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Genetics

*Eco*RI cleavage site variants of mitochondrial DNA molecules from rats

(restriction enzymes/electron microscopy/replication/maternal inheritance)

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ABSTRACT **The mitochondrial DNA (mtDNA) molecules of some rats obtained from the SASCO colony contain six *Eco*RI-sensitive sites while the mtDNA molecules of other rats obtained from the same source contain only four *Eco*RI sites. By mapping the positions of the *Eco*RI sites on the molecules relative to the D-loop it was determined that mtDNA molecules from all rats have four *Eco*RI sites in common, but all the mtDNA molecules of some rats have two extra *Eco*RI sites. *Eco*RI sensitivity of mtDNA molecules was shown to be maternally inherited. Construction of heteroduplex molecules failed to reveal evidence of gross nucleotide sequence changes associated with differences in *Eco*RI sensitivity.**

Circular mitochondrial DNA (mtDNA) molecules of various metozoans are sensitive to cleavage by the *Escherichia coli* restriction endonuclease *Eco*RI (1–5). The mtDNA molecules from an individual organism are usually homogeneous in regard to the *Eco*RI-sensitive sites they contain. However, Robberson *et al.* (1) reported that some mtDNA molecules derived from each of two mouse tissue culture lines are not cleaved at one of two sites cleaved in other molecules. Recently, Dawid *et al.* (6) also found that mtDNA molecules from one goat contained an *Eco*RI site not present in mtDNA molecules of a second goat.

We have found that the mtDNA molecules from some albino rats obtained from the SASCO colony have six *Eco*RI-sensitive sites, while the mtDNA molecules from other rats have only four *Eco*RI sites. We have studied the relationship of the *Eco*RI-sensitive sites in the two kinds of mtDNA molecules and determined the mode of inheritance of *Eco*RI sensitivity.

MATERIALS AND METHODS

Rats used throughout this work, except where otherwise stated, were randomly bred albino rats obtained from SASCO, Inc., Omaha, NE.

Mitochondria were isolated from rat livers as described (7), except that mitochondria were pelleted by centrifugation at 15,000 × *g*, and DNA was prepared from mitochondria and separated into covalently closed circular molecules and open (nicked) circular molecules as described in Wolstenholme and Fauron (4).

The samples of restriction endonuclease *Eco*RI and *Hin*dIII (from *Haemophilus influenzae*) (8) used in these studies were obtained from Richard Negeher and Herbert W. Boyer, Department of Microbiology, University of California, San Francisco, CA, Dana Carroll, Department of Microbiology, University of Utah, and from Miles Laboratories Inc., Elkhart, IN.

Details of the methods used to either completely or partially cleave mtDNA molecules with *Eco*RI and analysis of digestion products by either agarose gel electrophoresis or electron microscopy were as described elsewhere (ref. 4; D. L. Fouts, D. R. Wolstenholme, and H. W. Boyer, unpublished data).

Replicative form DNA molecules [molecular weight, 4.05×10^6 (4)] of bacteriophage fd or whole, nicked, circular mtDNA molecules from rat liver were used as internal length standards in all electron microscope preparations.

Fragments resulting from either *Eco*RI or *Hin*dIII digestion (4, 9) were circularized as follows. The restriction enzyme was destroyed by heating to 63° for 5 min and the DNA fragment-containing solution was dialyzed against 33 mM Tris·HCl (pH 7.6). $MgCl_2$ was added to 6.6 mM, ATP to 0.06 mM, and dithiothreitol to 10 mM (10). One unit of DNA ligase isolated from T4-infected *E. coli* cells (Miles Laboratories) was added to 100 μl of solution containing DNA at a concentration of less than 10 μg/ml, and the mixture was incubated for 48 hr at 4° and prepared for electron microscopy at room temperature.

