

Analysis of a 120-Kilobase Mitochondrial Chromosome in Maize

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ABSTRACT

The organization of the mitochondrial genome in plants is not well understood. In maize mitochondrial DNA (mtDNA) several subgenomic circular molecules as well as an abundant fraction of linear molecules have been seen by electron microscopy. It has been hypothesized that the circular molecules are the genetic entities of the mitochondrial genome while the linear molecules correspond to randomly sheared mtDNA. A model has been proposed that explains the mechanism of generation of subgenomic circles (of a predictable size) by homologous recombination between pairs of large direct repeats found on a large (~570 kb for the fertile (N) cytoplasm) master circle. So far the physical entities of the mitochondrial genome, as they exist *in vivo*, and the genes they carry, have not been identified. For this purpose, we used two gel systems (pulsed field gel electrophoresis and Eckhardt gels) designed to resolve large DNA. Large DNA was prepared from the Black Mexican Sweet (BMS) cultivar. We resolved several size classes of mtDNA circles and designate these as chromosomes. A 120 kb chromosome was mapped in detail. It is shown to contain the three ribosomal genes (*rrn26*, *rrn18* and *rrn5*) plus two genes encoding subunits of cytochrome oxidase (*Cox1* and *Cox3*); it appears to be colinear with the 570-kb master circle map of another fertile cytoplasm (B37N) except at the "breakpoints" required to form the 120-kb circle. The presence of the 120-kb chromosome could not have been predicted by homologous recombination through any of the known repetitive sequences nor is it a universal feature of normal maize mitochondria. It is present in mitochondria of BMS suspension cultures and seedlings, but is not detectable in seedlings of B37N. No master genome was detected in BMS.

THE mitochondrial genomes of all Angiosperms investigated thus far can be represented as a single circular map derived as a montage of overlapping clones. In all but one species these "master" circular maps contain pairs of large inverted or direct repeat DNA (NEWTON 1988; LEVINGS and BROWN 1989). In B37N maize (cultivar B37 with a normal = N, fertile cytoplasm) the master chromosome map is 570 kb and contains 7 large pairs of repetitive DNA, 0.7 to 14 kb in size (LONSDALE, HODGE and FAURON 1984; LONSDALE *et al.* 1988; FAURON and HAVLIK 1988). It has been proposed that recombination within the repeats generates a myriad of subgenomic circles of predicted sizes (LONSDALE, HODGE and FAURON 1984; LONSDALE *et al.* 1988). To date, however, neither the master genome assembled from cosmid mapping nor the predicted subgenomic chromosomes have been identified by either electron microscopy or gel electrophoresis.

Two lines of evidence support the existence of multiple circular chromosomes in maize mitochondria. First, several size classes of circular molecules were observed by electron microscopy (LEVINGS *et al.* 1979), however, most of the mitochondrial DNA

(mtDNA) visualized was linear, possibly the result of shear-induced breakage of large molecules. Second, up to a 7-fold variation in the stoichiometry of restriction fragments visualized by ethidium bromide staining was reported (BORCK and WALBOT 1982); if only a single circular molecule exists, it is difficult to rationalize this variation.

There is less evidence to support the hypothesis that homologous recombination is the major mechanism which generates subgenomic molecules. Thus far, in maize, homologous recombination between direct repeats has been demonstrated by Southern blotting for only one (the 5-kb repeat) of the seven types of repeats (HOUGHINS *et al.* 1986). In most *Brassica* species, only one large repeat is found, and has been shown to be involved in recombination (PALMER, 1988). In this work PALMER showed indirect evidence for the existence of the smaller predicted subgenomic circle. However, this molecule, which was enriched for by isolating supercoiled *Brassica oleracea* mtDNA on a CsCl gradient, was not mapped. In both maize and other species, however, recombination events have been reported to occur outside of the large repetitive sequences (MAKAROFF, APEL and PALMER 1988; CHÉTRIT *et al.* 1984; FAURON, HAVLIK and BRETTELL 1990; NEWTON *et al.* 1990; SHUSTER *et al.* 1987; DEWEY, LEVINGS and TIMOTHY 1986). In order to

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test the "homologous recombination" model, subgenomic pieces, as they exist *in vivo* must be identified and compared to those predicted by the model.

