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STRICTLY MATERNAL INHERITANCE OF RAT MITOCHONDRIAL DNA

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SUMMARY

Maternal inheritance of rat mitochondrial DNA (mtDNA) was demonstrated in Sprague-Dawley rats, which have two types of mtDNAs with respect to their *Eco*RI cleavage patterns on agarose gel electrophoresis. Use of litters of rats with the two types of mtDNAs for interbreeding showed that the mtDNA type of the progeny was the same as that of the dam. Furthermore, examination using the electrophoresis system that can detect as little as 0.02 μg mtDNA showed that 237 progeny in at least 40 generations in the closed breeding colony had one or other type of mtDNA, but that no individual had both types. Thus it is concluded that maternal inheritance of rat mtDNA is strict.

INTRODUCTION

Mitochondrial DNA (mtDNA) from animals of different species gave different cleavage patterns with restriction endonucleases (1). In addition to this interspecific heterogeneity, intraspecific heterogeneity of mtDNA has been observed in human and horses (1), sheep and goats (2), and rats (3-5). From studies on these inter- and intraspecific heterogeneities of mtDNAs, maternal inheritance of mtDNAs has been reported in *Xenopus* (6), between horse and donkey (7), and in rats (4).

However, it is still uncertain whether paternal mtDNA contributes at all to the mtDNA population in the progeny, because it is known that after insemination, mitochondria of the rat sperm middle piece remain in the cytoplasm of the egg (8). Sprague-Dawley rats seemed useful for resolving this question, because they have two types of mtDNA, α-type and β-type (5) or

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type A and type B (3)*, and individual rats can be obtained from colony after many years of closed breeding. The results presented here show that these two type mtDNAs are completely isolated genetically: i.e., that the paternal mtDNA does not contribute at all to the inheritance of mtDNA.

MATERIALS AND METHODS

Preparation of mtDNA from Sprague-Dawley rat: Sprague-Dawley rats were purchased from CLEA JAPAN, INC. At least 40 generations of these rats have been raised in the breeding colony of CLEA JAPAN, INC. since the establishment of specific pathogen free rats. Rats were killed after overnight fasting and mtDNAs were prepared from the livers of individual animals by the procedure of Kasamatsu *et al.* (9). The various organs other than liver of 12 rats in the litter were pooled, and the mtDNAs were prepared from the pooled organs by the same procedure as that for liver. The concentration of mtDNA was determined optically at 260 nm.

Digestion of mtDNA with *Eco*RI: Restriction endonuclease, *Eco*RI, was purchased from Boehringer Mannheim. MtDNA was completely digested by treatment with an appropriate amount of *Eco*RI at 37°C for 2 h. The digestion was carried out in 100 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, and 50 mM NaCl, as reported previously (5, 10).

Electrophoresis: Agarose gel (1%) electrophoresis was performed in glass tubing (6 mm X 135 mm) in 0.036 M Tris base, 0.03 M NaH_2PO_4 and 1 mM EDTA, pH 7.5 (11). After electrophoresis, the gels were stained with ethidium bromide (0.1 μg/ml). The DNA bands were located under short-wave ultraviolet light and photographed through an orange filter (Nikon CP-3) using Kodak Tri-X film.

RESULTS

We reported previously that Sprague-Dawley rats have two types of mtDNA, the cleavage patterns of which are different when they are digested with *Eco*RI, *Hin*dII, or *Hae*III (5). As a preliminary experiment, we examined the liver mtDNAs in individual rats of twenty litters and their dams, and found that the mtDNA types of the litters were the same as those of their dams (data not shown). Thus if the mtDNA type of one individual in a litter was known, those of the other individuals of the same litter could be predicted.