Heteroduplexes were constructed as follows. A 1:4 mixture of nicked circular mtDNA molecules containing six *Eco*RI sites and fragments resulting from complete *Eco*RI digestion of mtDNA molecules containing four *Eco*RI sites were dialyzed against 95% formamide/10 mM EDTA (pH 8.2) for 1 hr to denature the DNA, then against 50% formamide/100 mM Tris·HCl (pH 8.2)/10 mM EDTA for 1 hr to achieve approximately 50% renaturation, and prepared directly for electron microscopy with a hypophase containing 20% formamide/10 mM Tris·HCl (pH 8.2)/1 mM EDTA (11). The lengths (as percentage circular genome) of double and single-stranded segments of heteroduplex molecules were determined by comparison with the mean lengths of totally double-stranded and totally single-stranded circular molecules contained in the same preparation.

All of the confidence limits given (±) are standard deviations, and the number of observations in each case is given by *n*.

RESULTS

A preparation of mtDNA obtained from the pooled livers of six rats was shown by electron microscopy to contain approximately 95% circular molecules. The molecular weights of these circular molecules had a unimodal distribution with a mean of $10.34 \pm 0.26 \times 10^6$ ($n = 30$). Analysis of this mtDNA (or of separated covalently closed and nicked circular molecules) by agarose gel (1.6%) electrophoresis after *Eco*RI digestion revealed eight bands at positions expected for fragments with mean lengths equivalent to approximately 3, 4, 12, 16, 18, 24,

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Abbreviations: mtDNA, mitochondrial DNA; *Eco*RI, *Escherichia coli* restriction endonuclease RI.

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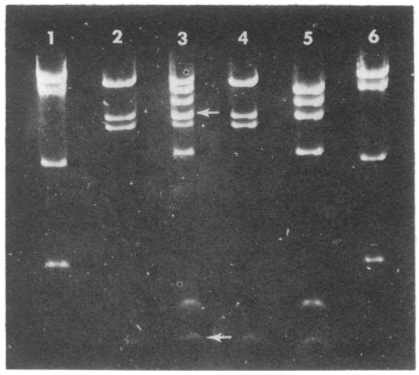

FIG. 1. Fluorescence photograph showing the distribution of ethidium-bromide-stained DNA bands comprising fragments resulting from complete digestion (30 min at 37°) with *Eco*RI in a 1.6% agarose slab gel after electrophoresis. Slots 1 and 6 contain the four *Eco*RI fragments of *Drosophila melanogaster* mtDNA, which have molecular weights of 7.3, 3.4, 1.14, and 0.57×10^6 (4). Slots 2, 4, and 5 contain mtDNA from three different rats. Four bands are visible in slots 2 and 4 at the positions expected for fragments with molecular weights of approximately 6.5, 1.9, 1.7, and 0.3×10^6 (corresponding to approximately 63, 18, 16, and 3% of the mitochondrial genome length). Six bands are visible in slot 5 at the positions expected for fragments with molecular weights of approximately 4.0, 2.5, 1.9, 1.3, 0.5, and 0.3×10^6 (corresponding to approximately 39, 25, 18, 13, 5, and 3% of the mitochondrial genome length). Slot 3 contains a mixture of the DNAs in slots 2 and 5. Only eight bands are visible, indicating that the digestion products of the two kinds of rat mtDNAs have two bands (arrows) in common.

