Alternative Promoters Are Used for Genes within Maize Chloroplast Polycistronic Transcription Units

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Many chloroplast genes are co-transcribed in polycistronic transcription units that give rise to numerous overlapping RNAs, but the significance of this pattern of transcript accumulation is not understood. An analysis of the transcripts of the adjacent and divergent maize psbE-psbF-psbl-ORF40 and ORF31- petE-ORF42 gene clusters indicates that transcription initiation at alternative promoters contributes to the generation of overlapping RNAs for both clusters. Furthermore, developmentally varying transcript ratios for the ORF31-petE-ORF42 gene cluster are determined at least in part by selective promoter usage. During light-induced plastid maturation, increased levels of primarily monocistronic petE transcripts accumulate from a promoter upstream of the internal petE gene. Dark-predominant and non-light-responsive bi- and tricistronic transcripts result from transcription initiation upstream of ORF31, the proximal gene of the cluster. In addition to the transcriptional overlap within gene clusters, divergent transcription units for the two gene clusters overlap and reciprocal antisense RNAs accumulate. The organization of the transcription units in this region raises the possibility of promoter interdependence or other functional interaction between transcription units.

INTRODUCTION

Many chloroplast polycistronic transcription units are characterized by the accumulation of numerous heterogeneous and overlapping RNAs. Gene sequences can be carried on both mono- and polycistronic transcripts in various patterns of transcript redundancy that are not understood. The overlapping RNAs of a polycistronic transcription unit may accumulate to different steady-state levels, and ratios of transcripts may vary during light-induced plastid development (Rodermel and Bogorad, 1985; Hudson et al., 1987; Rock et al., 1987; Gamble et al., 1988; Westhoff and Herrmann, 1988; Woodbury et al., 1988; for review, see Gruissem, 1989).

The current model used to explain the generation of complex transcript accumulation patterns for co-transcribed chloroplast genes is largely generalized from analyses of the transcription of the psbB-psbH-petB-petD gene cluster. The model assumes that heterogeneous transcripts accumulate as a result of extensive post-transcriptional processing of a large primary polycistronic transcript. A primary precursor transcript for the psbB gene cluster has been identified and mapped by in vitro transcription (Westhoff and Herrmann, 1988) and in vitro capping (Kohchi et al., 1988); multiple mRNA processing events, including splicing, endonucleolytic cleavage, and 5’ and 3’ end-trimming, have been deduced from transcript accumulation patterns for the cluster in maize, tobacco, and spinach (Rock et al., 1987; Tanaka et al., 1987; Westhoff and Herrmann, 1988). Transcripts of other complex transcription units have been mapped (e.g., Hudson et al., 1987; Gamble et al., 1988), but the origins of the transcripts have not been analyzed. A corollary to the transcript-processing model derived from analyses of the psbB gene cluster is the proposal (Gruissem et al., 1988) that developmentally regulated transcript processing or stability, rather than initiation, accounts for developmental changes in the transcript ratios of complex transcription units.

We have analyzed the transcripts of two adjacent and divergent maize gene clusters. The psbE-psbF-psbl-ORF40 cluster encodes three polypeptide subunits of the photosystem II photosynthetic electron transport complex of the thylakoid membrane (Westhoff et al., 1985; Widger et al., 1985; Ikeuchi et al., 1989; Webber et al., 1989; for review of photosystem II, see Rochaix and Erickson, 1988) and one unidentified polypeptide. The divergent ORF31-petE-ORF42 gene cluster encodes a polypeptide subunit of the photosynthetic electron transport cytochrome b₆-f complex (Haley and Bogorad, 1989; for review of the cytochrome b₆-f complex, see Hauska et al., 1983) and two unidentified polypeptides. We find that the 5’ heterogeneity of some of the overlapping transcripts of both gene clusters results from transcription initiation at more than one promoter; other 5’ termini probably derive from the processing of precursor transcripts. During light-induced chloroplast maturation, increased levels of several mono-
cistronic petE transcripts accumulate from a promoter internal to the transcription unit expressing the entire ORF31-petE-ORF42 gene cluster. Thus, developmentally varying transcript ratios within this polycistronic transcription unit are determined in part by selective promoter usage. The overlap of transcription units for the two gene clusters in this region raises the possibility of functional interaction between them, including promoter interactions that may influence transcription.

