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Differential transcription *in vivo* and *in vitro* of two adjacent maize chloroplast genes: The large subunit of ribulosebisphosphate carboxylase and the 2.2-kilobase gene

(RNA polymerase S factor/bundle sheath cell/mesophyll cell)

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ABSTRACT **The transcription of cloned maize plastid DNA sequences *in vitro* by maize plastid DNA-dependent RNA polymerase has been studied to expose the roles of the enzyme, polypeptide cofactors, and DNA sequences in the regulation of gene expression. The 4.35-kilobase pair *Bam*HI fragment 9 carries the maize plastid gene for the large subunit of ribulosebisphosphate carboxylase and part of the gene for a 2.2-kilobase RNA. These two genes are separated by ≈330 base pairs and are transcribed divergently. Transcripts of the gene for the large subunit of ribulosebisphosphate carboxylase are abundant in bundle sheath cells of maize leaves and we show here that transcripts of the 2.2-kilobase RNA gene are present in both mesophyll cells and the adjacent bundle sheath cells. *In vitro*, in the presence of the S factor, maize chloroplast DNA-dependent RNA polymerase produces a transcript of the gene for the large subunit of ribulosebisphosphate carboxylase with a 5′ terminus like that of the corresponding mRNA isolated from plastids, transcribes chloroplast DNA sequences of *Bam* fragment 9 in a chimeric plasmid in preference to the vehicle RSF 1030 and, in a ratio of 3:1, preferentially transcribes the gene for the large subunit of ribulosebisphosphate carboxylase over the 2.2-kilobase RNA gene from supercoiled chimeric plasmid DNA.**

Ribulosebisphosphate carboxylase [RbuPCase; 3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39] is found in chloroplasts of bundle sheath but not of mesophyll cells in leaves of the C_4 plant *Zea mays* (1, 2). The small subunit of this enzyme is the product of a nuclear gene (3) and the large subunit (LS) is encoded by a chloroplast gene (4, 5). The latter has been mapped physically on maize chloroplast DNA *Bam* fragment 9. This fragment also carries ≈1.4 kilobase pairs (kbp) of a gene that is transcribed *in vivo* into a 2.2-kilobase (kb) RNA of undetermined function. The two genes are separated by an untranscribed intercistronic "gap" 330 base pairs long and are transcribed from opposite strands (6). DNA sequences coding for the LS are contained in the chloroplast DNA of both mesophyll and bundle sheath cells but LS mRNA is found only in bundle sheath cells (7). On the other hand, as shown here, transcripts of the 2.2-kb RNA gene are present in both cell types. Thus, *Bam* fragment 9 is interesting for the study of the role of DNA sequences in transcriptional regulation of gene expression.

In the presence of the S factor (a 27.5-kilodalton polypeptide purified from maize chloroplasts), maize chloroplast DNA-dependent RNA polymerase preferentially transcribes cloned maize chloroplast DNA sequences (*Eco*RI fragment ℓ or a) in supercoiled plasmids (8). Such reconstituted *in vitro* systems provide powerful tools for analyzing the roles of proteins involved in transcription and of DNA sequences in the regulation of gene transcription.

In the present paper, we show that the chloroplast DNA fragment B (Fig. 1, a 2.7-kbp subfragment of the *Bam* 9 DNA that contains all the LS gene and 0.77 kbp of the gene for the 2.2-kb transcript) is preferentially transcribed over vehicle DNA (RSF 1030) by the *in vitro* transcription system, that the nucleotide sequences at the 5′ termini of the *in vitro* transcript of the LS gene and of LS mRNA are alike, and that transcription of the LS RbuPCase gene is strongly favored over that of the 2.2-kb RNA *in vitro* in the presence of the S factor.

MATERIALS AND METHODS

DNA. The plasmid pZmc37 is a chimera of RSF 1030/*Bam*HI fragment 9 of maize chloroplast DNA. *Bam* fragment 9 (Fig. 1) contains the LS RbuPCase gene and 1.4 kbp of the gene for a 2.2-kb transcript (4–6). The plasmid pZmc37-11 is a chimera of RSF 1030/fragment F of *Bam* 9 DNA. pZmc465 is a chimera of pBR322/fragment E of *Bam* 9 DNA.

