Unusual type of mitochondrial DNA in mice lacking a maternally transmitted antigen

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ABSTRACT

Mice that lack a maternally transmitted antigen (Mta) on the cell surface share a distinctive type of mitochondrial DNA. This is evident from restriction analyses of mitochondrial DNAs from 25 strains of mice whose antigenic state is known. One hundred sixty-eight cleavage sites have been mapped in the mitochondrial DNA of Mta$^-$ mice. Detailed maps for the 8 other types of mitochondrial DNA detected in the survey have also been prepared. The Mta$^-$ mice are estimated to differ from those expressing the antigen by 108 to 141 base substitutions at widely scattered points in the mitochondrial genome.

INTRODUCTION

Most strains of laboratory mice, Mus domesticus (1), bear a cell-surface antigen, Mta, that is transmitted maternally and noninfectiously (7,8). Since mitochondrial DNA (mtDNA) is also known to be transmitted maternally and non-infectiously in mammals (9), it might carry genetic information specifying the presence or absence of Mta. We have therefore examined mtDNA from various laboratory mice to find out whether those few strains lacking the maternally transmitted antigen have a unique type of mtDNA.

Restriction analysis has shown that in the wild there is a high level of mtDNA polymorphism among mice of the species Mus domesticus (5,6). By contrast, all the "old inbred" strains (10) tested have the same mtDNA map (5,11); they differ in this respect not only from most wild members of the species but also from several of the newly established strains of inbred mice (5,6). The present paper reports detailed restriction maps for the mtDNAs of 25 strains of mice whose antigenic state has also been determined. This survey reveals a strict association between mtDNA type and the state of the maternally transmitted antigen.

MATERIALS AND METHODS

Mice. The NZB/Füll strain has been described previously (12). Breeding pairs of NZO were provided by Dr. L. Herberg, Diabetes Forschungs-Institut,

Düsseldorf. Inbred NMRI mice obtained directly from the Naval Medical Research Institute at Bethesda are termed NMRI/Navy in this paper. They differ at several loci from the NMRI/Lac inbred strain, which was supplied by Dr. F. Lehmann-Grube, Heinrich-Pette Institut, Hamburg. The sources of the nine old inbred strains and the Hov mice are as described in Ferris et al. (5,6). NZB/BINJ, RIIIS/J, IS/Cam/J, and SF/Cam/J were obtained from the Jackson Lab, Bar Harbor. Milano II (Mil II), Cittaducale (CD), and Zadar were provided by A. Gropp, Medizinische Hochschule, Lübeck. Peru mice were obtained from two sources: the Peru-Atteck mice tested for Mta came from the Jackson Lab, Bar Harbor; the Peru-Coppock mice, whose mitochondria were examined, originate from the same farm in Peru as the Peru-Atteck mice (13) and were supplied by M. E. Wallace, Cambridge University. Other mice (NZB/Bom, NMRI/Bom, and NZW/Ola) were purchased from commercial breeders.

Analysis of MtDNA. One or two mice were sacrificed per strain and mtDNA was highly purified as described elsewhere (5,14). MtDNAs were digested with 11 different restriction enzymes (New England Biolabs), electrophoresed in agarose or polyacrylamide gels after labeling the fragments with ^{32}P (15), and autoradiographed. Estimates of fragment sizes were made by comparing them to the known sizes of the fragments of old inbred mtDNA. Cleavage maps were then constructed by the sequence comparison method (16). The sites required to account for each fragment pattern are mapped by comparison with the known base sequence from an old inbred mouse (17). Site gains are mapped by searching for potential sites in the relevant region, assuming an error of $\pm$50 bp for fragments less than 500 bp and $\pm$100 bp for fragments greater than 500 bp; in three instances where a single potential site could not be chosen by this criterion, the greater likelihood of transitions and, in protein-coding genes, of silent changes (18) was also considered. Site losses are located unambiguously with respect to the old inbred map.