RESULTS

Transcripts of the Divergent psbE-psbF-psbL-ORF40 and ORF31-petE-ORF42 Gene Clusters

Figure 1 shows the gene organization and the position in the maize chloroplast genome of the divergent psbE-psbF-psbL-ORF40 and ORF31-petE-ORF42 gene clusters. A series of DNA probes spanning the 4.0-kb region containing the divergent, adjacent gene clusters was used for RNA gel blot analyses of transcript accumulation in maize seedling leaves. Figure 2 shows RNA gel blots of transcripts complementary to DNA probes A to H, which accumulate in leaves of dark- and light-grown seedlings ("D" and "L" lanes, respectively, top panel). The diagram in Figure 2 (bottom panel) indicates the approximate size, location, and relative abundance of the detected transcripts as well as their relative accumulation in dark- and light-grown seedling leaves. The transcripts are assigned to groups I to VI based on the positions of their 5' termini as established by RNA gel blot and S1 nuclease mapping analyses shown in Figures 2 and 3, respectively. More than 25 transcripts of varying abundance and varying ratios of accumulation in the leaves of dark- and light-grown plants hybridize to this region. The eight transcripts in groups II and III contain sequences of the psbE-psbF-psbL-ORF40 gene cluster, whereas the 17 transcripts in groups I, IV, V, and VI contain sequences of the ORF31-petE-ORF42 cluster. Two transcripts in group VII map downstream of ORF42 and are not analyzed here. The tRNAs are transcribed divergently from the ORF31 gene cluster.

The 1.0-kb intergenic sequence between the proximal genes of the divergent clusters, psbE and ORF31, is transcribed from both strands and is carried on several dark-predominant, low-abundance transcripts that traverse the entire region in both directions and proceed through the flanking coding regions of both clusters (Figure 2, probes B to D, transcript groups I and II). Thus, the initial 1.0-kb leader sequences of the overlapping group I and II transcripts are reciprocal antisense RNAs that accumulate primarily in the etioplasts of dark-grown leaves. After dark-grown seedlings have been illuminated for about 72 hr, these transcripts decrease in abundance to levels fourfold to fivefold lower than those found in dark-grown leaves and similar to the levels in chloroplasts of light-grown leaves (Figure 2, probes B to D, compare "D" and "L" lanes; greening series not shown).

A number of more abundant and overlapping (same-sense) transcripts hybridize to the coding regions of the two clusters. The six tetracistronic transcripts of group III (Figure 2, diagram) contain the entire psbE gene cluster and have 5' ends that map near the proximal psbE gene at a position approximately 1 kb downstream of the 5' termini of the group II psbE cluster transcripts. The major transcript of group III, the 1.1-kb transcript, accumulates to about the same level in leaves of both dark- and light-grown plants (probes A and B) and exhibits only a minor
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Figure 2. RNA Gel Blot Analyses and Schematic Diagram of Transcripts.

Total leaf RNA (5 µg) isolated from dark-grown (D) or light-grown (L) maize seedling leaves was separated by gel electrophoresis and analyzed with a series of double-stranded DNA probes (A to H) containing different regions of the two gene clusters (shown at top and center). The schematic diagram (bottom) shows transcripts organized into groups I to VII based on the positions of their 5’ termini and their direction of transcription [determined by single-stranded M13 probes (data not shown) and designated by arrows]. Sizes of transcripts (in kilobases) are shown at left (for psbE-psbF-psbL-ORF40 gene cluster transcripts transcribed to the left) or at right (for ORF31-perE-ORF42 cluster transcripts transcribed to the right). The approximate relative abundance of transcripts is indicated by the thickness of arrow lines and the size of circles representing 5’ transcript termini, both of which increase with increased relative abundance. The 5’ circle also indicates whether a transcript accumulates predominantly in dark-grown leaves (●), predominantly in light-grown leaves (○), or to similar levels in both dark- and light-grown leaves (●).
Figure 3. **S1 Nuclease Protection Fine-Mapping Analysis of the 5' Termini of Transcripts.**