To determine the actual physical organization of the mitochondrial genome, we have utilized techniques for analyzing and mapping large DNA pieces. Large DNA was prepared from the Black Mexican Sweet (BMS) cultivar. BMS was chosen because a suspension culture was available from which DNA could be gently prepared. We report here on the identification of a 120-kb subgenomic circular molecule which contains five mitochondrial genes. This 120-kb circle is colinear with the cosmid map from another fertile cytoplasm (B37N), except at the "breakpoints" (the sequences at which BMS and B37N diverge). Sequences found at the breakpoints are 120 kb apart in the B37N master circle map, but appear to be adjacent in BMS. The mechanism responsible for the formation of this circular chromosome, and the general organization of the mitochondrial genome in BMS are discussed.

MATERIALS AND METHODS

Resolution of large mtDNA was optimized using mitochondria prepared from a Black Mexican Sweet (BMS) suspension culture. Mechanical shearing of DNA was avoided by preparing mtDNA from protoplasts. Protoplasts were prepared (FROMM *et al.* 1987), resuspended in two volumes of homogenization buffer (KEMBLE, GUNN and FLAVELL 1980), and disrupted by sieving through a 15 μ m nylon mesh. Mitochondria were separated from nuclei and cell debris by differential centrifugation (KEMBLE, GUNN and FLAVELL 1980). The resulting pellet was mixed with an equal volume of 1% low gelling temperature (LGT) agarose. Agarose plugs were prepared, lysed, washed, and digested as described by KENWRICK *et al.* (1987). mtDNA was also extracted from seedlings of BMS and B37N. Mitochondria were prepared as described by MULLIGAN, MALONEY and WALBOT (1988), immobilized in agarose and lysed as for BMS suspension culture.

Large linear mtDNA fragments from BMS suspension cultures were resolved with a Contour-Clamped Homogeneous Electric Field apparatus (CHEF) (CHU, VOLLRATH and DAVIS 1986). Supercoiled circular molecules were resolved on ECKHARDT (1978) gels using a horizontal rather than vertical agarose gel (GARDELLA *et al.* 1984). *Rhizobium leguminosarum* strain 897 (HIRSCH *et al.* 1980) and various derivatives of *Agrobacterium* strain 348 (harboring plasmids of known sizes) were used as size standards. Specifically, strains containing the plasmids pTiA6 (180 kb) (KNAUF and NESTER 1982), pVK102 (23 kb) (KNAUF and NESTER 1982), pSM304 (60 kb) (STACHEL *et al.* 1985), and pAL4404 (100 kb) (OOMS *et al.* 1982) were used (data not shown). A series of maize mitochondrial probes was hybridized to Southern blots prepared from CHEF and Eckhardt gels (Table 1). All probes were derived from the B37N cytoplasm.

RESULTS

Our initial experiments addressed the physical state of maize mtDNA—does it exist as linear, circular or supercoiled molecules? We analyzed the electropho-

retic mobility of untreated mtDNA as visualized by ethidium bromide staining of Eckhardt and CHEF gels (Figure 1). Although the mobility of DNA molecules in these two gel systems does not constitute a proof of their structure, it provides a rough assessment of genome organization. On CHEF gels, no bands corresponding to discrete size classes of linear molecules were observed with undigested DNA [Figure 1A, lane a, and as observed under various conditions of pulse time and voltage (data not shown)]. About 20% of the undigested DNA migrates as a smear from ~30–150 kb, typical of linear molecules (data not shown). The rest of the DNA remains in the well and hence probably includes large relaxed circular molecules whose migration is strongly retarded on pulse-field gels (LEVENE and ZIMM 1987; BEVERLEY 1988). Eckhardt gel analysis also showed a majority of staining in the well, suggestive of relaxed circular molecules (ECKHARDT 1978). There is staining at the electrophoretic front indicative of linear pieces but there is no visible staining corresponding to supercoiled DNA (Figure 1B, lane b). These observations suggest that the putative circular molecules of the mtDNA in BMS suspension culture cells exist in a relaxed configuration. Although our Eckhardt gel conditions allowed resolution of supercoiled circular molecules of up to 1.5 Mb (Figure 1B, lane a) in this and other gels (data not shown) we never observed the putative 570-kb master circle by ethidium bromide staining. The exact size of the BMS mitochondrial genome is not known, but is likely to be between 500 to 600 kb as in other maize cytoplasms, and is probably close to that of B37N (see DISCUSSION).