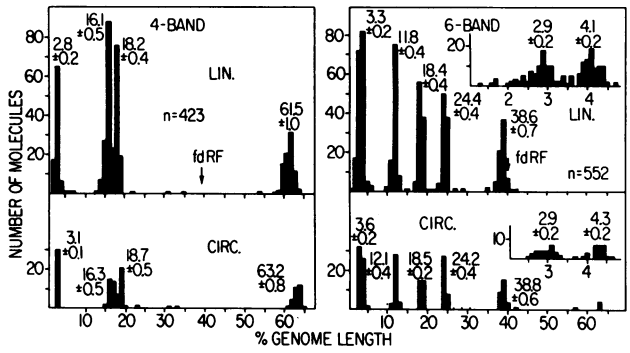

FIG. 2. Frequency distributions of the lengths of linear (LIN.) and circularized (CIRC.) fragments of mtDNA molecules in *Eco*RI-digested (30 min at 37°) preparations from two individual rat livers. Electrophoresis (Fig. 1) displayed four bands for the digest on the left and six for that on the right. The mean ±SD of each size group is given. The distributions shown in the upper frames in each case represent all of the molecules found within two 25 μm^2 sections of a grid square. The distributions shown in the lower frames were in each case obtained by measuring molecules at random from a collection of photographs of circular molecules until 30 molecules of each size class (60 molecules of the two central overlapping size classes in the left frame, and 60 molecules of the two smallest overlapping size classes in the right frame) were obtained. (*Insets*) The frequency distributions of the lengths of fragments in the range 0–4.7% plotted on a scale expanded 10 times. For both linear and circularized fragments, two distinct length classes are apparent, which correspond to the two smallest bands visible in the agarose gel of this digest (Fig. 1).

38, and 62% (total = 177%) of the rat circular mtDNA molecule (genome). These results would be expected if the mtDNA molecules examined were heterogeneous in regard to the number of *Eco*RI-sensitive sites they contain.

In order to determine the origin of the mtDNA molecules with different numbers of *Eco*RI sites, we prepared mtDNA from the liver of each of 32 rats (21 females and 11 males). Each mtDNA was then analyzed by agarose gel electrophoresis (1.6%) after *Eco*RI digestion. Four bands (Fig. 1) were apparent for mtDNAs of 20 of the rats (14 female and 6 male) and six bands (Fig. 1) were apparent for the mtDNAs of the remaining 12 rats (7 female and 5 male). In each case the lengths of the fragments determined by comparison with DNA fragments of known molecular weights coelectrophoresed in an adjacent slot totaled approximately 100% of the genome length. Agarose gel electrophoresis of an approximately equimolar mixture of *Eco*RI-digested mtDNA, which separated into four bands, and *Eco*RI-digested mt DNA, which separated into six bands, revealed a pattern of eight bands (Fig. 1), identical to that produced when mtDNA from pooled livers was similarly analyzed. Examination of the gel pattern indicated that the two kinds of mtDNA have two bands (equivalent to 3.0% and 18.5% of the genome length) in common.

Electron microscopy of *Eco*RI-digested mtDNA that showed four bands after agarose gel electrophoresis also revealed four size groups of fragments (Fig. 2) with lengths equivalent to 2.8 ± 0.2%, 16.1 ± 0.5%, 18.2 ± 0.4%, and 61.5 ± 1.0% of the genome length (sum = 98.6%). Circularized *Eco*RI fragments, which provide more accurate estimates of *Eco*RI fragment lengths (4), fell into four similar groups (3.1 ± 0.1%, 16.3 ± 0.5%, 18.7 ± 0.5%, and 63.2 ± 0.8% of the genome length; sum = 101.3%). Electron microscopy of *Eco*RI-digested mtDNA that showed six bands after agarose gel electrophoresis also revealed six size groups of fragments (Fig. 2) with lengths equal to 2.9 ± 0.2%, 4.1 ± 0.2%, 11.8 ± 0.4%, 18.4 ± 0.4%, 24.4 ± 0.4%, and 38.6 ± 0.7% of the genome length (sum = 100.2%). Again circularized fragments fell into six similar size groups (2.9 ± 0.2%, 4.3 ± 0.2%, 12.1 ± 0.4%, 18.5 ± 0.2%, 24.3 ± 0.4%, and 38.8 ± 0.6% of the genome length; sum = 100.9%). These data clearly suggest that mtDNA molecules from all rats tested have four *Eco*RI-sensitive sites in common (cleavage of which would result in the 3, 16.5, 18.5, and 63% fragments) and that all of the mtDNA molecules from some rats have two extra *Eco*RI sites (cleavage of which results in production of the 24 and 39% fragments from the 63% fragment, and of the 12 and 4.5% fragments from the 16.5% fragment).