Next we used the other individuals in these litters, whose mtDNA type had been predicted, as parents for the four cross-breedings shown in Table I. The liver mtDNAs were prepared from the parents (21 week old) and progeny

* We first named the two mtDNA types α- and β-types (5), but here we have adopted the nomenclature of Francisco and Simpson, of type A and type B (3) in order to avoid confusion: Type A and B correspond to α- and β-type, respectively.

Table I Liver mtDNA type of the progeny of cross-breedings of Sprague-Dawley rats with typa A and B mtDNA

Parents*			Progeny	
mtDNA type			numbers of individuals	
dam		sire	type A	type B
A	X	A	45	0
A	X	B	48	0
B	X	A	0	47
B	X	B	0	51

* Each test was made on four pairs of rats.

(7-8 week old) of these crosses, and their mtDNA types were determined by agarose gel electrophoresis after digestion with *Eco*RI. Results showed that the mtDNA types of the progeny were the same as those of the dams in all four cross-breedings. In these cross-breedings there was no essential difference in the birth rate (Table I) and the mtDNA contents of all preparations of the progeny were about the same; the DNA contents of type A and type B per preparation were 13.5 ± 2.6 μg and 12.3 ± 1.8 μg, respectively.

Figure 1 shows that even the mtDNAs of other organs of the progeny, such as the kidney, heart, testis, muscle, brain, thymus, or intestine, were the same as that of the liver: no organ specificity was observed.

Two experiments were performed to test for the possible existence of a small amount of paternal type mtDNA. First, type B mtDNA was prepared from the liver of an individual obtained by cross-breeding a type A rat (sire) and a type B rat (dam), and 10 μg of type B mtDNA was cleaved with *Eco*RI and subjected to electrophoresis in agarose gel. In this electrophoresis system, as little as 0.02 μg of type A mtDNA could be detected (Figure 2, No. 1). However, no band corresponding to the type A mtDNA was observed in the preparation (Figure 2, No. 2). Thus even if paternal mtDNA existed in the preparation, it amounted to less

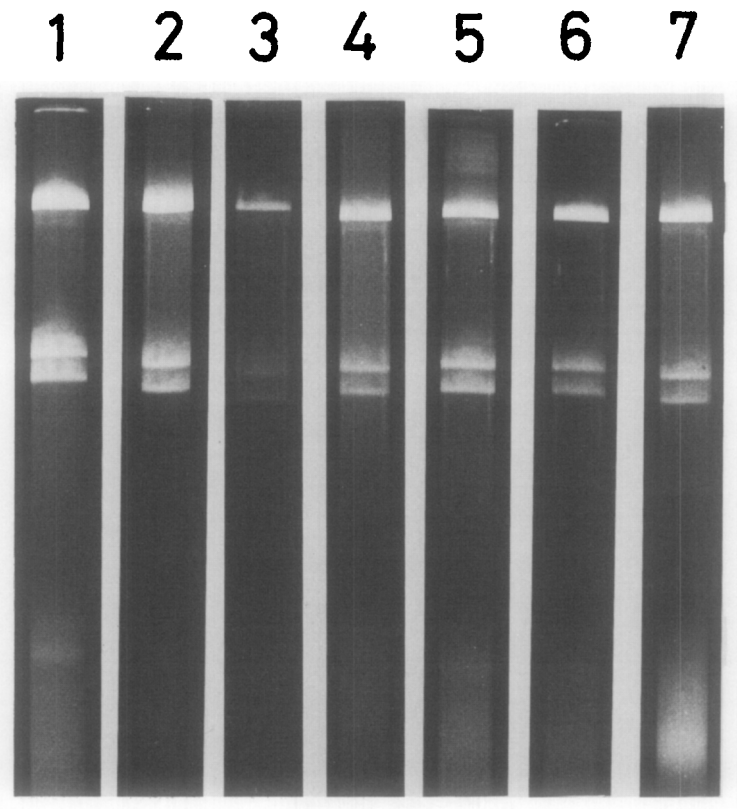

Figure 1. *Eco*RI cleavage patterns of mtDNAs prepared from various organs of a litter. Results on progeny of cross-breeding between type B (dam) and type A (sire) are shown. The various organs of the 12 rats in the litter were pooled, and the mtDNAs prepared from the pooled organs were cleaved with *Eco*RI and subjected to agarose gel electrophoresis. 1, kidney; 2, heart; 3, testis; 4, muscle; 5, brain; 6, thymus; 7, intestine.