Total leaf RNA (50 μg) isolated from either dark-grown (DARK) or light-grown (LIGHT) maize seedling leaves was hybridized with excess 5‘ end-labeled DNA probe as shown. Protected fragments were mapped against sequencing ladders; 5‘-terminal bases of mapped transcripts are aligned with the complementary ladder sequences.

(A) 1.1-kb psbE gene cluster transcript.
(B) Light-induced group V transcripts.
(C) 1.35-kb group IV transcript (?).
(D) Dark-predominant group / transcripts.
(E) 0.53-kb and 1.35-kb group IV transcripts.
(F) 0.26-kb group VI transcript.

1985), *Oenothera* (Carrillo et al., 1986), and wheat (Webber et al., 1989). Figures 3A and 4A show that the 5‘ terminus of the maize 1.1-kb transcript maps to a position 138 nucleotides (nt) upstream of the psbE initiation codon near sequences resembling chloroplast promoter elements. A similar 5‘ terminus has been established for the wheat 1.1-kb transcript (Webber et al., 1989). The proximity of promoter-like sequences suggests that the mapped 5‘ terminus represents the transcription initiation site of the 1.1-kb transcript.

Northern-Cross hybridization analysis (Graham et al., 1986) of in vitro capped chloroplast and etioplast RNA was used to identify and map primary transcripts. The hybridization of in vitro capped (32P-GTP-labeled), electrophoretically separated transcripts to unlabeled DNA probes permitted the identification of cappable transcripts by the simultaneous determination of their sizes, map positions, and approximate locations of their capped 5‘ termini. Figure 5A shows that the major 1.1-kb psbE gene cluster transcript can be capped in vitro and is, therefore, a primary transcript initiated at the site of its mapped 5‘ terminus: an abundant, capped 1.1-kb chloroplast transcript hybridizes uniquely to chloroplast DNA probes 1 and 2, which contain the psbE gene cluster (Figure 5A, −RNase panel; probes shown in 5D); the capped 1.1-kb transcript co-migrates with the 1.1-kb psbE cluster transcript detected by probe 1 in a standard RNA gel blot assay (at left). In the second part of the experiment (shown in Figure 5A, +RNase panel), the 5‘-labeled capped terminus of the 1.1-kb transcript is seen to be protected from RNase digestion by hybridization with DNA probe 2, which extends upstream of probe 1 and contains the region of the S1-mapped 5‘ terminus of the 1.1-kb group III transcript, but not by hybridization with DNA probe 1 lacking this 5‘
Figure 4. Nucleotide Sequences (RNA-Like Strand) Containing S1 Nuclease Protection Mapped 5’ Transcript Termini of the Two Gene Clusters.

Arrows above the sequence indicate the mapped termini shown in Figure 3. Sequences resembling “−35” and “−10” chloroplast promoter elements (Hanley-Bowdoin and Chua, 1987) are underlined. The heptanucleotide sequence ATGTA/TATT located near processed 5’ termini is doubly underlined. Nucleotides are numbered in (A) and (C) beginning with the first base of the group III and group I transcripts, respectively.

(A) Sequence upstream of the psbE gene containing the 5’ terminus of the major 1.1-kb group III transcript; the BamHI site used for S1 mapping (Figure 3A) is shown.

(B) Sequences in the region of the adjacent ORF31 and petE genes containing 5’ termini of group IV and the group V light-induced transcripts.

