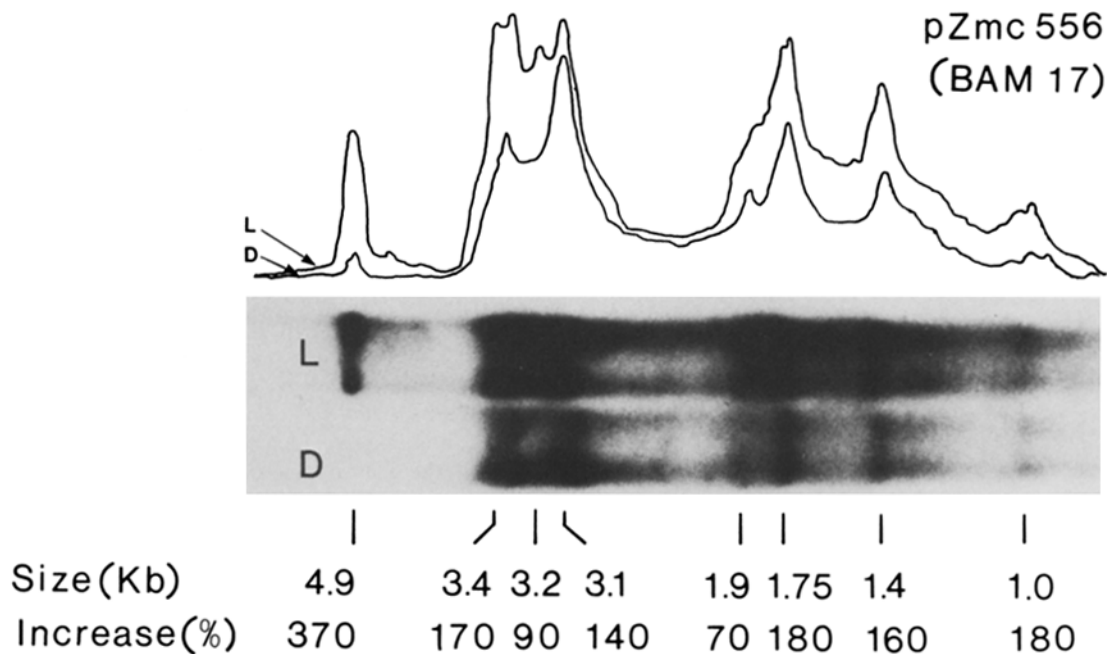
FIGURE 11   Hybridization of nick-translated pZmc556 to 0- and 16-h RNA. See the legend to Fig. 8 for further details.

(vs. etiolated) seedlings. Similar Northern hybridizations of pZmc404 (containing BamHI 21′) and pZmc415 (containing BamHI 24) to filters containing 0-h and 16-h RNA reveal that these fragments are also complementary to multiple transcripts that increase to differing extents in the light (data not shown); on the basis of both similar size and similar levels of increase in 16-h-greened (vs. etiolated) seedlings, many transcripts are common to BamHI fragments 17, 21′, and 24. As depicted schematically in Fig. 10, some transcripts are complementary to all three fragments (the 4.9-, 1.9-, 1.75-, 1.4-, 1.0-, and 0.9-kb mRNAs), several (on the basis of hybridization intensity) are complementary primarily to the contiguous BamHI fragments 17 and 21′ (the 3.4-, 3.2-, and 3.1-kb mRNAs), and one transcript (1.6 kb) is complementary to the contiguous BamHI fragments 21′ and 24. The data thus indicate that *ps1A1* and *ps1A2* are complementary to 9 and 10 light-stimulated transcripts, respectively, and that these mRNAs do not increase in abundance uniformly during light-induced plastid development.

