THE OCCURRENCE OF TWO TYPES OF MITOCHONDRIAL DNA IN RAT POPULATIONS AS DETECTED BY *Eco*RI ENDONUCLEASE ANALYSIS

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1. Introduction

Recently, a restriction enzyme study of the mitochondrial DNA (mtDNA) of a number of mammalian species showed that more than one type of mtDNA can occur in a single species [1]. While twelve diverse species, including the rat, showed only a single type of mtDNA when examined with *Hae*III endonuclease, each of the ten horses studied yielded one of five different patterns, and each human source yielded a distinct pattern. Thus, these results and the results of two more recent studies, in which several restriction endonucleases, including *Eco*RI, were used, have not indicated the occurrence of more than one type of mtDNA in the rat population [2,3].

Our investigations into the origins of '8 S' mtDNA [4,5] led to the *Eco*RI analysis of mtDNA obtained from the pooled livers of Sprague Dawley rats. The gel patterns obtained suggested the presence of two full size mtDNA circles. Extension of these studies to individual livers and to two additional rat strains showed that, while the individual rat liver appears to contain a single type of mtDNA, there occur two types of mtDNA both of which are distributed throughout each rat strain examined.

2. Materials and methods

All rats were male and weighed between 150 g and 250 g. They were of the species *Rattus norvegicus*, and consisted of the Sprague Dawley, Long Evans Hooded, and Wistar strains. They were purchased from: Charles River Laboratories (CR), North Wilmington, Mass. 04601; Taconic Farms (TF), Germantown, NY 12526; Microbiological Associates (MA), Walkersville, Md., 21793; Maryland Breeding Farms (MBF), Hewitt, NJ 07421. Special contact was made with Charles River Laboratories and Taconic Farms to insure against accidental mixing of strains. *Eco*RI and *Hin*dIII endonucleases were obtained from Miles Laboratories, Elkhart, Indiana, 46514. DNA markers were gifts of Dr Eiichi Ohtsubo.

Intact mitochondria were isolated under conditions of minimal bacterial contamination and the bulk DNA was extracted as described previously [5]. After purification (see legends) the DNA was subjected to exhaustive digestion with endonuclease *Eco*RI or *Hin*dIII [6,7]. The digests were electrophoresed on 0.7% agarose gels in a vertical slab-gel apparatus [8] by a slight modification [9] of the procedure of Mickel and Bauer [10]. DNA bands were visualized by ethidium bromide staining [11].

Fragment sizes were determined by employing *Eco*RI digests of phage λ DNA (wild type) [12] and F factor plasmid DNA (wild type) [13] as markers.

3. Results

Our early experiments were done on mtDNA from twenty pooled livers obtained from Sprague Dawley rats. The *Eco*RI pattern (fig.1) shows eight clearly visible bands (fig.1, Channel 2). Four of these (bands 2, 3, 6, 7) are present in equimolar ratios. Each of two bands (bands 4 and 8) is present at 1.57 times this ratio and two (bands 1 and 5) at 0.57 times the

