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Binding and transcription of relaxed DNA templates by fractions of maize chloroplast extracts

(RNA polymerase/*psbA* gene/*rbcL* gene/topoisomers/*Zea mays*)

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ABSTRACT **Preparations of partially purified chloroplast DNA-dependent RNA polymerase from maize and some other plants transcribe cloned chloroplast genes preferentially and much more actively from appropriately negatively supercoiled templates than from relaxed templates. We have found that the polymerase in such fractions does not bind to promoter regions of the maize chloroplast genes *psbA* and *rbcL* on small linear DNA fragments but that some protein(s) in unfractionated chloroplast extracts does bind. DEAE chromatography of the extracts has permitted the separation of a DNA-binding fraction from the bulk of the RNA polymerase activity. The binding fraction contains plastid RNA polymerase activity that is relatively independent of template topology.**

Chloroplast DNA-dependent RNA polymerase has been extensively purified from maize (1–3), pea (4), and spinach (5). Preparations of partially purified maize polymerase transcribe cloned homologous maize chloroplast DNA sequences in preference to bacterial vector sequences from supercoiled plasmids when a 27-kDa polypeptide, the S factor, is present (6). Such enzyme preparations transcribe cloned maize chloroplast genes *rbcL* (for the large subunit of ribulose-bisphosphate carboxylase) and *atpBE* (for the β and ε subunits of the coupling factor for photophosphorylation) about 50 times more actively from optimally negatively supercoiled templates than from relaxed templates, but the relative transcription of the two genes from a single bacterial plasmid varies with negative superhelicity of the template (7, 8). Transcription of cloned maize chloroplast genes *rpS4* (for ribosomal protein S4) and *psbA* (for the Q_B protein of photosystem II) *in vitro* by maize plastid RNA polymerase preparations is also much greater from negatively supercoiled templates than from relaxed templates (9). Negative superhelicity of the template has been shown to affect transcription by RNA polymerase preparations from chloroplasts of other higher plant species as well (10, 11).

Relative roles of transcription, postranscriptional processing, and translation in chloroplast differentiation are not well defined and appear to differ among plants. The abundance of transcripts of a single maize chloroplast gene can be substantially different in adjacent mesophyll and bundle-sheath cells and in etiolated vs. greening or green leaves (12–14); differences in transcription may contribute to such variations. Activity of maize chloroplast RNA polymerase increases substantially during light-induced greening of dark-grown maize although commensurate increases in major polypeptides are not seen (15).

The present work was undertaken to examine interactions between cloned maize chloroplast gene sequences and maize chloroplast proteins. We have identified a region of the maize plastid gene *psbA* to which a protein (or proteins) in chloroplast extracts binds. We have also found that DEAE fractions of chloroplast extracts with the most DNA-binding material contain RNA polymerase activity that transcribes chloroplast genes from relaxed templates about as well as from supercoiled templates. As reported earlier (6, 8), relaxed chloroplast DNA is a very poor template for transcription by the bulk of the RNA polymerase in plastid extracts.

MATERIALS AND METHODS

Preparation of Maize Plasmid Extracts and RNA Polymerase. Gradient-purified chloroplasts were prepared from 7- or 8-day-old dark-grown maize seedlings (FR9cms × FR37, Illinois Foundation Seeds, Champaign, IL) illuminated for 16 hr (1, 6). Sucrose density gradient-purified maize plastids were extracted with high-salt (0.5 M KCl) buffer (2) or by the EDTA method (1). The supernatant from high-speed centrifugation of a plastid extract was applied, at 0.3 ml/min, to a Protein Pak Glass DEAE-5PW anion-exchange column (8.0 mm × 7.5 cm, Nihon Waters, Tokyo) previously equilibrated with 40 mM Tris·HCl, pH 8/100 mM KCl/1 mM dithiothreitol/0.14 mM phenylmethylsulfonyl fluoride/0.1 mM EDTA/10% (vol/vol) glycerol on a Waters 650 Advanced Protein Purification System (Millipore). Fractions eluted with a linear 0.1–0.5 M KCl gradient in the above buffer at 0.5 ml/min were collected on ice, made 50% (vol/vol) glycerol, and assayed for RNA polymerase (6) and DNA-binding activities. Both activities were stable for at least 6 months in fractions stored at −80°C.

