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**Characterization of two new plasmid DNAs found in mitochondria of wild-type *Neurospora intermedia* strains**

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**ABSTRACT**

Mitochondria from two *Neurospora intermedia* strains (P405-Labelle and Fiji N6-6) were found to contain plasmid DNAs in addition to the standard mitochondrial DNA species. The plasmid DNAs consist of monomeric circles (4.1-4.3 kbp and 5.2-5.3 kbp for Labelle and Fiji, respectively) and oligomers in which monomers are organized as head-to-tail repeats. DNA-DNA hybridization experiments showed that the plasmids have no substantial sequence homology to mtDNA, to each other, or to a previously characterized mitochondrial plasmid from *N. crassa* strain Mauriceville-1c (Collins *et al.* Cell 24, 443-452, 1981). The intramitochondrial location of the plasmids was established by cell fractionation and nuclease protection experiments. In sexual crosses, the plasmids showed strict maternal inheritance, the same as *Neurospora* mitochondrial DNA. The plasmids may represent a novel class of mitochondrial genetic elements.

**INTRODUCTION**

Mitochondrial (mt) DNAs from many organisms have been shown to encode a subset of proteins associated with the electron transport system and rRNA and tRNA species that function in mitochondrial protein synthesis (1). We have recently found that mitochondria from certain *Neurospora* strains contain additional, closed-circular plasmid DNAs which have virtually no sequence homology to the standard *Neurospora* mtDNA. We have previously reported the characterization of a 3.6 kbp plasmid found in mitochondria of *N. crassa* strain Mauriceville-1c (ref. 2). Here, we report the characterization of two additional, closed-circular plasmid DNAs (4.1-4.3 kbp and 5.2-5.3 kbp) which are found in mitochondria of *N. intermedia* strains P405-Labelle and Fiji N6-6. DNA-DNA hybridization experiments showed that the Labelle and Fiji plasmids are not related to each other or to the previously

described plasmid from Mauriceville-1c. The plasmids may represent a novel class of mitochondrial genetic elements.

### MATERIALS AND METHODS

#### Strains of Neurospora and Growth Conditions

The Neurospora strains used in this study were 74-OR23-A (74A), Mauriceville-1c (FGSC #2225), P405-Labelle (FGSC #1940), Fiji N6-6 (FGSC #235), and Taipei-1c, 1g (FGSC #'s 1766 and 1767). 74A and Mauriceville are N. crassa strains. Labelle, Fiji and Taipei are N. intermedia strains. Maintenance of strains, preparation of conidia, and sexual crosses were carried out by standard procedures (3). Mycelia were grown in liquid culture for 14 h (25°) as described previously (4).

#### Isolation of Mitochondria

Mitochondria were isolated by the flotation gradient method (5) with modifications (6).

#### Isolation of Nucleic Acids

Three different methods were used for the isolation of mtDNA. For large scale preparations, mtDNA was extracted from nucleoprotein pellets using the phenol-ribonuclease-protease procedure (7) with modifications (2). For isolation of closed-circular DNA, mitochondrial lysates were centrifuged directly in CsCl-ethidium bromide gradients as described previously (2). Finally, a new, small-scale procedure was used to analyze mtDNAs from progeny in sexual crosses. Mitochondria were isolated from 2 liter cultures by differential centrifugation as described (6). The mitochondrial pellet was dissolved in 1.5 to 2.0 ml of a solution containing 1% SDS, 100 mM NaCl, 2 mM EDTA, and 100 mM Tris-HCl, pH 8.2. Insoluble material was pelleted at 17,000 x g for 15 min, and the supernatant was extracted 2 to 3 times with phenol and then twice with ether. DNA was ethanol precipitated and resuspended in a solution containing 150 mM NaCl, 1 mM EDTA, and 100 mM Tris-HCl, pH 7.1. The DNA was then incubated with RNase A (Type XII-A; Sigma Chemical Company, St. Louis, Missouri; 50 µg per ml, 30 min, 37°) and protease K (Protease XI; Sigma Chemical Co., St. Louis, Missouri; 200 µg per ml, 40 min, 37°), extracted with phenol and ether and finally ethanol precipitated. The yield of mtDNA from this procedure was sufficient for 5-15

restriction enzyme digests.

RNA was extracted from flotation gradient-purified mitochondria by the SDS-diethylpyrocarbonate procedure (5,6).

Nucleic acids were extracted from nuclear and cytosolic fractions as described (2).

#### Cloning of Plasmid DNAs

Closed-circular Labelle and Fiji plasmids were linearized with Pst I and cloned into the single Pst I-site of *E. coli* plasmid pBR322 (8). The recombinant plasmids containing the cloned Labelle and Fiji plasmids are designated pLSP1 and pLSP24, respectively.

#### Restriction Enzyme Analysis

Restriction enzymes used in this study, Eco RI, Hind III, Hinc II, Bgl II, Bam HI, Hpa II, and Pst I, were obtained from Miles Laboratories (Elkhart, Indiana), New England Biolabs (Beverly, Massachusetts), and Bethesda Research Laboratories (Gaithersburg, Maryland). DNA (1 to 3  $\mu$ g) was digested using reaction conditions specified by the suppliers. Enzyme concentrations were 2 to 10 units per  $\mu$ g DNA, and reaction times varied from 5 to 15 h. Restriction enzyme digests were analyzed by electrophoresis in 5 mm horizontal slab gels containing 0.8 to 1.4% agarose in 90 mM boric acid, 2.5 mM EDTA, 90 mM Tris. For preparative purposes, restriction digests were electrophoresed in horizontal slab gels containing 0.8 to 1.0% low melting point agarose (Bethesda Research Laboratories, Gaithersburg, Maryland). DNA was extracted from the gels according to methods recommended by the supplier.