BamHI FRAGMENT 8:   Hybridization of nick-translated pZmc427 (containing a 2.0-kbp portion of BamHI 8) to 0-h and 16-h RNA reveals the presence of two major transcripts whose levels increase during light-induced plastid development (data not shown). One of these is the 1.2-kb *ps2B* transcript, previously localized within this plastid DNA fragment (16), and the other is a 2.5-kb mRNA whose level increases approximately threefold more than that of the *ps2B* transcript in etiolated vs. 16-h-greened seedlings (320 vs. 109%). In an effort to localize the 2.5-kb transcript within pZmc427 (Fig. 12), 0.7- and 1.3-kbp plastid DNA-containing subfragments of the plasmid (see Fig. 2B) were nick-translated and hybridized to 0-h (lanes *a* and *c*) and 16-h RNA (lanes *b* and *d*). Lanes *a* and *b* show the hybridization of the 1.3-kbp HpaII–EcoRI subfragment, and lanes *c* and *d* the hybridization of the 0.7-kbp BamHI–HpaII subfragment. The data indicate, as expected, that the 1.2-kb *ps2B* transcript is complementary to both subfragments (see Fig. 2B), but that the 2.5-kb transcript is complementary only to the 0.7-kbp

subfragment. It is concluded that the 2.5-kb mRNA is not an extension of the 1.2-kb mRNA, but is rather the transcript from a separate photogene whose 5′ or 3′ end lies within the 0.7-kbp fragment. The localization of two major light-stimulated transcripts within both of these subfragments of pZmc427 is consistent with the data from the competitive hybridization experiments (above).

BamHI FRAGMENT 9:   *rcL* and *cf1BE* (the fused gene for the β- and ε-subunits of CF₁) of maize have been sequenced (34, 40) and mapped (see Fig. 2C and 4) within BamHI fragments 9 and 18 (7, 20); 1.6- and 2.2-kb transcripts have been found to be complementary to the *rcL* and *cf1BE* genes, respectively, in greenhouse-grown plants (39). In Fig. 13, nick-translated pZmc37 (containing BamHI 9) was hybridized to a filter containing the same samples of total maize cell RNA as were used in the dot-blot analyses (Fig. 5). Densitometric analysis of the data (Fig. 14) reveals that (*a*) both the 1.6- and 2.2-kb transcripts show slightly increased levels by 2-h illumination; (*b*) both transcripts increase in abundance up to ~20-h illumination, with the *cf1BE* transcript showing an overall increase of ~100%, and the *rcL* transcript an overall increase of ~50%; and (*c*) both transcripts decrease in abundance to approximately preillumination levels after a 20-h illumination, with the rate of decrease in those seedlings returned to darkness greater than the rate of decrease in those seedlings maintained in the light. Another light-stimulated transcript (1.8 kb) showing the same general pattern of increase and decrease as the 1.6- and 2.2-kb mRNAs (i.e., a maximum level at 20 h, followed by a decline) is also evident in Fig. 13; this transcript has been identified as an ~237-bp 5′ extension of the 1.6 *rcL* transcript (19). Densitometric analysis (Fig. 14) reveals that the 1.8-kb mRNA is three- to fourfold less abundant than the 1.6-kb mRNA in etiolated seedlings, and that it shows a noticeably increased level by 2 h of illumination, with an overall increase of two- to threefold in 20-h-illuminated seedlings.

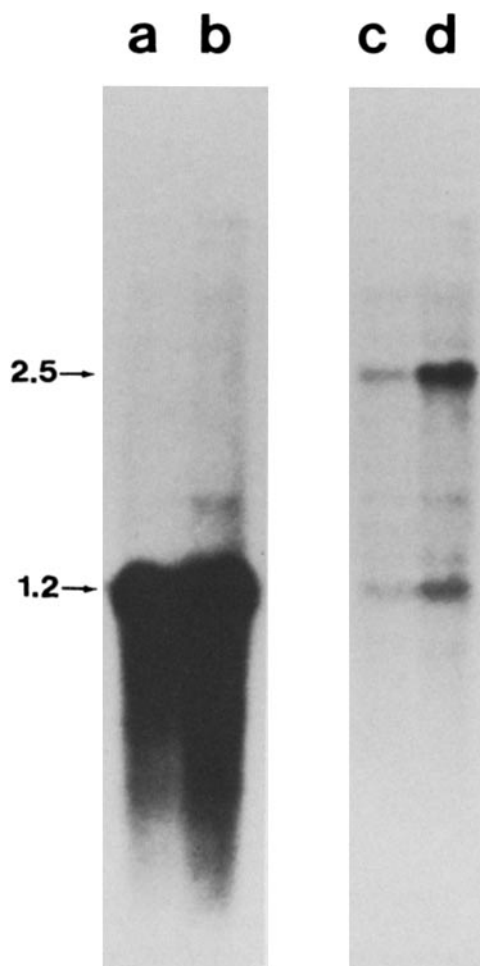BamHI FRAGMENTS 18 AND 20:   Based upon their behavior in competitive hybridization experiments, BamHI