S1 Nuclease RNA Polymerase Assays. [^{32}P]RNA synthesized *in vitro* from cloned maize plastid genes was measured as described (16) except for the inclusion of 20 units of RNasin (Promega Biotec) in each 20-μl transcription mixture. Single-stranded probes for S1 nuclease protection were prepared from mp19-427, which contains a 545-base fragment of maize *psbA* from pZmc427 (17) extending from the *Sma* I site (residues 1–6, Fig. 1) to an *Alu* I site (not shown) inserted between the *Sma* I and *Hin*cII sites of M13mp19 (18), or from mp8-pZ3 (16), which contains a 925-base *Sma* I–*Ava* I fragment of the 5′ region of maize *rbcL* in m13mp8.

DNA Manipulations. Recombinant plasmid pUC18-PG, constructed by D. R. Russell, consists of a 245-base-pair (bp) *Sma* I–*Bgl* II DNA fragment (nucleotides 1–245, Fig. 1) of *psbA* from pZmc427 inserted into pUC18 (18). A "290-bp" *Eco*RI–*Hin*dIII fragment, containing the 245-bp 5′ region of *psbA* (Fig. 1) plus 15 bp of upstream and 28 bp of downstream vector sequence, was excised from pUC18-PG for use in the DNA–protein binding experiments.

For 3′-end labeling, 1–2 μg of pUC18-PG plasmid DNA (0.5–1 pmol) was digested with *Eco*RI and *Hin*dIII. The DNA was labeled with [α-^{32}P]dATP (New England Nuclear; 4–5 μM) by the Klenow fragment of *Escherichia coli* DNA

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Abbreviations: BF, binding fraction; PF, polymerase peak fraction.

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polymerase (19). The 290-bp fragment was separated from vehicle DNA by electrophoresis in a 5% (wt/vol) polyacrylamide gel (29:1 acrylamide/*N,N'*-methylenebisacrylamide) in TBE (89 mM Tris base/89 mM boric acid/2 mM EDTA). Labeled DNA was eluted from a slice of the gel (20), precipitated with 2-propanol, washed with 70% ethanol, dried under vacuum, and dissolved in water.

For 5'-end labeling, 1–2 μg of pUC18-PG DNA was digested with *Eco*RI or *Hin*dIII and dephosphorylated with alkaline phosphatase (Boehringer Mannheim). DNA was labeled with [γ-^{32}P]ATP (New England Nuclear; 0.6 μM) by 10 units of phage T4 polynucleotide kinase (New England Biolabs) (19). After inactivation of the kinase at 65°C, NaCl was adjusted to 50 mM and the complementary restriction endonuclease (*Hin*dIII or *Eco*RI) was added. The mixture was then electrophoresed in a 5% polyacrylamide gel and the 290-bp DNA fragment was eluted and purified as described above.

DNA–Protein Binding Assays. DNA–protein complexes were detected by a mobility-shift assay (21, 22). End-labeled DNA fragments (1–2 ng, 5–10 fmol, 0.5–1 × 10^7 dpm/μg) were incubated with 5 μl of unfractionated or DEAE-fractionated chloroplast extract (≈5 μg of protein) in 20 μl of 10 mM Tris·HCl, pH 8/70–140 mM KCl/5 mM EDTA/10% glycerol containing 2 μg of bovine serum albumin and 1 μg of poly(dI-dC)·poly(dI-dC) (Pharmacia) at 25°C for 20 min. Reaction mixtures were electrophoresed at 300 V for 5 hr in 4% polyacrylamide gels (14 × 20 cm), containing 0.5× TBE and 5% glycerol, that had been pre-run at 100 V for 1 hr.

RESULTS

Chloroplast DNA-Binding Proteins. The gene *psbA* codes for a 32-kDa thylakoid membrane polypeptide (23, 24) that binds triazine herbicides (25) and that is a component of the reaction center of photosystem II. mRNA for this protein is barely detectable in etiolated maize leaves but is one of the most abundant messages in plastids of greening and green mesophyll maize leaf cells (15, 23). DNA sequences required for *in vitro* transcription of mustard *psbA* (26) and maize *psbA* (L. Crossland and L.B., unpublished data) have been defined. Sequences thought to function as −10 and −35 components of the maize *psbA* promoter are indicated in Fig. 1.

