Vol. 98, No. 4, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 27, 1981 Pages 936-941

LENGTH POLYMORPHISMS OF RESTRICTION FRAGMENTS OF RAT MITOCHONDRIAL DNAS

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Received December 19,1980

SUMMARY

Differences were found in the HpaII cleavage patterns of two types of rat (*Rattus norvegicus*) mtDNA, types A and B. One HpaII fragment, Hpa5, of type A was about 30 base pairs smaller than that of type B, but no 30-base pair fragment was detectable in an HpaII digest of type A mtDNA. Moreover, one HaeIII fragment, which is overlapped by Hpa5 in the cleavage map, was also about 30-base pairs smaller in type A than in type B. Thus, the length polymorphism of Hpa5 in the two types is probably not caused by HpaII site gain or loss, but by sequence deletion or insertion.

INTRODUCTION

The existence of inter- and intraspecific heterogeneity of animal mtDNAs has been deduced from variations in restriction endonuclease cleavage patterns (1-9). These variations can be explained by cleavage site gains or losses. Using a small HindIII fragment of rat types A and B mtDNA, de Vos et al. (10) showed that at least a part of the cleavage site variations found in types A and B is caused by point mutations; no deletion or insertion was found in the fragment.

In contrast, Reilly and Thomas (11) reported that intraspecific variations of restriction cleavage patterns found in mtDNAs of *Drosophila melanogaster* are not due to cleavage site variations, but to length polymorphism of a single restriction fragment that includes an adenine and thymidine-rich (AT-rich) region.

In this paper we report the presence of length polymorphism of a single restriction fragment in rat types A and B mtDNA that is probably not caused by cleavage site gains or losses.

0006-291X/81/040936-06$01.00/0

MATERIALS AND METHODS

Preparation of mtDNAs from individual rat organs: Mitochondria were prepared as described in (12), and mtDNAs of liver were prepared by the procedure of Kasamatsu et al. (13) with slight modifications (6). MtDNAs of small organs, such as thymus and testis, were prepared as described in (14).

Restriction endonuclease digestion and electrophoresis: The mtDNAs were completely digested with HpaII, HaeIII (Miles Laboratories), and MspI (New England BioLabs) under the conditions reported previously (15). Polyacrylamide gel (4%) electrophoresis was carried out using slab gel (2 X 12 X 14mm) in 0.09M Tris, 0.09M boric acid, and 2.5mM EDTA, pH 8.5. Polyacrylamide gel (12%) electrophoresis was used to detect small fragments (<200 base pairs). After electrophoresis, gels were stained with ethidium bromide (0.1μg/ml) and the DNA bands were located under short-wave ultraviolet light and photographed through a filter (Nikon CP-3) using Kodak Tri-X film. The sizes of restriction fragments were determined using lambda DNA digested with EcoRI (16) and pBR322 DNA digested with HpaII (17) as size standards.

RESULTS

The HpaII cleavage patterns of two types of rat mtDNA (types A and B) prepared from the livers of individual Sprague-Dawley rats were compared (Fig. 1). As reported previously (6), the two types can be distinguished by differences in the cleavage sites of EcoRI, HindII, and HaeIII. On 4% polyacrylamide gels (Fig. 1a), mtDNAs of both types gave 9 HpaII bands numbered in the order of size (Hpa1-Hpa9). However, a slight difference in the mobility of one HpaII fragment,

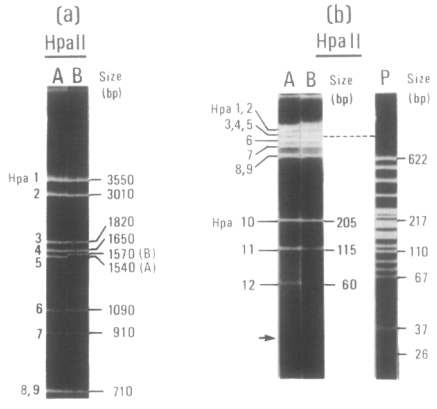


Figure 1. Comparison of HpaII cleavage patterns and sizes of the HpaII fragments of type A and B mtDNA. (a) Polyacrylamide gel (4%) electrophoresis of HpaII digests. A, mtDNAs of type A; B, mtDNAs of type B. HpaII fragments are numbered in order of molecular size (Hpa1-Hpa9) (from top, cathode, to bottom, anode, of the gel). (b) Polyacrylamide gel (12%) electrophoresis of HpaII digests. A, mtDNAs of type A; B, mtDNAs of type B; P, pBR322 DNA digested with HpaII as size standards (17). A small amount of 4%-polyacrylamide gel was mounted on 12%-polyacrylamide gel to allow fragments with large molecular size (Hpa1 and 2) to enter the gel. The boundary of the 4% and 12% gels is indicated by a broken line. The arrow indicates the position where a fragment of 30-bp should migrate.

Hpa5, was observed: the Hpa5 of type A (1540 base pairs (bp)) was about 30 bp smaller than that of type B (1570 bp). Since similar cleavage patterns were also observed on MspI digestion (data not shown), HpaII sites of both types may not be methylated (18).

This length polymorphism might be explained by supposing the presence of an additional HpaII site in type A mtDNA, resulting in cleavage of the 1570-bp fragment into a large (1540 bp) and a small (30 bp) fragment, but the latter fragment could not be detected under the conditions of electrophoresis used. In an attempt to detect the 30-bp fragment in the HpaII digest of type A, we carried out electrophoresis in 12% polyacrylamide gels using HpaII digests of pBR322 DNA as size standards (Fig. 1b). Under these conditions, even the 26-bp fragment of pBR322 DNA (17) can be detected. HpaII digests of types A and B mtDNA showed three small additional bands (Hpa10-Hpa12) on the 12% polyacrylamide gel. However, no band of about 30 bp was detectable in the digest of either type A or type B (Fig. 1b). Thus, the length polymorphism of Hpa5 does not seem to be caused by gain or loss of an HpaII site.

