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TWO DIFFERENT MOLECULAR TYPES OF RAT MITOCHONDRIAL DNAS

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SUMMARY

Experiments with restriction endonucleases showed that Sprague-Dawley strain rats have two types of mitochondrial DNAs (mtDNAs), named α- and β-types, which differ in primary structure. Individual rats have only one type. Individuals of other strains, such as Wistar, Fischer, and Donryu, also all have one of these types of mtDNA. Thus irrespective of their strain, all rats can be classified into one of two groups according to the type of their mtDNA.

INTRODUCTION

Mitochondria of animals have closed circular DNA consisting of 15,000-18,000 base pairs. This DNA contains genes encoding about 20 mt-tRNAs (1, 2), poly(A)-containing RNAs (3-5), and two mt-rRNAs (2, 6, 7).

We have reported differences in the primary structures of mtDNAs from rat liver (Donryu strain) and Ascites-Hepatoma 130 (AH-130) obtained from Donryu rats (8). During our previous work, we found that when mitochondrial DNA (mtDNA) prepared from pooled livers of Sprague-Dawley rats was cleaved with *Eco*RI and subjected to electrophoresis, in addition to the major bands, two faint bands were detected even under conditions for complete digestion. These bands were observed in all mtDNA preparations tested, but their intensities relative to those of the major bands varied in different preparations. Thus we supposed

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that the additional bands were derived from different kinds of mtDNA from that producing the major bands. To substantiate this possibility we prepared mtDNAs from the livers of individual Sprague-Dawley rats and compared their *Eco*RI cleavage patterns. The results showed that individual rats have one of two types of mtDNA, which differ in primary structure.

MATERIALS AND METHODS

Preparation of mtDNAs from individual rat livers: Albino rats (Sprague-Dawley, Wistar, Fischer, and Donryu strains) weighing 200-500 g were killed after overnight fasting. MtDNAs were prepared from the livers of individual animals by the procedure of Kasamatsu *et al.* (9).

Enzymes and DNAs: Restriction endonucleases, *Eco*RI, *Hin*dII, and *Hin*dIII, were purchased from Boehringer Mannheim, while *Hae*III and *Hpa*II were obtained from Miles Laboratories. λDNA was prepared as described by Yabuki *et al.* (10). Col El plasmid DNA was a gift from Dr. Y. Sakakibara of the National Institute of Health.

Digestion with restriction endonuclease: The mtDNAs were completely digested by treatment at 37°C with appropriate amounts of the restriction endonucleases, *Eco*RI, *Hin*dII, *Hin*dIII, and *Hpa*II for 2 h, and of *Hae*III for 16 h. The digestions were carried out in 100 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$ and 50 mM NaCl for *Eco*RI; 10 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 50 mM NaCl and 14 mM dithiothreitol for *Hin*dII and *Hin*dIII; 6 mM Tris-HCl (pH 7.4), 6 mM $MgCl_2$ and 6 mM β-mercaptoethanol for *Hae*III; and in 10 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 6 mM KCl, 1 mM dithiothreitol and 0.1 mg/ml gelatin for *Hpa*II.

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). Polyacrylamide gel (4%) electrophoresis was carried out in 0.09 M Tris, 0.09 M boric acid, 2.5 mM EDTA, pH 8.5. 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.

Molecular weight determination: The apparent molecular weights of the products with restriction endonuclease were determined by agarose gel electrophoresis, using Col El (12) and λDNA (13) digested with *Eco*RI as molecular weight standards.

Electron microscopy: Liver mtDNA was treated with glyoxal and digested with *Eco*RI or *Hin*dII, and the location of the D-loop was determined as descirbed by Brown and Vinograd (14).