Crude maize chloroplast extracts as well as certain DEAE fractions contain material that retards the electrophoretic migration of the ^{32}P-labeled 290-bp fragment (Fig. 2) containing bp 1–245 of *psbA* (Fig. 1). (Such fractions are designated BFs—i.e., binding fractions—in this paper.) A 222-bp *Ava* II fragment of the *bla* gene of pBR322 was not retarded after incubation with these fractions (Fig. 2). Thus, the DNA binding is sequence-specific.

The slower-migrating band was not observed when SDS or proteinase K was included in the binding reaction mixtures (data not shown), indicating that the band is composed of labeled DNA plus one or more plastid proteins.

Mapping the Binding Site on the *psbA* Gene. To locate the 3' ends of the sequence protected by the plastid DNA-binding protein(s), either of the two strands of the 290-bp *Eco*RI–*Hin*dIII fragment from pUC18-PG was 5'-end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. The fragment with one 5' end labeled was purified by polyacrylamide gel electrophoresis, incubated with DEAE fractions of a plastid extract, and digested with exonuclease III (New England Biolabs) at 25, 100, 500, or 1000 units/ml. A prominent doublet of bands judged to terminate at nucleotides +16 and +17 (Figs. 1 and 3*C*) of the sense strand was present even after treatment with 1000 units of exonuclease III per ml (Fig. 3*A*). These bands were identical to a pair that remained after exonuclease III digestion of the labeled fragment in the presence of purified *E. coli* RNA polymerase (data not shown). Novel bands arising from the other DNA strand were missing after treatment of the binding reaction mixtures with higher concentrations of exonuclease. The plastid DNA-binding protein(s) may associate mainly or more strongly with the sense strand than with the antisense strand of *psbA*.

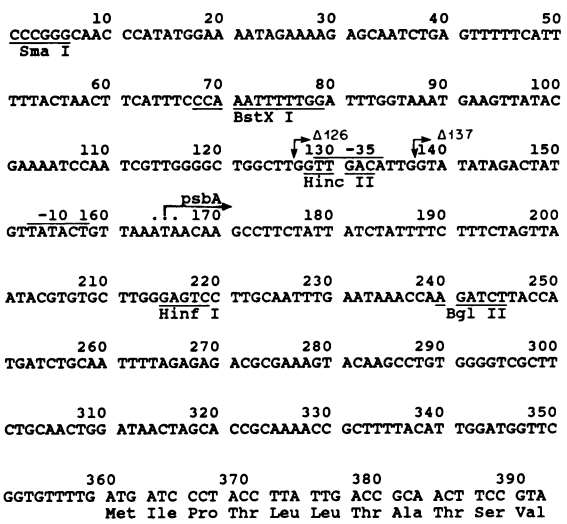

FIG. 1. Nucleotide sequence of the 5' region of the maize *psbA* gene from pZmc427 (18). The −10 and −35 elements of the maize plastid *psbA* promoter are overlined. The transcription start site is at nucleotide 164, 165, or 166. The 290-bp fragment used in the DNA binding experiments contains nucleotides 1–245. Double-headed arrows show the deleted forms of *psbA* used in the experiment of Fig. 4.

To locate the 5' end of the region protected by the plastid protein(s), the 290-bp DNA fragment was 3'-labeled at the *Hin*dIII end with [α-^{32}P]dATP by the Klenow fragment of *E. coli* DNA polymerase, purified by gel electrophoresis, and incubated with the maize plastid extract fraction under conditions used for the gel mobility-shift assay, and 5' → 3' λ exonuclease (Bethesda Research Laboratories) was added to 125 units/ml. DNA was digested at 37°C at pH 8.0, 8.5, and 9.1 to be sure to be within the enzyme's alkaline pH optimum. The 5' end of the protected region is represented by bands on the autoradiograph corresponding to termination at positions

