Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp. 4309–4313, December 1976
Biochemistry

Endonuclease recognition sites mapped on *Zea mays* chloroplast DNA

(chloroplast rRNAs/*Sal* I/*Bam* I/*Eco*RI/hybridization)

JOHN R. BEDBROOK AND LAWRENCE BOGORAD

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Contributed by Lawrence Bogorad, September 27, 1976

ABSTRACT **The closed-circular DNA molecules of 85 × 10^6 daltons from *Zea mays* chloroplasts were isolated, digested with the restriction endonucleases *Sal* I, *Bam* I, and *Eco*RI, and the resulting fragments sized by agarose gel electrophoresis. A map of maize chloroplast DNA showing the relative location of all the *Sal* I recognition sequences and many of the *Bam* I and *Eco*RI recognition sites was determined. A DNA sequence representing approximately 15% of the *Zea mays* chloroplast genome is repeated. The two copies of this sequence are in an inverted orientation with respect to one another and are separated by a nonhomologous sequence representing approximately 10% of the genome length. The inverted repeats contain the genes for chloroplast ribosomal RNAs.**

The DNA of higher plant chloroplasts can be isolated as a covalently closed-circular molecule with a molecular weight, depending on the species, of 85 to 95 × 10^6 (1, 2). The molecular size of chloroplast DNA is about the same whether determined by electron microscopy or renaturation kinetics and suggests that the circular DNA is a single "unique" sequence. Denaturation mapping of chloroplast DNA by electron microscopy (3) further supports this view.

In this paper, we report the physical mapping of chloroplast DNA fragments from *Zea mays* produced by using restriction endonucleases. DNA fragments produced by restriction enzymes were fractionated by agarose gel electrophoresis. The order of fragments produced by one enzyme was determined by finding overlapping fragments produced by another enzyme. The order of all the fragments resulting from digestion with *Sal* I, is given. Genes for chloroplast ribosomal RNAs have been located on the physical map.

MATERIALS AND METHODS

DNA Isolation. Chloroplast DNA was isolated from young leaves of *Zea mays* (WFG TMSx BS7 Illinois Foundation Seeds, Inc.) by methods described by Kolodner and Tewari (4). Lambda phage DNA was prepared from C1857S7 (5).

Restriction Endonucleases. The endonucleases *Sal* I, *Bam* I, *Eco*RI, and *Hae* III were prepared and assayed according to published procedures (6–9). Enzyme activities were determined by using lambda phage DNA as a substrate. DNA at 10–40 μg/ml was digested with endonucleases in reaction mixtures of 10–50 μl. Chloroplast DNA was incubated with 2 to 10 times more enzyme than was needed to complete the digestion in 2 hr at 37°. Digestion with *Sal* I was in 6 mM Tris·HCl at pH 8.3, 120 mM NaCl, 5 mM $MgCl_2$, 60 μg/ml of bovine serum albumin, and 8% glycerol (vol/vol). The *Bam* I reaction was carried out in 10 mM Tris·HCl at pH 8.0, 80 mM KCl, 7 mM $MgCl_2$, and 1 mM 2-mercaptoethanol. *Eco*RI incubations were in 45 mM Tris·HCl at pH 7.4, 85 mM NaCl, 5 mM $MgCl_2$, 0.04% Triton X-100 (vol/vol), and 14% glycerol (vol/vol). Reactions were stopped by making the solution 25 mM EDTA.

Agarose Gel Electrophoresis of DNA Digested with Restriction Enzymes. Agarose gels were prepared, stained with ethidium bromide, and photographed as previously described (10, 11). DNA samples in volumes of 10–50 μl containing 5% glycerol and 0.005% bromophenol blue were layered under the electrophoresis buffer (40 mM Tris, 5 mM sodium acetate, and 1 mM EDTA at pH 7.8). Electrophoresis was for 16 hr at 1 mA per gel.

Stoichiometry of DNA Restriction Endonuclease Fragments from Chloroplast DNA. The relative amounts of DNA in individual bands in agarose gels was determined by scanning the gels in a recording fluorimeter using 305 nm as the excitation wavelength and 600 mn as the measuring wavelength. The amount of DNA per band was estimated by calculating the area under the scan and comparing it to standards.

DNA Isolation from Agarose Gels. DNA fragments were eluted from agarose gels using the procedure described by Tanaka and Weisblum (12).