In contrast to our findings concerning *Eco*RI cleavage, we found that all rat liver mtDNAs examined were cleaved by *Hin*dIII into six fragments which were determined by electron microscopy to have mean lengths equivalent to 2.1 ± 0.2%, 5.0 ± 0.2%, 12.7 ± 0.4%, 15.8 ± 0.5%, 25.5 ± 0.7%, and 39.7 ± 0.9% (sum = 100.8%) of the genome length.

Many rat mtDNA molecules contain a D-loop, a region of the molecule apparently produced by replication of approximately 3.5% of the H-strand (12). To permit mapping of the position of D-loop sites relative to *Eco*RI-sensitive sites, we fixed samples of 4-*Eco*RI-site mtDNA and 6-*Eco*RI-site mtDNA with glyoxal (2) and digested them to completion with *Eco*RI. D-loops were found (Fig. 3) at a constant distance (approximately 8.5% of the genome length) from one end of the 63% fragment of 4-*Eco*RI-site mtDNA molecules and the same distance from one end of the 39% fragments of 6-*Eco*RI-site mtDNA molecules is homologous to the D-loop-containing end of the 63% fragment of 4-*Eco*RI-site mtDNA molecules.

The 63% fragments and the 39% fragments included double-forked molecules (Fig. 3) with characteristics of size and structure consistent with their being partially replicated segments of circular molecules (7, 13, 14). In each of the replicated fragments examined, one fork was located approximately 8.5% of the genome length from one end of the fragment. These findings indicate that, as has been found for other mammalian mtDNAs (1, 2, 15, 16), the D-loop is the site at which replication

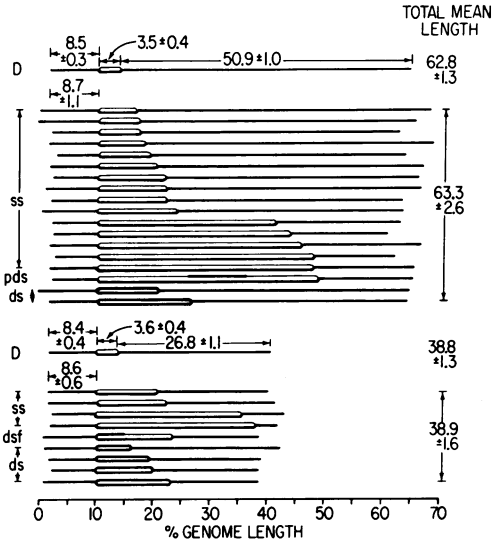

FIG. 3. Fragments containing D-loops or other double-forked replicating regions seen after complete *Eco*RI digestion of mtDNA molecules with (*Upper*) four *Eco*RI sites or (*Lower*) six *Eco*RI sites. The mean lengths of the various segments of 30 D-loop-containing fragments (D) found in each of the two mtDNA preparations are shown. The remaining fragments were aligned at the fork bounding the daughter segments with the parental segment closest in length to 8.5% genome length. One daughter segment of all replicating fragments was totally double stranded. The other daughter segment was completely single stranded (ss), totally double stranded (ds), double stranded in a central region only (pds), or double stranded at one fork and single stranded at the other fork (dsf) (14).