#### Electron Microscopy

DNA was spread for electron microscopy as described (9). Closed-circular pBR322 DNA was used as the molecular weight standard (8).

#### Southern Hybridization

DNA gel patterns were transferred to nitrocellulose strips (Schleicher and Schuell BA85) as described by Southern (10). DNA fragments to be used as probes were labeled by nick translation (11) using  $^{32}$ P-ATP or  $^{32}$ P-CTP (ca. 1000 Ci per mmole; New England Nuclear, Boston, Massachusetts). Specific activities were  $10^7$  to  $10^8$  dpm per  $\mu$ g for whole mtDNA or cloned restriction fragments and  $10^6$  to  $10^7$  dpm per  $\mu$ g for restriction fragments excised from

gels. Hybridization conditions were as described (2).

### RNA Gel Transfer Hybridization

Mitochondrial nucleic acids were denatured with glyoxal and electrophoresed in 1.4% agarose gels containing 10 mM sodium phosphate buffer, pH 7.0 (ref. 12). Gel patterns were transferred to aminophenylthioether (APT)-paper and hybridized with nick translated DNA probes as described previously (2).

### Analysis of Mitochondrial Translation Products

Mitochondrial translation products were labeled in vivo with  $^{35}\text{S}$ -sulfate in the presence of cycloheximide and analyzed by SDS-polyacrylamide gel electrophoresis followed by autoradiography (2).

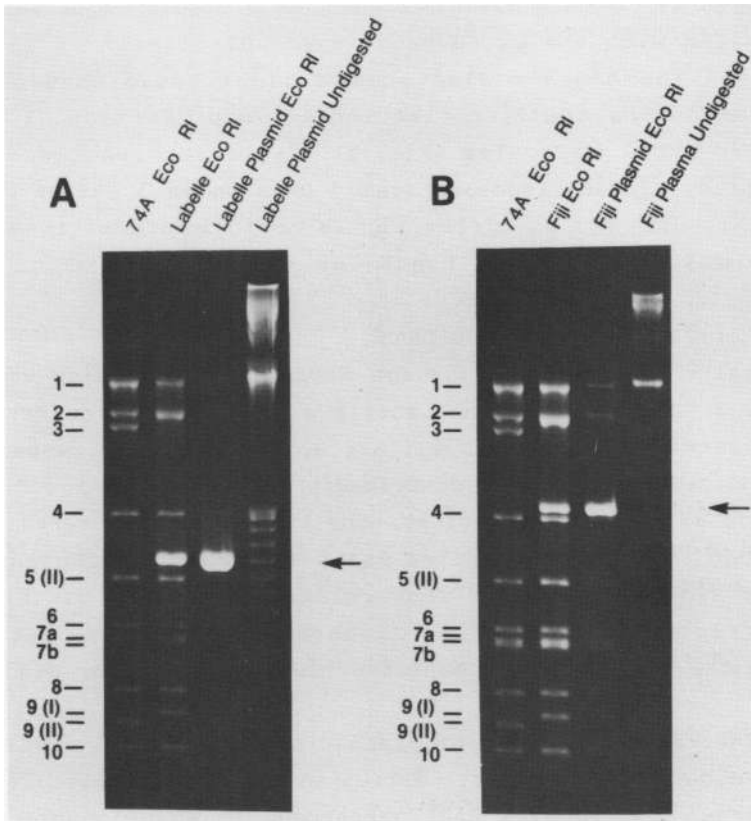
## RESULTS

### Identification of Plasmid DNAs

N. crassa wild-type strains commonly used in the laboratory contain either of two mtDNA types (I and II) which can be distinguished by Eco RI-digest patterns (13,14). The differences between the two mtDNA types are represented as insertions, one of 1200 bp in Eco RI-5 of type II mtDNA and the other 50 bp in Eco RI-9 of type I mtDNA (13,14).

Fig. 1 shows Eco RI-digests of type II mtDNA from N. crassa wild type 74A and DNA extracted from mitochondria of the plasmid-containing N. intermedia strains P405-Labelle and Fiji N6-6. The plasmid DNAs appear as extra bands (4.1 and 5.2 kb in the Labelle and Fiji strains, respectively) which are redundant compared to the neighboring mtDNA bands. Otherwise, the N. intermedia mtDNAs are remarkably similar to N. crassa mtDNAs as judged by comigration of all but two Eco RI-fragments. The exceptions are Eco RI-3 which is 1.1 kbp larger in Labelle and Fiji and comigrates with Eco RI-2 (Collins and Lambowitz, manuscript in preparation) and Eco RI-9 which is ca. 50 bp larger in Labelle and Fiji and appears to be the form characteristic of N. crassa type I mtDNA.

Closed-circular Labelle and Fiji plasmid DNAs were separated from mtDNA by centrifugation in CsCl-ethidium bromide gradients. Although Neurospora mtDNA itself is assumed to be circular in vivo, preparations consist predominantly of linear molecules (M<sub>w</sub>s = 20 to 40 Mdal), reflecting one or more double stranded breaks



**Figure 1.** Gel electrophoretic analysis of DNAs from *Neurospora* mitochondria. **A**, (1) Eco RI-digest of DNA from 74A mitochondria; (2) Eco RI-digest of DNA from Labelle mitochondria; (3) Eco RI-digest of closed-circular DNA from Labelle mitochondria; (4) closed-circular DNA from Labelle mitochondria, undigested. **B**, (1) Eco RI-digest of DNA from 74A mitochondria; (2) Eco RI-digest of DNA from Fiji mitochondria; (3) Eco RI-digest of closed-circular DNA from Fiji mitochondria; (4) closed-circular DNA from Fiji mitochondria, undigested. Closed-circular DNA was purified from mitochondrial lysates by centrifugation in CsCl-ethidium bromide gradients. The gel contained 0.8% agarose. The numbers at the left indicate Eco RI-fragments. Eco RI-5(II), 9(I) and 9(II) refer to the fragments characteristic of type I or II mtDNA. Arrows indicate Eco RI-fragments derived from the plasmids.