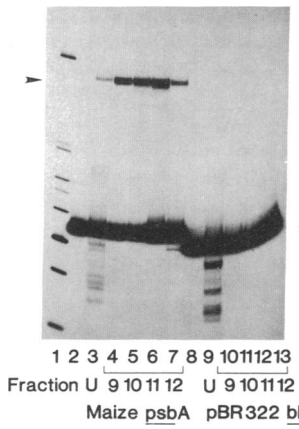

FIG. 2. Sequence specificity of the maize plastid DNA-binding component(s). No plastid extract (lanes 2 and 8), unfractionated (U) plastid extract, or aliquots of DEAE column fractions 9–12 of plastid extracts were mixed with end-labeled DNA fragments (*psbA*, lanes 2–7; *bla*, lanes 8–13) and electrophoresed. The gel was dried under vacuum and exposed to Kodak XAR-5 film for 12–15 hr at −80°C with an intensifying screen. A band of reduced mobility (arrowhead) is generated by labeled *psbA* DNA associated with plastid protein. pBR322 *bla* is a control DNA fragment. End-labeled *Hin*fI fragments of pBR322 served as size markers (lane 1).

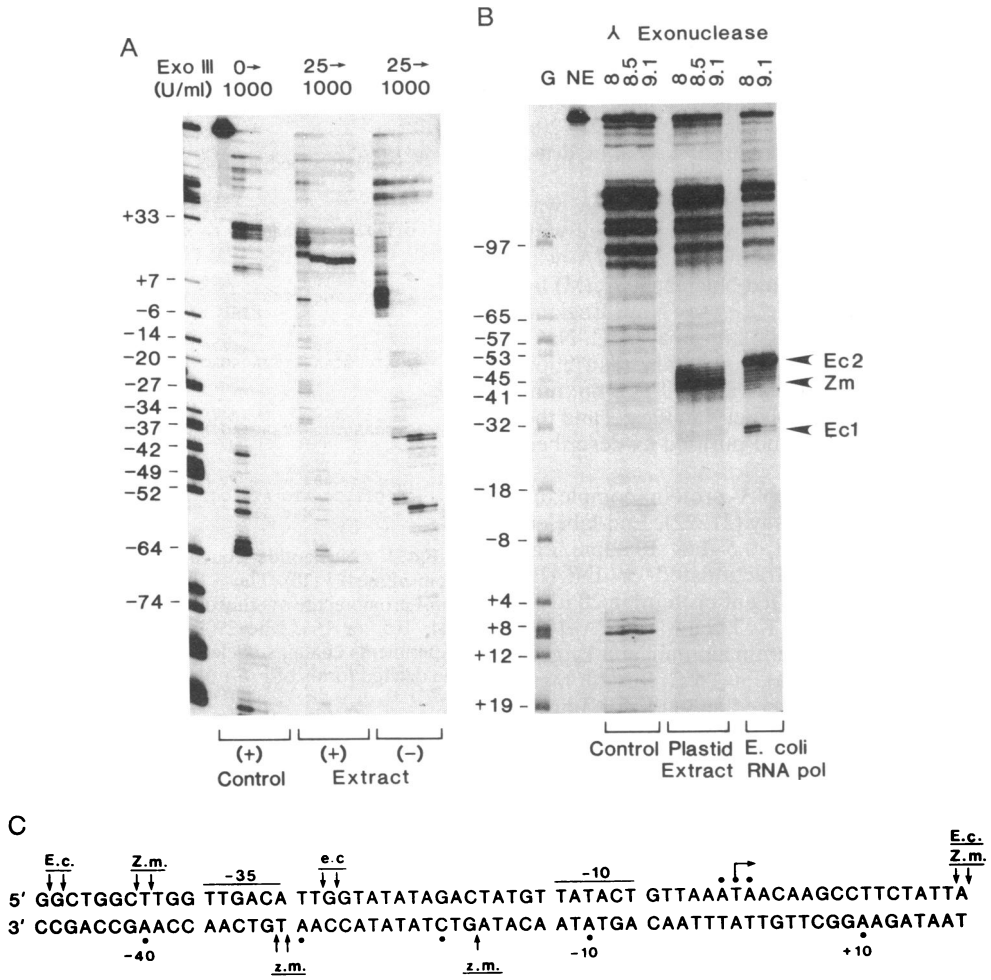

FIG. 3. Exonuclease mapping of the region of the 245-bp *psbA* gene fragment protected by *E. coli* RNA polymerase and by plastid BF protein(s). Exonuclease-resistant DNA was denatured in 95% formamide and electrophoresed in a 7% polyacrylamide gel (40:1 acrylamide/*N,N'*-methylenebisacrylamide ratio) containing 7 M urea. (*A*) Exonuclease III mapping of the 3' endpoints. Lane 1: products of G-specific partial chemical cleavage (20) of the 290-bp DNA fragment 5'-end-labeled at the *Eco*RI site. Lanes 2–5: control reactions containing 5'-end-labeled top strand (see Fig. 3*C*) DNA and exonuclease III at 0, 25, 500, or 1000 units (U)/ml in the absence of maize plastid extract. Lanes 6–9: end-labeled top strand digested with exonuclease III at 25, 100, 500, or 1000 U/ml after prior incubation with maize plastid BF. Lanes 10–13: end-labeled bottom strand digested in the presence of maize plastid extract. (*B*) λ exonuclease mapping of the 5' endpoints of the protected region. Lane 1: products of G-specific partial chemical cleavage of the *psbA* gene fragment 3'-end-labeled at the *Hin*dIII site. Lane 2: 3'-end-labeled top strand incubated without exonuclease. Lanes 3–5: end-labeled top strand incubated with λ exonuclease (125 U/ml) at pH 8, 8.5, or 9.1 in the absence of maize plastid extract. Lanes 6–8: end-labeled top strand digested with λ exonuclease (125 U/ml) after incubation with maize plastid extract. Lanes 9 and 10: digestion of end-labeled top strand/*E. coli* RNA polymerase complexes with λ exonuclease (125 U/ml) at pH 8 and pH 9.1. (*C*) Limits of exonuclease digestion on the maize plastid *psbA* gene sequence determined from the data in *A* and *B*. Z.m., endpoint of protection by maize plastid extract; E.c., end of region protected by *E. coli* RNA polymerase. Locations of weaker end signals are designated z.m. and e.c.