Plasmid DNA was purified from bacterial lysates by $CsCl_2$/ethidium bromide density gradient centrifugation (9). Supercoiled DNA was further separated from relaxed circular DNA by sucrose gradient centrifugation on 10–40% ultrapure sucrose (Schwarz–Mann) in 10 mM Tris·HCl, pH 8.0/1 mM EDTA in a Beckman SW40 rotor at 28,000 rpm for 20 hr at 4°C. Fractions containing supercoiled DNA were identified by electrophoresis in 0.8% agarose gels. Supercoiled DNA was concentrated by centrifugation using ultrafiltration membrane cones (CF 40, Amicon). Work with recombinant plasmids was carried out at the P-1 level of physical containment as specified in National Institutes of Health guidelines on recombinant DNA research.

Endonuclease Digestions, Agarose Gel Electrophoresis, and Filter Hybridizations. Restriction endonucleases (Bethesda Research Laboratories) were used as specified by the supplier. DNA digested with restriction endonucleases was fractionated by agarose gel electrophoresis and transferred (10) to strips of Millipore filter paper (HAWP00010). Hybridization with radioactive RNAs was in 0.3 M NaCl/0.03 M Na citrate at 66°C for 16 hr. Hybridization was revealed by autoradiography.

Quantitative hybridization was carried out as described (8).

Chloroplast RNA Polymerase and S Factor. Maize chloroplast DNA-dependent RNA polymerase and S factor were prepared through the DEAE-cellulose column chromatography stage (8).

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Abbreviations: RbuPCase, ribulosebisphosphate carboxylase; LS, large subunit; kb, kilobase(s); kbp, kilobase pair(s).

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Transcription. The standard reaction mixture (0.1 ml) for transcription was 5 μmol of Tris·HCl, pH 8.0, 4 μmol of 2-mercaptoethanol, 2.5 μmol of $MgCl_2$, 0.1 μmol each of ATP, CTP, and GTP, [^{3}H]- or [α-^{32}P]UTP as indicated, DNA as indicated, and 10 μl of RNA polymerase with or without S factor. Incubation was for 30 min at 37°C.

DNase. RNase-free DNase was prepared from deoxyribonuclease I (DPFF, Worthington) by passage through a column of agarose-5-(4-aminophenylphosphoryl) uridine 2′(3′) phosphate (Miles) as described by Maxwell *et al.* (11).

Plant Material and Isolation of Leaf Cell Types. Maize seeds ($FR9^{C}_{MS}$ × FR 37) were obtained and grown as described earlier (7, 8). Preparation of mesophyll cells and strands of bundle sheath cells has been described (7).

RESULTS

Differential Transcription of LS and 2.2-kb RNA Genes *in Vivo*. Chloroplast DNA fragment *Bam* 9 is hybridized by RNA from either mesophyll or bundle sheath cells while subfragment B (Fig. 1) is strongly hybridized only by RNA of the latter cell type (7). To determine the relative abundance of transcripts of the 2.2-kb RNA and LS RbuPCase genes—1.4 kbp of the former and all of the latter are on *Bam* 9—RNA was prepared from the two cell types, labeled, and hybridized to chloroplast DNA fragments (7) generated from plasmid pZmc37 by digestion with *Bam*HI/*Eco*RI (Fig. 2).

Fragment G (Fig. 1), on the right-hand side of *Bam* 9 and containing most of the LS gene, is hybridized strongly by bundle sheath cell RNA but only very weakly by RNA from mesophyll cell preparations. Hybridization to the latter may be accounted for by ≈3% contamination of mesophyll cell preparations by bundle sheath cells (7). Fragment H, which is part of the LS gene, is hybridized by bundle sheath but not by mesophyll cell RNA. In contrast, fragment E, which contains a 1.4-kbp portion of the 2.2-kb RNA gene, is hybridized by RNA from both cell types with about equal intensity. These results confirm and extend our earlier conclusion (7) that genes located on chloroplast DNA fragment *Bam* 9 are differently expressed in mesophyll and bundle sheath cells.

Initiation of Transcription of the LS RbuPCase gene *in Vitro*. Transcripts of a cloned maize $tRNA^{His}$ gene produced *in vitro* by maize chloroplast DNA-dependent RNA polymerase and S factor have 5′ termini indistinguishable from those of *in vivo* transcripts (14). Similar analyses have been carried out comparing *in vitro* transcripts of the LS RbuPCase gene and the