(15,16). Consequently, more than 90% of the mtDNA is found in the band corresponding to linear DNA in CsCl-ethidium bromide gradients. The Fiji and Labelle strains gave additional bands corresponding to closed-circular DNA. In both cases, the Eco RI-

digests of the closed-circular DNA show a single major band which comigrates with the extra band in Eco RI-digests of total DNA (Fig. 1). The digests also show a light background of mtDNA restriction fragments, reflecting a low proportion of closed-circular mtDNA molecules (Fig. 1). Gel electrophoresis of the undigested, closed-circular plasmid DNAs shows a series of bands which are assumed to correspond to conformational isomers and higher molecular weight bands due to oligomers (Fig. 1; see below).

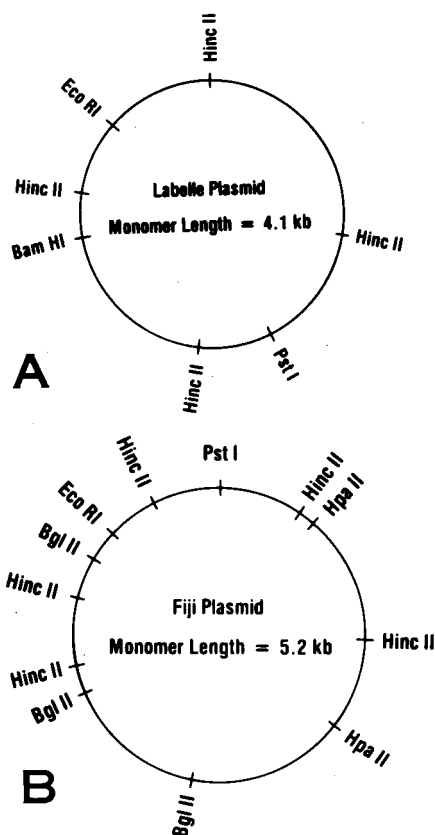
#### Characterization of Plasmid DNAs

Restriction enzyme cleavage maps of the plasmids are shown in Fig. 2. The sizes of the Labelle and Fiji plasmids determined by restriction enzyme analysis are 4.1 and 5.2 kbp, respectively. The Mauriceville plasmid, described previously, was found to contain two extraordinary regions, one containing clustered Eco RI-sites and the other clustered Pst I-sites (2). By contrast, the Labelle and Fiji plasmids show relatively uniform distributions of restriction sites with no comparable Eco RI- and Pst I-site clusters. Neither plasmid contains cleavage sites for Hind III or Sal I.

Electron micrographs of plasmid DNAs purified by CsCl-ethidium bromide gradient centrifugation show circular molecules whose contour lengths in each case correspond to an oligomeric series (Figs. 3 and 4). The monomers have lengths of 4.3 and 5.3 kbp, for the Labelle and Fiji plasmids, respectively, in close agreement with the sizes estimated from the restriction enzyme analysis. In both cases, molecules containing as many as six monomer units were observed by electron microscopy. Because the sum of the lengths of the restriction enzyme fragments is equivalent to the monomer length determined by electron microscopy, we can infer that the repeat units in the oligomers are organized head-to-tail rather than head-to-head.

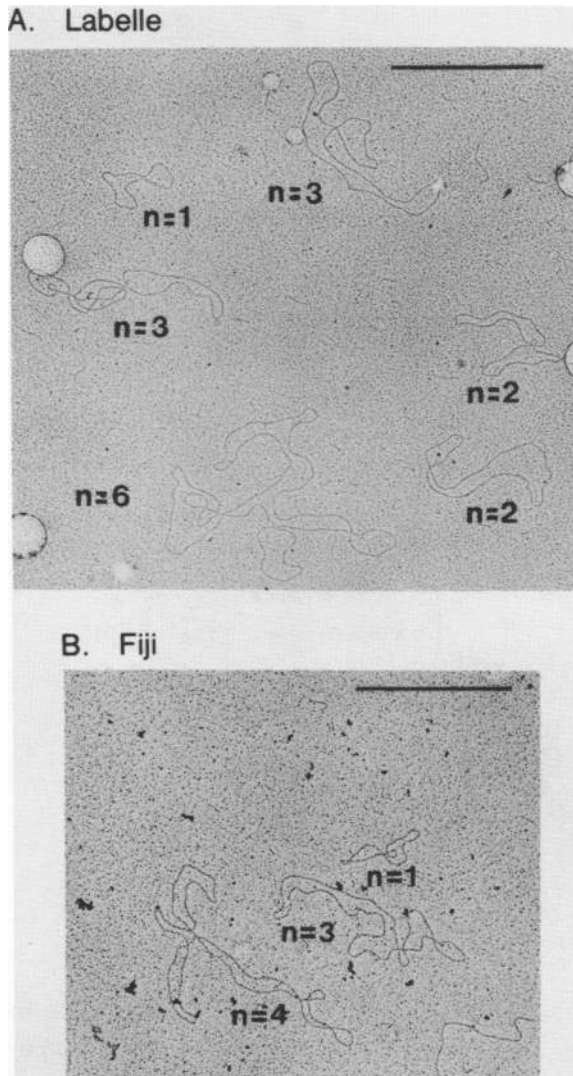
#### Relation of the Plasmid DNAs to Other DNA Species