Copy RNA Transcribed from Isolated DNA Fragments. *E. coli* RNA polymerase (RNA nucleotidyltransferase, nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) was prepared according to Berg *et al.* (13). Copy RNA was made in 150 μl reaction mixtures containing 0.2 μg of DNA, *E. coli* RNA polymerase, and 10 μCi of [^{32}P]UTP (specific activity 100 Ci/mmol, New England Nuclear) in 50 mM KCl; 50 mM Tris·HCl at pH 8.0; 0.1 mM dithiothreitol; 10 mM $MgCl_2$: 2 mM each CTP, GTP, ATP; and 10% glycerol (vol/vol). The incubation mixture, at 37° for 60 min, was terminated by addition of 40 μg of yeast tRNA and 0.30 ml of 2 M ammonium acetate. The mixture was homogenized with 0.5 ml of water-saturated phenol and RNA was precipitated from the aqueous phase with 2.5 volumes of 95% ethanol. The precipitate was washed twice with 65% ethanol, dried in a vacuum desiccator and dissolved in 0.70 M NaCl and 0.07 M sodium citrate at pH 7.0 containing 50% formamide (vol/vol).

Chloroplast Ribosomal RNA. Chloroplast rRNA was prepared from purified chloroplast ribosomes (14) and radiolabeled *in vitro* by exchanging the 5′ OH with ^{32}P by using [α^{32}P]ATP and polynucleotide kinase (polynucleotide 5′-hydroxyl-kinase, ATP:5′-dephosphopolynucleotide 5′-phosphotransferase, EC 2.7.1.78) (N. Maizels, personal communication).

Transfer of DNA from Agarose Gels to Nitrocellulose Filters. Chloroplast DNA fragments fractionated on agarose gels were transferred directly to strips of Millipore filter (HAWP00010) (15).

Filter Hybridizations. ^{32}P-Labeled cRNA transcribed from chloroplast-DNA fragments and ^{32}P-labeled chloroplast rRNA were hybridized to chloroplast DNA fragments on nitrocellulose

Abbreviations: cRNA, complementary RNA; *Sal* I, restriction endonuclease from *Streptomyces albus garcia*; *Bam* I, restriction endonuclease from *Bacillus amyloliquefaciens* H; *Eco*RI, restriction endonuclease from *E. coli*; *Hae* III, restriction endonuclease from *Haemophilus agyptius*.

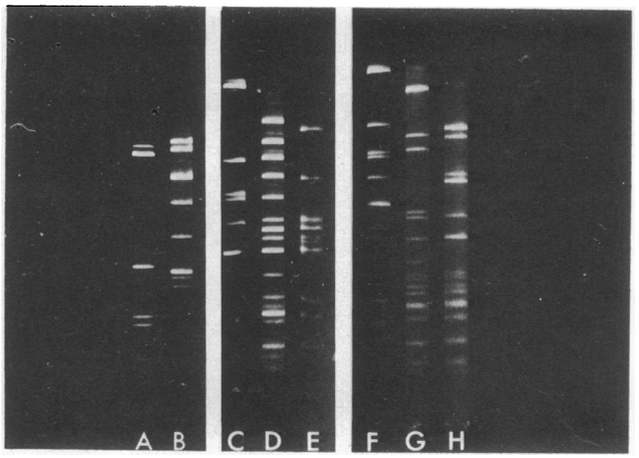

FIG. 1. Fractionation of chloroplast-DNA fragments by agarose gel electrophoresis. (A) Lambda phage DNA digested with *Eco*RI, 0.75% agarose gel. (B) Chloroplast DNA digested with *Sal* I, 0.75% agarose gel. (C) Lambda phage DNA digested with *Eco*RI plus lambda phage DNA digested with *Hae* III, 0.85% agarose gel. (D) Chloroplast DNA digested with *Bam* I, 0.85% agarose gel. (E) Chloroplast DNA digested with both *Bam* I and *Sal* I, 0.85% agarose gel. (F) DNA as in (C), 1.0% agarose gel. (G) Chloroplast DNA digested with *Eco*RI, 1.0% agarose gel. (H) Chloroplast DNA digested with *Eco*RI and *Sal* I, 1.0% agarose gel.

filters. RNA in 0.15 ml of 0.7 M NaCl and 0.07 M sodium citrate at pH 7.0 containing 50% formamide was used to wet the filter strips. The filters were wrapped in Saran wrap (self adhesive plastic), incubated at 42° for 24 hr, and then washed exhaustively with 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0, at 65°.