FIGURE 12 Localization of light-stimulated transcripts within pZmc427. The plasmid was digested with BamHI, HpaII, and EcoRI, generating 0.7-kbp BamHI-HpaII and 1.3-kbp HpaII-EcoRI plastid DNA-containing subfragments. After electrophoresis and electroelution, these fragments were nick-translated and hybridized to separate filters containing 0- (lanes a and c) and 16-h (lanes b and d) RNA. (Lanes a and b) Hybridization of the 1.3-kbp subfragment; (lanes c and d) hybridization of the 0.7-kbp subfragment.
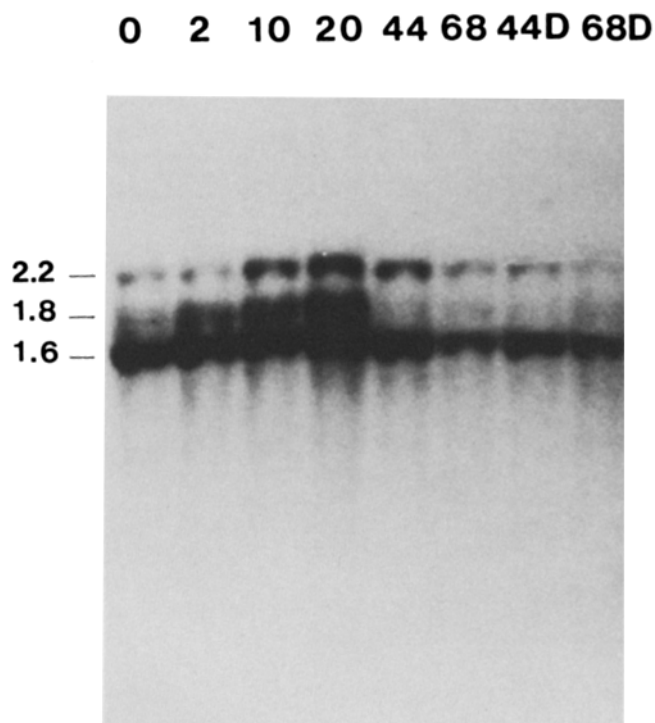

FIGURE 13 The change in the abundance of transcripts (in kilobases) complementary to BamHI fragment 9 during greening. Nick-translated pZmc37 was hybridized to a Gene Screen filter containing RNA from each of the samples described in Fig. 5. RNA samples (20 μg each) were electrophoresed in adjoining lanes of a 1.4% Tris-Borate-EDTA agarose gel before transfer. 2.2-kb RNA, cf1BE mRNA; 1.8- and 1.6-kb RNAs, rcL mRNAs.
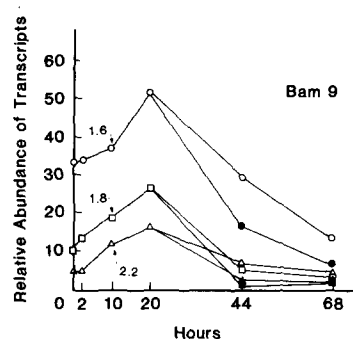

FIGURE 14 The relative abundance of transcripts complementary to BamHI fragment 9 during greening. Densitometric scans were performed upon several autoradiographic exposures of the Northern filter shown in Fig. 13. The relative abundance of each transcript is indicated: the 1.6-kb mRNA (circles), the 1.8-kb mRNA (squares), and the 2.2-kb mRNA (triangles). Seedlings were maintained in the light (open symbols) or returned to darkness (closed symbols) after 20 h illumination.

