Mitochondrial Genotype Segregation in a Mouse Heteroplasmic Lineage Produced by Embryonic Karyoplast Transplantation

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ABSTRACT

Mitochondrial genotypes have been shown to segregate both rapidly and slowly when transmitted to consecutive generations in mammals. Our objective was to develop an animal model to analyze the patterns of mammalian mitochondrial DNA (mtDNA) segregation and transmission in an intraspecific heteroplasmic maternal lineage to investigate the mechanisms controlling these phenomena. Heteroplasmic progeny were obtained from reconstructed blastocysts derived by transplantation of pronuclear-stage karyoplasts to enucleated zygotes with different mtDNA. Although the reconstructed zygotes contained on average 19% mtDNA of karyoplast origin, most progeny contained fewer mtDNA of karyoplast origin and produced exclusively homoplasmic first generation progeny. However, one founder heteroplasmic adult female had elevated tissue heteroplasmy levels, varying from 6% (lung) to 69% (heart), indicating that stringent replicative segregation had occurred during mitotic divisions. First generation progeny from the above female were all heteroplasmic, indicating that, despite a meiotic segregation, they were derived from heteroplasmic founder oocytes. Some second and third generation progeny contained exclusively New Zealand Black/BINJ mtDNA, suggesting, but not confirming, an origin from an homoplasmic oocyte. Moreover, several third to fifth generation individuals maintained mtDNA from both mouse strains, indicating a slow or persistent segregation pattern characterized by diminished tissue and litter variability beyond second generation progeny. Therefore, although some initial lineages appear to segregate rapidly to homoplasmy, within two generations other lineages transmit stable amounts of both mtDNA molecules, supporting a mechanism where mitochondria of different origin may fuse, leading to persistent intraorganellar heteroplasmy.

INFORMATION on the patterns of mitochondrial segregation during development in mammals is limited and somewhat conflicting. On one hand are the studies on the inheritance patterns of mitochondrial DNA (mtDNA) in cattle maternal lineages, showing a very rapid alteration in a restriction site polymorphism within only one or a few generations (HAUSWIRTH and LAIPIS 1982; ASHLEY *et al.* 1989; KOEHLER *et al.* 1991). Rapid switch in mtDNA patterns has been attributed to a phenomenon known as mitochondrial meiosis, as several mtDNA molecules per mitochondrion in most somatic cells are reduced to a single mtDNA molecule during the reduction in size of the oocyte mitochondria. The presence of a single genome within a mitochondrion enables the organelle itself to be the unit of segregation of mtDNA. Moreover, mitotic divisions during embryogenesis up to the establishment of germ cells enables stringent segregation of mitochondria with the same genome allowing for fast fixation of mtDNA polymorphisms in the following generation (HAUSWIRTH and LAIPIS 1985; LAIPIS *et al.* 1988; KOEHLER *et al.* 1991). In contrast to the above findings are studies of mtDNA polymorphism in human maternal lineages showing much slower segregation patterns, in many cases with persistent heteroplasmy. Lethality of the homoplasmic mutant organelle together with replicative advantage of deleted mtDNA molecules could lead to positive selection favoring heteroplasmy in human lineages causing deleterious diseases (HOLT *et al.* 1990; LOTT *et al.* 1990; SHOFFNER *et al.* 1990; HARDING *et al.* 1992). However, persistent homoplasmy occurs also in lineages showing silent point mutations and mutations leading to only mild clinical symptoms (VILKKI *et al.* 1990; HOWELL *et al.* 1992; LARSSON *et al.* 1992). Moreover, heteroplasmic profiles of molecules with various length of a homopolymeric tract of cytosines in the control region of human mtDNA have shown that, while unrelated individuals have different proportion of length variants, all maternally related individuals studied have very similar proportions of polymorphic mtDNA molecules (BENDALL and SYKES 1995). Although possible mechanisms have been suggested, it is yet unclear how identical heteroplasmic profiles are stably maintained in maternally related individuals.