−39, −40, and perhaps −41. The 5' end(s) of the DNA fragments incubated with *E. coli* RNA polymerase are at different positions (Fig. 3*B*). Other bands seen on the sequencing gel, in both the control and experimental lanes, may reflect locations of exonuclease pause sites.

Results of the exonuclease experiments are summarized in Fig. 3*C*. The DEAE fraction of the maize plastid extract used here contains one or more proteins that protect a continuous expanse of 55–57 bases (from −40/−39 to +16/+17) on the *psbA* sense strand from digestion by exonuclease III and the λ exonuclease. Purified *E. coli* RNA polymerase holoenzyme protects the region from −27/−28 to +16/+17, and protected fragments extending from −45 to +16/+17 are also seen. The results are comparable to those (27) regarding protection in the adenovirus 5 promoter region by *E. coli* RNA polymerase in an open complex (i.e., in the presence of heparin), and the −20 to +25 protection by this *E. coli* enzyme of sequences around the maize *rbcL* promoter under lower salt conditions (28).

Analysis of *psbA* Promoter Regions by Testing Protein Binding to Deleted Genes. The capacity of protein(s) in BFs to bind to the 290-bp fragment containing nucleotides 1–245 (Fig. 1) was compared with binding to a fragment deleted 5' to nucleotide 126 (Δ126) and to another fragment deleted through nucleotide 137 (Δ137). Standard protein binding assay and polyacrylamide gel electrophoresis conditions were used. As seen in Fig. 4, deletion of sequences starting three nucleotides upstream of the −35 region (Δ126, Fig. 1) does not interfere with binding, but deletion of the −35 region (Δ137, Fig. 1) completely abolishes binding. In contrast, BFs transcribe equally well from cloned supercoiled Δ126 DNA and from the normal gene (in pZmc427), but Δ126 is about one-third as good a template as pZmc427 for most of the RNA polymerase in the extract (data not shown). We do not understand the basis for these transcription differences.