To increase sensitivity, a battery of maize mtDNA probes (Table 1) was hybridized to Southern blots prepared from mtDNA resolved by the Eckhardt gel technique. Again we failed to detect the proposed master molecule in supercoiled form. We did, however, observe five discrete bands in the region in which supercoiled molecules are well resolved. Three of these are illustrated in Figure 2C: a 115-kb supercoiled molecule hybridizing to *Cob*, a 120-kb molecule containing five genes, and a 70-kb molecule containing *Atp1*. Two other molecules of approximately 30 and 60 kb, respectively, hybridized to *Cox2* (data not shown).

Although maize mtDNA lacks *NotI* sites (data not shown), it does have a few *SfiI* sites, and these large *SfiI* restriction fragments are readily visualized on CHEF gels by ethidium bromide staining (Figure 1A, lanes b and c). If a single chromosome exists, then a partial *SfiI* digestion in which only a single site is digested should yield a 500–600-kb linear fragment. A fragment of this size was never observed on CHEF after partial digestion with *SfiI* in conditions where

TABLE 1
Probes used in this study

Probe	Source	Sites	Size (kb)	Content	Coordinates
117	*	<i>Hind</i> III/ <i>Eco</i> RI	0.6	<i>Cob</i>	312.3– 312.9
159H2.5	*	<i>Hind</i> III	2.5	See Figure 3	333.5– 336.0
2.7XS	Cosmid N8D4	<i>Xho</i> I/ <i>Sfi</i> I	2.7	Region between <i>ct-12</i> and <i>ct-LS</i>	339.0– 341.7
AT153	Plasmid BN6601	<i>Eco</i> RI/ <i>Bam</i> HI	3.9	<i>Cox</i> 1	353.5– 357.4
171	*	<i>Bam</i> HI	8.8	Region between <i>Cox</i> 1 and <i>rnn26</i>	365.9– 374.7
185	*	<i>Ava</i> I	1.0	<i>rnn26</i>	399.5– 400.5
206	*	<i>Xho</i> I/ <i>Hind</i> III	1.6	<i>rnn18</i>	418.4– 420.0
76	*	<i>Bgl</i> I/ <i>Xba</i> I	3.9	<i>Cox</i> 3	439.3– 443.2
59a	*	<i>Xho</i> I/ <i>Bam</i> HI	2.6	See Figure 2	450.9– 453.5
59XS2.5	*	<i>Xho</i> I/ <i>Sma</i> I	2.5	See Figure 2	450.9– 453.4
59b	*	<i>Bam</i> HI	3.6	<i>Atp</i> 1	453.5– 457.1

Probes were obtained from a clone library prepared in our laboratory [*(MULLIGAN, MALONEY and WALBOT 1988)] or provided by others: cosmid N8D4 was provided by CHRISTIANE FAURON; plasmid BN6601 was provided and described by ISAAC, JONES and LEAVER (1985). Coordinates refer to the map of FAURON and HAVLIK (1988).

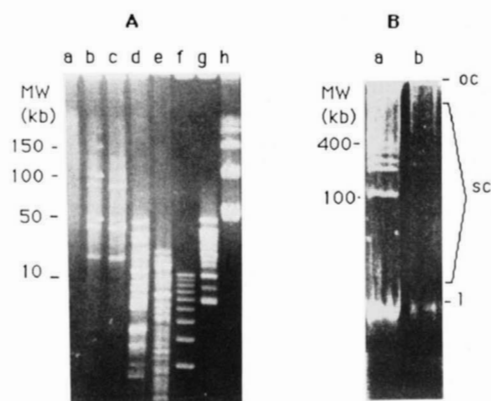


FIGURE 1.—Ethidium bromide staining of undigested mtDNA from BMS tissue culture cells displayed on CHEF and Eckhardt gels. (A) mtDNA was electrophoresed on a CHEF gel at 180 V with a 1–10-sec ramped pulse in 1% agarose. Lane a, undigested mtDNA; lane b, partial *Sfi*I digest; lane c, complete *Sfi*I digest; lane d, complete *Sma*I digest; lane e, complete *Bam*HI digest. Molecular weight markers are indicated along the left margin and were derived from a 1-kb ladder (lane f, BRL), a high MW DNA ladder (lane g, BRL) and a lambda ladder (lane h). (B) An ECKHARDT gel electrophoresed at 5 V/cm with 0.6% agarose. Megaplasmids of known size from various *Rhizobium leguminosarum* strain 897 were run as MW markers (lane a) next to undigested mtDNA (lane b). In other gels megaplasmids from *Agrobacterium* were utilized as size markers (not shown). oc = open circular DNA; sc = region in which supercoiled molecules are resolved by size; l = linear DNA.