Mta Typing. At least two mice of each strain were typed for expression of Mta by a killer cell assay as described (8,12).

RESULTS

Table 1 summarizes the fragment patterns produced by 11 restriction enzymes for the mtDNAs of 20 laboratory strains of known antigenic state. Five types of mtDNA are evident, one of which, type I, is diagnostic for mice lacking Mta. Type I has been found before only in wild mice at a frequency of 0.08 (5), and their antigenic state is unknown.

Five additional mouse strains, namely Mil II, Zadar, Peru, Hov, and CD,

Table 1. Fragment Patterns for MtDNA from 20 Strains* of Mice

Mouse strain and mtDNA type	Fragment Pattern†										
	Xba I	Hpa II	Ava II	Hinc II	Acc I	Hae III	FnuD II	Hind III	Taq I	Mbo I	Hinf I
Antigen absent											
Type I											
NZB/Bom	A	A	A	A	A	A	B	B	D	C	H
NZB/BINJ	A	A	A	A	A	A	B	B	D	C	H
NZO/Dus	A	A	A	A	A	A	B	B	D	C	H
NMRI/Bom	A	A	A	A	A	A	B	B	D	C	H
Antigen present											
Type II											
SF/Cam/J	A	A	C	B	A	E	A	B	H	J	N
Type III											
IS/Cam/J	A	A	A	A	D	C	A	B	D	F	M
Type IV											
NMRI/Navy	A	A	A	A	C	A	A	A	A	A	A
Type V											
NZW/Ola	A	A	A	A	A	A	A	A	A	A	A
NZB/Füll	A	A	A	A	A	A	A	A	A	A	A
NMRI/Lac	A	A	A	A	A	A	A	A	A	A	A
Old inbreds‡	A	A	A	A	A	A	A	A	A	A	A
RIIIS/J	A	A	A	A	A	A	A	A	A	A	A

*The mtDNAs of five additional strains, all of them Mta^+, were examined with the two most discriminating enzymes, MboI and HinfI. This revealed 4 additional types of mtDNA as follows: VI (Mil II, YV), VII (Zadar, AC), VIII (Peru, RC), and IX (Hov and CD, IL). The two letters given in parentheses after the strain name refer to the fragment patterns obtained with MboI (first letter) and HinfI (second letter).

†Each fragment pattern is given a capital letter, the letter A being reserved for the pattern appearing in old inbred mice.

‡The nine strains examined by Ferris et al. (5).

all of them Mta^+, were subjected to a less thorough mtDNA examination. They were tested with the two most discriminating enzymes, MboI and HinfI, which sufficed to establish that these mtDNAs differ from the five mtDNA types found in the more detailed survey of the first 20 strains. Since Hov and CD were