Transcriptional Activity of Chloroplast Extract BF Preparations. Fig. 5 shows a plot of the radioactivity incorporated from [α-^{32}P]UTP into trichloroacetic acid-precipitable RNA

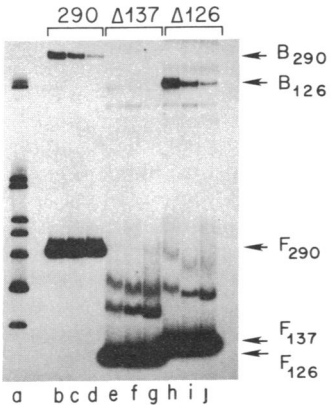

FIG. 4. Autoradiograph of a polyacrylamide gel showing the effects of deletions of *psbA* 5′ sequences on binding of maize chloroplast protein. Extract fraction 9 prepared as for the experiment shown in Fig. 2 was incubated with labeled 290-bp (lane b), Δ 137, or (lane e), Δ 126 (lane h) *psbA* DNA fragments (see text and Fig. 1) excised from the cloning vectors with *Bam*HI and *Bgl* II in the standard DNA binding assay conditions. Supercoiled competing DNAs, pBR322 (lanes c, f, and i) and pZmc427 (lanes d, g, and j), were included at 5 times the molarity of the test DNAs. B_{290} and B_{126}, retarded 290-bp and Δ 126 fragments; F_{290}, F_{137}, and F_{126}, free 290-bp, Δ137, and Δ126 fragments. End-labeled *Hin*fI fragments of pBR322 served as size markers (lane a).

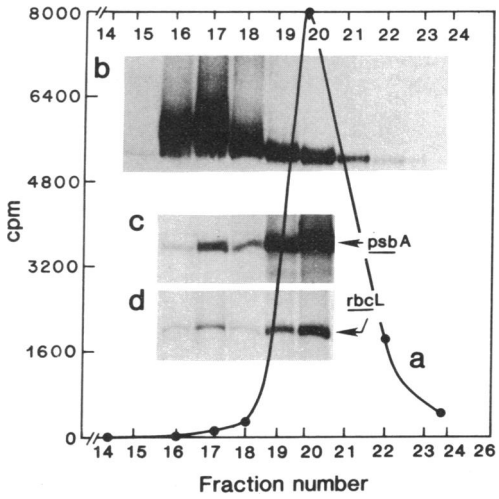

FIG. 5. DEAE fractions of a maize chloroplast extract were assayed for RNA-synthesizing activity with calf thymus DNA template (curve a), binding to a 290-bp DNA fragment containing the 5′ region of *psbA* (*Inset* b), transcription from the cloned maize *psbA* gene in a supercoiled template (*Inset* c), and transcription of cloned maize *rbcL* in a supercoiled template (*Inset* d). Curve a represents ^{32}P cpm incorporated into trichloroacetic acid precipitable RNA from [α-^{32}P]UTP by various fractions; template was calf thymus DNA, and standard assay conditions and incubation time were used. (*Inset* b) Autoradiograph of a gel electrophoresis assay for DNA-binding activity in the DEAE fractions. ^{32}P-labeled 290-bp DNA fragments retarded by interaction with protein in the fractions (compare Fig. 2) are shown. Unbound labeled DNA, which moves faster in the gel, is not shown. (*Inset* c) Part of an autoradiograph of an S1 nuclease/gel electrophoresis assay for gene-specific transcription activity (16). Aliquots of DEAE fractions were incubated with ribonucleotides, including [α-^{32}P]UTP, and supercoiled pZmc427 plasmid (*psbA*) DNA (σ about −0.06) (18). The radioactive RNA synthesized was hybridized with an excess of mp19-427 single-stranded DNA complementary to *psbA* transcripts but extending beyond the 5′ transcription start site. After S1 nuclease digestion, aliquots containing surviving DNA·[^{32}P]RNA hybrids were subjected to gel electrophoresis under nondenaturing conditions. A peak of activity occurs in fraction 17 (BF peak). The second peak, seen in fraction 20 (PF peak), is at the peak of the incorporation activity with calf thymus DNA template plotted in curve a. (*Inset* d) As in *Inset* c except that the template was *rbcL* and the mp8-pZ3 single-stranded DNA was used for hybridization before S1 nuclease treatment.

transcribed from calf thymus DNA by aliquots of maize plastid extract fractions eluted from a DEAE-5PW column. The peak of activity was in fraction 20 but the maximum DNA-binding activity peak was in fraction 17 (Fig. 5 *Inset* b). (Smaller, and therefore more, fractions were taken here than in the experiment shown in Fig. 2. Consequently, the fraction numbers do not correspond in the two experiments.)