fragments 18 and 20 were classified as photogene-containing regions of the maize plastid genome. DNA sequence analysis of BamHI fragment 18 reveals that it contains 1.5 kbp of the 3' end of the cf1BE gene and a 700-bp split tRNA^{Val} gene (35)—see Fig. 4. Northern hybridizations of nick-translated pZr48 (containing EcoRI fragment e, which includes BamHI 18) to 0-h and 16-h RNA show that the levels of both the 2.2-kb cf1BE and the 700-bp tRNA^{Val} transcripts increase in the light (data not shown); the increase in the abundance of the former transcript is consistent with the data presented in Fig. 13, while the increase in the abundance of the latter is consistent with the results of Haff and Bogorad (29) showing an increase in the abundance of a plastid-encoded tRNA^{Val} during greening.

Hybridization of nick-translated pZmc569 (containing BamHI fragment 20) to 0-h and 16-h RNA reveals the presence of two transcripts whose levels increase differently during light-induced plastid development (Fig. 15); the level of a 2.4-kb mRNA is ~220% higher, and that of a 1.7-kb transcript ~285% higher, in 16-h-greened vs. etiolated seedlings.

DISCUSSION

If changes in RNA pool sizes are taken at face value, i.e., as reflecting alterations in rates of transcription, the transcription of ~19% of the unique DNA of the maize chloroplast chromosome (i.e., counting DNA in the two large, inverted repeats only once) is stimulated upon illumination of dark-grown maize seedlings. Although we have no direct evidence for or against changes in rates of RNA destruction during greening, RNA pool size reductions for all genes of the early-max, mid-max, and late-max classes must involve degradation. How-

pZmc 569
(BAM 20)