Southern hybridization experiments were carried out to determine (i) whether the plasmid DNAs contain sequences homologous to mtDNA, and (ii) whether the plasmid DNAs are homologous to each other or to the previously described plasmid from Mauriceville-1c. Fig. 5 shows Southern blots in which each <sup>32</sup>P-labeled



**Figure 2.** Restriction enzyme cleavage maps of **A**, Labelle and **B**, Fiji plasmid DNAs. The maps are based on sizes of restriction fragments in partial and double digests.

plasmid DNA was hybridized to Eco RI-digests of DNA from mitochondria of *N. crassa* wild-type 74A, Mauriceville, Labelle, and Fiji. In each case, the plasmid DNA shows detectable hybridization only to itself and to no other band in any of the Southern blots. Fig. 6 shows reciprocal experiments in which Eco RI-digests of DNA from Labelle and Fiji were hybridized with  $^{32}\text{P}$ -labeled mtDNA from wild-type 74A. Now, the blots show hybridization to every band except for those derived from the plasmid DNAs. A similar experiment was reported previously for the Mauriceville plasmid (2). We conclude that the three plasmid DNAs have no strong sequence homology to mtDNA or to each other.



**Figure 3.** Electron micrographs of closed-circular DNA fractions from A, Labelle and B, Fiji. Micrographs show monomers ( $n = 1$ ) and oligomers (up to  $\bar{n} = 6$ ). Bar in each panel equals 1 micron.

We carried out additional Southern hybridization experiments in which the probes were cloned plasmid DNAs (recombinant plasmids pLSP1 and pLSP24; see Materials and Methods) which had been  $^{32}\text{P}$ -labeled by nick translation to high specific activity. In very long exposures, we detected weak hybridization between the



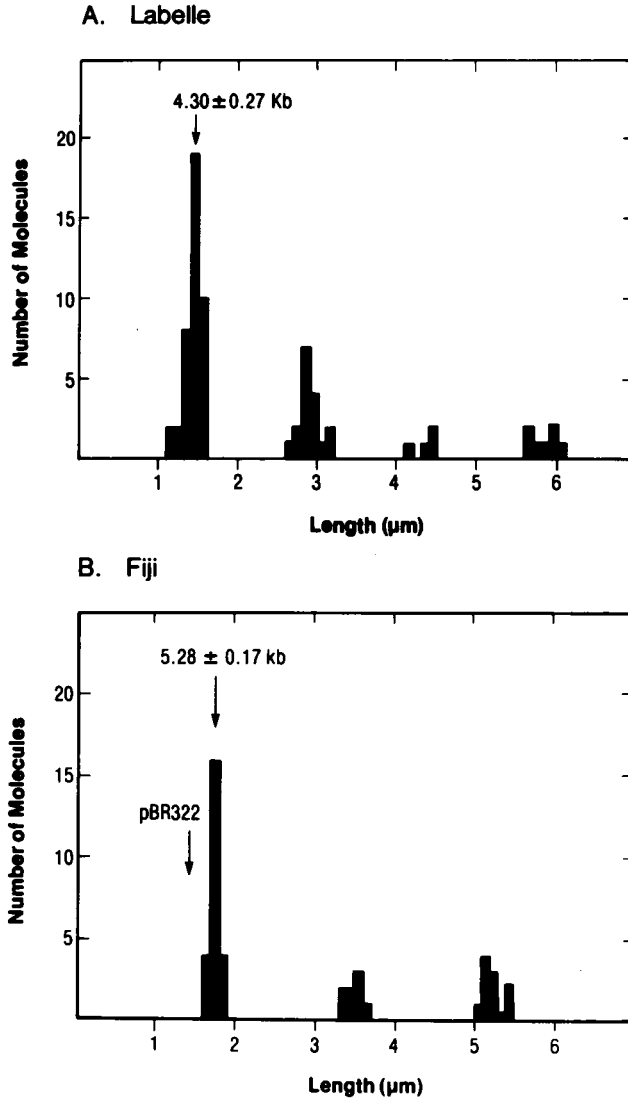
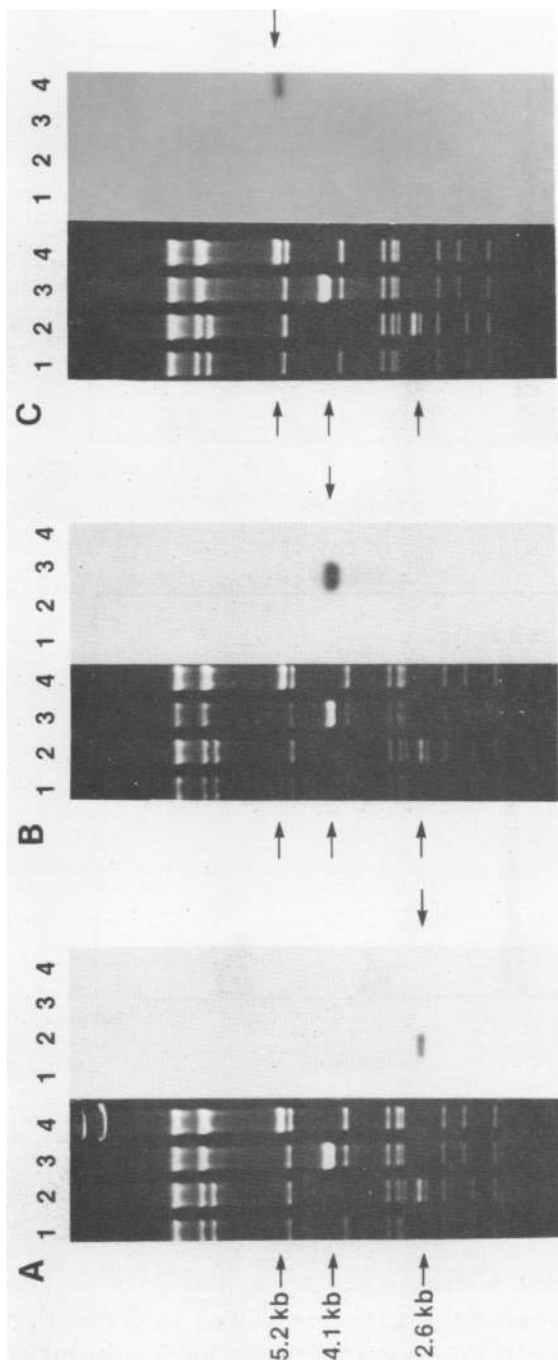
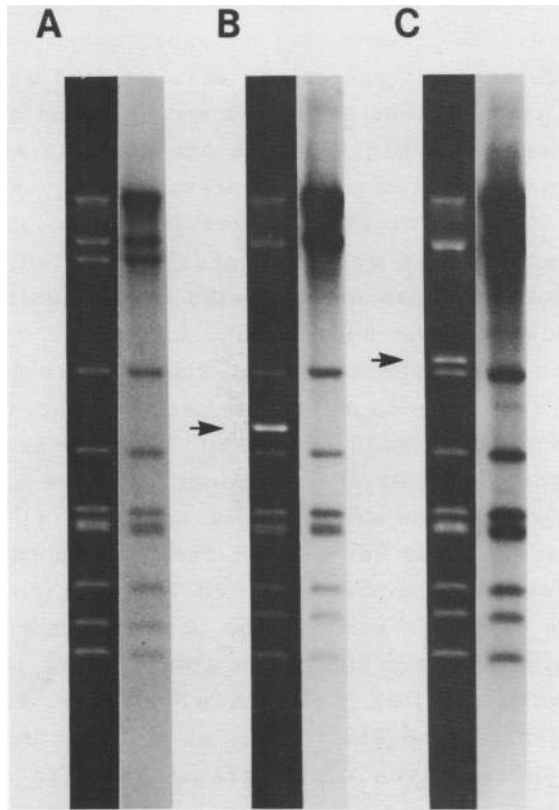