This idea was supported by comparing the molecular sizes of products of the two mtDNAs with another restriction endonuclease (HaeIII). Figure 2 shows the HaeIII cleavage patterns of the two types; 17 (type A) and 18 (type B) fragments

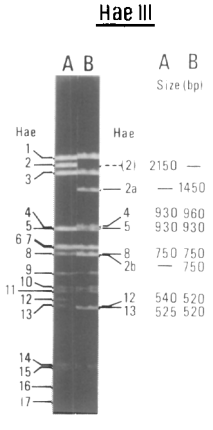
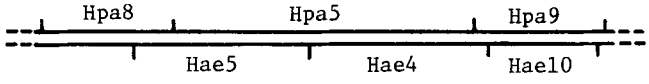


Figure 2. Comparison of HaeIII cleavage patterns and sizes of fragments of type A and B mtDNA. Polyacrylamide gel (4%) electrophoresis of HaeIII digests. A, mtDNAs of type A; B, mtDNAs of type B. HaeIII fragments are numbered in order of molecular size (Hae1-Hae17, Hae2a and 2b).

were obtained and were numbered in order of size (Hae1-Hae17, Hae2a and 2b). From the HaeIII and HpaII cleavage maps of rat mtDNAs reported by Feldmann and Grosskopf (19), two HaeIII fragments, Hae4 and Hae5, were aligned on the maps to include Hpa5 as follows;



If length polymorphism of Hpa5 is caused by a site gain or loss, no polymorphism should be observed in the HaeIII fragments that cover the same region as Hpa5. However, as shown in Fig. 2, Hae4 of type A (930 bp) was about 30 bp smaller than that of type B (960 bp). Thus, the Hpa5 and Hae4 fragments which overlapped each other on the cleavage maps were about 30 bp smaller in type A than in type B. This suggests that the length polymorphism of Hpa5 and Hae4 is probably caused by deletions or insertions of sequences of about 30 bp within the fragments.

Several HaeIII fragments showed similar length polymorphism to that of Hae4 (Fig. 2): Hae12 was about 20 bp larger in type A than in type B, and the mobilities of Hae13, 14 and 15 seemed to be slightly different in the two types. In contrast, a single site gain or loss can well explain the presence of one unique fragment in type A, Hae2 (2150 bp), and two unique fragments in type B, Hae2a (1450 bp) and Hae2b (750 bp) (Fig. 2).

The type A mtDNAs prepared from different individuals of the Sprague-Dawley, Fischer, and Wistar strains all had an Hpa5 fragment of 1540 bp, while the type B mtDNAs from all Sprague-Dawley and Wistar strain rats had an Hpa5 fragment of 1570 bp. Inheritance of length polymorphism of Hpa5 was maternal and mtDNAs of all organs tested (liver, thymus, kidney, testis, and brain) of individual rats had the same length of Hpa5 fragment. Strictly maternal inheritance of site variations of types A and B is known (20). Thus, the length polymorphism was always linked to the site variations of types A and B. In preliminary experiments, we found that the Hpa5 of type C mtDNAs (6) prepared from two individual wild rats trapped in Japan (Gotenba and Sendai) were

different from each other but similar to those of Hpa5 of type A and type B, respectively.

DISCUSSION

Previously (6) we found at least four types of mtDNA (types A-D) in rats (*Rattus norvegicus*) that differed in their cleavage patterns with EcoRI, HindII, and HaeIII. The differences in these types of mtDNAs were well explained by cleavage site gains or losses (6). Based on these site variations, the sequence divergence between types A and B was calculated to be about 1% (21). In contrast, the present results suggested that the variations observed in the HpaII cleavage patterns of types A and B cannot be explained by HpaII site gain or loss, but are probably due to insertion or deletion of about 30-bp sequences within the fragments.

Similar length polymorphism has been observed in HaeIII fragments. Since HaeIII cuts rat mtDNA into many small fragments, small length variations of about 5-20 bp can easily be identified; in addition to Hae4, several HaeIII fragments such as Hael2 and 13 differed slightly in mobility in types A and B. Thus, the occurrence of insertion or deletion of sequences in rat mtDNAs may not be restricted to the Hpa5 fragment.

Using heteroduplex analysis between sheep and goat mtDNAs, Upholt and Dawid (2) have observed duplicated regions close to the origin of replication. Reilly and Thomas (11) found intraspecific length polymorphism in restriction fragments of *Drosophila melanogaster* mtDNAs, the fragments including the AT-rich region that contains the replication origin. In contrast, the rat Hpa5 fragment is separated from the replication origin (D-loop region) by about 400 bp and contains parts of ribosomal RNA genes (22). The Hpa9 that includes the replication origin did not differ in size in the two types of rat mtDNA (Fig. 1).

Only very limited regions of the nucleotide sequences of rat types A and B mtDNA are known; de Vos et al. (10) determined the sequences of the smallest restriction fragment of HindIII, named "F", of both types. Three point mutations

were found, but no sequence was deleted or inserted. However, this fragment is very small (about 150 bp) (22), and its position in the cleavage map does not overlap that of Hpa5 or Hae4 (19, 22). Thus, determination and comparison of the Hpa5 sequences of types A and B is necessary for precise location of the region of deletion or insertion and for determination of the characteristics of the sequence. This will also provide useful information on the problem of how animal mtDNAs have evolved.

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