RESULTS

Fragmentation of *Zea mays* Chloroplast Genome with Restriction Endonucleases. Fig. 1 shows gel electrophoresis of maize chloroplast DNA fragments produced by terminal digestion with *Sal* I (Fig. 1B), *Bam* I (Fig. 1D), and *Eco*RI (Fig. 1G). Lambda-phage DNA digested with *Eco* RI (16) and with *Hae* III (11, 17) is also shown (Fig. 1A, C, and F). Table 1 gives the estimated molecular size of fragments produced by the three endonucleases. In the following discussion, the fragments produced by *Sal* I are called: A, B, C, etc.; by *Bam* I: 1, 2, 3, etc.; and by *Eco*RI: *a, b, c,* etc. Chloroplast DNA fragments produced by complete digestion with *Sal* I are termed "*Sal* I fragments", those produced by digestion with *Bam* I, "*Bam* I fragments" and those produced by digestion with *Eco*RI are termed "RI fragments". The *Sal* I fragments shown in Fig. 1B represent the entire chloroplast genome because the sum of the fragment molecular weights is equivalent to the molecular weight of the *Zea mays* chloroplast genome determined by electron microscopy (1). The *Bam* I fragments shown in Fig. 1 represent approximately 80% of the genome and the RI fragments approximately 60%. Many size classes of fragments produced by *Bam* I and RI are small and are lost from the bottom of these gels during electrophoresis. Fractionation of the *Bam* I and RI fragments on higher percentage agarose gels reveals these smaller size classes.

Table 1 shows that size class C produced by digestion with *Sal* I is represented twice and size class F three times per genome. *Bam* I digestion of C (not shown) produces fragments the molecular weights of which sum to twice the molecular weight of C itself. C, therefore, is two different sequences of identical length named C and C′. Similarly, the size class F is comprised of two different sequences of equal length, one of which is represented twice per genome, named F, and another present once and named F′. *Sal* I fragment G was found to be present in less than 1 copy per genome in three independent estimations. The *Bam* I fragments 7, 9, and 15 are present twice per genome. Digestion of these fragments with *Eco*RI shows each is a single sequence. Size class 13 is composed of two sequences, one present twice per genome and called 13 and one present once per genome and named 13′. *Eco*RI fragment *a* which is present twice per genome was found by digestion with *Sal* I and with *Bam* I (not shown) to be a single sequence. The size class *j* has been found by digestion with *Sal* I to include two different sequences of equal length; one, *j*, with and one, *j′*, without a *Sal* I recognition site. The sequence complexity of *Eco*RI bands *k, l, m,* and *o* has not been determined.

Table 1. Size and stoichiometry of chloroplast DNA fragments produced by three restriction endonucleases

Sal I			*Bam* I*			*Eco* RI*		
Fragment	Size†	Copies‡	Fragment	Size	Copies	Fragment	Size	Copies
A	25	1	1	15.0	1	a	12.5	2
B	21	1	2	11.0	1	b	6.3	1
C	16	1	3	9.7	1	c	5.6	1
C′	16	1	4	7.1	1	d	2.85	1
D	12.7	1	5	6.0	1	e	2.8	1
E	9	1	6	4.9	1	f	2.2	1
F	6.7	2	7	4.6	2	g	2.0	1
F′	6.7	1	8	4.3	1	h	1.9	1
G	6.3	0.8	9	3.9	2	i	1.85	1
H	6.1	1	10	3.2	1	j	1.8	2
			11	2.5	1	k	1.7	2
			12	2.4	1	l	1.65	3
			13	2.3	2	m	1.6	2
			13′	2.3	1	n	1.5	3
			14	2.1	1	o	1.25	—§
			15	2.0	2	p	1.2	—
			16	1.8	1			
			17	1.5	2			

Chloroplast DNA was digested with restriction enzymes and the DNA fragments fractionated on agarose gels. The sizes of the chloroplast DNA fragments were estimated relative to *Eco*RI (20) and *Hae* III (30) fragments of lambda phage DNA. The number of copies of a fragment per genome was estimated as described in *Materials and Methods.*

* Only those size classes of fragments shown in Fig. 1 are tabulated.

† Molecular sizes of fragments are in kilobases.

‡ The number of copies of given fragment per genome. Average value from three independent estimations.

§ Not determined.

***Bam* I and RI Fragments with Recognition Sequences for *Sal* I.** Fig. 1E illustrates gel electrophoresis of chloroplast DNA digested with *Bam* I and *Sal* I together and Fig. 1H chloroplast DNA digested by both RI and *Sal* I. By comparing Fig. 1D and E and Fig. 1G and H, it was determined that several *Bam* I fragments and RI fragments are further fragmented by *Sal* I. Fig. 1 demonstrates that *Bam* I fragments 1, 2, 3, 4, 5, and 12 contain recognition sites for *Sal* I. *Sal* I digestion of isolated *Bam* I fragments (not shown) demonstrated that fragments 7 and 13′ were further digested by *Sal* I. Fig. 1G and H show that fragments *a, c, d,* and *g* are fragmented by *Sal* I.

