Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3423–3427, June 1981
Biochemistry

Overlapping divergent genes in the maize chloroplast chromosome and *in vitro* transcription of the gene for $tRNA^{His}$

(DNA sequence analysis/1.6-kilobase RNA and $tRNA^{His}$ genes/S1 nuclease mapping/chloroplast RNA polymerase/transcription initiation site)

ZSUZSANNA SCHWARZ*, SETSUKO O. JOLLY, ANDRE A. STEINMETZ, AND LAWRENCE BOGORAD

The Biological Laboratories, Harvard University, Cambridge Massachusetts 02138

Contributed by Lawrence Bogorad, March 2, 1981

ABSTRACT **In the presence of the S polypeptide, maize chloroplast DNA-dependent RNA polymerase preferentially transcribes sequences within the 2200-nucleotide-pair-long maize chloroplast chromosome fragment *Eco ℓ* from a supercoiled chimeric plasmid cloned in *Escherichia coli* [Jolly, S. O. & Bogorad, L. (1980) *Proc. Natl. Acad. Sci. USA* 77, 822–826]. *Eco ℓ* contains one gene for $tRNA^{His}$ and one for a 1.6-kilobase RNA that includes an open reading frame. These two genes overlap by at least a few nucleotides and are transcribed divergently from complementary DNA strands. This indicates possible transcriptional regulation of chloroplast DNA at the nucleotide level. The 5′ end of $tRNA^{His}$ (G-U-G) isolated from maize chloroplasts is indistinguishable from that of the transcript produced from *Eco ℓ in vitro* by maize chloroplast DNA-dependent RNA polymerase. This purified system initiates RNA synthesis faithfully and exhibits preference for some chloroplast genes. Maize chloroplast DNA for $tRNA^{His}$ lacks the sequence C-C-A at its 3′ terminus; it is presumably added post-transcriptionally. Maize $tRNA^{His}$ has both prokaryotic and eukaryotic features.**

The S factor, a 27.5-kilodalton polypeptide isolated from maize chloroplasts, stimulates transcription of circular DNA by maize plastid DNA-dependent RNA polymerase (1, 2) and causes it to preferentially transcribe certain cloned chloroplast DNA (cpDNA) sequences—e.g., *Eco ℓ* (3)—in supercoiled chimeric DNA molecules *in vitro* (4). Three fragments of the *Eco ℓ* size class occur in the maize chloroplast chromosome (3). Two of these are identical, only lying just inside each of the two large inverted repeated sequences that contain genes for rRNAs (3, 5). We have identified two genes on one of the duplicated fragments and used this information to determine whether the 5′ end of a transcript made by the chloroplast polymerase *in vitro* corresponds to the 5′ end of the same RNA as well as to facilitate the search for DNA sequences that may be recognized by the polymerase, the S factor, or both.

The gene for $tRNA^{His}$, found to lie within fragment *Eco ℓ*, overlaps for at least a few nucleotides a gene on the complementary strand of DNA that is transcribed into a 1.6-kilobase (kb) RNA that includes an unidentified open reading frame. Transcription of one of these genes is likely to interfere with and limit transcription of the other.

MATERIALS AND METHODS

Isolation of Nucleic Acids. Total RNA was prepared (6) from plastids of dark-grown *Zea mays* seedlings illuminated for 16 hr. Etioplast and cRNA preparations were gifts of G. Link.

tRNAs were prepared from leaves of maize seedlings (e.g., 500 g) homogenized in the cold in 10 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$. After extraction with phenol and 1% sodium dodecyl sulfate, the RNA was precipitated with ethanol in the presence of 2% potassium acetate. The pellet was resuspended in 5 ml of 2 M $(NH_4)_2SO_4$. After centrifugation the supernatant was applied to a 1.5 × 20 cm Sepharose 4B column, washed with 2 M $(NH_4)_2SO_4$, and eluted with distilled water. After dialysis, the tRNAs were concentrated by ethanol precipitation and stored in water at −20°C.

Supercoiled DNAs of plasmids pZmc150 and pZmc450, which consist of *Eco ℓ* inserted into plasmids pMB9 and pBR322, respectively, were prepared from *Escherichia coli* (7). This work is covered under Section III-0 of the National Institutes of Health recombinant DNA guidelines for performance at P-1 physical containment level.

Labeling of Nucleic Acids. After chemical cleavage (8) of their 3′-terminal pA, 10–40 μg of tRNAs were labeled at 37°C for 45 min with [α-^{32}P]ATP by tRNA nucleotidyltransferase (CCA enzyme) from yeast (kindly provided by R. Giege and G. Burkard, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France) in a 20-μl reaction mixture of the following composition: 200 μCi of [^{32}P]ATP (Amersham, 400 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels), 8 mM dithiothreitol, 10 mM $MgCl_2$, 25 mM Tris·HCl at pH 8.0, and 50 mM CTP (9). The mixture was then loaded onto a Sephadex G-50 column (5-ml disposable pipette filled to the top) and the labeled tRNAs were eluted with 4× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate).