Table 2. Locations of 30 Variable Restriction Sites in Nine Types of Mouse MtDNA

Site	Enzyme	Location of Site			Presence of Site*								
		Start of Site	Base Change	Functional Region	I	II	III	IV	V	VI	VII	VIII	IX
1	HinfI	232	232-3 or 235-6	12S RNA	+	+	+	+	+	+	+	+	–
2	HinfI	2999	2999, A - G	Urf 1	–	–	+	–	–	–	–	–	–
3	HinfI	3360	3360-1 or 3363-4	Urf 1	+	+	–	+	+	+	+	+	+
4	MboI	3597	3597-600	Urf 1	+	–	+	+	+	+	+	+	+
5	MboI	4045	4045, A - G	Urf 2	+	+	–	–	–	–	–	–	–
6	MboI	4065	4065-8	Urf 2	–	–	+	+	+	+	+	+	+
7	MboI	4187	4188, G - A	Urf 2	–	–	+	–	–	–	–	–	–
8†	HinfI	4275	4276, G - A	Urf 2	+	+	–	–	–	+	–	–	–
9†	MboI	4276	4276, G - A	Urf 2	–	–	–	+	+	–	+	+	+
10	MboI	5016	5019, A - C	$tRNA^{Ala}$	+	–	–	–	–	–	–	–	–
11	HaeIII	5900	5900, A - G	Co 1	–	–	+	–	–	n	n	n	n
12	MboI	5988	5988-91	Co 1	+	+	+	+	+	+	+	+	–
13	HinfI	7973	7973-4 or 7976-7	ATP 6	+	+	+	+	+	+	+	+	–
14	TaqI	8357	8358, T - C	ATP 6	+	–	+	–	–	n	n	n	n
15	HincII	8637	8642, T - C	Co 3	–	+	–	–	–	n	n	n	n
16	HindIII	9136	9136-41	Co 3	–	–	–	+	+	n	n	n	n
17	HinfI	9526	9526-7 or 9529-30	Urf 3	–	–	–	+	+	–	–	–	–
18‡	HinfI	9574	9577-8	Urf 3	–	–	–	+	+	–	–	–	–
19‡	TaqI	9577	9577-8	Urf 3	–	–	–	+	+	n	n	n	n
20	FnudII	9595	9596, A - G	Urf 3	+	–	–	–	–	n	n	n	n
21	MboI	9793	9795, A - T	Urf 3	–	–	+	–	–	–	–	–	–
22	MboI	12028	12028-31	Urf 5	+	–	+	+	+	+	+	+	+
23	AvaII	12515	12515-9	Urf 5	+	–	+	+	+	n	n	n	n
24	HinfI	12520	12520-1 or 12523-4	Urf 5	+	–	+	+	+	+	+	+	–
25	HinfI	13225	13225-6 or 13228-9	Urf 5	+	–	+	+	+	–	+	+	–
26	MboI	13400	13400-3	Urf 5	+	–	+	+	+	–	+	–	–
27	HaeIII	13479	13482, T - C	Urf 5	–	+	–	–	–	n	n	n	n
28	AccI	13856	13861, T - C	Urf 6	–	–	+	–	–	n	n	n	n
29	MboI	14239	14240, G - A	Cyt b	–	–	–	–	–	–	–	–	+
30	AccI	15924	15925, C - T	D loop	–	–	+	+	–	n	n	n	n

*n means not scored.

†To explain the variation at sites 8 and 9, which overlap, we postulate two mutations. Mutation of G to A at position 4276 in the MboI site (GATC) of the old inbred type would eliminate this site and form a HinfI site (GAATC), thereby explaining the differences between mtDNA types I, II, and IV-IX. A second mutation could account for the absence of either site in type III.

‡A single mutation at 9577 or 9578 can account for the simultaneous presence or absence of both the HinfI and TaqI sites.

alike, the net result of the fragment survey is that nine types of mtDNA were found, one of which is unique to Mta^- mice (19).

Cleavage maps were made from the fragment patterns by relating them to the known base sequence of the old inbred type (V) of mtDNA. The nine maps appear to differ from one another only by the presence and absence of certain cleavage sites, which implies that all of the differences may be due to base

Table 3. Matrix of Mutational Differences among Five Types of Mouse MtDNA

		I	II	III	IV	V
I	NZB etc.	–	11	12	10	9
II	SF/Cam	133	–	19	15	14
III	IS/Cam	141	229	–	11	12
IV	NMRI/Navy	116	177	128	–	1
V	Old inbred	108	169	137	8	–

Upper right: Number of site differences taken from Table 2. The mouse strains are designated as in Fig. 1 and Table 1.
Lower left: Conservative estimate of the number of point-mutational differences per genome, calculated with equation 16 of Nei and Li (21) and a genome size of 16295 bp (17). This method of calculation assumes that there is heterogeneity among restriction sites as regards the probability of base substitutions.

substitutions. We can exclude large length mutations but not the possibility that these mtDNAs differ by small length mutations (i.e., less than 20 bp long) in addition to the base substitutions. Table 2 lists the variable sites, their locations in the genome, and those mtDNA types in which the sites are present; it does not include the constant sites, since they can be inferred from the published base sequence for the old inbred type (17).