A common model used to investigate mitochondrial replicative segregation in somatic tissues is to analyze

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changes in mtDNA ratios in heteroplasmic cell lines during mitotic divisions *in vitro* (HUSTON *et al.* 1985; YONEDA *et al.* 1994; MATTHEWS *et al.* 1995). Although some studies show rapid loss of the heteroplasmic state within a few generations, others have shown established cell lines with stable levels of mtDNA heteroplasmy. These *in vitro* studies have originated several hypotheses on the mechanisms controlling mitochondrial segregation *in vivo*. We hereby describe an *in vivo* model to study mitochondrial segregation, in which we employ a founder heteroplasmic female derived from a zygote reconstructed using the technique of karyoplast transplantation (MCGRATH and SOLTER 1983). Analysis of heteroplasmy levels of the progeny over five generations has identified rapid and slow patterns of heteroplasmy loss similar to those observed in bovine and human lineages. Moreover, we describe a gradual change in the variability of patterns of mtDNA heteroplasmy in which homogeneity increases initially in tissues within animals in second generation progeny, followed by higher homogeneity within litters in the following generations.

MATERIALS AND METHODS

Source of mice and zygotes with different mtDNA background: Zygotes containing mtDNA of *Mus musculus domesticus* (C57BL/6) origin were obtained from F_1 females derived from a cross between males of the C3H strain and C57BL/6 females (B6C3F1; Charles River Canada Inc., St-Constant, PQ, Canada). Embryos containing mtDNA of New Zealand Black (NZB) origin were obtained from females from a NZB/BINJ backcross line derived by mating founder pure NZB/BINJ strain females (Jackson Laboratories, Bar Harbor, ME) to C57BL/6 (Charles River Inc.) males and mating the female progeny to C57BL/6 males for four generations. The restriction pattern length polymorphism (RFLP) pattern of the NZB/BINJ mtDNA differs from C57BL/6 mtDNA (YONEKAWA *et al.* 1982). Animals from NZB/BINJ backcross line contained ~94% of their nuclear genome of C57BL/6 origin and, due to predominant maternal inheritance of mitochondria (GYLLENSTEN *et al.* 1985, 1991), mtDNA of NZB/BINJ origin.

Females averaging 4–7 weeks of age were superovulated by intraperitoneal injection of 5 i.u. of pregnant mare's serum gonadotrophin (PMSG) (Folligon; Ayerst, Montreal, PQ, Canada) and 5 i.u. of human chorionic gonadotrophin (hCG; Ayerst) given 44–48 hr apart. After hCG injection, females were paired with C57BL/6 males and inspected the following morning for copulation plug. Pronuclear stage embryos were flushed at 24 hr post hCG from the oviducts using a modified Hepes buffered CZB medium (CHATOT *et al.* 1989). Embryos were cultured in 40-μl droplets of glucose free, bicarbonate-buffered CZB medium under paraffin oil at 38° in a humidified atmosphere of 5% CO_2 in air until microsurgery.

Microsurgery, culture and embryo transfer: Embryos were placed in CZB medium with cytoskeleton inhibitors for microsurgery (1 μg cytochalasin D ml^{-1} and 0.3 μg nocadazole ml^{-1}; Sigma, St. Louis, MN). Karyoplast transplantations were carried out using a technique similar to that described previously (MCGRATH and SOLTER 1983; SMITH *et al.* 1988). Briefly, this method involves removing membrane-bound pronuclei and some cytoplasm (karyoplast) and its subsequent introduction into the perivitelline space of another embryo from which a karyoplast was previously removed. The karyoplast was fused to the recipient zygote by electrofusion in a 0.3 M Mannitol solution employing a Electro Cell Manipulator 200 (BTX, San Diego, CA) using a 1 kV cm^{-1} pulse for 70 μsec. Successfully reconstructed zygotes were cultured for 3.5 days to the blastocyst stage followed by transfer into the uterine horn of CD1 (Charles River) albino females mated to CD1 males. Female progeny derived from the reconstructed embryos were raised to puberty at which stage they were mated to C57BL/6 males to obtain first generation progeny.