Products of *Sal* I-*Bam* I, and *Sal* I-RI Digestion of the Chloroplast Genome. *Bam* I and RI fragments containing *Sal* I recognition sites were eluted from agarose gels, after electrophoresis. The isolated fragments were digested with *Sal* I and the products were fractionated and sized by agarose gel

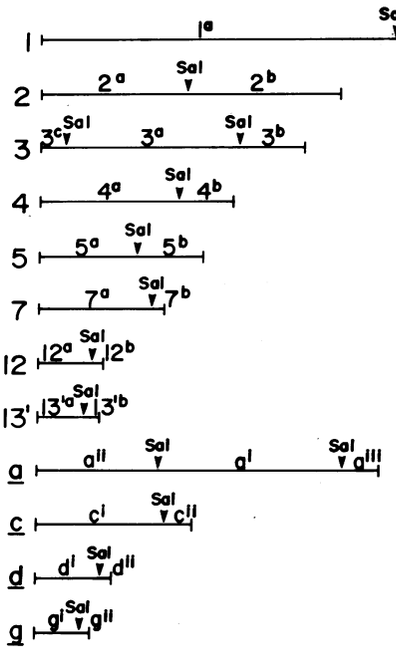

FIG. 2. Location of *Sal* I recognition sites in *Bam* I and *Eco*RI chloroplast DNA fragments. *Bam* I and *Eco*RI fragments known to contain *Sal* I recognition sites were extracted from agarose gels, digested with *Sal* I, and fractionated on 1% agarose gels. The size of the resulting DNA fragments was estimated relative to lambda phage DNA digested with *Eco*RI or *Hae* III. The location of each *Sal* I site is indicated by an arrow.

electrophoresis. Fig. 2 shows the position of the *Sal* I recognition sites in these *Bam* I and RI fragments. The fragments resulting from *Bam* I and *Sal* I digestion are denoted by the *Bam* I fragments of origin and given superscripts a, b, etc. The RI/*Sal* I fragments are denoted in the same way with superscripts i, ii, etc.

Comparison of the sizes of the *Bam* I digestion products of isolated *Sal* I fragments with the sizes of the *Sal* I digestion products of the *Bam* I fragments, permits identification of those *Sal* I fragments from which the *Bam* I/*Sal* I digestion products arise. *Sal* I fragments containing *Sal* I/RI fragments were found in the same way. *Sal* I fragment G is not further digested by *Bam* I and is entirely within *Bam* I fragment 3. Similarly *Sal* I fragment F is not further digested by RI and is within RI fragment *a*. Fragment 1^a is in C; 2^a in B; 2^b in E; 3^a in G; 4^a in H; and 5^a in D. Fragment a^i is in F; a^{ii} is in H and C, and c^i in E, d^i in E, and g^i in A.

Comparative sizing cannot be used to establish the origin of the small double-digestion products because they are difficult to resolve from single-digestion products; consequently we have used an alternative method to determine which *Sal* I fragments overlap which *Bam* I and RI fragments.

^{32}P-Labeled cRNA was prepared by using the isolated *Bam* I and RI fragments as templates which can be further digested with *Sal* I. Chloroplast DNA was terminally digested with *Sal* I and the fragments were separated by electrophoresis on agarose gels. Fragments were transferred directly to a filter strip (15); [^{32}P]cRNA was hybridized to the filters. Radioautographs show the results of the hybridizations (Fig. 3).

cRNA from *Bam* I (fragment 1) hybridizes to *Sal* I fragments C, F′, and H (Fig. 3A); fragment 2 hybridizes to B, E, and G (Fig. 3B); fragment 3 hybridizes to A, C′, and G (Fig. 3G); fragment 4 hybridizes to C, F, and H (Fig. 3C); fragment 5 hybridizes to A and D (Fig. 3D); fragment 7 hybridizes to B, C′ and F (Fig. 4E); fragment 12 hybridizes to E and F′ (Fig. 3E); fragments 13 and 13′ hybridize to C, F, and H. cRNA to RI fragment *a* hybridizes to B, C, C′, F, and H (Fig. 3H); *c* hybridizes to E (Fig. 3I), *d* hybridizes to A and D (Fig. 3J); and *g* hybridizes to B and E (Fig. 3K).