The 5′ ends of DNA restriction endonuclease fragments were labeled (10) with [γ-^{32}P]ATP (New England Nuclear, 3300 Ci/mmol) by polynucleotide kinase (Bethesda Research Laboratories, Rockville, MD). After precipitation with ethanol, the 5′-end-labeled DNA fragments were digested with a restriction enzyme and the subfragments were separated electrophoretically.

Nick translations of DNA probes were performed (11) in a volume of 50 μl.

Preparation of DNA Restriction Fragments. Restriction enzymes were from Bethesda Research Laboratories. Preparative and analytical electrophoretic separations of restriction fragments were performed in 0.7–1.5% agarose slab gels or in 5–8% polyacrylamide gels (12, 13). DNA fragments were eluted from gels electrophoretically (14).

Filter Hybridizations. Identification of cpDNA restriction fragments containing tRNA genes: After digestion with restriction enzymes, cpDNA fragments were separated electrophoretically on 0.7–1.5% agarose slab gels and transferred to nitrocellulose filters (15). Hybridization with labeled tRNAs was in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: cp-, chloroplast; kb, kilobase; tDNA, DNA complementary to the strand coding for tRNA; NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate; DBM-, diazobenzyloxymethyl-.

* Present address: Max-Planck-Institut für Züchtungsforschung, 5 Köln 30, Federal Republic of Germany.

2× NaCl/Cit containing 50% (vol/vol) formamide at 37°C in Seal-N-Save plastic bags (Sears Roebuck).

Identification of cpRNAs complementary to Eco ℓ cpDNA: Between 1 and 5 μg of total plastid RNA was treated with glyoxal and subjected to electrophoresis on 1% agarose gels (16). The RNAs were transferred from the agarose to diazobenzyloxymethyl (DBM)-paper (17). Covalently bound RNA was hybridized to nick-translated DNA probes in 5× NaCl/Cit, 50% formamide, and Denhardt's solution for 24 hr at 42°C (17).

S1 Nuclease Mapping of the 5′ Ends of Chloroplast Transcripts. DNA sequences protected from digestion with S1 nuclease after hybridization to total cpRNAs were identified by a minor modification of the method of Berk and Sharp (18): Between 0.5 and 1 ng of end-labeled DNA restriction fragments was hybridized with 50 μg of total cpRNA at 53°C for 16 hr. After digestion with 5 units (19) of S1 nuclease (Miles Laboratories) per 100-μl reaction mixture for 30 min at 42°C, the sample was precipitated with ethanol, dissolved in the sample buffer used for sequencing reactions, and loaded onto a sequencing gel.

***In Vitro* Transcription of Fragment for S1 Nuclease Experiments.** [^{3}H]RNA was synthesized *in vitro* by using supercoiled pZmc 450DNA (2.5 μg) as template in a 250-μl reaction mixture containing 12.5 μmol of Tris·HCl (pH 8.0), 5 μmol of $MgCl_2$, 12.5 μmol of KCl, 10 μmol of 2-mercaptoethanol, and 25 μl of maize chloroplast RNA polymerase with or without S factor (4). After 30 min at 37°C, 25 μg of yeast tRNA and 5 μg of RNase-free DNase (20) were added to the reaction mixture and it was maintained at 0°C for 30 min. After addition of 120 μmol of ammonium acetate the mixture was extracted with phenol. RNA was precipitated with ethanol.

Hybridizations of *in vitro* synthesized [^{3}H]RNA (23 ng of RNA transcribed in the presence of the S factor, 17 ng of RNA transcribed in the absence of the S factor) with [^{32}P]DNA fragment *Hha* D (0.5–1 ng) and the S1 nuclease experiments were performed as described above.

Sequence Analysis of DNA Fragments. Nucleotide sequences were determined (10) by using thin (0.5 mm × 15 cm × 40 cm) polyacrylamide gels containing 8 M urea (21).

RESULTS

Location and Organization of *Eco*RI Fragment ℓ. Maize cpDNA fragments *Bam* 6 and 8 overlap the large unique region of the chloroplast chromosome and the ends of the two 22-kb-long inverted repeats (3). Each of these *Bam* fragments contains one copy of *Eco* ℓ, the last *Eco*RI-generated fragment within each repeated unit (unpublished data). A refined physical map of *Eco* ℓ is shown in Fig. 1*C*.

***Eco* ℓ Contains a tRNA^{His} Gene.** A number of *Bam* fragments, including *Bam* 6 and *Bam* 8, contain tRNA genes (Fig. 1 *A* and *B*). *Hpa* II-generated fragments of *Eco* ℓ (data not shown) hybridized only to *Hpa* fragment B_2.