(C) Sequence containing the 5’ terminus of dark-predominant group I transcripts; the BglII site used for S1 mapping (Figure 3D) is shown. At top is shown the sequence of the opposite strand containing the 5’ terminus of the divergent 1.1-kb group III transcript for the psbE gene cluster [(A)].

(D) Sequence upstream of the ORF42 gene containing the 5’ terminus of the 0.26-kb group VI transcript.

Figure 6. Nucleotide Sequences (RNA-Like Strand) Containing S1 Nuclease Protection Mapped 5’ Transcript Termini of the Two Gene Clusters.
Figure 5. Northern-Cross Hybridization Analysis of in Vitro Capped Chloroplast and Etioplast RNA.

Capped, $^{32}$P-GTP-labeled RNA (75μg) was fractionated by gel electrophoresis and blotted without fixing onto a GeneScreen membrane; uncapped (unlabeled) total leaf RNA from dark-grown (D) or light-grown (L) seedlings was separated simultaneously in a companion lane and analyzed with labeled DNA probes as for standard RNA gel blots. (RNA gel blots are shown aligned at the left of each hybridization analysis above.) Unlabeled DNA probes 1 to 6, containing regions of the two gene clusters, were separated on a second gel, blotted, and fixed to Zeta-Probe membranes. Contact hybridization of the two membranes, oriented at right angles to each other, permitted the diffusion of labeled, capped RNAs and their hybridization to the fixed DNA probes. After autoradiography of the “cross-hybridized” blot, the membrane was treated with RNase A to digest the capped 5’ termini of transcripts that were not protected because the hybridizing DNA probes lacked sequences complementary with the termini; cap signals remaining after RNase treatment are considered indicative of hybridization-protected 5’ caps.

(A) Autoradiogram of capped chloroplast RNA “cross-hybridized” to DNA probes 1 and 2 before (−RNase) and after (+RNase) RNase A treatment of the membrane; RNA gel blot aligned at left is assayed with labeled DNA probe 1.

(B) Autoradiogram of capped chloroplast RNA “cross-hybridized” to DNA probes 3 and 4 before (−RNase) and after (+RNase) RNase A treatment of the membrane; RNA gel blot aligned at left is assayed with labeled DNA probe 4.

(C) Autoradiogram of capped etioplast RNA “cross-hybridized” to DNA probes 2, 5, and 6 before (−RNase) and after (+RNase) RNase A treatment of the membrane; RNA gel blot aligned at left is assayed with labeled DNA probe containing the psbE-ORF31 intergenic region (corresponding to probes B to D, Figure 2).

(D) Map of gene organization, indicating subregions contained in DNA probes 1 to 6 used in the “cross-hybridization” analyses shown in (A) to (C). The S1-mapped origins of group I, group III, and group V transcripts are shown (see Figures 3 and 4). Probes 1 and 2 contain the psbE gene cluster; they differ in length by a region containing the mapped 5’ termini of group I and group III transcripts ($\Delta = 360$ bp).
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**Figure 6.** Schematic Diagram of Locations of Promoter Regions Deduced from Transcript Mapping and Capping Analyses.

PI, PV, and PIII represent promoters shown to exist by the capping of members of transcript groups I, V, and III, respectively (Figure 5). PII and the tRNA promoter are implied by the existence of group II transcripts and the tRNAs, respectively (Figure 2).

existence of an upstream promoter (PII) (not mapped here) that initiates transcription of the group II transcripts through the sequence of the downstream PIII promoter.