RESULTS

MtDNAs were prepared from seven individual Sprague-Dawley rats and were cleaved with *Eco*RI and *Hin*dII under the conditions for complete digestion. Two types of cleavage pattern were observed on agarose gel electrophoresis: one type of mtDNA, named α-type, showed at least six *Eco*RI fragments and four *Hin*dII

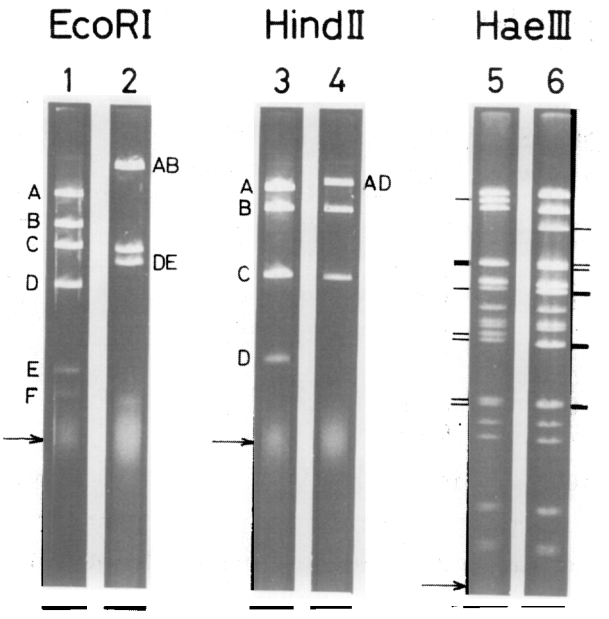

Figure 1. Comparison of the restriction endonuclease cleavage patterns of the α- and β-type mtDNAs from Sprague-Dawley strain rats. 1-4, 1% agarose gel electrophoresis; 5 and 6, 4% polyacrylamide gel electrophoresis. 1, 3, 5, the α-type, 2, 4, 6, the β-type, digested with *Eco*RI, *Hin*dII, and *Hae*III, respectively. Electrophoresis was performed from top (cathode) to bottom (anode). Arrows show the positions of Bromphenol Blue, with which contaminating origonucleotides comigrated on agarose gel.

fragments (Fig. 1, Nos. 1 and 3), and the other type, named β-type, showed four *Eco*RI fragments and three *Hin*dII fragments (Fig. 1, Nos. 2 and 4). Five of the seven rats had the α-type and the other two had the β-type. The molecular weights of the fragments showed that the β-type lacks two of the sites attacked by *Eco*RI (between fragments A and B, and D and E), and one of the sites cleaved by *Hin*dII (between fragments A and D) of the α-type (Table I).

The two types of mtDNA were also each digested with the other restriction endonucleases, *Hin*dIII, *Hpa*II and *Hae*III. Different cleavage patterns were observed when they were treated with *Hae*III (Fig. 1, Nos. 5 and 6). *Hae*III cleaved the mtDNAs into numerous small fragments and since some of these had very similar mobilities, it was not possible to locate some of the fragments accurately and to determine their molecular weights. However, the mobilities of some bands

Table I Molecular weights of *Eco*RI and *Hin*dII fragments of the α- and β-type mtDNAs

*Eco*RI fragment	Mol. wt ($\times 10^{-6}$) α-type	β-type	*Hin*dII fragment	Mol. wt ($\times 10^{-6}$) α-type	β-type
AB	—	6.23	AD	—	5.1
A	3.80	—	A	4.55	—
B	2.43	—	B	3.35	3.35
C	1.85	1.85	C	1.43	1.43
DE	—	1.58	D	0.55	—
D	1.14	—	E*	0.15	0.15
E	0.44	—			
F	0.31	0.31			
G*	0.05	0.05			
Sum	10.02	10.02		10.03	10.03

*, These fragments can be detected on 4% polyacrylamide gel electrophoresis.

of products from the α- and β-types were different. It was clear that the β-type had at least one additional *Hae*III cleavage site, because the β-type lacked the band corresponding to the second band of the α-type.