Maize cpDNA fragment *Eco* ℓ was shown to contain tDNA^{His} (DNA complementary to the strand coding for tRNA^{His}) by the method described in the legend to Fig. 2.

Fig. 2*A* shows the position of maize chloroplast tRNA^{His} on a two-dimensional electropherogram (23). Fig. 2*B* shows that tRNA^{His} hybridizes with pZmc150 DNA. The presence of tDNA^{His} was confirmed by direct sequence analysis of a portion of the *Hpa* B_2 fragment of *Eco* ℓ DNA as shown in Fig. 3. The sequencing strategy is shown in Fig. 1C. The histidine anticodon sequence GTG occurs at positions 36–38 (Figs. 3 and 4). Unlike genes for tRNAs of *E. coli*, the universal 3′-terminal tRNA triplet C-C-A is not encoded in *Eco* ℓ.

Fragment *Eco* ℓ Encodes Transcripts Other than tRNAs. RNA from maize chloroplasts or synthesized *in vitro* by chloroplast RNA polymerase using pZmc 150 as a template hybridizes to both the faster and slower migrating strands of *Eco* ℓ (4), indicating that this fragment contains information for the synthesis of more than one RNA. To determine the size of cpRNA(s) other than tRNAs encoded within fragment *Eco* ℓ, nick-translated DNA of *Eco* ℓ was hybridized to electrophoretically separated total cpRNA bound to DBM-paper. The probe hybridized to a major band of about 1.6 kb (Fig. 5). The possibility that the band contains more than one RNA species remains to be investigated. Any tRNAs present would have run off the gel.

The 5′ End of Chloroplast tRNA^{His} Is Complementary to the 5′ End of a 1.6-kb cpRNA. As shown in Fig. 5, *Eco* ℓ codes for at least one 1.6-kb RNA in addition to tRNA^{His}. The 5′ end of

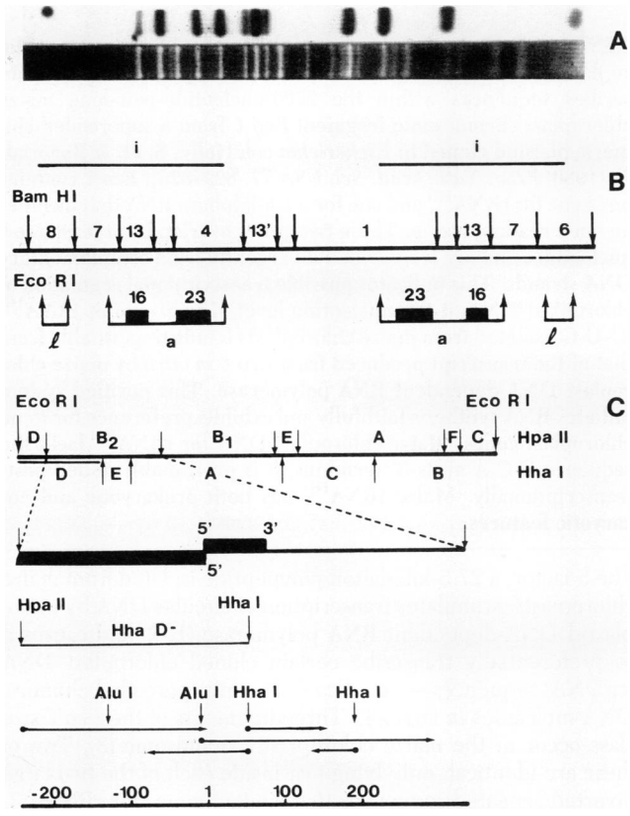

FIG. 1. Location of the gene for tRNA^{His} in the maize chloroplast chromosome. (*A*) Lower: ethidium bromide-stained 0.75% agarose gel showing electrophoretic separation of *Bam*HI restriction fragments of total cpDNA. Upper: autoradiograph of Southern (15) blots after hybridization with total radioactive leaf tRNAs. (*B*) Physical map of the region within the circular chloroplast chromosome that contains the large inverted repeats (i) carrying the genes for rRNAs. Heavy bars indicate positions of genes for the 16S and 23S rRNAs. Recognition sites for *Bam*HI are indicated by arrows pointing downward, and for *Eco*RI by arrows pointing upward. The size classes of the restriction fragments are given by numbers for *Bam*HI and lower-case letters for *Eco*RI. Only fragments of known position are shown. *Bam*HI restriction fragments that hybridize with chloroplast tRNAs are underlined. (*C*) Refined physical map of one copy of fragment *Eco*RI ℓ showing *Hpa* II (downward arrows) and *Hha* I (upward arrows) recognition sites. The letters are for size classes (decreasing size from A to F) observed after electrophoresis of the restriction fragment mixtures on a 6% polyacrylamide gel. Each fragment is designated by the (abbreviated) name of the restriction enzyme used to generate it and its size (given by number or letter)—e.g., *Bam* 7, *Eco* ℓ, *Hpa* B, *Hha* D. Chloroplast tRNA hybridizes to *Hha* fragment D. It is underlined. Fragment *Hpa* B_2, part of which was used for sequence analysis, is shown enlarged and the positions of the tRNA^{His} gene and the gene for the 1.6-kb transcript are indicated with heavy bars. The sequencing strategy used is shown by horizontal arrows below the physical map. The dots under the restriction sites show the 5′ labeled end of the fragment and the lengths of the arrows indicate the DNA region sequences. The scale under these fragments is in base pairs.