200–1000-kb molecules could be resolved (data not shown).

Mapping the 120-kb circular molecule: One of the

bands observed in Southern blots of Eckhardt gels, corresponding to a 120-kb circular DNA fragment, was of particular interest because it hybridized to five mitochondrial genes including all three ribosomal genes (Figure 2C). Moreover, these genes were linked on the original cosmid map of the N (fertile) cytoplasm (Wf9N) (LONSDALE, HODGE and FAURON 1984) and on maps of other normal genotypes (B37N) (FAURON and HAVLIK 1988). We hypothesized that the observed band corresponded to a 120-kb circular molecule—a chromosome—whose sequence is colinear with that described on the cosmid map, except at the sites where circularization occurs. We refer to these sites as “breakpoints.”

To construct a detailed map of the 120-kb chromosome and to define the breakpoints more precisely, we compared the restriction map derived from CHEF gels to that of the B37N cosmid map (FAURON and HAVLIK 1988). We used a series of probes located in the region between *Cob* (map coordinate 315, see Figure 2B) and *Atp*1 (map coordinate 454). *Cob* hybridized to the same 115-kb fragment in both partial and full *Sfi*I digests, indicating that it maps to a 115-kb circular chromosome with only one *Sfi*I site. On Eckhardt gels this chromosome comigrates with the 120-kb circle. All probes between map coordinates ~333 and ~453 hybridized to a 120-kb supercoiled molecule on Eckhardt gels (Figure 2C) and to a 120-kb partial *Sfi*I fragment (Figure 2A); these are presum-