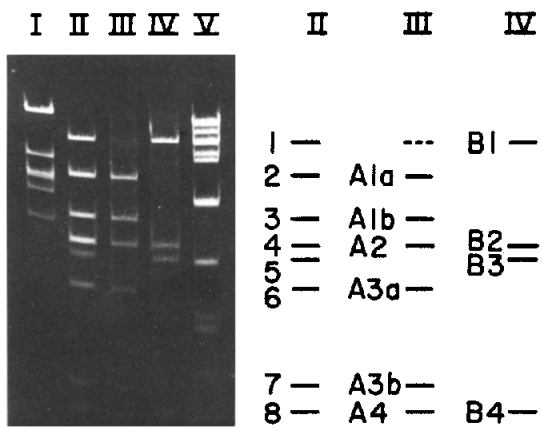

Fig.1. Agarose gel electrophoresis of a complete *Eco*RI endonuclease digest of mtDNA from the livers of Sprague Dawley rats. For the pooled liver experiments (Channel II), the extracted mtDNA from 20 livers was purified by sucrose density-gradient centrifugation [5] followed by CsCl isopycnic centrifugation as described previously [14]. In the individual animal experiments, the latter step was omitted. A diagrammatic representation of the gel patterns is shown to the right of the gel photograph since the fainter bands may not reproduce clearly in half tone. The dashed line in Channel III represents a 'partial' digest fragment (see text). For preparation of marker fragments, see text. Channel I, marker fragments from λ DNA (wild type); Channel II, mtDNA from pooled livers; Channel III, type A mtDNA from an individual liver; Channel IV, type B mtDNA from an individual liver; Channel V, marker fragments from F factor DNA.

equimolar ratio. When the sizes of all the bands are totalled (counting bands 4 and 8 twice), a value of 31 kilobases (kbases) is obtained (table 1). This figure corresponds to two complete molecules, types A and B, of 15.5 kbases each, with 1.7 molecules of A present for every molecule of B.

Several controls were run in the above experiment. First, treatment of phage λ DNA under identical conditions yielded a band pattern in complete accord with published data [12]. Second, additional enzyme was added after one hour and the incubation continued for an additional hour, with no difference found in the gel patterns. These results indicate both that the reaction conditions led to complete digestion and that nuclease activity was absent from the enzyme preparation. Third, further purification of the mtDNA by addition of a CsCl–ethidium bromide centrifugation step did not alter the gel patterns.

Table 1
Fragment sizes of products of complete *Eco*RI endonuclease digestion of types A and B rat liver mtDNA

Type A DNA		Type B DNA	
Fragment	Size (kbases)	Fragment	Size (kbases)
A1a (2)	6.03	B1 (1)	9.79
A1b (3)	3.76	B2 (4)	2.89
A2 (4)	2.88	B3 (5)	2.45
A3a (6)	1.77	B4 (8)	0.41
A3b (7)	0.67		
A4 (8)	0.42		

Numbers in parenthesis refer to the nomenclature used for the fragments in the pooled liver experiment (fig.1, Channel II).

The results of the pooled sample experiment might be explained in either or both of two ways: (a) two types of mtDNA occur in an individual animal or (b) a single type of mtDNA occurs in an individual animal but two types occur in the strain. To confirm the presence of the two types of DNA and to distinguish between these possibilities, we examined the liver mtDNAs of a number of individual Sprague Dawley rats obtained from a single supplier (CR). The two *Eco*RI patterns of types A and B mtDNA, each type obtained from an individual rat, are shown in fig.1, Channels III and IV, respectively. The results confirm the occurrence of two types of DNA and, in addition, clearly support possibility (b). Moreover, these results are in accord with the results of the pooled sample runs in fig.1, Channel II, recalling that bands 4 and 8 were counted twice. Calculation of the sizes of the two DNA types shows them to be 15.5 kbases each, in excellent agreement with the values for the complete mtDNA circle [15].

These results raised the additional question of whether every strain of rat possesses its own distinct types of mtDNA. Thus, individual animals of two additional strains were examined and rats of each of the three strains used were procured from two different suppliers. The results showed that two types of liver mtDNA occur in both the Long Evans (fig.2) and the Wistar strains (data not shown) and that these DNA types yield *Eco*RI patterns identical to those seen for Sprague Dawley rats. Moreover, the patterns obtained appear to be independent of the commercial source of the rats (table 2); Sprague Dawley rats from