For mapping, the mtDNA of the α-type was partially digested with *Eco*RI and *Hin*dII, and the possible fragment compositions of the partial digestion products were determined by comparison of their molecular weights. The order of the fragments on the α-type was concluded to be -A-B-C-F-G-E-D- in *Eco*RI products, and -D-A-C-E-B- in *Hin*dII products. These fragments were arranged setting the D-loop in a fixed position, as reported by Brown and Vinograd (14) (Fig. 2). The physicap map of the β-type was easily constructed from the results described above (Table I and Fig. 2).

To investigate whether the α- and β-types exist in mtDNAs of other strains, we prepared liver mtDNAs from five individual rats of the Wistar, Fischer, and Donryu strains, respectively, and cleaved the mtDNAs with *Eco*RI, *Hin*dII, or *Hae*III. All the mtDNA preparations from Wistar strain rats showed the same cleavage pattern as the β-type, and those from the Fischer and Donryu strains showed the same cleavage pattern as the α-type. The *Eco*RI cleavage patterns are shown in Fig. 3. No individuals had both mtDNA types.

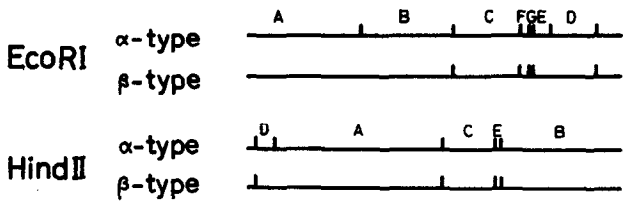

Figure 2. Physical maps of the α- and β-type mtDNAs of Sprague-Dawley strain rats. Both ends of the maps are the positions of the D-loop. The direction of mtDNA replication is from left to right.

Table II Distribution of the α- and β-type mtDNAs in rat strains

	Rat strain			
MtDNA type	Sprague-Dawley	Wistar	Fischer	Donryu
α-type	+	ND*	+	+
β-type	+	+	ND	ND

ND, not detected in the individual rats we used.
ND*, not detected in the individual rats we used, but seen in the reports of Kroon *et al.* (7, 15).

DISCUSSION

The present work with restriction endonucleases showed that Sprague-Dawley strain rats have two types of mtDNA that differ in primary structure, but that all individuals have only one of the two types. No strain specificity in rat mtDNAs was found. Five rats of the Wistar strain had the β-type, and five rats each of the Fischer and Donryu strains all had the α-type; however, the *Eco*RI cleavage pattern of Wistar rat mtDNA, reported by Kroon *et al.* (15, 7) was the same as that of the α-type. Thus the Wistar strain may also have both types of mtDNA (Table II), and furthermore, if more individuals of the Fischer and Donryu strains were examined, the β-type might be found in these strains also.

The cleavage map of Sprague-Dawley rat mtDNA reported by Parker and Watson (16) is the same as that of the β-type, whereas the *Eco*RI cleavage pattern of mtDNA from rat tissue culture cells described by the same authors is similar to

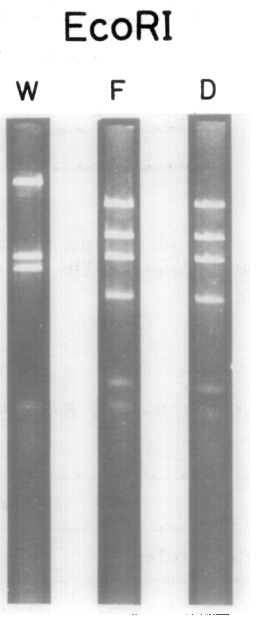

Figure 3. Agarose gel electrophoresis of mtDNAs from three other strains digested with *Eco*RI. W, Wistar strain; F, Fischer strain; D, Donryu strain.

that of the α-type. This discrepancey may be explained by supposing that their tissue culture cells were derived from Sprague-Dawley rats with the α-type mtDNA.