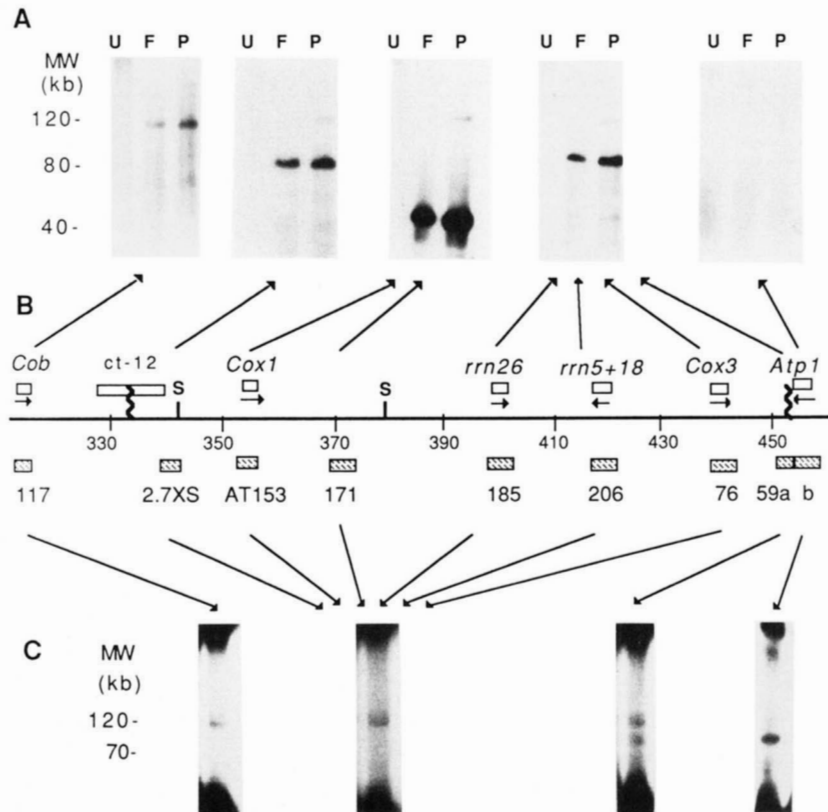


FIGURE 2.—Hybridization analysis of mtDNA. The general strategy of the analysis was to successively probe Southern blots prepared after CHEF (A) or Eckhardt (C) gel electrophoresis with a battery of probes spanning map coordinates 310 to 470. The hybridization pattern obtained by each probe is indicated by the arrows connecting a probe to a blot. Only one panel is shown with a cluster of arrows when several probes yielded identical fragments. (A) Southern blots were prepared of mtDNA from BMS tissue culture separated on CHEF gels. Each panel contains mtDNA that is undigested (U), partially (P) or fully (F) digested with *Sfi*I. (B) The line presents the map coordinates in kb of the master circle assembled for B37N (FAURON and HAVLIK 1988). Listed above the line are the approximate positions (accurate to about 1 kb) of known mtDNA genes (open boxes) and a region of chloroplast DNA homology (*ct-12*); the direction of transcription is indicated by the arrow. Below the line, the locations of the additional probes (shaded boxes; Table 1) lacking known genes are indicated. The deduced breakpoints (zigzag line) and *Sfi*I sites (S) are indicated on the line. Note that probe 59, near coordinate position 450 is bisected by a breakpoint; subclone 59a hybridizes with both the 120- and 70-kb chromosomes while probe 59b, containing part of *Atp1* copy 1, is present only on the 70-kb chromosome. (C) Southern blots were prepared from undigested mtDNA resolved on ECKHARDT gels.

ably the same molecule. On the other hand, *Atp1* hybridized to a ~70 kb supercoiled circle but not to a *Sfi*I fragment; we interpret this as evidence that *Atp1* exists on a 70-kb circular chromosome that lacks *Sfi*I sites. Moreover, probe 59a hybridized to both the 120- and the 70-kb circles (Figure 2C), suggesting that it contains sequences present on both chromosomes. These data indicate that at least three different chromosomes exist and that the 120-kb chromosome diverges from B37N by recombination events near *Cob* and *Atp1*.

In Southern blots of our CHEF gels all probes hybridized to the location corresponding to the plugs in the well. The wells are not shown in Figure 2 because plugs fell out of some wells during gel denaturation and hence there is no hybridization in some lanes. This hybridization signal could correspond to DNA still bound to proteins or membranes, or to secondary structures of the DNA (*e.g.* replication loops or forks), to DNA tethered in the gel matrix, or

to other factors which prevent DNA from entering the gel.

In complete *Sfi*I digests, it is clear that the 120-kb region between map coordinates ~333 and ~453 has two *Sfi*I sites. The ~40-kb *Sfi*I fragment includes the *Cox1* gene and probe 171, placing the *Sfi*I sites on either side. The presence of these sites was independently confirmed by mapping them on clones derived from B37N mtDNA to coordinates 342–343 and 378–379 (data not shown). The presence of *Sfi*I sites at the same locations in B37N and BMS mtDNAs supports our assumption of colinearity between the two cytoplasts. The 80-kb *Sfi*I fragment hybridized to probes on either side of the 40-kb *Sfi*I fragment, that is at both ends of the 120-kb region (Figure 2B), and hence it is not colinear with the B37N cosmid map. We propose that this 80-kb fragment is generated as a result of breakpoints and circularization near *Atp1* and *Cob*.