Probably no more than a few percent of the total maize chloroplast RNA polymerase activity was in the major BFs 16–18. The failure of most of the plastid polymerase in the PFs (RNA polymerase DEAE peak fractions, e.g., fractions 19–22 in Fig. 5) to bind to the linear 290-bp DNA fragment was not surprising, since this enzyme preparation requires supercoiled template DNA for substantial transcription (6, 8) but, because of the contradictions—the DEAE fractions with almost all of the polymerase activity (fractions 19–21) contain a very small part of the binding activity but the protein(s) in BFs protects the promoter sequence of *psbA*—we were interested in qualitative comparisons of the RNA polymerase activities in the BFs and PFs. Unfortunately, fractions near the front of the DEAE elution peak, and even within the peak of RNA polymerase activity, contain topoisomerase and nuclease activities that can interfere with assays for the relation of transcription to template topology. The PF-derived enzyme used in previous experiments designed to study quantitative relationships between transcription and template topology in detail was purified through a second column to remove toposiomerases and nucleases (8). BFs or PFs were not purified further because of possible loss of important components; however, we observed no changes in the supercoiled template after incubation for 2½ min with BFs or PFs that had been stored at −80°C for a few weeks. After 10 min, some of the supercoiled DNA had been converted to another form—probably linear—but most of the template remained supercoiled (data not shown). Polymerase assay times in the present experiments were therefore 10 min.

Fig. 5 shows that BFs have the capacity to correctly initiate and transcribe the genes *psbA* and *rbcL* from supercoiled templates and that this activity is eluted from DEAE in parallel with the binding activity (fractions 16–18) but is separated from the peak of RNA polymerase activity. However, Fig. 6 shows that transcription by BFs from a relaxed circular template is about as strong as from a negatively supercoiled template of σ value about −0.06. [Detailed experiments showed slightly stronger transcription by BFs from templates of intermediate negative superhelicity and a decline at higher values (unpublished data). Thus, the BF polymerase is not entirely topologically neutral but is much less sensitive than the PF polymerase in this regard.] Transcription from a linear template was substantially lower and an unidentified band of a larger protected fragment also appeared. The two peaks of transcription from supercoiled templates, fractions 17 and 20 (Fig. 5 *Insets* c and d), correspond to the BF and PF peaks, respectively.

When a relaxed template was incubated with BF or PF under RNA polymerase assay conditions, negatively supercoiled DNA was not formed (data not shown)—i.e., the preparations were not contaminated with DNA gyrase and the approximately equal RNA polymerase activity of BF on relaxed and supercoiled (σ value about −0.06) templates cannot be accounted for by gyrase activity. The capacity of BF to transcribe relaxed as well as supercoiled cloned chloroplast DNA has been tested with maize *psbA*, *rbcL* of maize and rice, and maize *atpBE* with similar results. As expected (6, 8), PF transcribes cloned maize chloroplast