Tissue mtDNA analysis: Genomic DNA was extracted from tissues obtained from animals derived from reconstructed zygotes and several matrilineal generation progeny thereafter. Occasionally entire newborn pups were analyzed to determine whole body mtDNA content. Briefly, tissue was chilled to 4° in homogenization buffer (0.32 M sucrose, 1 mM K-EDTA, 10 mM Tris-HCl pH 7.4), minced and homogenized with five strokes in a loose-fitting Potter-Elvejhem homogenizer followed by centrifugation at 1000 × *g* for 5 min. The supernatant was centrifuged at 13,000 × *g* for 20 min, and the pellet containing mitochondria was suspended in lysis buffer (0.02 M Tris HCl, 0.01 M EDTA, 1% SDS, 0.15 M NaCl and 0.01 M DTT, final pH 8.0) with gentle stirring. Proteinase K (100 μg/ml) was added, and lysate was incubated at 37° for 2–8 hr with occasional stirring. Purified DNA was recovered from the lysate using standard phenol:chloroform extraction and ethanol precipitation (SAMBROOK *et al.* 1989).

A mtDNA region consisting of a 1126-bp fragment encoding part of the ND1 gene, the entire isoleucine, glycine and methionine tRNA genes, and part of the ND2 gene (position 3401–4527; according to BIBB *et al.* 1981) was amplified by PCR from an initial 0.5 μg of DNA in 100 μl of reaction mixture. The reaction mixture consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 0.01% gelatin, 200 μM each dGTP, dATP, dTTP, and dCTP (dNTP; Pharmacia, Piscataway, NJ), 100 pmol of each oligonucleotyde primer, and 2.5 U of Taq polymerase (Pharmacia). PCR was performed for 30 cycles each consisting of denaturation for 45 sec at 94°, annealing for 55 sec at 50° and extension at 72° for 120 sec in a temperature cycler (TwinBlock EasyCycler, Ericomp, San Diego, CA). Oligonucleotide primer sequences were as follows: mtNZB.F2, 5′ CGGCCCATTCGCGTTATTC 3′ (forward) and mtNZB.R2, 5′ AGGTTGAGTAGAGTGAGGGA 3′ (reverse). With the exception of using 1.2 mM $MgCl_2$ in the reaction mixture and 40 cycles of PCR, the content mtDNA in reconstructed zygotes was assessed as described above.

Mitochondrial DNA from most *Mus musculus domesticus* strains, including C57BL/6, and NZB/BINJ can be differentiated by digestion with *Bam*HI (Promega, Madison, WI). The 1126-bp fragment contains one or two *Bam*HI restriction sites in NZB/BINJ and C57BL/6 mtDNA, respectively, producing a nonspecific 166-bp fragment and either 709- and 251-bp C57BL/6-specific fragments or a 960-bp NZB/BINJ-specific band (YONEKAWA *et al.* 1982). The amplification product of some tissues was also double-digested with *Bam*HI and *Hin*fI (New England Biolabs, Mississauga, ON, Canada). The ratio between C57BL/6 and NZB/BINJ mtDNA was determined by densitometry of the respective strain-specific restriction fragments and corrected for fragment size and heteroduplexes.

Cloning and sequencing of NZB/BINJ mtDNA fragments: Purified NZB/BINJ mtDNA obtained from liver was digested with *Bam*HI and ligated into the plasmid vector Bluescript (Promega). Clones were obtained containing a fragment spanning the region from nucleotide 3572 to 11068 (nucleotide position based on sequence by BIBB *et al.* 1981), and one of these, NZB9, was sequenced with the dideoxy chain termination method and T7 polymerase (Sequenase I, U.S. Biochemical) (SANGER *et al.* 1977). The sequencing template