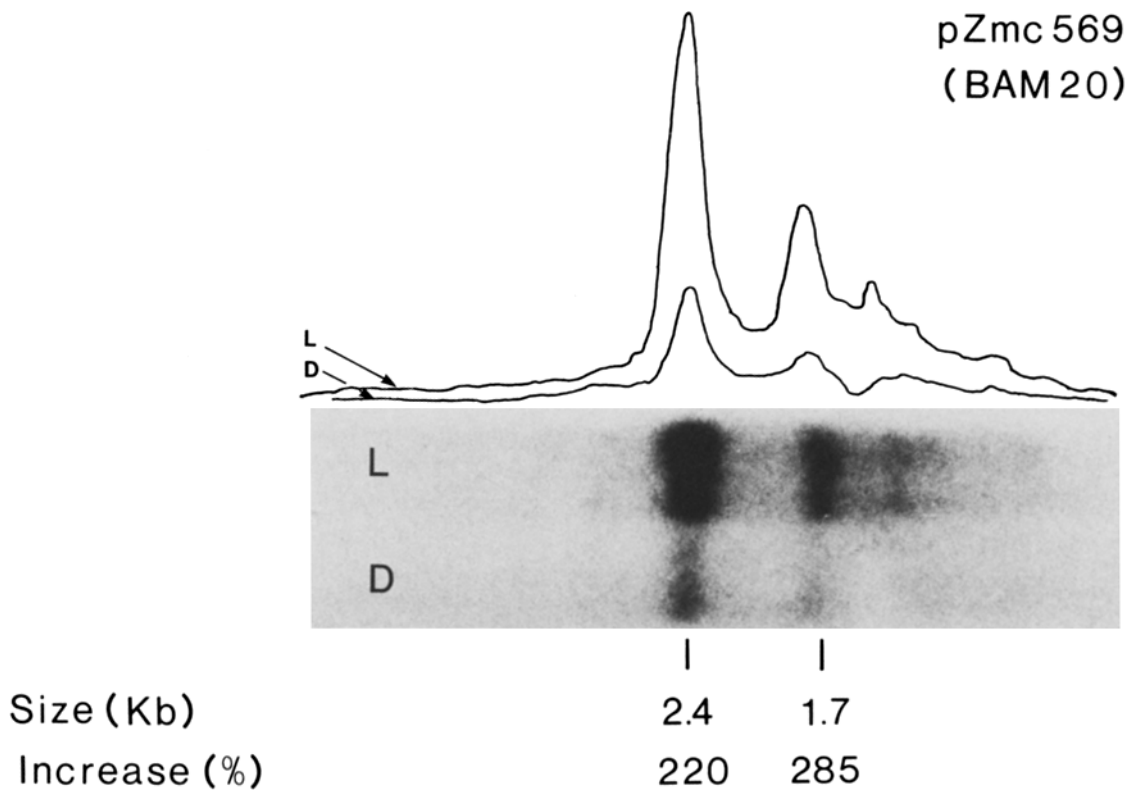| Size (Kb)	2.4	1.7
Increase (%)	220	285

FIGURE 15   Hybridization of nick-translated pZmc569 to 0- and 16-h RNA. See the legend to Fig. 8 for further details.

ever, the following observations make it very hard to explain the present data solely on this basis without postulating increases and decreases in members of complex sets of highly specific RNAases: first, transcripts of ~80% of the maize plastid chromosome do not change in abundance significantly during greening; second, the patterns of change in the sizes of transcript pools are quite different from gene to gene—one especially striking example is the behavior of the *ps2B* mRNA that rises continuously during illumination (in contrast to the increases and decreases in transcript pools for all the other photogenes examined); and, third, transcripts of at least one region of the chromosome fall when dark-grown plants are illuminated (Lukens, J., and L. Bogorad, unpublished observation). Unfortunately, direct pulse-chase experiments are not possible with maize seedlings or leaves.

The data presented here show that photogenes are scattered on the maize plastid chromosome. Photogene regions include some clusters of genes that are transcribed in polycistronic messages and other genes that are transcribed individually. Competitive hybridization data that have identified positively photoregulated regions of the maize plastid chromosome have been confirmed by dot-blot assays and by Northern analyses of specific-sized transcripts. However, we have not employed any independent method to confirm whether competitive hybridization data that identify "constantly expressed" or "photo-neutral" regions are correct except in the case of rRNAs for which a transitory increase of ~10-20% has been observed ~3 h after illumination of etiolated maize seedlings using detailed quantitative measurements (Barton, J., and L. Bogorad, unpublished observation). Thus, it is possible that other (minor) photogenes will be found in the maize plastid genome.

One of the objectives of this investigation was to determine whether photogenes might fit into different classes with regard

to the time of pool size increases, decreases, etc. By our assay methods, we have found that the pools of transcripts of all photogenes increase at about the same time, i.e., sometime within the first 2 h of illumination. These results are highly reproducible but it is difficult to measure the small early changes quantitatively; it has not been possible to distinguish whether there are early and late responses. Thus, from our present data it seems reasonable to conclude that the transcription of photogenes may be stimulated by a single signal, by multiple signals that are all set off at the same time, or by signals separated in time by such short periods that we cannot measure them. Transcription of all of the photogenes shown in Fig. 4 are regulated by phytochrome (Zhu, Y. S., and L. Bogorad, unpublished observation).

One of the most surprising findings of the present study has been that transcript pools for most photogenes reach a maximum size and later, during continuous illumination, drop back to levels that frequently are at or near those in unilluminated plants. According to the times after illumination that their transcript pools reach a maximum size, maize photogenes can be classified as either early-max (showing an early maximum in pool size), mid-max (showing a middle maximum), or late-max (showing a late maximum). Furthermore, within the 68-h time span of our experiments, no maximum could be seen in the level of transcripts for *ps2B*, and thus it can be classified as a no-max (showing no maximum pool size) photogene. The reduction in pool sizes after reaching the maximum may involve positive or negative controls on transcription rates after completion of specific parts of the photosynthetic apparatus, set against the lifetime of specific transcripts. In this context, it is worth noting that the apoprotein(s) of CPI are encoded by early-max photogenes, whereas the α-, β-ϵ-, and proteolipid subunits of the coupling factor are encoded by mid-max photogenes. Moreover, the B protein

of photosystem II is encoded by a no-max photogene, though whether the continual increase in the level of the *ps2B* mRNA is a characteristic of photogene-encoded photosystem II components in general, or is rather due to the need of an expanding thylakoid membrane during greening for the rapidly turned-over *ps2B* gene product (23) has yet to be determined.