**Group V and Group I ORF31 Gene Cluster Transcripts Arise by Transcription Initiation**

The S1 nuclease protection analysis shown in Figure 3B maps a heterogeneous 5' terminus with a light-induced protection pattern approximately 40 nt upstream of the petE gene (Figure 4B). This position corresponds to the location of the 5' terminus of group V light-induced transcripts as established by RNA gel blot analyses (Figure 2, probe E, and additional probes not shown). Another weakly light-induced 5' terminus maps approximately 45 nt upstream of the strongly light-induced terminus (Figures 3C, 4B); this terminus may correspond to the origin of a second 1.35-kb group IV transcript that maps in this general region according to RNA gel blot analysis (Figure 2, increase in signal at 1.35 KB for probes F to H over signal for probe E). Promoter-like sequences, including several overlapping

"-10" as well as redundant "-35" elements, are found upstream of both termini. Figure 5B shows that the abundant 0.22-kb light-induced group V transcript is capable in vitro and is, therefore, a primary transcript that arises at a promoter in front of the petE gene. A capped chloroplast transcript that co-migrates with the 0.22-kb light-induced transcript (shown in the RNA gel blot at left) hybridizes to DNA probes 3 and 4, which contain the petE gene (Figure 5B, -RNase panel); its 5'-labeled capped terminus is protected from RNase digestion by hybridization to DNA probe 4, which contains the region of the S1-mapped light-induced terminus (Fig. 5B, +RNase panel), but not by hybridization to DNA probe 3, which lacks this region. Figure 5B shows three other less abundant, larger, in vitro cappable chloroplast transcripts that also map to this region and that correspond in size to transcripts mapped at this location by RNA gel blot analysis. These include two other group V transcripts, the 0.95-kb transcript and probably the low-abundance 0.55-kb transcript (Figure 2, probe F), and a 1.35-kb transcript that may arise just upstream of the strongly light-induced transcripts or may represent a co-migrating light-induced transcript. None of the four capped transcripts hybridizes to DNA probes specific for ORF31, and only the 1.35-kb capped transcript hybridizes to ORF42 coding sequences (data not shown). We conclude that there is at least one functional promoter (PV) in this region (shown in Figure 6) from which the 0.22-kb transcript and probably several other light-induced group V transcripts encoding primarily the petE gene are transcribed. The PV promoter is internal to the transcription unit(s) that promote the synthesis of group I and group IV transcripts through the PV promoter region. The heterogeneity of the mapped light-induced terminus representing the origin of PV transcription initiation (Figure 3B) may result from multiple initiation sites, although 5' end mRNA trimming or experimental artifact cannot be ruled out.

Figure 3D shows that the dark-predominant, low-abundance group I transcripts encoding the ORF31 gene cluster (Figure 2, probes B to D) share a 5' terminus that maps by S1 nuclease protection to a position 3 nt from the 5' terminus of the divergent 1.1-kb primary group III transcript containing the psbE gene cluster (Figure 4C). Redundant promoter-like sequences lie immediately upstream of the mapped terminus. A Northern-Cross hybridization analysis of in vitro capped etioplast RNA (Figure 5C, -RNase panel) detects two cappable transcripts corresponding in size to the group I dark-predominant 2.6-kb and 1.55-kb transcripts (shown in the RNA gel blot at left in Figure 5C) that are not detected in capped chloroplast RNA (compare the

**Figure 5.** (continued).

Probes 3 and 4 contain a portion of the ORF31 gene cluster including the petE gene and downstream sequences; they differ in length by a region containing the mapped 5' terminus of the light-induced group V transcripts that extends 120 nt upstream of the petE gene (Δ = 155 bp). Probes 5 and 6 contain the entire ORF31 gene cluster; they differ in length by a region containing the mapped 5' terminus of group I transcripts (Δ = 230 bp).
hybridization of capped etioplast RNA to DNA probe 2 in Figure 5C with that of capped chloroplast RNA to the same probe 2 in Figure 5A). The 5' labeled cap of the 2.6-kb etioplast transcript is largely protected from RNase digestion by hybridization with DNA probes 2 and 5, which contain the region of the mapped dark-dominant terminus of the group I transcripts, but is unprotected by hybridization with DNA probe 6 lacking this sequence (Figure 5C, +RNase panel). The cap signal of the 1.55-kb transcript hybridized to probe 6 is not abolished by RNase treatment, but it is somewhat diminished by comparison to the 1.55-kb signal protected by hybridization with probes 2 and 5; secondary RNA structure may protect the cap from RNase digestion or there may be more than one co-migrating primary 1.55-kb transcript. We conclude that at least one dark-dominant group I transcript (2.6 kb) is initiated at a promoter (PI) (Figure 6). Figure 4C shows that the PI promoter may be located in the region of DNA transcribed from the divergent PII promoter to yield the initial bases of the 1.1-kb psbE cluster primary transcript. Thus, adjacent and divergent PI and PII promoters for the two gene clusters may each initiate transcription within a region complementary to the promoter sequence of the other.