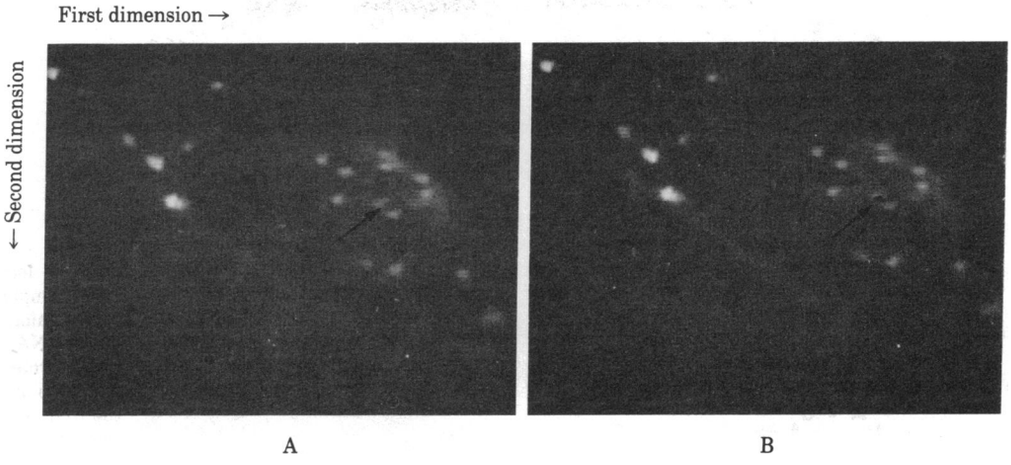

FIG. 2. Identification of the tRNA coded by *Eco* ℓ. (*A*) Maize chloroplast tRNAs after two-dimensional electrophoresis (23). The arrow points to $tRNA^{His}$. (*B*) The arrow points to the position of the radioactive spot resulting from this experiment: DNA of pZmc150 (10–20 μg) was bound to 25-mm-diameter nitrocellulose filter discs (0.45-μm pore size, Sartorius). Labeled tRNAs in 2× NaCl/Cit and 50% formamide were hybridized to the DNA for 20 hr at 37°C. Filters were washed at least four times with 50 ml of 2× NaCl/Cit and once with distilled water at room temperature. RNA was eluted by soaking discs in 1 ml of 95°C distilled water for 1 min. Each filter disc could be used for several hybridizations. After addition of 10 μg of unlabeled total chloroplast tRNAs, the tRNAs were concentrated by freeze drying or precipitation with ethanol. The tRNA mixture in 20 μl of loading buffer (22) was introduced on a 10% polyacrylamide gel (0.8 mm × 47 cm × 23 cm; 1-cm slots) and electrophoresed at 600 V in the cold until the xylene cyanol dye marker reached the bottom of the gel. For separation in the second dimension, a track of the 10% polyacrylamide gel slab was embedded horizontally across the top of a 20% polyacrylamide gel (0.8 mm × 47 cm × 23 cm) and electrophoresed in a 4°C room at 600 V until the marker reached the bottom (22). The positions of maize chloroplast tRNAs in this two-dimensional gel system are known (23). The gel was labeled at its edges with radioactive ink and covered with Saran Wrap. After autoradiography overnight (Kodak XR-5 film), the spot showing the location of radioactivity was cut out of the film, which was then oriented on the gel in its original position to guide removal of the radioactive portion of the gel. The gel was stained with ethidium bromide (25 μg/250 ml) for 5 min and exposed to ultraviolet light to visualize tRNAs.

the 1.6-kb RNA is located within subfragment *Hpa* B_2 of *Eco* ℓ and is encoded by the DNA strand complementary to that containing the gene for $tRNA^{His}$ (data not shown).