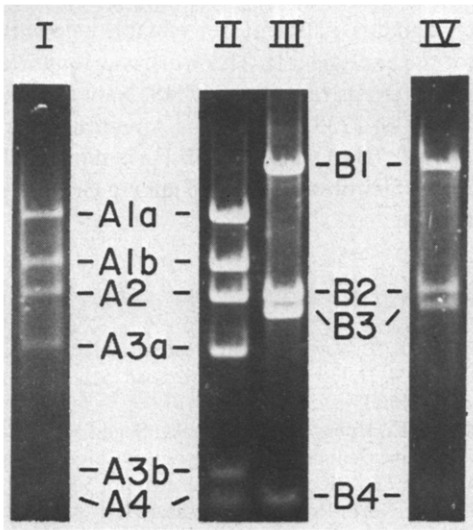

Fig.2. Agarose gel electrophoresis of a complete *Eco*RI endonuclease digest of mtDNA from the livers of Long-Evans Hooded rats. Conditions of the experiment are described in the legend to fig.1 except that, for direct comparison, the marker fragments used were an *Eco*RI digest of either type A or type B liver mtDNA obtained from Sprague Dawley rats. Faint bands may not appear on half tone reproduction. Channel I, Sprague Dawley type A marker fragments; Channel II, Long Evans Hooded type A mtDNA; Channel III, Long Evans Hooded type B mtDNA; Channel IV, Sprague Dawley type B marker fragments.

both CR and TF, Wistar rats from MA, and Long Evans rats from M, all possess the two DNA types. A possible exception is the result of the examination of the nine Wistar rats from CR which showed the occurrence of only the B type of DNA but more Wistar animals from this source need to be surveyed. (Also, only one Long Evans rat from CR was examined.)

Of the 60 individual rats which were examined, 65% were type A (table 2). If we include the inferred results from the pooled samples, a total of 80 rats were examined, 62% being type A. However, a sufficient number of rats has not yet been examined to assess the statistical significance of this figure or to determine whether the ratio of the two DNA forms differs in different rat strains.

It should be mentioned here that an additional band, very faint and fast moving, is sometimes, detected in the *Eco*RI mtDNA digests. It is present in both types of DNA and its size is less than 0.3 kbases. It is not the 7 S fragment from D-loop DNA as demonstrated by control gel runs of this fragment. This band has thus far not been reported in studies on the physical mapping of rat liver mtDNA [2,3] and is under further study.

4. Discussion

The results of these experiments show that two types of liver mtDNA, which we term types A and B, occur in each of the three strains of *Rattus norvegicus* which we have examined. Type A, as determined by *Eco*RI analysis, has been reported [2,3]. We report here the existence of a second type, type B, which we find to be present in the rat population with surprisingly high frequency. Of the 60 individual rats examined, however, in no case was a single animal found to have more than one type of liver mtDNA.

Table 2
The occurrence of types A and B DNA in the liver mitochondria of different rat populations

Rat strain	Commercial source	Number of rats possessing	
		Type A DNA	Type B DNA
Sprague Dawley	CR	26	4
	TF	6	2
Wistar	CR	0	9
	MA	3	3
Long Evans Hooded	CR	0	1
	MBF	4	2

Analysis of rat liver mtDNA by renaturation kinetics showed only a single species of DNA [16]. Such results could have been obtained had only a single DNA type been present in the sample examined owing to the small number of rats which were used. Additionally, if the two DNA types differ only slightly in primary structure, the sensitivity of this technique would be insufficient to detect this. That the differences are probably small is suggested by *Hin*dIII analysis which showed the two fragment patterns to be identical. Indeed, the minimum difference in the *Eco*RI fragment patterns between type A and type B DNA could be two *Eco*RI-specific sites. Both Kroon [3] and Koike [2] have shown that fragments Ala and Alb are adjacent on the physical map as are A3a and A3b. If the sizes of Ala and Alb and of A3a and A3b are summed, the two resultant fragments sizes would be equal to fragments B1 and B3 respectively. This is supported by the type A DNA 'partial' digest fragment seen in fig.1, channel III (dashed line) which comigrates with type B DNA, band B1. In any case, we are studying the question of whether the difference between the two DNA types is one of sequence, base modification, or both.

It was difficult to draw any generalized conclusion as to the extent of intraspecific mtDNA heterogeneity in mammals from the results of Potter et al. [1] in which only two of the fourteen species examined showed more than one type of mtDNA. However, the results reported here show that two types of mtDNA exist in the rat population and recent findings by Upholt and Dawid [17] demonstrate such intraspecific heterogeneity in goats and sheep. These results in combination suggest that intraspecific heterogeneity of mtDNA in mammals is a widespread phenomenon.

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