5' Processing of Transcripts Encoding the ORF31-petE-ORF42 Gene Cluster

Several major non-light-responsive or dark-dominant ORF31 cluster transcripts are not capped in vitro, and their 5' termini, therefore, may well be determined by post- or co-transcriptional processing. Transcripts in groups IV and VI, whose 5' termini map upstream of the ORF31 and ORF42 genes, respectively, fall into this category. Figures 3E and 3F show the major 5' termini mapped in these regions of the gene cluster by S1 nuclease protection. RNA gel blot analyses with probes extending upstream from within the ORF31 gene (data not shown) allow the termini mapped in Figure 3E to be assigned to the 0.53-kb and one of the 1.35-kb group IV transcripts (Figure 4B). The terminus mapped in Figure 3F corresponds to the 0.26-kb group VI transcript encoding ORF42 (Figure 4D). Upstream promoter-like sequences are not found near the 5' termini of these transcripts. Although RNA gel blot analyses clearly indicate that a 1.55-kb dark-dominant transcript must also originate in the region just upstream of the ORF31 gene (Figure 2, increase in signal at 1.55 kb for probes E to H over that for probes B to D), no dark-dominant terminus was identified by S1 nuclease or primer extension mapping. RNA secondary structure may interfere with the mapping of this terminus.

The heptanucleotide sequence ATGA/TATT is found in the DNA sequence near the mapped locations of the 5' termini of major noncappable transcripts as well as near other minor S1-mapped termini (double-underlined sequences in Figures 4B and 4D). The heptanucleotide is not found elsewhere in the 4.0-kb DNA region, and its correlation with the position of 5'-processed transcript termini suggests that it may have a role in their processing. This sequence has some resemblance to the hexanucleotide YGGAA/TY associated with the 5' termini of psbB gene cluster transcripts thought to be generated by endonucleolytic cleavage (Westhoff and Herrmann, 1988).

DISCUSSION

The results presented here indicate that the multiple heterogeneous transcripts that encode chloroplast gene clusters can arise by transcription initiation from more than one promoter. This is a new finding for chloroplast gene transcription and it not only has interesting implications for the expression of the genes analyzed here but also permits a refinement of the operative concept of the chloroplast polycistronic transcription unit. Each of the gene clusters we describe is not transcribed solely as one large transcription unit defined by a single proximal promoter but rather as overlapping units defined by tandem promoters. The use of more than one promoter for the transcription of chloroplast genes may be unique to the expression of the two gene clusters analyzed here but it seems likely to occur more generally. Thus, it may be instructive to analyze the transcript families within other polycistronic transcription units. This would include, for example, such transcripts as the internal light-induced transcripts of the psbD-psbC cluster (Gamble et al., 1988) and the families of transcripts arising upstream of atpI and atpH in the rps2-atpl-atph-atpF-atpA cluster (Rodermel and Bogorad, 1985; Hudson et al., 1987).