A notable feature of eukaryotic cell biology is the great dependence of mitochondria and plastids on products of nuclear genes (e.g., 12, 14). In fact, multimeric organelle components are usually—perhaps always—composed of a mixture of products of nuclear and organelle genes. It would not be surprising to find that the nuclear–cytoplasmic system is a source of signals for the regulation of transcription of plastid photogenes. However, the reduction in the levels of plastid photogene transcript pool sizes at the time of completion of a portion of the photosynthetic apparatus leads us to ask about the behavior of pools of transcripts of nuclear genes for plastid components. The levels of transcripts of *rcS*, the nuclear gene for the small subunit of RuBPCase, and of *rcL*, the plastid gene for the large subunit, rise in parallel upon illumination of etiolated maize seedlings. However, *rcS* transcripts continue to accumulate well after the *rcL* transcript pool declines sharply between the 20th and 44th h of illumination. The *rcS* transcript pool declines only after 44 h of illumination. It seems reasonable to conclude that transcription of *rcS* in the nucleus may be regulated by plastid metabolism.

One finding that opens a number of questions is that transcripts of several discreet sizes are complementary to most maize plastid photogenes. For example, the region of the chromosome bearing BamHI fragments 3 and 24 contains at least two genes—those for the α-subunit of CF_1 and subunit III of CF_0—and all nine transcripts complementary to *cf1A* and all 11 complementary to *cf0III* increase upon illumination of dark-grown seedlings (Figs. 9 and 10); the amounts of increase in these transcripts are not uniform. Comparably, the region of the chromosome bearing BamHI fragments 17 and 21' contain the duplicated genes for the apoprotein(s) of CPI, *ps1A1*, and *ps1A2* (Fish, L., U. Kück, and L. Bogorad, article in press), and all nine different-sized transcripts complementary to *ps1A1* and all 10 complementary to *ps1A2* increase in abundance during light-induced plastid development (Figs. 10 and 11); again, the increases are not uniform. Another, simpler case is that of the two different-sized transcripts complementary to the *rcL* gene of BamHI fragment 9; the level of each in relation to time after illumination is shown in Fig. 13 and 14—the larger transcript is abundant during but not before or after greening. It remains to be demonstrated (*a*) whether the transcripts from a single polycistronic region of the chromosome have common 5' ends and/or common 3' ends (for all photogenes except *rcL*); (*b*) whether the different-sized transcripts result from the use of internal transcription initiation sites and/or termination sites; or (*c*) whether the different-sized transcripts result from processing and/or degradation of the largest transcripts seen. It also remains to be determined whether differences in the relative abundance of certain-sized transcripts during greening reflect regulatory mechanisms for the expression of specific genes within regions that can be transcribed as single polycistronic units (owing to, for example, increased transcription from internal promoters during early greening), or whether the differences are inconsequential and related to rates of RNA destruction (e.g., decreased rates during early greening).

The data presented in this report suggest that a common DNA sequence element and RNA polymerase component might function in the accelerated accumulation of maize plastid photogene transcripts, and that some distinctive sequence signals and regulatory proteins might be involved in the decline of transcription of early- and mid-max photogenes. The DNA sequences of the maize *rcL* and *cf1BE* genes have been reported (34, 40) and those of *ps1A1* and *ps1A2* have been completed (24a). But thus far no common features to account for the apparent simultaneous promotion of transcription have been identified. Detailed analyses of DNA sequences of all maize plastid photogenes await availability of the data. Of particular interest will be relationships between the 5' ends of transcripts that are complementary to the same region of the chromosome and that differ in abundance during photoregulated development and the surrounding gene sequences.

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