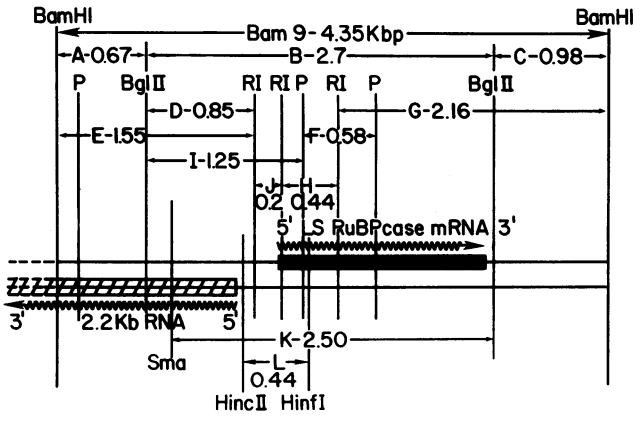

FIG. 1 Map of the restriction endonuclease recognition sites on maize plastid DNA fragment *Bam* 9 (4, 5) and locations of the LS RbuPCase and 2.2-kb RNA genes (6). Recognition sites are given for *Pst* (P), *Bgl* II, *Eco*RI (RI), *Sma*, *Hinc*II, and *Hin*fI.

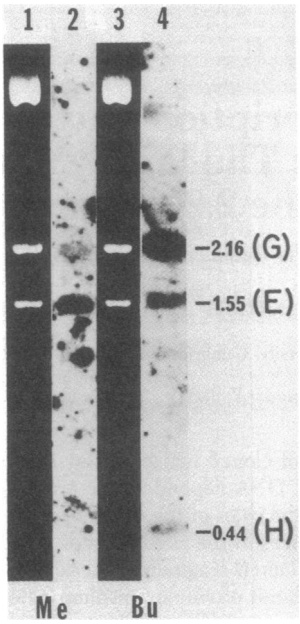

FIG. 2. Hybridization of RNAs from maize mesophyll (Me) and bundle sheath (Bu) cells against fragments of maize chloroplast DNA sequence *Bam* 9. pZmc37 DNA (1 μg) was digested with *Bam*HI/*Eco*RI, and the products were separated electrophoretically in a 1.5% (wt/vol) agarose gel. The positions of the resulting fragments were visualized by staining with ethidium bromide (lanes 1 and 3). The heavy band at the top of each gel is the vehicle. The fragments of *Bam* 9 visible on the gel are designated by their size in kbp and by letter relating them to Fig. 1. Fifty nanograms of [^{32}P]RNA (1 × 10^7 cpm/μg) from either Me or Bu preparations was used to hybridize DNA transferred to each nitrocellulose strip (10). Autoradiographs of the nitrocellulose sheets are shown in lanes 2 and 4.

LS mRNA prepared from maize plastids.

The 5′ terminus of maize RbuPCase LS mRNA has been shown to fall between nucleotide positions 53 and 59 upstream of the translation start site, based on nuclease S1 mapping (13). Nuclease S1 experiments of the same type were carried out here. RNA was prepared either from plastids (6,13) or from *in vitro* transcription reaction mixtures (12) and hybridized against fragment L (Fig. 1) labeled at its 5′ *Hin*fI end—i.e., at the 5′ end of the coding strand. After hybridization and treatment with nuclease S1, the surviving fragments were subjected to gel electrophoresis alongside a similarly labeled fragment L treated for the "G" reaction in DNA sequence analysis, as described previously (13). The data show (Fig. 3) that the 5′ terminus of the *in vitro* transcript of the LS gene (lanes B, C, and E) is the same as that of LS mRNA isolated from plastids (lanes A and F). The protection of fragments of four different sizes in the nuclease S1 experiment could be the result of multiple initiation start sites or of "breathing" of the RNA·DNA hybrid during the nuclease S1 treatment. Regardless of the cause, *in vitro*-synthesized transcripts and mRNA isolated from plastids protected LS RbuPCase gene fragment L DNA in the same way.

Selective Transcription of Cloned Maize Plastid *Bam* 9 Sequences. Experiments were designed to determine whether all portions of the chimeric plasmid pZmc37, or its derivative pZmc37-11, are equally transcribed *in vitro* and whether the S factor has any influence on preferential transcription.

pZmc37 DNA was cleaved by *Bam*HI/*Bgl* II. The fragments were separated by agarose gel electrophoresis and transferred (10) to nitrocellulose filter sheets. [α-^{32}P]UMP-labeled RNA produced by chloroplast RNA polymerase in the presence or absence of S factor with pZmc37 as a template was hybridized to the DNA fragments on the filters. Fig. 4 shows that chloroplast RNA polymerase preferentially transcribes chloroplast DNA sequences rather than those of the vehicle and suggests that this preference is increased by the S factor. Furthermore, the S factor promotes transcription of the fragment containing the LS RbuPCase gene and a portion of the 2.2-kb gene (fragment B, Fig. 1) over that of fragment A (Fig. 1)—only 2.2-kb RNA gene sequences—transcription of A appears to be relatively repressed.