For the ORF31-petE-ORF42 gene cluster, where transcript ratios vary during the maturation of chloroplasts induced by the illumination of dark-grown seedling leaves, there is a general correlation between promoter usage and the developmental pattern of accumulation of the transcripts. That is, transcripts that accumulate predominantly in the etioplasts of dark-grown leaves arise from proximal promoters, whereas at least one major transcript that becomes more abundant during light-induced chloroplast maturation originates at a distal promoter. The major light-induced transcript encodes the internal petE gene, and—although we have no direct evidence on this point—its accumulation may serve to uncouple the expression of petE from that of ORF31 and ORF42 during and following plastid maturation. It has been suggested that the light-induced accumulation of barley chloroplast psbD-psbC transcripts during plastid maturation is required to maintain translation of the psbD and psbC gene products in mature chloroplasts (Gamble et al., 1988). A similar case may obtain for the expression of the 4-kD cytochrome-b}_{6}-f polypeptide encoded by petE.
It is less clear how dual promoter usage may influence the expression of genes in the psbE-psbF-psbL-ORF40 cluster. However, an intriguing characteristic of the transcripts that accumulate from the upstream PI promoter is their complementarity, over their initial 1 kb, to transcripts initiated at the divergent PI promoter of the ORF31 cluster. It is not known whether double-stranded RNAs of the complementary transcript sequences form in vivo; if so, they may provide a functional link between the two overlapping transcription units that is in some way involved in their regulation.

The light-induced increase in accumulation of primary transcripts from the internal promoter of the ORF31 gene cluster may result from developmental regulation of either transcript initiation or transcript stability. Currently, it is not known how chloroplast promoters are regulated or to what extent the specific regulation of initiation at a promoter determines the levels and patterns of transcript accumulation. It has been proposed that the differential developmental accumulation of chloroplast transcripts can be accounted for entirely by changes in transcript stabilities due to the interaction of developmentally regulated proteins with stem-loop structures at the 3' transcript termini (Gruissem et al., 1988). The shortest and most abundant of the light-induced family of petE transcripts, the 0.22-kb transcript, may contain a predicted stem-loop structure at its 3' end (ΔG = −20.9 kcal) that could function in such a regulatory scenario for differential stability. However, other light-induced transcripts do not contain stable predicted stem-loop structures near their 3' termini. If differential stability accounts for the light-induced increase in accumulation of group V transcripts, the mechanism for conferring this stability might be more likely to involve the 5' sequences (or secondary RNA structures) that are common to all the transcripts. There are, in fact, data from other systems that indicate that 5' transcript sequences may influence transcript stability (for review, see Braverman, 1989).

The accumulation of the petE gene-encoded 4-kD polypeptide during greening (Haley and Bogorad, 1989) parallels rather closely the accumulation of the light-induced family of primary petE transcripts. There is a relatively low level of the petE polypeptide in etioplasts, despite the association of many petE-encoding transcripts with polynes (data not shown). The petE gene is distal to the ORF31 gene on all the transcripts that accumulate in etioplasts except the relatively scarce group V monocistronic transcripts. Assuming that the 4-kD polypeptide is not turned over rapidly in etioplasts, the low level of its accumulation may be due to inefficient translation from transcripts on which it is not the proximal gene.

The extensive overlapping of transcription units in the region of the two chloroplast gene clusters analyzed here raises several possibilities of promoter-promoter interactions that may regulate transcription initiation. In both prokaryotic and eukaryotic systems, interactions between tandem or adjacent divergent promoters have been shown to regulate transcription initiation and affect transcript abundance (Adhya and Gottesman, 1982; Proudfoot, 1986; Biswas and Getz, 1988). These interactions include the steric hindrance of RNA polymerase binding at a promoter by its binding at a closely situated promoter and the inhibition of a promoter by transcription through it. In the region analyzed here, the adjacent promoters for group I and group III transcripts (PI and III, Figure 6) appear to have mutually exclusive RNA polymerase binding sites that might preclude the simultaneous use of both promoters on the same individual template. Furthermore, transcription through the distal promoters of both gene clusters (PIII and PV) might be expected to interfere with initiation at these promoters. Simultaneous convergent transcription of group I and group II transcripts and of the tRNAs and ORF31 cluster transcripts might also be expected to be incompatible. It is possible, however, that if promoter-promoter interactions do occur, they may operate differently in plastids containing many copies of a genome than in cells having unicity genomes. For example, interference between plastid promoters may play a role in determining how many individual chloroplast DNA templates in the total multicopy population are used for transcription at each promoter. It is not known whether all chloroplast chromosomes are functionally equivalent with respect to the transcription of any one gene or cluster at any one time.