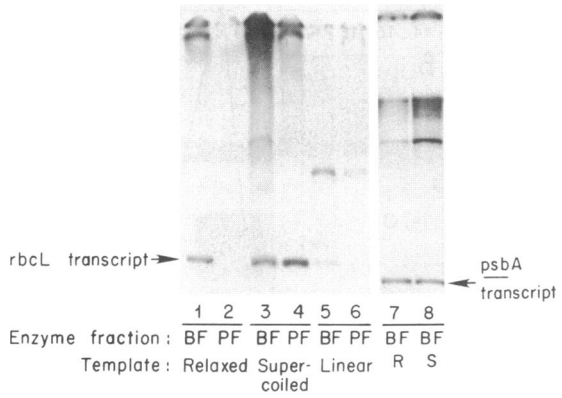

FIG. 6. Autoradiograph showing the relation between template conformation and transcription of cloned maize chloroplast genes *psbA* and *rbcL* by BF and PF. Templates were pZmc427 DNA for transcription of *psbA* and pZmc460 for transcription of *rbcL*. Relaxed DNA was prepared by incubation of supercoiled DNA with topoisomerase I (8). pZmc460 DNA was linearized with *Xba* I. Transcription was assayed by the S1 nuclease method (16). S1-resistant hybrids of single-stranded DNA probes and ^{32}P-labeled transcripts of *rbcL* and *psbA* are indicated. R, relaxed; S, supercoiled.

DNA actively from a supercoiled template but almost undetectably from a relaxed template (Fig. 6).

DISCUSSION

Partially purified preparations of the maize chloroplast RNA polymerase preferentially transcribe cloned chloroplast DNA sequences from supercoiled DNA but not from relaxed DNA (6), and the rate of transcription of cloned maize chloroplast genes *in vitro* by such preparations is much greater from supercoiled than from relaxed circular templates (8). We report here that fractions of plastid extracts that are eluted from DEAE with KCl ahead of the bulk of the RNA polymerase activity transcribe three maize chloroplast genes and one rice chloroplast gene from relaxed templates about as well as from negatively supercoiled templates, in contrast to the bulk of the polymerase, which prefers negatively supercoiled to relaxed chloroplast DNA templates by more than 50:1. Further, the BF protein binds to the promoter region of *psbA*, whereas fractions with the maximum polymerase activity (PFs) contain very little DNA-binding material.

It remains to be determined whether (*i*) the BFs contain the PF type of multimeric RNA polymerase (2) mixed with a free protein factor that coincidently is eluted with it and alters the behavior of PF enzyme, or (*ii*) the BFs contain a complex of all the subunits of the PF enzyme plus one or more additional, perhaps dissociable, components that alter its behavior; or (*iii*) the BF and PF enzymes are composed of totally distinct sets of polypeptides. If *i* or *ii* is true, have chloroplast RNA polymerase preparations that preferentially transcribe supercoiled template DNA lost a component that normally confers the capacity for using relaxed templates? Is all of the polymerase equally capable of transcribing relaxed and supercoiled DNAs *in situ*? If the PFs and BFs contain substantially different RNA polymerases, we must ask whether they have distinctive roles with regard to their natural templates and differential gene transcription as well as whether their activities can be regulated in different ways. It has been argued that *Euglena* chloroplasts contain two physically and functionally distinct polymerases (29).

With PF preparations free of nuclease and topoisomerase activity (8), the transcription rate/negative superhelicity profiles of maize chloroplast genes *rbcL* and *atpBE* were found to differ from one another, although both genes are transcribed much more actively from optimally negatively supercoiled templates than from relaxed templates. These results are in accord with earlier, less precise, experiments (6). Comparably, Borowiec and Gralla (30, 31) have shown that the rate of formation of open complexes by the *lac* P^s promoter and *E. coli* RNA polymerase holoenzyme is 40-fold greater when the template is moderately supercoiled than when it is relaxed, and that mutations in the *lac* P^s region affect the extent of stimulation and the amount of superhelicity associated with maximal stimulation. [*E. coli* holoenzyme transcribes maize *atpBE* >15-fold more actively from a template of σ value -0.065 than from a relaxed template (J.H., unpublished data).] Thus, transcription from the maize chloroplast promoters enumerated above by both maize PF and *E. coli* holoenzymes is strongly responsive to template topology; the BF enzyme is different from both of these in being much less responsive to the topology of plastid DNA templates in circular plasmids over the range of maximal differences for PF and *E. coli* enzymes. The basis for this difference between PF and BF polymerase activities remains to be determined but does not correspond to that between holoenzyme and core forms of *E. coli* RNA polymerase. The bacterial core polymerase does not initiate transcription correctly from cloned maize *rbcL* regardless of the topology of the plasmid template (J.H. and L.B., unpublished data).

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