The relationship of *Sal* I fragments C or C′ and F or F′ to *Bam* I and RI fragments was determined in a similar manner. cRNA from 1, 4, 7, 13 and 13′, or *a* was hybridized to filters containing fragments of C and C′ after further digestion with *Bam* I. Likewise, cRNA from 1, 3, 4, 7, 12, and *a* was hybridized to filters containing RI digestion products of *Sal* I fragments F and F′ (not shown).

The Two Copies of the *Eco*RI Fragments *a* Are in an Inverted Orientation. The RI fragment *a* yields three pieces designated a^i, a^{ii}, and a^{iii} on digestion with *Sal* I (Fig. 2). Fig. 4 shows the relationship between a^i, a^{ii}, a^{iii}, and the *Sal* I fragments. cRNA from a^{iii} hybridizes B and C′. By using these data and those shown in Figs. 2 and 3, we determined the order of the *Sal* fragments overlapped by RI fragment *a* as diagrammed in Fig. 5. One copy of *a* extends from H through F

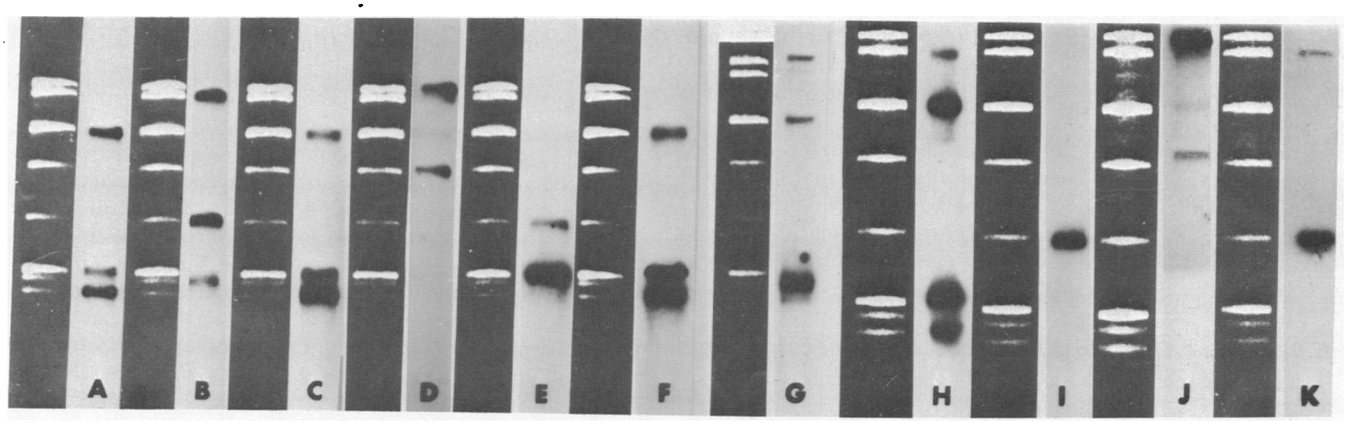

FIG. 3. Homology between *Bam* I or *Eco*RI fragments, with *Sal* I sites, and the *Sal* I fragments of chloroplast DNA. Chloroplast DNA digested with *Sal* I was fractionated by electrophoresis on 0.75% agarose gels (0.5 μg of DNA per gel). The DNA was denatured and transferred to Millipore filters (15). ^{32}P-Labeled cRNA of *Bam* I and RI fragments known to contain *Sal* I recognition sites were hybridized to the *Sal* I fragmented DNA on filters. Hybridization was detected by autoradiography. The stained gels and the autoradiographs were photographed at the same magnification and aligned. (A) cRNA from *Bam* I fragment 1; (B) cRNA from *Bam* I fragment 2; (C) cRNA from *Bam* I fragment 4; (D) cRNA from *Bam* I fragment 5; (E) cRNA from *Bam* I fragment 12; (F) cRNA from *Bam* I fragments 13 and 13′; (G) cRNA from *Bam* I fragment 3; (H) cRNA from *Eco*RI fragment *a;* (I) cRNA from *Eco*RI fragment *c;* (J) cRNA from *Eco*RI fragment *g;* and (K) cRNA from *Eco*RI fragment *d* all hybridized to the *Sal* I fragments.