We reported previously that the primary structures of mtDNAs from Donryu rat liver and ascites hepatoma AH-130 cells obtained from Donryu rats were different (8). The *Eco*RI cleavage pattern of AH-130, however, is not the same as that of either type of mtDNA. Thus the primary structure of mtDNA from AH-130 cells may be unique.

MtDNAs from animals of different species gave different cleavage patterns with restriction endonucleases (17). Moreover, intraspecific heterogeneity of mtDNAs has been observed in horse (17), sheep, and goats (18), and higher plants (19). The present work shows that rats do not have strain specific mtDNAs, but that there are two types of mtDNA, the α- and β-types, one or other of which is present in all strains. Thus, irrespective of their strain, rats can be classified into two groups according to the type of their mtDNA. This heterogeneity of

rat mtDNAs should be useful in investigating the mode of mtDNA inheritance in rats of the same strain. Maternal inheritance of mtDNAs has been reported in *Xenopus* (20), and between horse and donkey (21), however, these observations were obtained by the interspecific hybridization. In our preliminary experiments, a litter of Sprague-Dawley rats composed of about ten individuals had the same type of mtDNA as its dam.

It is reasonable to consider that since the primary structures of these two mtDNA types have been conserved in rats throughout evolution, the different primary structures of rat mtDNAs found in this work must have some functional importance. Thus the difference might be expressed as different phenotypes.

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REFERENCES

1. Lynch, D.C. and Attardi, G. (1976) J. Mol. Biol. 102, 125-141.
2. Angerer, L., Davidson, N., Murphy, W., Lynch, D., and Attardi, G. (1976) Cell 9, 81-90.
3. Hirsch, M., Spradling, A., and Penman, S. (1974) Cell 1, 31-35.
4. Ojala, D. and Attardi, G. (1974) J. Mol. Biol. 88, 205-219.
5. Lewis, F.S., Rutman, R.J., and Avadhani, B.G. (1976) Biochemistry 15, 3367-3372.
6. Dawid, I.B. (1972) J. Mol. Biol. 63, 201-216.
7. Kroon, A.M., Pepe, G., Bakker, H., Holtrop, M., Bollen, J.E., Van Bruggen, E.F.J., Cantatore, P., Terpstra, P., and Saccone, C. (1977) Biochim. Biophys. Acta 478, 128-145.
8. Hayashi, J.-I., Yonekawa, H., Gotoh, O., Motohashi, J., and Tagashira, Y. Cancer Lett., in press.
9. Kasamatsu, H., Robberson, P.L., and Vinograd, J. (1971) Proc. Natl. Acad. Sci. USA 68, 2252-2257.
10. Yabuki, S., Gotoh, O., and Wada, A. (1975) Biochim. Biophys. Acta 395, 258-273.
11. Hayward, G.S. and Smith, M.G. (1972) J. Mol. Biol. 63, 385-395.
12. Oka, A. and Takanami, M. (1976) Nature 264, 192-196.
13. Thomas, M. and Davis, R.W. (1975) J. Mol. Biol. 63, 385-395.
14. Brown, W.M. and Vinograd, J. (1974) Proc. Natl. Acad. Sci. USA 71, 4617-4621.
15. Kroon, A.M. Bakker, H., Holtrop, M., and Terpstra, P. (1977) Biochim. Biophys. Acta 474, 61-68.
16. Parker, R.C. and Watson, R. (1977) Nucleic Acids Res. 4, 1291-1299.
17. Potter, S.S., Newbold, J.E., Hutchison III, C.A., and Edgell, M.H. (1975) Proc. Natl. Acad. Sci. USA 72, 4496-4500.
18. Upholt, W.B. and Dawid, I.B. (1977) Cell 11, 571-583.
19. Quetier, F. and Vedel, F. (1977) Nature 268, 365-368.
20. Dawid, I.B. and Blackler, A.W. (1972) Develop. Biol. 29, 152-161.
21. Hutchison III, C.A., Newbold, J.E., Potter, S.S., and Edgell, M.H. (1974) Nature 251, 536-538.