Twenty of the 30 variable sites (Table 2) lie in five unidentified reading frames (Urfs 1, 2, 3, 5, and 6) though these regions account for only 30% of the length of mouse mtDNA. For many of the variable sites it is possible to infer the nature of the base substitution that produced the site change. Twelve of the 14 inferred substitutions are transitions, consistent with the transitional bias detected by sequencing cloned mtDNA fragments from rats and primates (18,20). 83% of the inferred substitutions in protein-coding regions are silent, as compared with 78% for closely related primate mtDNAs (18).

Quantitative comparisons were made for mtDNA types I through V, i.e., those mapped for all 11 enzymes. The number of site differences between each pair of maps ranges from one to 19, as shown in Table 3 (upper right). From these numbers, the number of sites compared, and the known length of mouse mtDNA (17), we estimated the approximate number of point mutational differences for each pair of mitochondrial genomes by equation 16 of Nei and Li (21). The mice lacking the antigen consistently differ from those expressing the antigen by more than 100 base substitutions in mtDNA (Table 3, lower left).

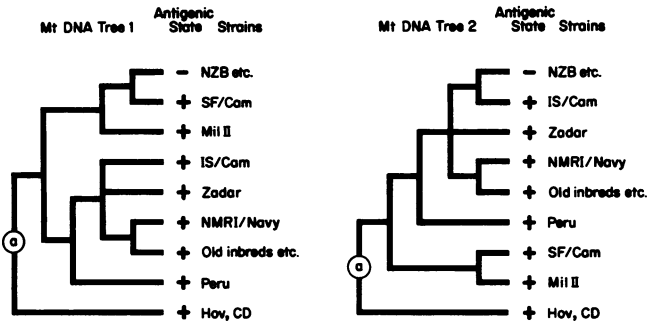

Fig. 1. Genealogical relationships among nine maternal lineages, inferred from mtDNA comparisons of 25 mouse strains, all of which have been tested for the presence (+) or absence (-) of a maternally transmitted antigen. The trees were built by the parsimony method (22) from restriction maps whose variable sites are listed in Table 2. This method, it should be emphasized, does not assume that the rate of point-mutational evolution is constant. The trees show the order of branching of lineages leading from a common ancestral mtDNA, designated a, to the nine types of mtDNA found in the survey of 25 mouse strains; both trees require the same number of events (Table 4). In each tree the three-way split represents a consensus of three equally parsimonious, bifurcating counterparts. The position of the Hov, CD lineage was assigned in accordance with Ref. 6. The mouse strains having the same mtDNA types as NZB and the old inbreds are listed in Table 1.

Tree analysis was conducted to find out how the nine maps might be related to one another genealogically. The parsimony method (22) produced the trees in Fig. 1, which shows two possible orders of branching of lineages leading from a common ancestral mtDNA, designated a, to the nine maps found in the survey of 25 mouse strains. No other branching order allows the nine mtDNA maps to evolve from a with fewer mutations than do those in Fig. 1 (see Table 4). The closest relatives of type I are types II, III, and VI, found in the SF/Cam, IS/Cam, and Mil II strains.

By considering the results in Table 2 in relation to the trees (Fig. 1), one infers that a minimum of two mutations occurred in the lineage leading to type I mtDNA. One, attributed to a substitution at site 10, alters the structure of alanine transfer RNA. The other change, at site 20, is apparently due to a silent substitution in Urf3, a gene coding for an unidentified protein (17).