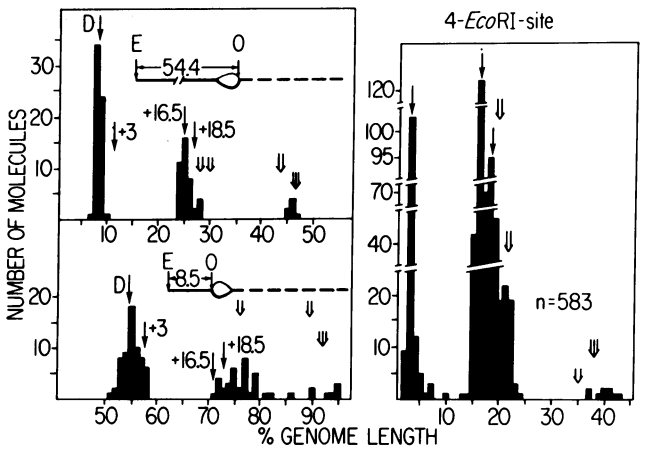

FIG. 4. Frequency distribution of the lengths of specific segments of D-loop-containing fragments (*Left*) or whole fragments (*Right*), resulting from incomplete *Eco*RI digestion of 4-*Eco*RI-site mtDNA molecules. (*Upper left*) D-loop-containing fragments resulting from cleavage at the *Eco*RI site (E) 54.4% of the genome length from the replication origin (O). The distribution represents the lengths of the double-stranded segment shown as the broken line measured from O. The single-shafted arrows indicate the mean lengths expected for this segment if the *Eco*RI site proximal to the D-loop is cleaved (D) or if this segment includes one of the 3%, 16.5%, or 18.5% fragments. The double-shafted arrows (left to right) indicate the mean lengths expected if the segment includes one of the fragment combinations, 3% + 16.5%, 3% + 18.5%, or 16.5% + 18.5%. The triple-shafted arrow indicates the length expected if all three fragments are included. (*Lower left*) D-loop-containing fragments resulting from cleavage at the *Eco*RI site (E) 8.5% of the genome length from the replication origin (O). The distribution represents the combined lengths of the D-loop and the double-stranded segment shown as the broken line. Double-shafted arrows indicate values for fragment combinations 18.5% + 3% and 18.5% + 16.5%. Other details are as given above. (*Right*) *All* fragments measuring less than 50% of the genome length. Such fragments should include those free of uncleaved *Eco*RI sites with expected mean lengths of 3%, 16.5%, and 18.5% (single-shafted arrows); those containing a single uncleaved *Eco*RI site (double-shafted arrows, left to right combinations of the 3% + 16.5%, 3% + 18.5%, and 16.5% + 18.5% fragments); and fragments containing two uncleaved *Eco*RI sites and comprising the 3%, 16.5%, and 18.5% fragments (triple-shafted arrow).

of the molecule begins, and replication of all molecules is unidirectional around the molecule as a whole and always proceeds in the same direction. The origin of replication is taken to be represented by the fork of the D-loop located at a mean distance of 8.5% of the genome length from the *Eco*RI site nearest to the D-loop (2).

To determine the arrangement of the fragments in the uncleaved 4-*Eco*RI-site and 6-*Eco*RI-site mtDNA circular molecules, we analyzed the products of incomplete digestion of these DNAs (4) after glyoxal fixation (2). These analyses were based on the assumption that the rate of cleavage is approximately the same for all the *Eco*RI-sensitive sites. In each case, a digested preparation that contained mainly fragments, but in which some circular molecules were still apparent, was chosen for study. Four-*Eco*RI-site mtDNA was examined first. Fragments containing a D-loop were photographed and measured. Molecules resulting from cleavage at the *Eco*RI site located 54.4% of the genome length (Fig. 3) from the origin were selected and the distribution of distance from the origin to the other end of the molecule was examined (Fig. 4 *upper left*). A distinct length group expected for fragments resulting from cleavage at the *Eco*RI site located 8.5% of the genome from the origin was apparent. A second distinct group occurred with a mean length expected if the 16.5% fragment, rather than 3% or 18.5% fragment, shared the *Eco*RI site of the 63% fragment proximal to the D-loop. The distribution of the remaining lengths were more consistent with the 3%, rather than the 18% fragment, being contiguous with the 16.5% fragment. Molecules resulting from cleavage at the *Eco*RI site located 8.5% of the genome (Fig. 3) from the origin were next selected; the distribution of distances from the origin to the other end of the molecule is shown in Fig. 4 *lower left*. In agreement with the conclusion drawn above, these data indicate that the 18.5% fragment is the most likely one to share the *Eco*RI site of the 63% fragment distal to the D-loop. The distribution of lengths of *all* fragments in the incomplete *Eco*RI digest measuring less than 50% of the genome was also examined (Fig. 4). While fragments with the length expected for the combinations 3% + 16.5% and 3% + 18.5% were abundant, fragments with the length expected for the combination 16.5% + 18.5% were absent. These data indicate the fragment arrangement 63% − 18.5% − 3% − 16.5% (see Fig. 7) in the circular 4-*Eco*RI-site molecule.