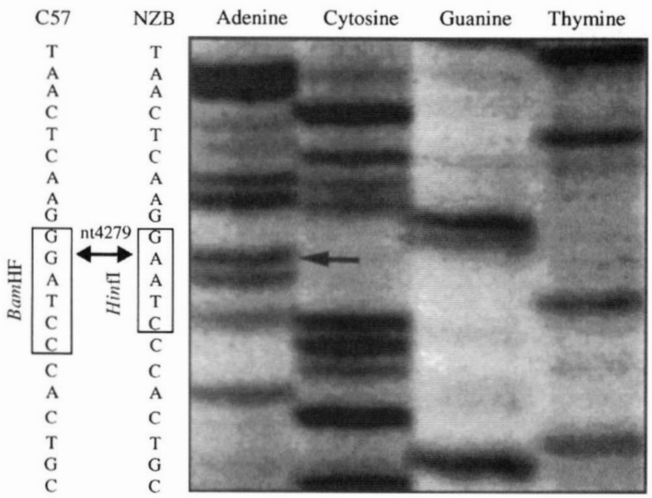

FIGURE 1.—Sequence autoradiogram showing the polymorphic site found in NZB/BINJ mtDNA used to determine NZB/BINJ and C57BL/6 mitochondrial content in tissues and zygotes. This guanine to adenine transition located at nucleotide 4279 (BIBB *et al.* 1981) causes loss of a *Bam*HI site and gain of a *Hin*fI site by NZB/BINJ mtDNA, enabling double digestions to determine precise heteroplasmic ratios without interference of artifactual heteroduplex structures formed during PCR reactions.

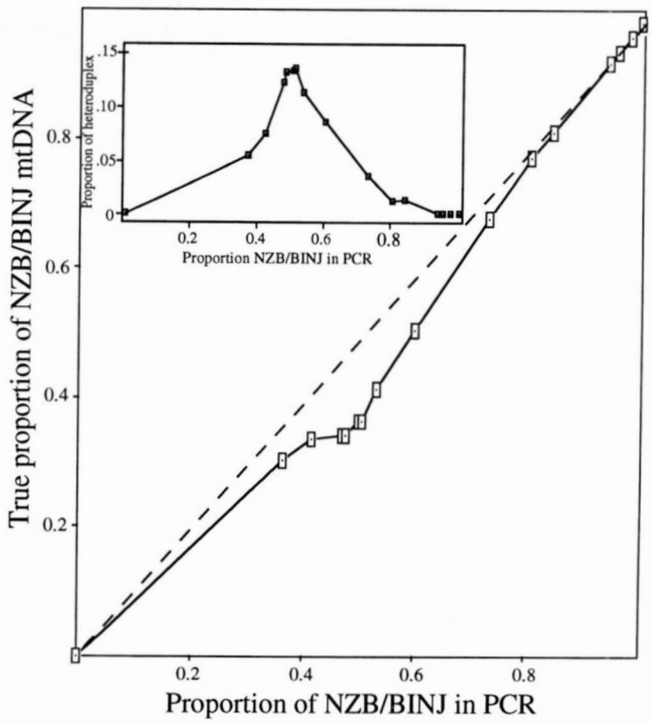

FIGURE 2.—Comparison between real and artifactual NZB/BINJ mtDNA content (large panel) and heteroduplex formation (inset) in PCR reactions using single digestions with *Bam*HI. The true NZB/BINJ ratios were obtained by subtracting heteroduplex band obtained from *Bam*HI-*Hin*fI double digestion from the PCR NZB/BINJ band obtained with *Bam*HI alone. Note that maximal heteroduplex component was obtained at 1:1 NZB/BINJ C57BL/6 ratios.

was double-stranded plasmid DNA purified on a solid phase column matrix (Magic Minipreps, Promega). Radionucleotide 35S dATP (Amersham, Arlington Heights, IL) was incorporated into the polymerizing chain for detection. The sequence reaction was performed with a primer NZB.R2 and size-fractionated by electrophoresis through a 8% polyacrylamide urea gel.