Figure 4. Size distribution of circular DNAs in A, Labelle and B, Fiji. Fields were photographed at random and all circular molecules that could be distinguished were measured. pBR322 (4362 bp; ref. 8) was used as the molecular weight standard.

Labelle plasmid and mtDNA fragment Eco RI-6, which is believed to contain a mtDNA replication origin (17), and between the Fiji plasmid and the Mauriceville plasmid. These weak hybridizations



**Figure 5.** Southern hybridization experiments. MtDNA was digested with Eco RI and electrophoresed in 0.8% agarose gels. Gel patterns were transferred to nitrocellulose strips and hybridized with plasmid DNAs which had been  $^{32}$ P-labeled by nick translation. The plasmid DNAs used as probes were purified by CsCl-ethidiumbromide gradient centrifugation, linearized by digestion with appropriate restriction enzyme (Bgl II for Mauriceville and Eco RI for Labelle and Fiji), and then repurified by gel electrophoresis. Probes were: A, Mauriceville plasmid; B, Labelle plasmid; C, Fiji plasmid. In A-C, (1) 74A, (2) Mauriceville, (3) Labelle, (4) Fiji. In each part, gel stained with ethidium bromide is shown on the left and autoradiogram is shown on the right. The arrows to the left indicate Eco RI-fragments derived from plasmid DNAs. The arrows to the right indicate hybridizations.



**Figure 6.** Southern hybridization experiments. DNAs from A, 74A, B, Labelle, and C, Fiji mitochondria were digested with Eco RI and electrophoresed in 0.8% agarose gels. Gel patterns were transferred to nitrocellulose strips and hybridized with mtDNA from 74A which had been  $^{32}\text{P}$ -labeled by nick translation. In each part, gel stained with ethidium bromide is shown on the left, and autoradiogram is on the right. The arrows to the left in B and C indicate Eco RI-fragments derived from the Labelle and Fiji plasmids, respectively.

could reflect very short sequences having perfect homology or longer sequences having partial homology.

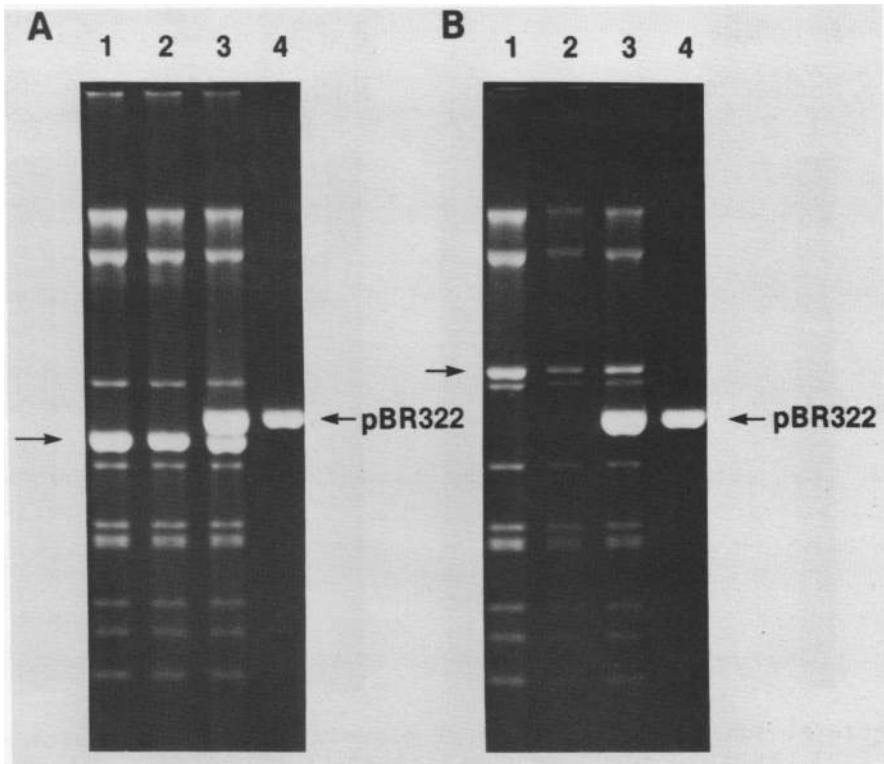
#### Plasmid DNAs are Located in Mitochondria