To study preferential transcription in detail, [^{3}H]UMP-labeled RNA was synthesized by chloroplast RNA polymerase in the presence or absence of S factor using supercoiled pZmc37-

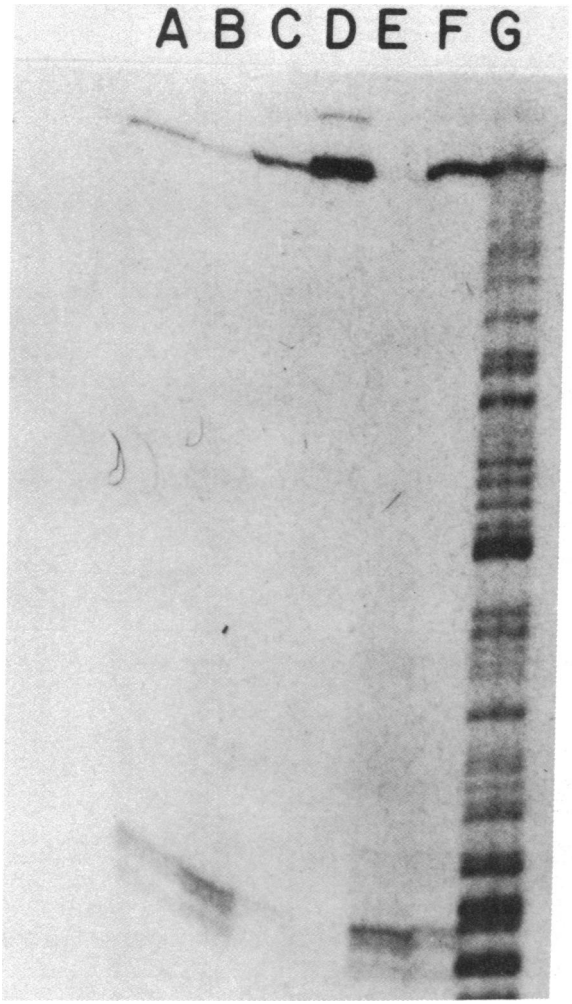

FIG. 3. Localization of the 5′ termini of LS RbuPCase mRNA and of the *in vitro* transcript of this gene on its coding strand. Maize chloroplast DNA fragment L (Fig. 1) labeled with ^{32}P at its 5′ *Hin*fI restriction site was prepared as described (13), and 27 pmol (≈3000 cpm) was hybridized with either RNA isolated from maize chloroplasts (6) or with transcripts prepared *in vitro* with pZmc37 as the template. Hybridization conditions, nuclease S1 treatment, and gel electrophoresis were as described (13). Lanes: G, fragments generated by the "G" sequence analysis reaction (15) for fragment L (Fig. 1); A and F, hybridization with 30 μg of chloroplast RNA; B and E, hybridization with RNA transcribed from pZmc37-11 DNA *in vitro* by maize plastid RNA polymerase in the presence of S-factor; C, hybridization with RNA from pZmc37-11 in the absence of S factor.

11 as the template. This 11-kbp chimeric plasmid is composed of the vehicle RSF 1030 (8.3 kbp) and subfragment B (2.7 kbp) of the maize chloroplast DNA sequence *Bam* 9. Denatured pZmc37-11 or RSF 1030 DNA was fixed to nitrocellulose filter disks and hybridized with [^3H]RNA transcribed from pZmc37-11. The same amount of radioactivityof [^3H]RNA was used in each hybridization. As shown in Fig. 5, 115 cpm of [^3H]RNA synthesized in the absence of S factor hybridized to pZmc37-11 DNA and 30 cpm hybridized to RSF 1030 DNA. [^3H]RNA produced in the presence of S factor hybridized to RSF 1030 at not more than background levels but 250 cpm of this [^3H]RNA hybridized to pZmc37-11.

The ratio of transcription of the chloroplast DNA sequences to vehicle RSF 1030 DNA was calculated by the formula {[RNA (cpm) hybridized to pZmc37-11 − RNA (cpm) hybridized to RSF 1030 DNA]/size of chloroplast DNA} fragment (kbp) ÷ [RNA (cpm) hybridized to RSF 1030 DNA] size of RSF 1030