A similar analysis was next carried out for the D-loop-containing fragments resulting from incomplete cleavage of 6-*Eco*RI-site mtDNA molecules. For fragments produced by a cleavage at the *Eco*RI site located 30.3% of the genome length (Fig. 3) from the origin, the distribution of distances from the origin to the other end of the molecule (Fig. 5) clearly indicates that the 12% fragment shares the *Eco*RI site proximal to the D-loop. These data are also consistent with the fragment arrangement 12% − 4.5% − 3%. The frequency distribution (Fig. 5) for fragments produced by cleavage at the *Eco*RI site 8.5% from the origin indicates that the fragment sharing the *Eco*RI site 30.3% from the origin is the 24% fragment. These data indicate the fragment arrangement 39% − 24% − 18.5% − 3% − 4.5% −12.1% in the circular 6-*Eco*RI-site molecule (see Fig.

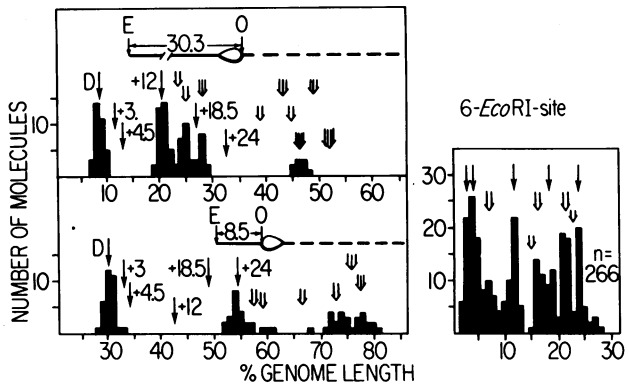

FIG. 5. Frequency distribution of the lengths of specific segments of D-loop-containing fragments (*Left*) or whole fragments (*Right*) resulting from incomplete *Eco*RI digestion of 6-*Eco*RI-site mtDNA molecules. Details are as given in the legend of Fig. 4, except that the arrows (left to right for each series) indicate the mean lengths expected for the segments which include various fragment combinations as follows. (*Upper left*) Double-shafted arrows 12% + 3%, 12% + 4.5%, 12% + 18.5%, and 12% + 24%; triple-shafted arrows, 12% + 4.5% + 3%, 12% + 4.5% + 18.5%, and 12% + 4.5% + 24%; quadruple-shafted arrow 12% + 4.5% + 3% + 18.5% and 12% + 4.5% + 3% + 24%. (*Lower left*) Double-shafted arrows 24% + 3%, 24% + 4.5%, 24% + 12%, and 24% + 18.5%; triple-shafted arrows 24% + 18.5% + 3%, and 24% + 18.5% + 4.5%. (*Right*) *All* fragments measuring less than 30% of the genome length. Such fragments should include those free of uncleaved *Eco*RI sites, with expected mean lengths of 3%, 4.5%, 12%, 18.5%, and 24% (single-shafted arrows), and those containing a single uncleaved *Eco*RI site (double-shafted arrows, left to right, combinations of the 3% + 4.5%, 3% + 12%, 4.5% + 12%, 3% + 18.5%, and 4.5% + 18.5% fragments).

7). Further evidence in favor of this interpretation was obtained from the distribution of lengths of *all* fragments in this partial *Eco*RI digest measuring less than 30% of the genome length (Fig. 5). Fragments of lengths excepted for the combination 3% + 4.5%, 3% + 18.5%, and 4.5% + 12% were clearly more abundant than fragments of lengths expected for other possible combinations in the size range studied.