The identity of the nucleotide on *Eco* ℓ complementary to the 5′ end of chloroplast $tRNA^{His}$ was sought in S1 nuclease protection experiments (24–26). Fragment *Hha* D of *Eco* ℓ, whose ends are determined by recognition sites for *Eco*RI and *Hha* I (Figs. 1 and 3), was labeled at the 5′ end and then digested with *Hpa* II. The DNA fragment labeled at its *Hha* I end, designated *Hha* D−, was isolated; this fragment extends from nucleotide −231 to +49 in the lower strand (Fig. 3). DNA fragments surviving after hybridization and S1 nuclease treatment are indicated with arrows at the right-hand edge of Fig. 6*A*. Other lanes contain the same end-labeled original full-sized DNA fragment used in the hybridization but treated for DNA sequence analysis (10). From these data, and taking into account that fragments in the sequencing ladder are reported to migrate 1–1½ nucleotides faster than an S1 nuclease digestion product of the same size (26), it is concluded that protection of DNA against digestion by hybridization with $tRNA^{His}$ extends to positions −1, −2, −3, or −4 (Fig. 3, upper strand).

The conclusion that the 5′ end of mature $tRNA^{His}$ extends to position −4, −3, −2, or −1 conflicts with the prediction, based on the usual 8-base-pair aminoacyl stem of the known $tRNA^{His}$ sequences (32), that the 5′ end of this tRNA is at +1. The discrepancy could result from an exceptional type of mature $tRNA^{His}$, an extraordinarily abundant precursor at least four nucleotides longer than the mature form, or an unrecognized peculiarity of the S1 nuclease experiment, including electrophoresis artifacts. The first two possibilities seem unlikely. Our

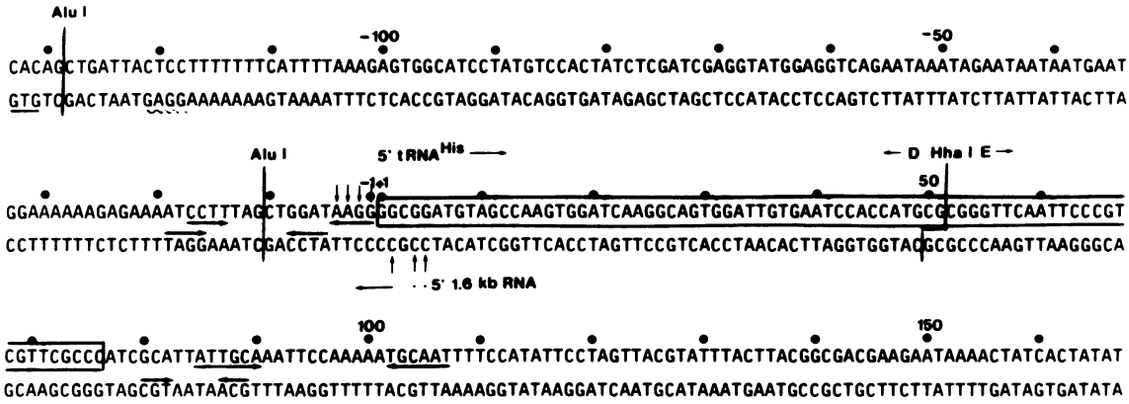

FIG. 3. The nucleotide sequence of the portion of *Eco* ℓ that contains the gene for $tRNA^{His}$. The sequence of $tDNA^{His}$ is boxed within the noncoding DNA strand and nucleotide number +1 corresponds to the deduced 5′ end of the mature $tRNA^{His}$. Negative and positive numbers indicate the distance of nucleotides upstream and downstream from +1, respectively. Vertical arrows point to nucleotides that are judged to be at 5′ ends of the mature transcripts for $tRNA^{His}$ (upper strand) and for the 1.6-kb transcript (lower strand). The amino acid sequence deduced from the nucleotide sequence starting at position −131 is: Met-Thr-Arg-Lys-Lys-Thr-Asn-Pro-Phe-Val-Ala-Arg-His-Leu-Ala-Lys-Ile-Glu-Lys-Val-Asn-Met-Lys-Gly-Glu-Lys-Glu-Ile-Ile-Val-Thr-Trp-Ser ... Only the translation initiation codon (−131 to −133) sequence is shown above.

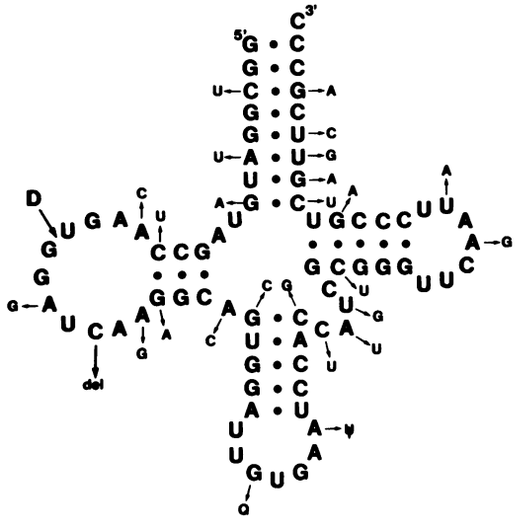

FIG. 4. The cloverleaf structure of maize chloroplast $tRNA^{His}$ as deduced from the tDNA sequence. The 5′-terminal G is considered to be at position 1. Small arrows pointing outward indicate nucleotides present at the position in the corresponding (unmodified) *E. coli* tRNA isoacceptor. The deletion of a nucleotide is shown by "del," and the boldface letter is an insertion as compared to the *E. coli* tRNA.