The restriction enzyme digests (Fig. 1) show that the plasmid DNAs are major DNA species in mitochondrial preparations that have been highly purified by flotation gradient centrifugation. The intramitochondrial location of the Labelle and Fiji plasmids

was confirmed by experiments similar to those reported previously for the Mauriceville plasmid. First, experiments were carried out in which mitochondrial preparations were treated with micrococcal nuclease, and then DNA was extracted and analyzed by gel electrophoresis. As shown in Fig. 7, both plasmid DNA and mtDNA were protected from nuclease digestion, presumably by enclosure within mitochondria, whereas closed-circular pBR322 added to the mitochondrial preparations was completely digested. Second, cell fractionation experiments were carried out in which plasmid and mtDNA sequences in the nuclear and cytosolic fractions were assayed by Southern hybridization. The nuclear fractions from Labelle and Fiji were found to contain small amounts of both plasmid DNA and mtDNA sequences (Fig. 8). In both strains, however, the ratios of Eco RI-fragments derived from the plasmid and mtDNAs were roughly the same as in mitochondrial preparations, indicating that most, if not all, of the plasmid DNA sequences in the nuclear fraction can be accounted for by mitochondrial contamination. In the Fiji strain, the post-mitochondrial supernatant showed no plasmid or mtDNA Eco RI-fragments in two independent experiments (Fig. 8). In Labelle, however, the post-mitochondrial supernatant showed the Eco RI-fragment corresponding to plasmid DNA, but no mtDNA Eco RI-fragments, even after extended exposure of the autoradiograms. The presence of the Labelle plasmid in the post-mitochondrial supernatant could reflect preferential leakage of plasmid DNA from the mitochondria. However, the results leave open the possibility that some proportion of the Labelle plasmid is located in the cytosol.

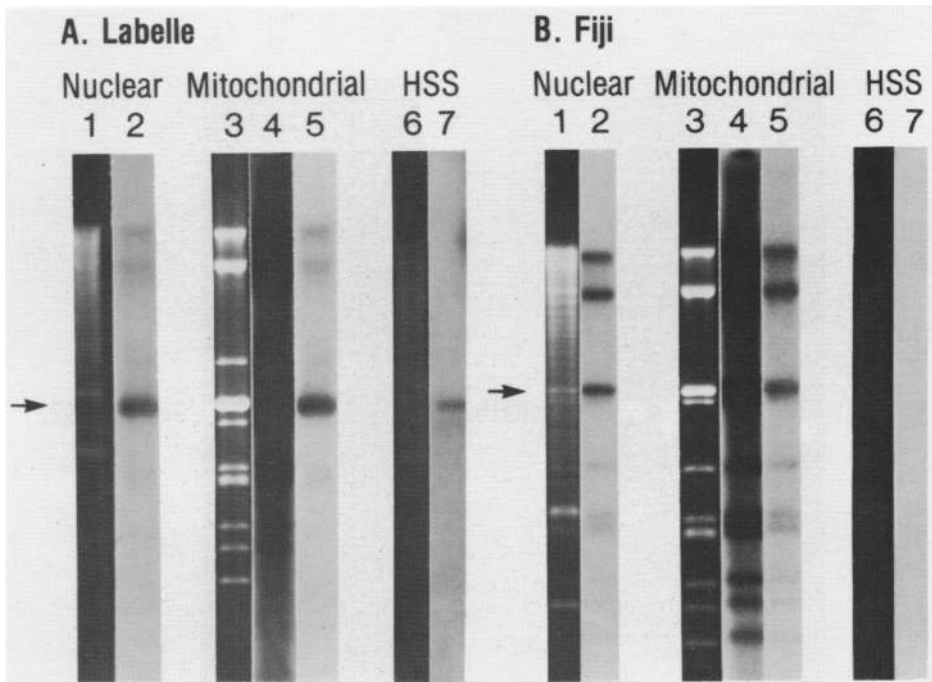
#### Inheritance of Plasmid DNA in Sexual Crosses

The pattern of inheritance of the plasmids was examined by carrying out crosses between the plasmid-containing strains and standard wild types. Random spores were collected and DNA was analyzed from 10 to 20 progeny of each cross. For Labelle and Mauriceville, the analysis of progeny from reciprocal crosses showed strict maternal inheritance of both plasmid DNAs--i.e., when the plasmid-containing strain was the maternal parent, all of the progeny contained the plasmid and when the plasmid-containing strain was the paternal parent, none of the progeny contained the plasmid (Fig. 9). The Fiji strain was inefficient