than 0.2% of the total mtDNA population. Second, using this electrophoresis system, we tested the mtDNA types of 237 progeny from at least 40 generations (see MATERIALS AND METHODS section). Results showed that all individuals had only one type of mtDNA and no individual with both types of mtDNA or a type other than type A or B was detected (Table II).

DISCUSSION

Our preliminary experiment showed that the mtDNA types of twenty litters of rats were the same as those of their dams. As shown in Table I, even when the mtDNA type of the sire was different from that of the dam, the mtDNA type

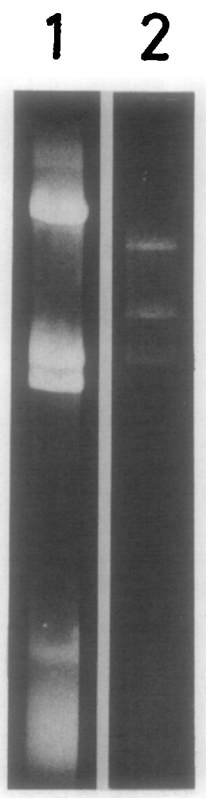

Figure 2. Detection of a small amount of type A mtDNA by agarose gel electrophoresis. 1, 0.02 μg of type A mtDNA; 2, 10 μg of type B mtDNA. Type B mtDNA was prepared from an individual obtained by mating between type B (dam) and type A (sire) rats and the sample was concentrated to 10 μg DNA/0.04 ml with Sephadex G-200 before digestion with *Eco*RI.

of the progeny was the same as that of the dam. This maternal inheritance of mtDNA could be observed in all organs tested. These findings suggest that during development, paternal mtDNA is not localized to a certain specific organ where it proliferates. Thus, the inheritance of rat mtDNA is maternal.

However, it was uncertain whether the paternal mtDNA made a minor contribution to the mtDNA population in the progeny. Since similar mtDNA contents were found in mtDNA preparations of the progeny of any of the four cross-breedings, the growth rates of type A and type B mtDNA in the liver are probably similar. Thus if the paternal mtDNA proliferated at a similar rate to the maternal mtDNA after introduction into the ovum in the sperm, after

Table II Classification of individual rats by their mtDNA type

Numbers of individuals				
No. Tested	type A	type B	Both types	Other types
237	196	41	0	0

breeding for many years some progeny should have both types of mtDNA and/or other types, such as recombinant types. However, using our electrophoresis system, which can detect very small amounts of mtDNA (as shown in Figure 2), we found that 237 individuals had exclusively either type A or B (Table II). These individuals were the progeny obtained in at least 40 generations in the closed breeding colony. Accordingly, it is very likely that type A and B mtDNAs are completely isolated genetically, and that the paternal mtDNA does not contribute at all to the mtDNA population of the progeny. This strictly maternal inheritance of mtDNA must have resulted in the stable continuation of type A and B mtDNAs throughout evolution. Our conclusion agrees well with the observation of Szollosi (8) that after penetration of the sperm into the rat ovum, the mitochondria of the sperm seem to disintegrate.

This conclusion raises some questions: First, why sperm mtDNA does not proliferate continuously during development?; Second, is the mode of the strictly maternal inheritance ubiquitous?; The last is how this intraspecific heterogeneity of mtDNA was established in spite of the facts that maternal inheritance of mtDNA is strict and the ovum has multicopies of mtDNA: that is, how mutated mtDNA, if it appeared, took over the prototype mtDNA completely in a certain population.

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