The data presented are fully consistent with the suggestion made above that the 24% and 39% fragments of 6-*Eco*RI-site mtDNA molecules are homologous to the 63% fragment of 4-*Eco*RI-site mtDNA molecules, and that the 12% and 4.5% fragments of 6-*Eco*RI-site mtDNA molecules are homologous to the 16.5% fragment of 4-*Eco*RI-site mtDNA molecules.

Heteroduplex molecules were constructed between the denaturation products of *Eco*RI fragments of 4-*Eco*RI-site mtDNA molecules and nicked circular molecules of 6-*Eco*RI-site mtDNA molecules. Eighteen circular molecules containing a double-stranded region with a mean length of 62.3 ± 2.5% of the genome length and 16 circular molecules containing a double-stranded region with a mean length of 16.6 ± 0.61% of the genome length were photographed (Fig. 6). In line with arguments presented above, the double-stranded regions of these molecules should contain the sequences differing in *Eco*RI sensitivity in the two kinds of mtDNA molecules. Examination of the heteroduplexes did not reveal strand separations in any of the double-stranded regions. This observation sets an upper limit to the degree of sequence heterogeneity existing between the two classes of molecule in the region that includes the sites differing in *Eco*RI sensitivity. A sequence of 50 or more mismatched bases should have been detectable under the conditions of this experiment (11).

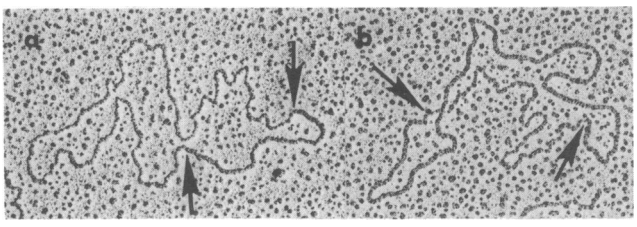

FIG. 6. Electron micrographs of circular, partially heteroduplex molecules produced by denaturation and renaturation of nicked circular 6-*Eco*RI-site mtDNA molecules and *Eco*RI fragments of 4-*Eco*RI-site mtDNA molecules. (*a*) A circular molecule containing a double-stranded region equal in length to approximately 16.5% of the genome. (*b*) A circular molecule containing a double-stranded region equal in length to approximately 63% of the genome. The arrows indicate the limits of the double-stranded regions. (Both micrographs × 34,000.)

Table 1. Data obtained from breeding experiments between rats with mtDNA molecules cleaved into four fragments by *Eco*RI and rats with mtDNA molecules cleaved into six fragments by *Eco*RI

Crosses defined by number of *Eco*RI sites of parents	Number of progeny			
	Females	Males	4 *Eco*RI sites	6 *Eco*RI sites
4♀ × 4♂	8	4	12	0
4♀ × 6♂	6	4	10	0
6♀ × 4♂	2	5	0	7
6♀ × 6♂	3	3	0	6

To gain information on the inheritance of the *Eco*RI sensitivity of mtDNA molecules, we bred individual males with groups of four virgin females. After weaning of the progeny at 21–25 days after birth, the sensitivity to *Eco*RI of the mtDNA of each parent was determined by agarose gel electrophoresis. Crosses were selected to give all four possible combinations (Table 1) in regard to mtDNA *Eco*RI sensitivity. mtDNA was prepared separately from each of the 35 to 44-day-old progeny of the selected crosses, and the sensitivity of the component molecules to *Eco*RI digestion was determined as for the parental mtDNAs. The results are summarized in Table 1. It is clear that mtDNA molecules of the progeny always contain the same number of *Eco*RI-sensitive sites as the mtDNA molecules of the mother. (In none of the digests from progeny of 6-*Eco*RI-site male × 4-*Eco*RI-site female, or 4-*Eco*RI-site male × 6-*Eco*RI-site female were the bands expected from the male mtDNA detected.) The simplest explanation of this finding is that the differences in number of *Eco*RI-sensitive sites occurring in mtDNA molecules in different individuals result from differences in nucleotide sequence at each of the two positions on the genome and that mtDNA molecules of progeny are derived only from the female parent. It appears less likely that the differences in sensitivity to *Eco*RI at the two positions result from base modifications that are under the influence of a nuclear gene.