DISCUSSION

The association between the state of the maternally transmitted antigen and a particular type of mtDNA, which we have observed in the comparison of

Table 4. Phylogenetically Informative Variation at 11 Locations in Mouse MtDNA

Site	Mutations per Tree		
	1	2	3
5	1	2	2
6	1	2	2
8-9	2	3	4
14	2	1	2
16	1	1	2
17	1	1	2
18-19	1	1	2
24	2	2	2
25	2	1	3
26	2	1	4
30	2	2	2
Total	17	17	27

Sites are listed as in Table 2. Trees 1 and 2 are shown in Fig. 1. Altogether, 36 trees were examined. Tree 3, which is the least parsimonious, is unstructured, meaning that all nine lineages diverged simultaneously from a common ancestor.

25 mouse strains, is consistent with the hypothesis that mtDNA carries genetic information affecting the structure or expression of this antigen. Our evidence does not, however, validate this hypothesis. The association could merely be a coincidence, especially if both mtDNA and the antigen are inherited strictly maternally.

The inheritance of mtDNA is known to be predominantly maternal in mammals (9,23) but the genetic tests conducted do not prove that it is strictly maternal. There are hints that mtDNA's genetic behavior is sometimes anomalous both in the laboratory and in the wild. Certain laboratory strains that were established within the past 30 years have recently acquired the old inbred type of mtDNA, while apparently retaining their original nuclear heritage (5). Similarly, wild M. musculus mice in Scandinavia possess the mtDNA of another species, M. domesticus (6). The genetic behavior of mtDNA in cattle also requires clarification (23).

This ability of mtDNA to turn up in unexpected places, apparently independent of nuclear DNA (5,6), should be borne in mind as we recall the evi-

dence that (a) not all the substrains of NZB have type I mtDNA, even though all of them and the NZO strain are thought to stem from a single female (24); (b) one of three NMRI substrains has type I mtDNA. The possibility of genetic contamination must be considered in these two cases. The non-inbred European NMRI mice (e.g., NMRI/Bom) differ at the H-2 complex from NMRI/Navy and originate from a stock imported in 1955 together with other albino mice to the Bundesanstalt für Viruskrankheiten der Tiere in Tübingen (25). The Zentralinstitut für Versuchstiere in Hannover obtained NMRI mice from there between 1959 and 1961 and later supplied Danish and French breeders (H. J. Hedrich, personal communication). We have been unable to find any direct evidence that a black NZB mouse contributed mtDNA to these albino Mta^- mice. The observed cotransmission of both type I mtDNA and the Mta^- state in these two possible cases of contamination could be viewed as strengthening the notion that mtDNA codes for Mta.

An apparent exception to the association between Mta and mtDNA types could be inferred from a report by Yonekawa et al. (11). At the 22 cleavage sites examined, the mtDNA of the NZC/Fgu strain resembled that of authentic NZB (and the related mtDNA types, II, III, and VI). The antigenic state of the NZC/Fgu mice would therefore be expected to be Mta^-. However, we have typed NZC mice from Otago University as Mta^+ (8). More recently, we have examined the WEHI 279 cell line, which comes from an irradiated NZC mouse. This line was Mta^+, and the mtDNA fragments obtained after digestion with BamHI and HindIII were those of standard inbred strains (M. Hirama, personal communication). Unfortunately, the NZC/Fgu mice cannot be tested for Mta because the substrain is now extinct. Most likely, substrains of NZC differ in their mtDNA (and Mta?) types just as NZB and NMRI substrains do.

Finally, we consider the possible time of origin of the Mta^- state. From the observations that point mutations accumulate at rather steady rates over evolutionary time (26) and that in mammalian mtDNA the average rate of sequence change is about 2 percent per million years (14,18,27), one estimates (from Table 3, lower left) that the type I mitochondrial lineage (leading to NZB in Fig. 1) could be hundreds of thousands of years old. Because the lack of Mta is peculiar to this mitochondrial lineage, the Mta^- state could have arisen this long ago or as recently as the formation of the NZO and NZB strains in 1948 (24). To narrow down the time at which this antigenic state was lost, it will be necessary to extend the antigenic survey to include mice whose mtDNAs are more closely related to type I than are the mtDNAs of SF/Cam, Mil II, or IS/Cam.

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