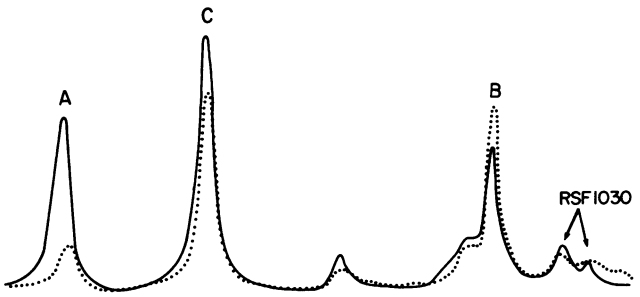

FIG. 4. Hybridization of RNA transcribed from pZmc37 DNA to fragments of the template. pZmc37 DNA was hydrolyzed with *Bam*HI/*Bgl* II. The DNA fragments (0.2 μg per gel) were separated by electrophoresis in 2% agarose gels (0.8 mA per gel for 15 hr), transferred to Millipore filter strips (10), and hybridized to [α-^{32}P]UMP-labeled RNA produced from [α-^{32}P]UTP (100 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and 10 μl of plastid RNA polymerase in the presence or absence of S factor. Supercoiled pZmc37 DNA (5 μg) was used as a template. The amounts of acid-precipitable products produced were 190 and 990×10^3 cpm in the absence (—) and presence (----), respectively, of S factor. After extraction with phenol and ethanol precipitation, RNA was dissolved in 0.3 M NaCl/0.03 M Na citrate. About 70×10^3 cpm of each RNA was used for hybridization to a nitrocellulose filter strip. Hybridization was at 66°C for 18 hr. The autoradiograph of the hybridization was scanned by a Zeineh Soft Laser Scanning Densitometer using a tungsten lamp.

(kbp)]. This ratio was 8.7 [(85/2.7) divided by (30/8.3)] in the absence of S factor but it was ∞ [(250/2.7) ÷ (0/8.3)] in the presence of the S factor—i.e., hybridization to RSF-1030 was not detected here.

Differential Transcription of Two Adjacent Plastid Genes. As noted above, the pattern of hybridizations shown in Fig. 4 also indicated that the S factor may affect the transcription of the two chloroplast genes on chloroplast DNA *Bam* 9 differently. The effect of the S factor on transcription of the two genes by chloroplast RNA polymerase was next compared quantitatively. As one probe for the quantitative hybridization, the 590 base pair *Pst*/*Pst* fragment F (Fig. 1) from within the structural gene for LS RbuPCase was cloned in pBR322; the chimeric plasmid is designated pZmc461. And, for the other probe, fragment E (Fig. 1) from maize *Bam* 9 DNA was cloned between the *Bam* and the *Eco*RI sites in pBR322 to produce pZmc465. Fragment E contains 1400 base pairs of the gene for the 2.2-kb transcript plus a little of the nontranscribed intercistronic region between the two genes present on *Bam* fragment 9. [^3H]UMP-labeled RNA was synthesized by maize chloroplast RNA polymerase in the presence or absence of S factor using as a template supercoiled pZmc37-11 [fragment B (Fig. 1) cloned in RSF 1030]. As in previous quantitative hybridization experiments, a constant amount of [^3H]UMP-labeled RNA was hybridized to DNA (pZmc461 or pZmc465) bound to nitrocellulose filters. As shown in Fig. 6, a maximum of 290 cpm of [^3H]RNA produced in the absence of S factor hybridized to pZmc461 and 400 cpm hybridized to pZmc465. Of the [^3H]RNA produced in the presence of the S factor, 195 cpm hybridized to pZmc461 but only 95 cpm hybridized to pZmc465. The ratio of transcription of the LS RbuPCase gene to that of the 2.2-kb gene was calculated as follows: [RNA (cpm) hybridized to pZmc461 DNA/size (kbp) of chloroplast DNA sequence F in pZmc461] ÷ [RNA (cpm) hybridized to pZmc465 DNA/size (kbp) of fragment D]. The length of fragment D was used in the denominator rather than that of fragment E, which is the actual probe, because transcription was from pZmc37-11, which contains D and not E. (pZmc37-11 was used as the template to keep the sizes of probes for transcription of the two genes as similar as possible.) The ratio of transcription of the LS RbuPCase gene to that the 2.2-