**Figure 7.** Micrococcal nuclease treatment of mitochondrial preparations. Mitochondria from **A**, Labelle and **B**, Fiji were treated with micrococcal nuclease as described previously (2). Mitochondria were then lysed, and DNA was extracted by the phenol-ribonuclease-protease method (2,7). DNA was digested with Eco RI and analyzed by electrophoresis in 0.8% agarose gels. In **A**, **B**, (1) mitochondria incubated with micrococcal nuclease; (2) mitochondria incubated with micrococcal nuclease; closed-circular pBR322 DNA (30  $\mu$ g) added prior to addition of nuclease; (3) mitochondria incubated with micrococcal nuclease; closed-circular pBR322 DNA (30  $\mu$ g) added after inactivation of nuclease; (4) pBR322 DNA digested with Eco RI. The arrows to the left in **A**, **B**, indicate the Eco RI-fragments derived from the Labelle and Fiji plasmids, respectively.

in the formation of protoperithecia and could only be used as the conidial (male) parent. In such crosses, none of the progeny contained plasmid, consistent with the strict maternal inheritance demonstrated in reciprocal crosses for the other plasmids. As expected, the mtDNA species in the different strains also showed strict maternal inheritance in the crosses (14,18). The



**Figure 8.** Subcellular location of plasmid DNAs in **A**, Labelle and **B**, Fiji. Nucleic acids were extracted from nuclear, cytosolic (high speed supernatant; HSS), and mitochondrial fractions, digested with Eco RI, and electrophoresed in 0.8% agarose gels. Digest patterns were transferred to nitrocellulose strips and hybridized with  $^{32}\text{P}$ -labeled DNA from mitochondria of the same strain. The  $^{32}\text{P}$ -labeled DNA included both plasmid and mtDNA. In each part, gel stained with ethidium bromide is shown to left and autoradiograms to right. In **A**, autoradiograms (2) and (4) were exposed for 48 h; (5) for 2 h (light exposure of (4)); and (7) for 90 h. In **B**, autoradiograms (2) and (4) were exposed for 60 h.; (5) for 5 h (light exposure of (4)); and (7) for 120 h.

pattern of inheritance of the plasmids shows that they are cytosolic and is consistent with their intramitochondrial location established by other approaches. However, it does not exclude the possibility that some proportion of the Labelle plasmid is cytosolic (see above).

#### Expression of Plasmid DNAs

In the previous study, RNA gel transfer hybridization experiments showed that the Mauriceville plasmid synthesizes a 3.3 to 3.4 kb RNA which is present in relatively high concentrations in

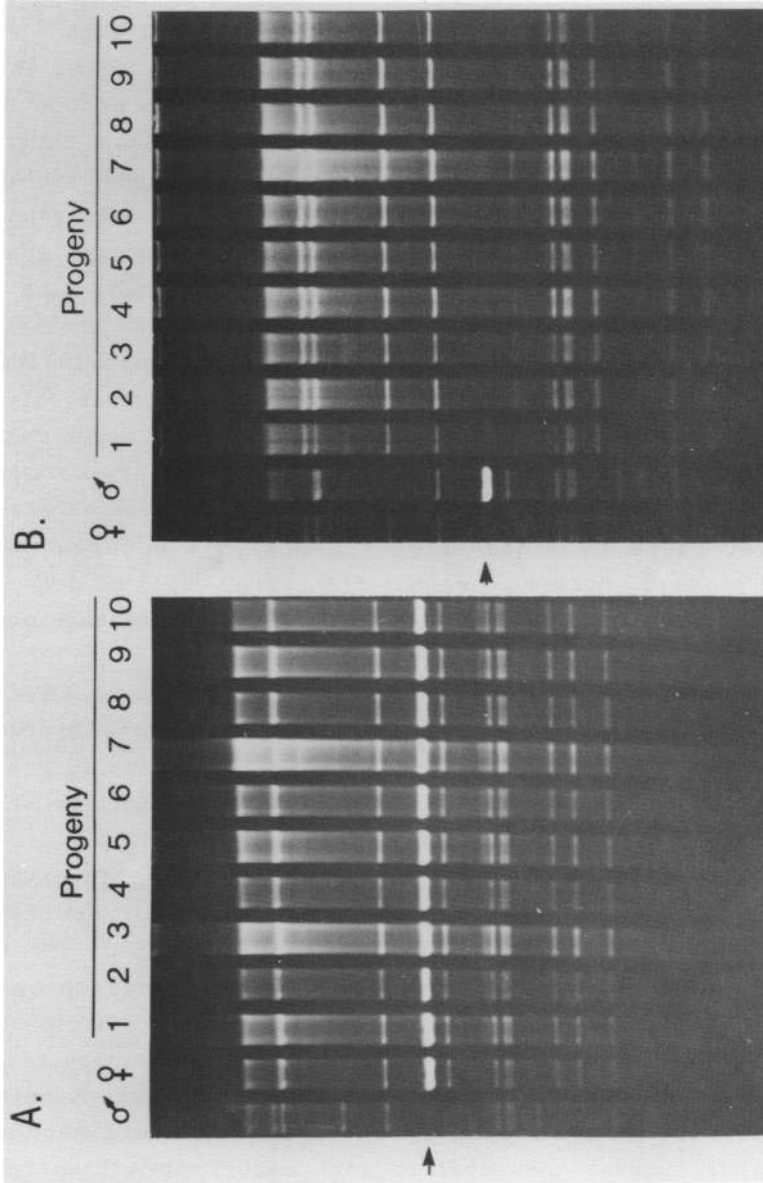


Figure 9. Eco RI-digests of mtDNA from progeny of reciprocal crosses between P405-Labelle and Taipei-1c. Taipei-1c is a wild-type *N. intermedia* strain which does not contain a mitochondrial plasmid. A, Labelle was female (protoperithecial) parent; B, Labelle was male (conidial) parent. Arrows indicate Eco RI-fragment derived from plasmid DNA. Gels in A and B were run for different lengths of time.