Additional probes were tested in the region of the

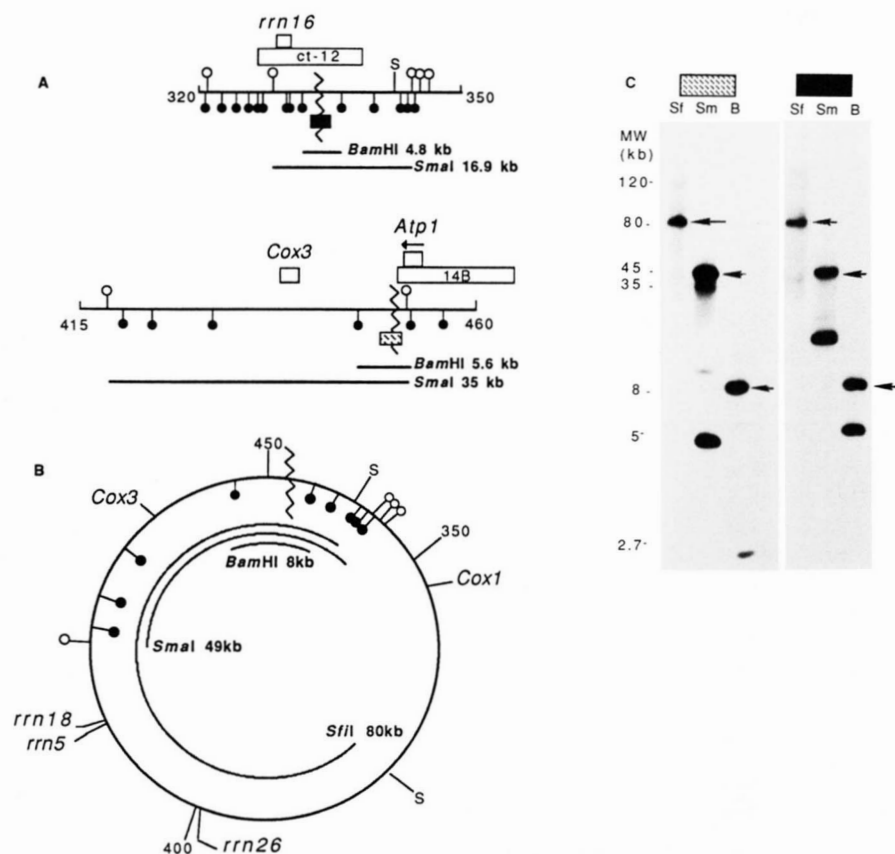


FIGURE 3.—Novel fragments detected at breakpoints. (A) Map coordinates and restriction sites in B37N (FAURON and HAVLIK 1988) for *Sma*I (empty circles) and *Bam*HI (filled circles) in the regions flanking the two breakpoints (zigzag line). The locations of probes spanning the breakpoints are indicated by a filled box (probe 159H2.5) in the chloroplast homology region (*ct-12*) and by a shaded box (probe 59XS2.5) at the edge of the 14-kb repeat (14B). (B) *Sfi*I (S) restriction map of the 120-kb chromosome generated after circularization at the breakpoints. *Bam*HI and *Sma*I sites are indicated only in the region flanking the breakpoints. Note that novel restriction fragments are generated as a result of circularization at the breakpoints. (C) Southern blots prepared from BMS mtDNA digested with *Sfi*I (Sf), *Sma*I (Sm) or *Bam*HI (B), after separation of fragments on CHEF. The blots were hybridized with the probes spanning the breakpoints (see panel A). Fragments observed with both probes are indicated by an arrow.

putative breakpoints. Probes which span a breakpoint are expected to hybridize to novel fragments on the 120-kb BMS chromosome compared to the B37N map. Moreover, as a result of circularization, breakpoint probes 120 kb apart in B37N will become physically linked on the same fragment in BMS. Using these criteria we narrowed the breakpoints down to the two 2.5-kb probes shown in Figure 3, A and B. Although these two probes are derived from regions 120 kb apart in B37N, they hybridize to the same 80-kb *Sfi*I fragment, 8-kb *Bam*HI fragment and 45-kb *Sma*I fragment in BMS mtDNA. These novel fragments are indicated by arrows in Figure 3C and are shown in Figure 3B on the 120-kb chromosome, while the expected fragments in the B37N configuration are shown above (Figure 3A). The two probes also hybridize to the same 12-kb *Apa*I, 30-kb *Bss*HIII and 5-kb *Hind*III fragments (data not shown).

Furthermore, each probe hybridized uniquely to some fragments (Figure 3C). In the case of 159H2.5, the additional unique fragments probably corresponded to chloroplast DNA (present at low levels in our mtDNA preparations) and/or to fragments from another mitochondrial chromosome. Interestingly, probe 59XS2.5 hybridized weakly to both a 35-kb *Sma*I and a 5.6-kb *Bam*HI fragment in BMS mtDNA (Figure 3C). These are precisely the fragment sizes on the B37N cosmid map. These results suggest that the

configuration of the genome present in B37N is also present in BMS but in very low stoichiometry.