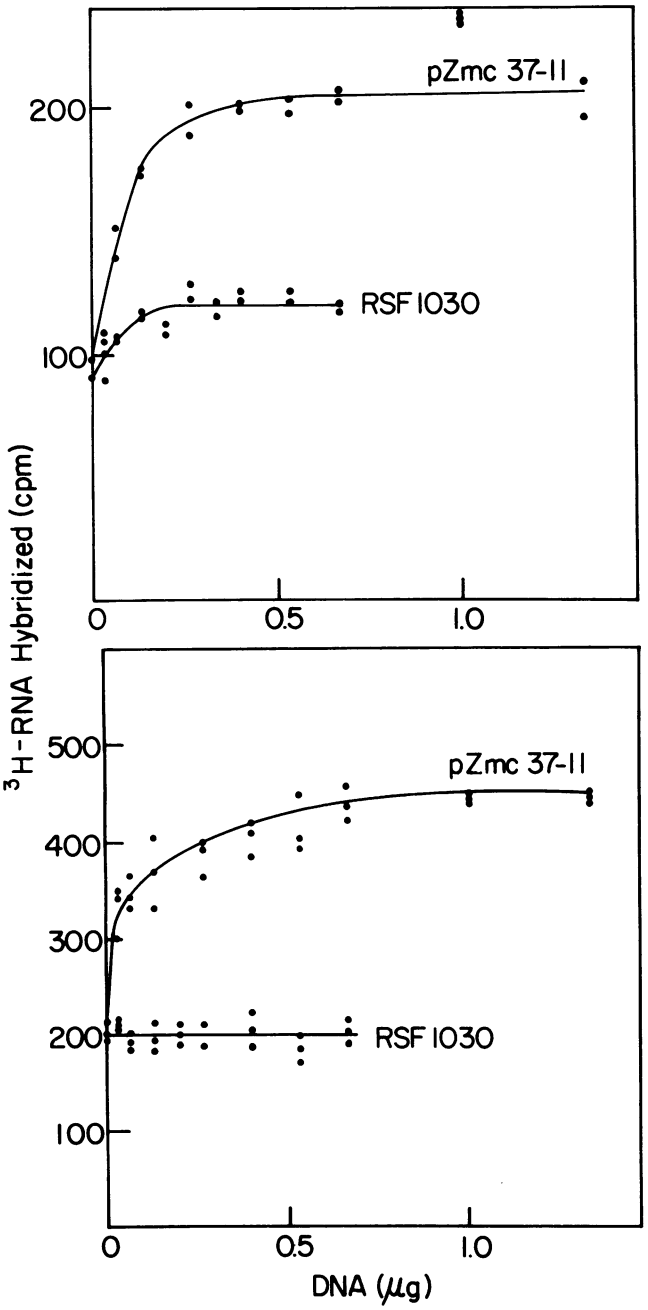

FIG. 5. Quantitative hybridization of plastid RNA polymerase products formed in the absence (*Upper*) or presence (*Lower*) of S factor to pZmc37-11 or RSF 1030 DNA. [^{3}H]RNA was made *in vitro* by using supercoiled pZmc37-11 DNA as the template with or without S factor and hybridized to pZmc37-11 or RSF 1030 DNA fixed on nitrocellulose filters. Inputs of [^{3}H]RNA for hybridization were 750 and 570 cpm per filter, respectively in the presence and absence of S factor.

kb RNA gene was 1.1 (290/0.59)/(400/0.92) in the absence of the S factor and 3.2 (195/0.59)/(95/0.92) in the presence of the S factor.

DISCUSSION

RNAs transcribed from the adjacent and divergently transcribed (6) 2.2-kb RNA and LS RbuPCase genes were both recovered from bundle sheath cells but RNA of only the former could be detected in mesophyll cells. The DNA fragment carrying both of these genes consequently appears to be useful for exploring possible contributions of DNA sequence to differential gene expression in maize leaf cell types. The remainder of the present work was a study of transcription of the 2.2-kb RNA and LS RbuPCase genes *in vitro* using maize chloroplast RNA polymerase and was undertaken to gain an understanding of mechanisms by which differential expression of genes in the chloroplast chromosome is effected.

The maize plastid RNA polymerase appears to initiate transcription of the LS gene "correctly" for the 5′ ends of the LS RbuPCase mRNA and of the transcript produced *in vitro* by the homologous plastid polymerase. Corresponding similarities have been seen between a maize chloroplast tRNA^{His} and the *in vitro* transcript of the cloned gene (12). Unless some fortui-