mitochondria (2). In the present work, similar RNA gel transfer hybridization experiments were carried out for the Labelle and Fiji plasmids. Total mitochondrial nucleic acids from these strains were treated with DNase I, electrophoresed through 1.4% agarose gels, transferred to APT-paper, and hybridized with  $^{32}\text{P}$ -labeled plasmid DNA probes. (Both the cloned plasmid DNAs and restriction fragments excised from gels were used as probes in these experiments.) Neither strain showed a major plasmid transcript comparable to that found in Mauriceville which was run in parallel as a positive control (data not shown). Prolonged exposure of the autoradiograms for Labelle and Fiji showed very weak hybridizations to RNA species having apparent lengths of 2 to 4 kb, but the significance of these weak hybridizations is unclear. They could indicate that plasmid transcripts are present in low concentrations or have unusually high AT-contents. Alternatively, they could reflect a small proportion of undigested plasmid DNA, or weak association between the plasmid DNA probes and normal mitochondrial RNA species. The main conclusion from these experiments is that there is a significant difference between the Mauriceville plasmid where a prominent transcript could be detected and the Labelle and Fiji plasmids where prominent transcripts could not be detected.

Mitochondrial translation products in the three plasmid-containing strains were labeled in vivo with  $^{35}\text{S}$ -sulfate in the presence of cycloheximide and analyzed by electrophoresis in SDS-polyacrylamide gradient gels followed by autoradiography. For all three strains, the gel patterns of mitochondrial translation products were indistinguishable from those of wild-type 74A (data not shown). We had previously reported that the Mauriceville strain contains a novel mitochondrial translation product ( $M_r = 41,000$  to  $44,000$ ) migrating just behind subunit 1 of cytochrome oxidase (two independent experiments; ref. 2). In two additional experiments, this novel polypeptide could no longer be detected, possibly because of comigration with cytochrome oxidase subunit 1. In general, proteins encoded by the plasmids could escape detection either because they overlap with major mtDNA translation products or because they are present in relatively low concentrations. Further insight into the coding potential of the



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plasmids should come from DNA sequence analysis which is now in progress.

### DISCUSSION

We have now identified three, closed-circular plasmid DNAs which are found in mitochondria of certain Neurospora strains, but which have virtually no sequence homology with the standard mtDNA. The three plasmids appear to be unrelated to each other, as judged by lack of significant cross hybridization. In addition, the Eco RI- and Pst I-site clusters which are found in the Mauriceville plasmid are not found in the Labelle and Fiji plasmids. Restriction enzyme digests (Fig. 1) showed that the plasmids are threefold or more redundant compared to mtDNA. Thus, there may be 100 or more copies of plasmid DNA per cell.

The intramitochondrial location of the plasmids was established by three complementary approaches: First, experiments in which mitochondrial preparations were treated with micrococcal nuclease showed that plasmid DNA and mtDNA are protected from digestion, whereas pBR322 DNA added to the preparations is completely digested. In the previous study of the Mauriceville plasmid, micrococcal nuclease digestion in the presence of increasing concentrations of Triton X-100 showed that the plasmid DNA and mtDNA become sensitive to digestion at the same concentration of detergent, suggesting that they are both protected by enclosure in the same membrane compartment (2). Second, cell fractionation experiments showed that the plasmids are major nucleic acid species in highly purified mitochondrial preparations, and that most, if not all, of the plasmid DNA sequences in other cell fractions could be accounted for by mitochondrial contamination. However, these experiments left open the possibility that some proportion of the Labelle plasmid is located in the cytosol. Finally, genetic analysis showed that the plasmid DNAs, like mtDNA, are maternally inherited in sexual crosses. The pattern of inheritance indicates that the plasmids are cytosolic and is consistent with their intramitochondrial location established by the other approaches.

The Neurospora mitochondrial plasmids may represent a novel class of genetic elements which are located in the mitochondria,

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but not directly related to the standard mtDNA. Male sterile lines of Maize contain novel DNA species which have been referred to as plasmids (19), but which differ from the Neurospora mitochondrial plasmids in that they hybridize to the mtDNA in normal (fertile) lines (20,21). Prowling (22) reported that normal sugarbeet mitochondria contain three small circular DNA species, whereas only one small circular DNA is present in mitochondria from male sterile sugarbeets. In this case, the relationship of the small DNA species to mtDNA was not reported (22). Finally, mitochondria from several different microorganisms have also been reported to contain small, circular DNA species, but in general these had been thought to be derived from mtDNA by processes analogous to those which give rise to defective mtDNAs in yeast petite mutants (see Discussion, ref. 2). In fact, such defective mtDNAs have been identified in Neurospora (16,23,24), Podospora (25,26), and Aspergillus (27) and may exist in other organisms as well (e.g., higher plants; 28,29). In light of our findings, we anticipate that reexamination of small, circular DNAs present in various mitochondrial preparations will reveal some which are analogous to the Neurospora mitochondrial plasmids.

Whether the plasmids have some biological function or whether they represent "selfish DNA" is not known. We found no differences between the plasmid-containing strains and standard wild-type strains in growth rates, cytochrome spectra, and mitochondrial rRNA profiles (data not shown). Previous work showed that the Mauriceville plasmid synthesizes a 3.3 to 3.4 kb RNA which is present in relatively high concentrations in mitochondria (2). The synthesis of a discrete transcript implies that the Mauriceville plasmid contains specific sites for the initiation and termination of transcription, consistent with some biological function. On the other hand, we could not detect major transcripts of the Labelle and Fiji plasmids, and it is still not certain whether any of the plasmids encodes a protein. Further insight into biological function may come from DNA sequence analysis.

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