The smallest fragments hybridizing to 59XS2.5, the 2.65-kb *Bam*HI and the 4.9-kb *Sma*I fragments, corresponded to the fragments expected in B37N for the second copy of *Atp1* (FAURON and HAVLIK 1988). This observation raised the possibility that one of the breakpoints in the 120-kb chromosome involved a known repetitive element. To determine whether the breakpoint at map coordinate ~453 occurred in the 14-kb repeat, we prepared a 2.65-kb *Bam*HI fragment from the second copy of *Atp1* copy; the clone contained a portion of the 14 kb repeat and the region immediately upstream of it (FAURON and HAVLIK 1988). When this fragment was used as a probe on the same gel as shown in Figure 3C, it did not hybridize to any of the recombinant fragments (80-kb *Sfi*I, 45-kb *Sma*I, 8-kb *Bam*HI; data not shown). This demonstrated that the breakpoint does not contain substantial homology to the 14-kb repeat.

To determine whether the 120-kb chromosome was generated in cell culture or also existed in plant tissues, we compared the restriction patterns of mtDNA from both sources (Figure 4). When digested with *Bam*HI and probed with 59XS2.5 (which spans the breakpoint), the 8-kb *Bam*HI fragment observed in tissue culture (Figure 3C, lane c) was also present in BMS seedlings (Figure 4, lane b). In contrast, the 5.6-

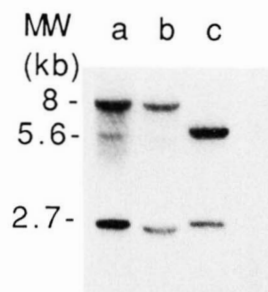


FIGURE 4.—Comparison of mtDNA in tissue cultures and plants by hybridization at the point of circularization in the 120-kb chromosome. A Southern blot was prepared from a *Bam*HI digest of mtDNA from BMS tissue culture (lane a), BMS seedlings (lane b) and B37N seedlings (lane c) and probed with 59XS2.5.

kb fragment was present in B37N plant tissue (Figure 4, lane c). All three sources contained the 2.65-kb fragment from the second copy of *Atp1*. Both the 8- and 2.65-kb bands in lane b (the BMS seedling sample) are shifted slightly downward, probably due to salt and/or DNA concentration differences. This shift was noticeable in the ethidium bromide stained gel (data not shown).

As noted earlier, mtDNA prepared from BMS tissue cultures contained both the 8.0- and 5.6-kb *Bam*HI fragments (Figure 4). Upon longer exposure, the 5.6-kb faint band (or sublimon) seen in BMS tissue culture (lane a) was also present, but weaker in BMS seedlings (data not shown). These observations indicate that the BMS cells contain both the 120-kb chromosome and a minor component in which the organization in map coordinates 415–460 is similar to the configuration of B37N. This second configuration is present in BMS at a low stoichiometry. On the other hand, the organization found in BMS is not detectable in B37N.

DISCUSSION

The size and complexity (NEWTON 1988; LEVINGS and BROWN 1989) of plant mtDNA has hampered elucidation of its physical structure *in vivo* despite the preparation of circular maps based on overlapping clones. To date, the physical organization is known only for the smallest components of the maize genome. These include the circular 1.4- and 1.9-kb plasmids (SMITH and PRING 1987; LUDWIG *et al.* 1985), a 2.3-kb linear plasmid (BEDINGER *et al.* 1986; LEON, WALBOT and BEDINGER 1989), and linear episomes, such as S1 and S2 and related molecules found in male sterile S and some normal maize lines (WEISSINGER *et al.* 1982). These small, apparently self-replicating molecules constitute but a tiny fraction of the genome.

We were unable to detect a master 500–600-kb genome in BMS that would contain the complexity of the genome, *i.e.*, to which any probe would hybridize. We conclude that either a master genome does not

exist in BMS, or that it is present at a very low stoichiometry, or that for some unknown reasons it is not released from the well. Although no cosmid map is available for BMS, the gene order is probably very similar to that of B37N (1) we demonstrated colinearity between BMS and B37N in the region of the 120-kb chromosome, and (2) we have demonstrated colinearity in all regions investigated so far (between coordinates ~10 to ~160 and ~540 to ~560; unpublished data). Therefore, it is likely that BMS is very similar to other N cytoplasms. It is important to note that circularity of a “master” circle, can be achieved with cosmid mapping by inappropriately linking clones together. For example, considering BMS, the existence of sublimons [a term coined to refer to mtDNA configuration detectable only by hybridization (SMALL, ISAAC and LEAVER 1987; SMALL, SUFFOLK and LEAVER 1989)] representing the B37N-type organization at map coordinates 440–460 would have made it possible to reconnect our breakpoint to a continuous circle. It is therefore important to be aware that although cosmid mapping yields a useful representation of the total complexity of the genome and of the local linkage of DNA sequences, it does not necessarily reflect the *in vivo* organization of the genome.