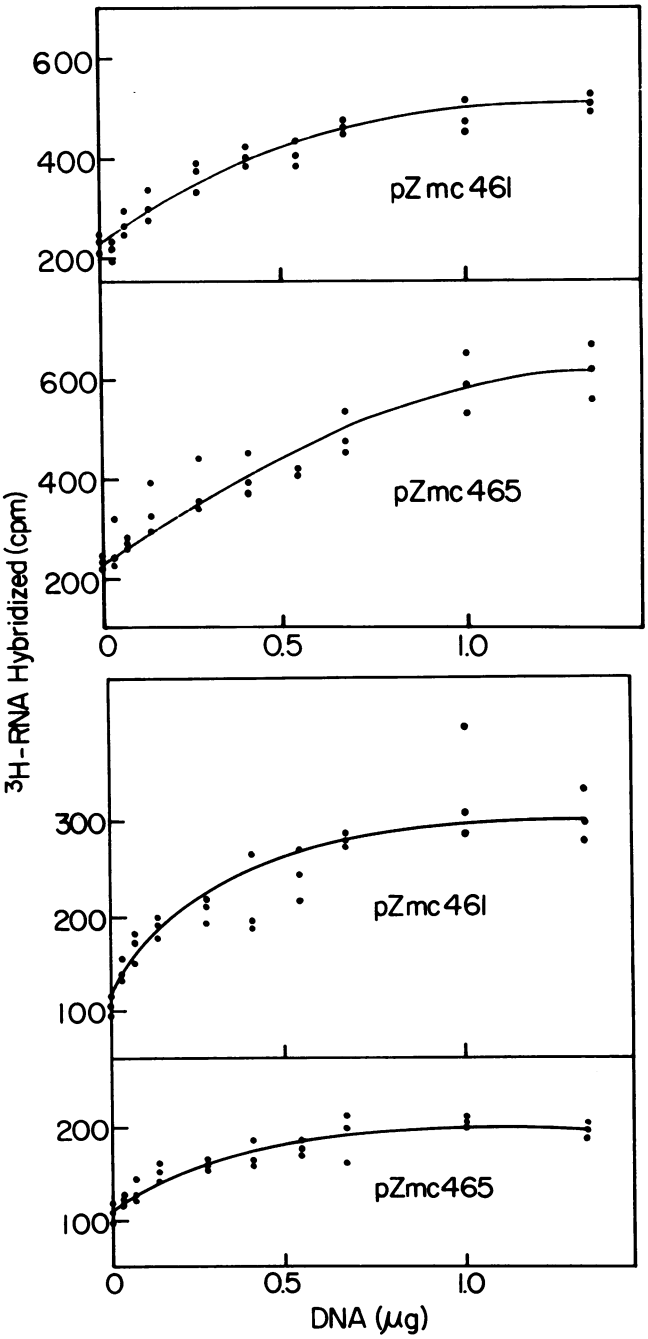

FIG. 6. Quantitative hybridization of plastid RNA polymerase products to pZmc461 or 465. [^{3}H]UMP-labeled RNA was synthesized *in vitro* by using supercoiled pZmc37-11 DNA as the template in the absence (*Upper*) or presence (*Lower*) of S factor. Inputs of [^{3}H]RNA for hybridization were 1070 and 2770 cpm per filter, respectively, in the presence, and absence of S factor.

tously identical processing occurs *in vivo* for both the $tRNA^{His}$ and the LS RbuPCase mRNA, transcription is initiated at the same site *in vitro* and *in vivo*. Fig. 3 (lanes B and C) suggests that there are more correct transcripts (and less radioactivity remaining at the top of the gel) when transcription is in the presence of S factor (lanes B and E) than in its absence (lane C). However, we have not determined directly whether the ratio of correctly to incorrectly initiated transcripts is altered by the presence of the S factor and cannot state whether the S factor is part of the apparatus required for correct initiation.

Examination of the DNA sequence upstream of the maize LS RbuPCase gene transcription initiation site (13) shows some homologies with prokaryotic promoter (16) and eukaryotic T-A-T-A (e.g., ref. 17) sequences at −23 to −28 (T-A-G-A-T-T) and at −40 to −45 (T-A-T-C-A-T)—i.e., in the regions at which sequences of those types are common for eukaryotic nuclear genes—but, like promoters for prokaryotic genes, a "−35 sequence" (T-T-G-A-T-A) is found (a considerable distance upstream of the normal location) at −50 to −55 (13). No experimental evidence is available that shows whether any of these sequences (Fig. 7) plays a role in DNA–protein interactions in the plastid system.

We found earlier (8) that preferential transcription of maize chloroplast DNA fragment Eco ℓ under the influence of the S factor occurs in supercoiled plasmids *in vitro* but is greatly reduced by unwinding the DNA to its closed circular form. To simplify the assay for the site of initiation of transcription, we examined whether a single-sized runoff fragment would be produced if fragment I or J (Fig. 1) was used as a template. Based on sequence data and nuclease S1 experiments (14), the runoff product using fragment I as a template should be 228 nucleotides long and that from fragment J would be 35 nucleotides long. We could not find RNAs of these sizes on the acrylamide/urea gels. This could result from the inability of the maize chloroplast RNa polymerase to initiate correctly on fragments of this size or from conformation; supercoiled or circular forms of DNA may be required.