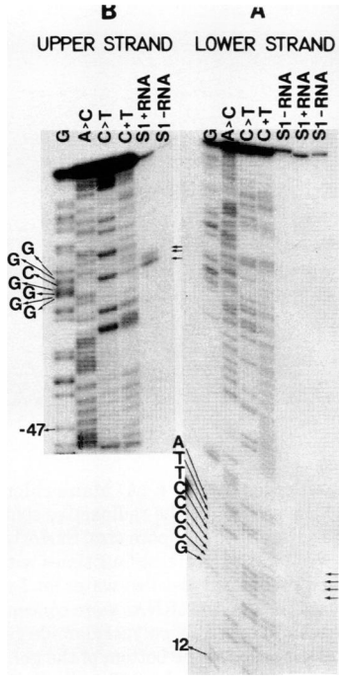

FIG. 6. Result of an experiment to locate nucleotides in *Eco* ℓ complementary to the 5′ ends of chloroplast $tRNA^{His}$ and 1.6-kb RNA. See text for S1 nuclease protection experiments. Numbers give the position of the first G visible on the autoradiograph within the nucleotide sequence in Fig. 3. The sequences in the vicinities of the 3′ ends of the DNA sequences protected by $tRNA^{His}$ (*A*) and 1.6-kb RNA (*B*) are indicated at the left in each case. The arrows at the right in *A* and *B* point to the protected DNA fragments seen in the "S1 + RNA" columns.

experience suggests that S1 nuclease may have some specificity for particular sequences or may slow down at certain nucleotides. For example, after partial digestion of a DNA fragment containing part of maize chloroplast rDNA (experiment not shown) some fragment size classes were overrepresented and some were entirely missing.

To locate the nucleotide complementary to the 5′ end of the 1.6-kb RNA, fragment *Hpa* D_2 (Fig. 2) was labeled at its 5′ ends and digested with *Hha* I to generate fragment *Hha* D− labeled at its *Hpa* II end. The locations of DNA fragments surviving after hybridization of *Hha* D− with tRNAs and S1 nuclease treatment are indicated on the sequencing gel (Fig. 6*B*). The conclusion that the complementarity between the RNA and DNA extends to positions +2, +4, or +5 is indicated in Fig. 3 on the lower, noncoding, strand. The genes for $tRNA^{His}$ and the 1.6-kb RNA overlap by three to nine nucleotides.

To determine whether the 5′ end of the chloroplast $tRNA^{His}$ corresponds to the 5′ end of the *in vitro* transcript of the same gene, an S1 nuclease protection experiment with 5′-end-labeled DNA fragment *Hha* D−, like that described above, was carried out. RNA used for hybridization was either total cpRNA or [^{3}H]RNA synthesized *in vitro*. DNA fragments of the same size are produced whether the *in vitro* transcript or chloroplast $tRNA^{His}$ is used to protect the cpDNA fragment (Fig. 7).

These data interdependently support the views that maize chloroplast RNA polymerase initiates transcription of the $tRNA^{His}$ gene at the same position *in vitro* as *in vivo* and that the *in vitro* transcription system initiates faithfully. Data shown in Fig. 7 do not permit us to conclude whether the S factor has a quantitative effect on correct initiation.

A Putative Signal for Translation Within the 1.6-kb cpRNA. The first possible initiation codon in the 1.6-kb RNA gene not soon followed by a stop codon is GTG at positions −131 to −133 (Fig. 3). This putative initiation codon is preceded, at positions −115 to −122, by the sequence A-A-A-G-G-A-G, which is complementary to the 3′ end of maize chloroplast 16S rRNA (27). A similar sequence has been found at a comparable location on the gene for the large subunit of ribulosebisphosphate carboxylase (24).

It is not known whether the 1.6-kb transcript is translated into a polypeptide *in vivo*.