RESULTS

Sequencing of the mtDNA region comprising the polymorphic site responsible for the difference in the restriction fragments obtained after digestion of NZB/BINJ mtDNA revealed a cytosine to adenine transition at nucleotide 4279 (BIBB *et al.* 1981), leading to the loss of a *Bam*HI site and appearance of a *Hin*fI site in NZB/BINJ mtDNA (Figure 1). The presence of two strain-specific restriction sites allowed for the screening of heteroduplex formation in PCR products by double digestions with *Bam*HI and *Hin*fI. Production of heteroduplex structures, formed by the denaturation and reannealing of partially complementary DNA strands during the PCR, followed a distribution in which maximal occurrence was present at 50% of either mtDNA (Figure 2). At this ratio, NZB/BINJ mtDNA was 13% overestimated whereas negligible amounts of heteroduplex molecules were observed at concentrations below 20% of either NZB/BINJ or C57BL/6 mtDNA. Thereafter, all NZB/BINJ proportions obtained through densitometric readings of *Bam*HI digestions alone were corrected following a curve obtained with samples digested with both enzymes.

Although several embryos derived from reconstructed zygotes were transferred to pseudopregnant recipients, only six developed to term. Of these, five animals were derived from zygotes reconstituted by the fusion of a karyoplast with C57BL/6 mtDNA to an enucleated zygote with NZB/BINJ mtDNA and one with the reciprocal combination. The amount of C57BL/6 mtDNA of karyoplast origin present in a group of reconstituted zygotes analyzed immediately after micromanipulation was 19%, ranging from 16 to 23%. However, the average amount of karyoplast originated mtDNA present in adult tissue varied considerably among progeny derived from reconstructed embryos (Table 1). With the exception of one female with high levels of heteroplasmy (Figure 3), all progeny obtained contained lower than ex-

TABLE 1

Percentage karyoplast-origin mtDNA in tissues of adult animals derived from reconstituted zygotes

Reconstructed animals	Brain	Heart	Lung	Liver	Muscle	Tail
Female 1	17.4	69.0	0.6	58.3	63.5	21.0
Female 2	NA	NA	NA	NA	NA	5.3
Male 1	None	None	None	7.0	None	None
Male 2	NA	4.1	9.8	4.5	1.4	NA
Male 3	None	None	None	None	4.0	NA
Male 4	None	None	NA	None	10.8	NA

NA, tissue was not analyzed.

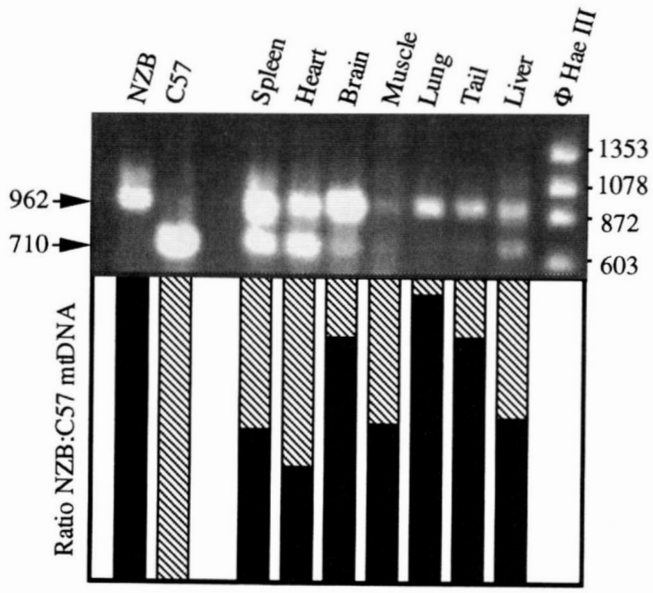

FIGURE 3.—Mitochondrial DNA patterns in tissues of heteroplasmic founder female derived from reconstructed zygote. PCR-amplified fragment digested with *Bam*HI to obtain upper (962 bp) and lower (710 bp) bands specific for NZB/BINJ and C57BL/6 mtDNA. The lower plot represents the densitometric readings of the above gel showing percentages of NZB/BINJ (■) and C57BL/6 (▨) mtDNA content.

pected average levels of tissue heteroplasmy, including several homoplasmic tissues.