METHODS

DNA Sequence

The DNA sequence of both strands of the maize chloroplast DNA fragment BamHI 15' (Larrinua et al., 1983; derived from plasmid pZmc503) and two neighboring BamHI fragments of 320 bp and 835 bp (Figure 1) was determined by chemical cleavage (Maxam and Gilbert, 1980) and dideoxy chain termination (Sanger et al., 1977). The full sequence has been deposited in the GenBank© EMBL Data Bank (accession no. J04502); partial sequences are shown in Figure 4. The tmP, tmW, and petE sequences have been published elsewhere (Lukens and Bogorad, 1988; Haley and Bogorad, 1989).

RNA Preparation and Gel Blot Analysis

Total leaf RNA was prepared from leaves of maize [Zea mays (FR9 cms x FR37) Illinois Foundation Seed] seedlings grown for 7 days in darkness or in the greenhouse. The apical 5 cm of leaves were harvested into liquid nitrogen and extracted with 4 M guanidinium thiocyanate (Mariat et al., 1982). RNA was separated on 1.2% agarose Mops-formaldehyde gels and transferred to Zeta-Probe membranes in 50 mM NaOH. RNA gel blots were hybridized with DNA probes labeled by random hexamer priming (Pharmacia LKB Biotechnology Inc.) in 250 mM sodium phosphate (pH 7.2), 7% SDS, and 1 mM EDTA at 65°C for 16 hr to 30 hr.
S1 Nuclease Protection Assays

S1 nuclease protection of the 5’ transcript termini was performed using kinased double-stranded (ds) DNA probes. Total leaf RNA was hybridized with excess denatured dsDNA probe in 80% formamide hybridization buffer (Favoloro et al., 1980). The RNA-DNA hybrids were treated with S1 nuclease, and protected DNA fragments were sized on denaturing acrylamide gels next to chemical cleavage sequencing ladders of the 5’ end-labeled protecting probes.

In Vitro Capping of RNA and Northern-Cross Hybridization Analysis

Etioplast or chloroplast RNA (75 μg) was capped in vitro in a 40-μL reaction mixture containing 50 mM Tris-HCl (pH 7.9), 1.25 mM MgCl₂, 6 mM KCl, 2.5 mM DTT, 80 units of RNasin, 350 μCi of α-33P-GTP (3000 Ci/mmol, Du Pont-New England Nuclear), and 10 units of guanyltransferase (Bethesda Research Laboratories) for 60 min at 37°C. A Northern-Cross hybridization method (adapted from that of Graham et al., 1986) was used to analyze the capped RNA. Capped, labeled RNA was separated electrophoretically across a 1.2% Mops-formaldehyde gel and transferred overnight in 10 x SSC to a GeneScreen membrane without protection probes.

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NOTE ADDED IN PROOF

Yao et al. [Nucl. Acids Res. (1989). 17, 9583–9591] have recently reported finding two transcription initiation sites in the psbD-psbC gene cluster of the tobacco chloroplast genome, and Woodbury et al. [Curr. Genet. (1989). 16, 433–445] have found that this is also the case for the same cluster in the pea chloroplast genome. Data in the latter paper also indicate that there may be more than one transcription initiation site for the rps2-atp1-atpH- atpF-atpA cluster in pea.

REFERENCES


Hudson, G.S., Mason, J.G., Holton, T.A., Keller, B., Cox, G.B.,


Lukens, J.H., and Bogorad, L. (1988). Nucleotide sequence containing the maize chloroplast proline (UGG) and tryptophan (CCA) tRNA genes. Nucl. Acids Res. 16, 5192.


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