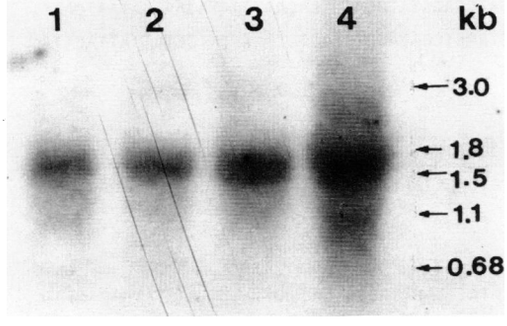

FIG. 5. Identification of cpRNAs with sequences complementary to *Eco* ℓ. Denatured maize cpRNA was subjected to electrophoresis on a 1% agarose gel and transferred to DBM-paper. It was then hybridized with ^{32}P-labeled nick-translated *Eco*RI fragment ℓ. The autoradiograph is shown here. Lane 1, 1 μg of etioplast RNA; lane 3, 1 μg of cpRNA; lanes 2 and 4, 1 and 5 μg, respectively, of plastid RNA of dark-grown plants illuminated for 16 hr.

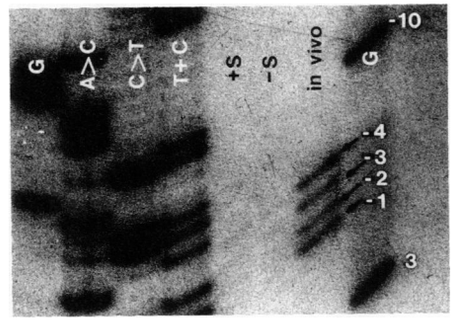

FIG. 7. DNA fragments protected from digestion with S1 nuclease by hybridization with chloroplast $tRNA^{His}$ or transcripts of the same gene made *in vitro* by maize chloroplast RNA polymerase in the absence (−S) and presence (+S) of S factor. See text for details. An enlargement of an autoradiograph of a sequencing gel is shown. DNA fragments protected from digestion by S1 nuclease are marked −1, −2, −3, and −4 (see Fig. 3).

DISCUSSION

***In Vitro* Transcription of tDNA^{His}.** Maize chloroplast DNA-dependent RNA polymerase (1, 2) together with a polypeptide that stimulates its activity and affects its specificity promotes the transcription of cpDNA sequences in supercoiled chimeric plasmid DNA containing the *Eco* ℓ fragment (4). It is shown here that the 5′ end of the *in vitro* transcript of tDNA^{His} is indistinguishable from that of tRNA^{His} from maize plastids. There is similar correspondence between the mRNA for the large subunit of ribulosebisphosphate carboxylase of maize and the *in vitro* transcript of the cloned gene (unpublished data).

Thus, this *in vitro* system is capable of preferential transcription and correct initiation. DNA sequences on the chloroplast chromosome recognized by the enzyme remain to be identified, but the availability of the maize chloroplast *in vitro* transcription system together with cloned and sequenced cpDNA fragments should permit recognition of sequences on cpDNA that influence gene expression through transcription.

Divergent Transcription from cpDNA. Overlapping genes in bacteria and bacteriophages have been observed most frequently either within the regulatory regions for transcripts on opposite strands of DNA or within transcripts of the same DNA strand (28, 29). The mature transcripts from the region of *Eco* ℓ we have studied are complementary and overlap at their 5′ termini; thus their transcription must be divergent. This feature resembles regions coding for oop RNA and the O protein in bacteriophage λ DNA (30).

Wherever RNA polymerase recognition sites for the tRNA^{His} and 1.6-kb RNA genes are located, transcription of both is not likely to occur simultaneously. Transcription of one would be expected to interfere with that of the other.

Comparison of Chloroplast tDNA^{His} with Genes and Isoacceptors from Other Sources. The ribonucleotide sequence deduced from chloroplast tDNA^{His} shows about 60% homology with the tRNA^{His} of *E. coli* (31) and about 48% homology to that of yeast mitochondria (32, 33). The absolute divergence among the three sequences is high: only 29 of 75 positions are common to all. All highly conserved positions (32) are maintained in the chloroplast tDNA^{His} sequence.

There are marked differences between the D loop and the D stem of maize plastid and *E. coli* tRNAs: deletion in the loop and shortening of the stem (Fig. 4). The sequence of the extra arm is also different from that of *E. coli* but its size (in contrast to the yeast mitochondrial tRNA^{His}) is the same.

Evolutionary Aspects of Plastid tDNA Structure. The evolutionary relationship between prokaryotic genomes and mitochondrial, plastid, and nuclear genomes of eukaryotic cells is perplexing and intriguing. The 3′-terminal C-C-A present on all tRNAs is encoded in the known structural genes in *E. coli* but it is added posttranscriptionally in coliphages and eukaryotic cytoplasmic tRNAs, maize chloroplast tRNAs for histidine, leucine, serine, and phenylalanine (unpublished data), and yeast mitochondrial tRNAs. However, unlike the mitochondrial tRNA^{His} (33, 34), the A+U content of chloroplast tRNA^{His} is similar to that of the *E. coli* tRNA (Fig. 4). In all, chloroplast tRNA^{His} has more nucleotides in common with the *E. coli* tRNA than with the mitochondrial isoacceptor; most of the 27 positions common to all three (excluding the C-C-A end) are those highly conserved in all tRNAs (32).