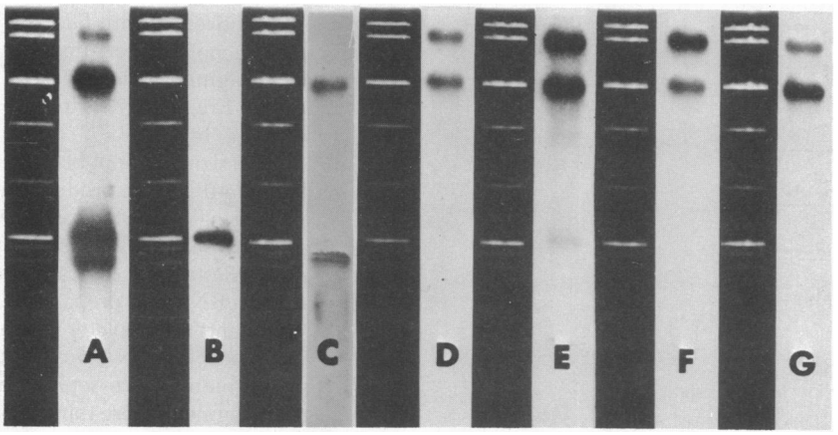

FIG. 4. Homology between *Eco*RI fragment *a* of maize chloroplast DNA and *Sal* I fragments and homology between *Bam* I fragments 6, 7, and 8, and *Sal* I fragments. *Eco*RI fragment *a* was digested with *Sal* I and fractionated on 1.5% agarose gels. The three resulting DNA fragments (a^{i}, a^{ii}, and a^{iii}) were extracted from gels and used as templates for the synthesis of ^{32}P-labeled cRNA. *Bam* I fragments 6, 7, and 8 were treated similarly. cRNA from fragments *a*, a^{i}, a^{ii}, a^{iii}, 6, 7, and 8 were hybridized to *Sal* I fragments. (A) cRNA from *Eco*RI fragment *a*; (B) cRNA from a^{i}, (C) cRNA from a^{ii}; (D) cRNA from a^{iii}; (E) cRNA from 7; (F) cRNA from 6; and (G) cRNA from 8 all hybridized to *Sal* I fragments.

into B and the other copy extends from C through F into C′. However, because H and C join via 13′, and not via *a*, the two copies of *a* are in an inverted orientation with respect to one another. The region of inverted repetition extends through *a* into C′ on the right and B on the left through the *Bam* I fragment 7. *Bam* I fragment 7 is present twice per genome and is common to B and C′ (Fig. 4E). Further, because *Bam* I fragment 6 in C′ and fragment 8 in B have sequences in common and both hybridize B and C′ (Fig. 4F and G), the inverted repeat is assumed to extend through 7 into 6 on the right and through 7 into 8 on the left. The extent of the inverted repeat is indicated by double thickness arrows in Fig. 5.

The Order of the *Sal* I Fragments. Fig 6 shows the order of all the *Sal* I fragments in the maize chloroplast genome based on our interpretation of the data in Figs. 2, 3, and 4. cRNA from *Bam* I fragment 2 hybridizes to *Sal* I fragments B and E as well as to G (Fig. 3). Because G is not digested with *Bam* I and *Bam* I fragment 2 does not contain G (Fig. 2), we assume that fragment 2 overlaps the *Sal* I site between B and E and contains a sequence common with *Sal* I fragment G. B joining E is confirmed by the data of Fig. 3 which show that cRNA from RI fragment *g* hybridizes to B and E. Fig. 3 shows that cRNA from *Bam* I fragment 12 hybridizes *Sal* I fragments E and F′, and implies that E joins F′. Fig. 3 shows that cRNA from *Bam* I fragment 3 hybridizes A, C′, and G. The data of Fig. 2 show that G is within *Bam* I fragment 3. These data imply that *Bam* I fragment 3 extends from *Sal* I fragment C′ through G into A. Fig. 3 shows that cRNA from *Bam* I fragment 5 and RI fragment *g* hybridized to *Sal* I fragments A and D. These data suggest that *Sal* I fragments A and D are contiguous. Because this chloroplast DNA is a covalently-closed circular molecule

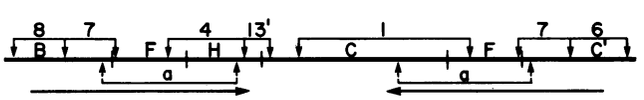

FIG. 5. Location of *Sal* I, *Bam* I, and *Eco*RI recognition sites in the repeated maize chloroplast DNA sequences with an inverted orientation. The *Sal* I recognition sequences are shown by vertical lines on the central horizontal line. The *Bam* I recognition sites are indicated by solid arrows from the upper horizontal line and the *Eco*RI recognition sites are indicated by arrows with dashed lines from the lower horizontal line. The letters and numbers on the three horizontal lines refer to the names of the DNA fragments between the arrows or vertical lines.