SASCO rats were derived from a group of approximately 45 Holtzman rats (Holtzman Company, Madison, WI) in 1967 and have been maintained as a randomly outbred colony since that time. mtDNA was prepared from each of 15 Holtzman rats (8 females, 7 males), digested to completion with *Eco*RI, and analyzed by agarose gel electrophoresis. In each case, a pattern of six bands, identical in position to those observed for 6-*Eco*RI-site mtDNA from SASCO rats, was observed.

DISCUSSION

The major conclusions concerning the distributions of *Eco*RI-sensitive sites in rat mtDNA molecules are summarized in Fig.

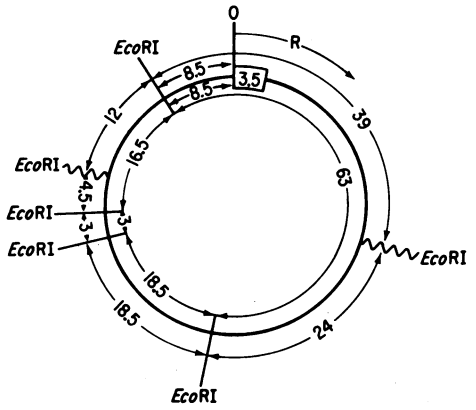

FIG. 7. The relative positions of the D-loop (box, 3.5) and *Eco*RI sites of 4-*Eco*RI-site molecules (inside) and 6-*Eco*RI-site molecules (outside), expressed as percentages of the length of the rat mitochondrial genome (heavy line). The *Eco*RI sites common to all molecules are indicated by straight lines. The two extra *Eco*RI sites of 6-*Eco*RI-site molecules are indicated by wavy lines. The direction of replication (R) from the origin (O) is indicated.

7. mtDNA from all rats contained four similarly located *Eco*RI sites. All of the mtDNA molecules of some individual rats, however, contained two extra *Eco*RI sites.

Kroon *et al.* (5) found that *Eco*RI digestion of rat mtDNA molecules resulted in six fragments with relative lengths similar to those found by us for 6-*Eco*RI-site mtDNA molecules, but concluded that the arrangement of the fragments in the uncleaved molecules was 39%–24%–3%–12%–4.5%–18.5%. We do not understand the reason for this discrepancy.

Previously reported variabilities in sensitivity to *Eco*RI cleavage of mtDNA molecules within a species were noted in the introduction. Differences have also been found in the patterns of fragments produced by *Hae* III digestion of mtDNAs obtained from different ponies, of mtDNAs of different humans and malignant human cell lines (17), and of mtDNAs of individual sheep and goats (6).

It is unclear whether in rats 4-*Eco*RI-site mtDNA molecules were derived from 6-*Eco*RI-site mtDNA molecules or vice versa. The differences in *Eco*RI sensitivity at each of the two positions on the molecules could result from small differences in nucleotide sequence arrangements, a single nucleotide difference, or nucleotide(s) modification.

Assuming that the differences in *Eco*RI sensitivity of rat mtDNAs are in fact a reflection of differences in nucleotides between the two kinds of molecules, then our data concerning the inheritance of *Eco*RI sensitivity indicate that the mtDNA molecules of rat are derived from the female parent. While evidence for maternal inheritance of mtDNA molecules between the closely related species *Xenopus laevis* and *X. mulleri* and *Equus caballus* and *E. asinus* have been presented (18, 19), the present data provide evidence for intraspecific maternal inheritance of mtDNA molecules. It cannot be ruled out, however, that [as has been noted by Hutchison *et al.* (19)] DNA molecules of sperm mitochondria, which in rats are known to enter the ovum (20, 21), make a minor contribution to the mtDNA complement of the organism.

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