Chloroplast tRNA^{His}, as well as chloroplast tRNAs for serine, leucine, and phenylalanine, does not show features as unusual as those of many mitochondrial tRNAs (33–35). Chloroplast tRNAs are unique and not only may be transitional with regard to prokaryotes and eukaryotes but also may have a separate place in organelle evolution if plastids and mitochondria are not on independent evolutionary lines.

We are indebted to Mrs. Linda Wolff and Elfi Schiefermayer for preparation of plasmid DNA. This research was supported principally by a research grant from the National Science Foundation. It was also supported in part by grants from the National Institute of General Medical Sciences, the Competitive Research Grants Office of the U.S. Department of Agriculture, and the Maria Moors Cabot Foundation of Harvard University. A.A.S. is Attache de Recherche at the Centre National de la Recherche Scientifique, France, and the recipient of a North Atlantic Treaty Organization Grant-in-Aide.

1. Smith, H. J. & Bogorad, L. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4839–4842.
2. Kidd, G. H. & Bogorad, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4890–4892.
3. Bedbrook, J. R. & Bogorad, L. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4309–4313.
4. Jolly, S. O. & Bogorad, L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 822–826.
5. Bedbrook, J. R., Kolodner, R. & Bogorad, L. (1977) *Cell* **11,** 739–746.
6. Link, G., Coen, D. M. & Bogorad, L. (1978) *Cell* **15,** 725–731.
7. Clewell, D. B. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* **62,** 1159–1166.
8. Neu, H. C. & Heppel, L. A. (1964) *J. Biol. Chem.* **239,** 2927–2934.
9. Rether, B., Bonnet, J. & Ebel, J. P. (1974) *Eur. J. Biochem.* **50,** 281–288.
10. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
11. Brown, K. D., Bennett, F. L., Schweingruber, M. E. & Yanofsky, C. (1978) *J. Mol. Biol.* **121,** 153–177.
12. Sharp, P. A., Sugden, B. & Sambrook, J. (1973) *Biochemistry* **12,** 3055–3063.
13. Jeppesen, P. G. N. (1974) *Anal. Biochem.* **58,** 195–207.
14. Wienand, U., Schwarz, Z. & Feix, G. (1979) *FEBS Lett.* **98,** 319–323.
15. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
16. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4835–4838.
17. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5350–5354.
18. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–732.
19. Vogt, V. M. (1973) *Eur. J. Biochem.* **33,** 192–200.
20. Maxwell, I. H., Maxwell, F. & Hahn, W. E. (1977) *Nucleic Acids Res.* **4,** 241–246.
21. Sanger, F. & Coulson, A. E. (1978) *FEBS Lett.* **87,** 107–110.
22. Fradin, A., Gruhl, H. & Feldman, H. (1975) *FEBS Lett.* **50,** 185–189.
23. Mubumbila, M., Burkard, G., Keller, M., Steinmetz, A., Crouse, E. & Weil, J. H. (1980) *Biochim. Biophys. Acta* **609,** 31–39.
24. McIntosh, L., Poulsen, C. & Bogorad, L. (1980) *Nature (London)* **228,** 556–560.
25. Moss, T. & Birnstiel, M. L. (1979) *Nucleic Acids Res.* **6,** 3733–3743.
26. Sollner-Webb, B. & Reeder, R. H. (1979) *Cell* **18,** 485–499.
27. Schwarz, Z. & Kossel, H. (1979) *Nature (London)* **297,** 520–522.
28. Rosenberg, M. & Court, D. (1979) *Annu. Rev. Genet.* **13,** 319–353.
29. Campbell, A. (1979) *Biological Regulation and Development,* ed. Goldberger, R. (Plenum, New York), 19–55.
30. Schwarz, E., Scherer, G., Hobom, G. & Kössel, H. (1978) *Nature (London)* **272,** 410–414.
31. Singer, C. E. & Smith, G. R. (1972) *J. Biol. Chem.* **247,** 2989–3000.
32. Gauss, D. H. & Sprinzl, M. (1981) *Nucleic Acids Res.* **9,** r1–r23.
33. Bos, J. L., Osinga, K. A., Van der Horst, G. & Borst, P. (1979) *Nucleic Acids Res.* **10,** 3255–3266.
34. Martin, N. C., Miller, D., Hartley, J., Moynihan, P. & Donelson, J. E. (1980) *Cell* **19,** 339–343.
35. Heckman, J. E., Alzner-Deweerd, B. & RajBhandary, U. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 717–721.