(11), and because the *Sal* I fragments shown in Fig. 1B represent the entire chloroplast genome, we assume that *Sal* I fragments D and F′ are joined.

Localization of the 16 and 23S Ribosomal RNA. Fig. 7 illustrates an experiment in which mixtures of the 16 and 23S maize chloroplast ribosomal RNAs, labeled with ^{32}P, were hybridized to the RI and *Sal* I fragments of chloroplast DNA. These RNAs hybridize to RI fragment *a* and to *Sal* fragments C, F, and H. Fig. 6 diagrams the general location of the ribosomal RNA genes.

DISCUSSION

These experiments show that we can ascribe a discrete order to the endonuclease digestion products of chloroplast DNA. This supports the view that the majority of the circular DNA molecules isolated from maize chloroplasts represent a single homogenous species. Two lines of evidence suggest that there may be some limited heterogeneity in this chloroplast DNA. First, less than one copy of *Sal* I fragment G appears to be present per genome. Second, RI fragment *c*, which contains a single recognition sequence for *Sal* I and overlaps *Sal* I fragment E (to the right in Fig. 6.), appears to overlap not only *Sal* I fragment F′ to the left, as predicted by the larger overlap with *Bam* I fragment 2, but it also overlaps other *Sal* I fragments. We do not understand the significance of this apparent heterogeneity.

Determination of DNA sequence homology between an overlapping fragment and the fragments being overlapped was the major method used to arrive at the fragment order shown in Fig. 6. If some significantly long identical DNA sequences occur at several places in the chromosome, then cRNA from the overlapping fragment may contain sequences complementary not only to the fragment from which it was prepared but also to some distant DNA fragment. For example, cRNA from *Bam* I fragment 2 hybridized to the *Sal* I fragments B and D and to fragment G as well. However, *Bam* I fragment 2 only overlaps the *Sal* I fragments B and D but has sequences in common with *Bam* I fragment 3 which overlaps A, G, and C′. In an extreme case, it is possible that a *Bam* I or RI fragment overlapping *Sal* I fragments is more extensively homologous with a *Sal* I fragment which it does not overlap. We have no reason to suspect that this is true in any of the given overlaps. However, we find low level hybridization of cRNA from most of the *Bam* I and

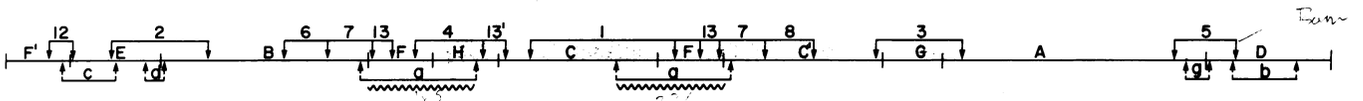

FIG. 6. Location of the determined *Sal* I, *Bam* I, and *Eco*RI recognition sites in *Zea mays* chloroplast DNA. *Sal* I fragments and recognition sites are shown in the central horizontal line by vertical lines. The *Bam* I fragments and recognition sites are indicated on the upper horizontal line by arrows with solid tails and the *Eco*RI fragments and recognition sites are shown on the lower line by arrows with dashed tails. The 16 and 23S ribosomal RNA genes are known to lie within the region of the squiggled horizontal line. The circular chromosome is shown in linear form only for convenience.

RI fragments to fragments other than those overlapped. This minor hybridization is specific, because in control experiments hybridization to lambda phage DNA fragments is not observed.

We estimate from analysis of the sequence complexity of the *Sal* I fragments that the chloroplast genome of *Zea mays* is at most 80% as complex as its length and most probably considerably less complex. This appears to contradict reassociation kinetics data for this genome which suggest that it is as complex as its length. It seems likely, however, that the reassociation kinetics of sequences repeated only two or three times per genome may be indistinguishable from the reassociation kinetics of unique sequences in the mixture of all the chloroplast DNA sequences.

The fragment map of the chloroplast DNA shown in Fig. 6 contains a large inverted repetition (shown in detail in Fig. 5). Inverted repetitions have been found in eukaryote chromosomal DNA (18, 19) and in bacterial DNAs (20, 21). Inverted repetitions are associated with certain transposable elements conferring various drug resistances (22, 23). An inverted DNA repeat is theoretically capable of mediating intramolecular recombination, without loss of genetic material, and thus would lead to the inversion of the orientation of DNA sequences flanking the repeat relative to those outside the repeats. Such an event may be important in gene regulation.