The present experiments confirm the observation that maize chloroplast RNA polymerase/S factor preferentially transcribes at least some chloroplast DNA sequences over those of the bacterial vehicle plasmid pMB9 (8). Here RSF 1030 in pZmc37 is transcribed little, even in the absence of the S factor, but its transcription appears to be virtually completely suppressed when the S polypeptide is included. We do not know whether differences in relative transcription of pMB9 and RSF 1030 DNA in these and the previous (8) experiments are due to properties of RSF 1030 vs. pMB9 promoters, to the extents of supercoiling of the DNA template used, or to the character of the chloroplast DNA sequences "competing" for transcription.

An interesting outcome of these experiments is the demonstration that transcription of the LS RbuPCase gene is favored ≈3:1 over transcription of the 2.2-kb RNA gene in the presence of S factor *in vitro*. It remains to be determined experimentally by modification of the DNA whether or which DNA sequences of the LS gene serve as signals for corrct initiation, preferential transcription, etc.

Does *in vitro* transcription of the 2.2-kb RNA and the LS genes in a 1:3 ratio duplicate RNA production from these genes in mesophyll or bundle sheath cells of maize? In neither the absence nor the presence of the S factor is the transcription of the LS RbuPCase gene suppressed as completely as in mesophyll cells. The situation *in vitro* may be comparable to that in bundle sheath cells. The LS RbuPCase message is one of the most abundant in those cells but quantitative estimates of the

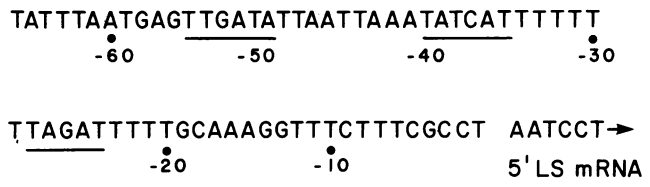

FIG. 7. Nucleotide sequence upstream of the transcription initiation site for the maize LS RbuPCase gene (13). Sequences reminiscent of those seen in prokaryotic "−10" and "−35" regions are underlined. Nucleotides are numbered with reference to the site of initiation of transcription.

ratio of 2.2-kb to LS RbuPCase RNAs in maize bundle sheath cells and in leaves of C-3 plants remain to be made. However, the S factor may simply be required to measure relative strengths of promoters for maize plastid genes and other factors may serve to suppress transcription—as may occur in maize mesophyll cells. Regulation of RNA levels by selective destruction *in vivo* cannot be excluded.

Note Added in Proof. Maize chloroplast DNA *Eco*RI fragment e contains all of the "2.2-kb gene" discussed here. The product of *in vitro* transcription and translation of this cloned sequence is a 58,000-dalton polypeptide that we judge, from its size and immunochemical behavior, to be the β subunit of the maize chloroplast coupling factor (unpublished data).

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1. Huber, S. C., Hall, T. C. & Edwards, G. E. (1976) *Plant Physiol.* **57**, 730–733.
2. Kirchanski, S. J. & Park, R. B. (1976) *Plant Physiol.* **58**, 345–349.
3. Wildman, S. G., Chen, K., Gray, J. C., Jung, S. D., Kwanyuen, P. & Sakano, K. (1979) in *Genetics and Biogenesis of Mitochondria and Chloroplasts*, eds. Birky, C. W., Perlman, P. S. & Byers, T. J. (Ohio State Univ. Press, Columbus, OH), pp. 309–329.
4. Coen, D. M., Bedbrook, J. R., Bogorad, L. & Rich, A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5487–5491.
5. Bedbrook, J. R., Coen, D. M., Beaton, A. R., Bogorad, L. & Rich, A. (1979) *J. Biol. Chem.* **254**, 905–910.
6. Link, G. & Bogorad, L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1832–1835.
7. Link, G., Coen, D. M. & Bogorad, L. (1978) *Cell* **15**, 725–731.
8. Jolly, S. O. & Bogorad, L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 822–826.
9. Clewell, D. D. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1159–1166.
10. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 508–517.
11. Maxwell, I. H., Maxwell, F. & Hahn, W. E. (1977) *Nucleic Acids Res.* **4**, 241–246.
12. Schwarz, Z., Jolly, S. O., Steinmetz, A. A. & Bogorad, L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3423–3427.
13. McIntosh, L., Poulsen, C. & Bogorad, L. (1980) *Nature (London)* **288**, 556–569.
14. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835–4838.
15. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
16. Rosenberg, M. & Court, D. (1979) *Annu. Rev. Genet.* **13**, 319–353.
17. Benoist, C. & Chambon, P. (1981) *Nature (London)* **290**, 304–310.