Using Eckhardt and CHEF gels, *Sfi*I digestion and a battery of probes, we have identified three circular chromosomes of 70, 115 and 120 kb in BMS maize mitochondria. Although these chromosomes constitute a significant fraction of the genome, more work needs to be done to determine the total number of different chromosomes and their relative stoichiometry. These chromosomes were resolved and characterized as physical entities as they exist *in vivo* rather than “rebuilt” from overlapping clones. Eckhardt gels in which supercoiled molecules are resolved, provided direct evidence for the circularity of all three chromosomes and indicated that they can exist in a supercoiled configuration. Circularity of the 120-kb chromosome was also deduced in CHEF gels from the partial and full restriction mapping with *Sfi*I and from the predicted novel fragments generated by circularization at the breakpoints.

We assume that the 120-kb fragments observed with CHEF and Eckhardt gels correspond to the same molecule because they are similar in size, they hybridize to the same probes, and they appear to be circular in both cases. Interestingly, there is a discrepancy between the high intensity of the bands corresponding to this chromosome with CHEF gels (the 40- and 80-kb full *Sfi*I fragments) and the faint 120-kb band obtained on Eckhardt gels. This suggests that the 120-kb chromosome is mostly in a relaxed configuration in our sample and does not resolve in Eckhardt gels (it probably remains in the well). Relaxed circles may

be the predominant form of plant mitochondrial chromosomes *in vivo*, as was found in human mtDNA (CLAYTON 1982) or nicks may be created during mtDNA preparation.

Using pulsed field gel electrophoresis, BENDICH and SMITH (1990) have found that the mitochondrial genome in watermelon seedlings consists almost exclusively of linear molecules ranging from 50 to 100 kb. It is not clear whether the abundance of linear molecules is a characteristic of watermelon or results from mtDNA preparation-induced shearing. An alternative explanation for our data is that the genome exists as circularly permuted linear molecules (BENDICH 1985). Our data do not support this model. The 120-kb chromosome appears to be in a circular configuration, as discussed above. Partial *Sfi*I digests yielded no fragments larger than 120 kb—larger fragments would be expected with a linear concatameric array of a 120-kb unit.

The process of tissue culture did not seem to affect the 120-kb chromosome; it is abundantly present in both the eight year old BMS cell line and in BMS seedlings. Both tissue sources also contained, in low stoichiometry, the sequence organization of B37N (the 5.6-kb *Bam*HI arrangement at one of the breakpoints). The maintenance of the primary and sublimon organization of the 120-kb chromosome in both cultured cells and seedling tissues suggests both are stable in the BMS genome. It may be significant that five genes, including the ribosomal genes, are clustered in this region (Figure 2B).

An interesting question is whether the presence of the 120-kb chromosome could have been predicted by homologous recombination in the known repeats as has been proposed (PALMER and SHIELDS 1984; LONSDALE, HODGE and FAURON 1984; LONSDALE *et al.* 1988). On the 120 kb chromosome the breakpoint near *Atp1* (coordinate ~453) is near the 14B repeat (FAURON and HAVLIK 1988), but the other breakpoint (coordinate ~333) is not found on a known repeat. Because no known repetitive DNA exists at one of the putative sites of recombination, we must consider that short repeats of 50–100 bp may exist at the breakpoints allowing homologous recombination. Alternatively, recombination may be site-specific (CRAIG 1988) with few, if any homologous bases necessary for a rearrangement; this model has also been proposed for the 7.3-kb circular molecule in *Oenothera* mitochondria (MANNA and BRENNICKE 1986). These possibilities will be explored by sequencing through the breakpoints of the three chromosomes we have identified.

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