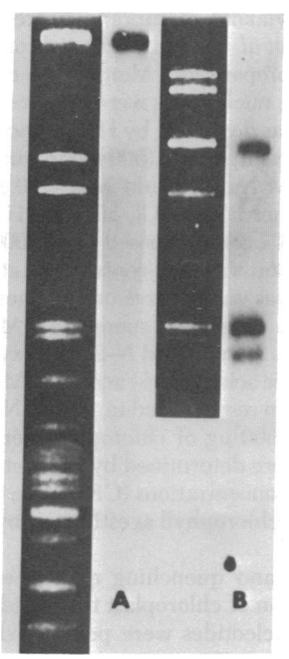

FIG. 7. *Sal* I and *Eco*RI fragments of chloroplast DNA with homology to 16 and 23S chloroplast ribosomal RNAs. Chloroplast ribosomal RNA was prepared and labeled *in vitro* with ^{32}P. The labeled RNA was hybridized to chloroplast DNA digested with *Sal* I and *Eco*RI. (A) ^{32}P-Labeled ribosomal RNA hybridized to the *Eco*RI fragments of chloroplast DNA. (B) ^{32}P-Labeled ribosomal RNA hybridized to the *Sal* I fragments of chloroplast DNA.

We thank Richard Kolodner for instruction in chloroplast DNA isolation, Jackie Upcroft for instruction in *Sal* I preparation, Nancy Maizels for unpublished methods of *in vitro* RNA labeling, Dean Hamer for *Bam* I, Lawrence Haff for ribosomal RNA and *Sal* I; we also thank Richard Kolodner, Peter Upcroft, and Fred Ausubel for helpful discussions. J.B. is supported by an Anna Fuller Postdoctoral Fellowship. This work was supported in part by the National Institutes of Health (GM20470), and in part by the Maria Moors Cabot Foundation of Harvard University.

1. Kolodner, R. D. & Tewari, K. K. (1975) *Biochim. Biophys. Acta* **402**, 372–390.
2. Herrmann, R. G., Bohnert, H-J., Kowallik, K. V. & Schmidtt, J. M. (1975) *Biochim. Biophys. Acta* **378**, 305–317.
3. Kolodner, R. D. & Tewari, K. K. (1975) *J. Biol. Chem.* **250**, 4888–4895.
4. Kolodner, R. D. & Tewari, K. K. (1976) *Biochim. Biophys. Acta*, in press.
5. Pirrotta, V., Ptashne, M., Chadwich, P. & Steinberg, R. (1971) in *Procedures in Nucleic Acid Research*, eds. Cantoni, G. & Davies, D. R. (Harper and Row, New York), pp. 703–715.
6. Hamer, D. H. & Thomas, C. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1537–1541.
7. Wilson, G. A. & Young, F. E. (1975) *J. Mol. Biol.* **97**, 123–125.
8. Hedgpeth, J., Goodman, H. M. & Boyer, H. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3448–3452.
9. Middleton, H. H., Edgell, M. H. & Hutchison, C. A., Jr. (1972) *J. Virol.* **10**, 42–50.
10. Hayward, G. S. & Smith, M. H. (1972) *J. Mol. Biol.* **63**, 383–395.
11. Hamer, D. H. & Thomas, C. A. (1975) *Chromosoma* **49**, 243–267.
12. Tanaka, T. & Weisblum, B. (1975) *J. Bacteriol.* **121**, 354–362.
13. Berg. D., Barrett, K. & Chamberlin, M. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York and London), Vol. 21D, pp. 506–519.
14. Vasconcelos, A. C. L. & Bogorad, L. (1970) *Biochim. Biophys. Acta* **228**, 492–502.
15. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
16. Thomas, M. & Davis, R. W. (1975) *J. Mol. Biol.* **91**, 315–328.
17. Allet, B. & Solem, R. (1974) *J. Mol. Biol.* **85**, 475–484.
18. Wilson, D. A. & Thomas, C. A. (1974) *J. Mol. Biol.* **84**, 115–144.
19. Schmid, C. W., Manning, J. E. & Davidson, N. (1975) *Cell* **5**, 159–172.
20. Adelberg, E. A. & Bergquist, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2061–2065.
21. Kleckner, N., Chan, R. K., Tye, B-K. & Botstein, D. (1975) *J. Mol. Biol.* **97**, 561–575.
22. Ptashne, K. & Cohen, S. N. (1975) *J. Bacteriol.* **122**, 776–781.
23. Kopecko, D. J. & Cohen, S. N. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1373–1377.