Progeny were obtained from both reconstructed females. All five progeny from the female with low karyoplast-derived mtDNA were homoplasmic for all tissues analyzed. In contrast, progeny derived from the female with high heteroplasmy levels were exclusively heteroplasmic to different degrees. Mitochondrial DNA heteroplasmy among generations varied substantially among tissues, animals and litters (Figure 4). With the exception of one animal showing five out of seven tissues homoplasmic to NZB/BINJ mtDNA, most first generation progeny had heteroplasmic tissues averaging between 37 and 82% NZB/BINJ mtDNA. Compared to the 58% average content found in maternal tissues, most progeny (three out of five) showed an average increase in NZB/BINJ mtDNA, indicating a slight drift toward mitochondria of host origin (NZB/BINJ). Second generation progeny were obtained from the two heteroplasmic first generation females. Whereas one female produced only heteroplasmic progeny, the other bred both heteroplasmic and homoplasmic progeny. The latter were all homoplasmic for NZB/BINJ mtDNA. Progeny from third generation showed less variable tissue and whole animal litter heteroplasmy. Whereas homoplasmic females bred consistently homoplasmic progeny, heteroplasmic females bred either only heteroplasmic progeny or both heteroplasmic and homoplasmic progeny. Although few animals from fourth and fifth generation were analyzed, heteroplasmy among tissues and litters was marginally higher than in third generation animals. These results indicate wide variation in the patterns of mtDNA segregation in consecutive generations with a tendency for persistent heteroplasmy in some lines and a drift toward homoplasmy in others.

The average proportion of NZB/BINJ mtDNA did not vary to a great extent between founder (53.9%), first (71.4%), second (72.9%), third (65.3%), fourth (43.0%) and fifth (57.6%) generations (Figure 5). Lower levels of NZB/BINJ mtDNA among generations were observed when only heteroplasmic animals were included, *i.e.*, 62.1% and 49.2% for second and third generation, respectively, showing a tendency for increased C57BL/6 mtDNA in the animals from lines that remain heteroplasmic. However, the variability in tissue heteroplasmy levels within animals was remarkably different among generations ($P < 0.05$). Whereas both the founder (coefficient of variation, = 45.9%) and heteroplasmic progeny in the first generation (CV = 47.1%) had highly variable mtDNA levels among analyzed tissues, second (CV = 9.4%), third (CV = 11.4%) and, to a lesser extent, fourth (CV = 29.5%) and fifth (CV = 19.0%) generation progeny contained less variable tissue heteroplasmy. These results indicate that changes to the patterns of tissue mtDNA segregation occurred during passage from first to second generation progeny.

DISCUSSION

Karyoplast and cytoplast embryo reconstructions between zygotes carrying mitochondria of *M. m. molossinus* and *domesticus* subspecies have previously been shown to enable the production of animals with low levels of mtDNA heteroplasmy (SMITH and ALCIVAR 1993). Results presented here, using a *M. m. domesticus* intraspecific model, show not only that higher levels of heteroplasmy can be obtained in animals derived from karyoplast reconstructions but also that the mtDNA transmission to the following generations coincides with segregation patterns observed naturally in human and bovine maternal lineages. Together, these findings demonstrate the validity of this model for the investigation of the mechanisms of mitochondrial gene transmission to successive generations in mammals.

Analysis of tissue heteroplasmy in the most heteroplasmic founder female provides some insight into mechanisms of segregation of mtDNA between zygote and adult somatic tissue. Since NZB/BINJ mitochondria predominated in the reconstructed zygote, the probability of C57BL/6 mtDNA drifting toward disappearance due to replicative segregation of organelles in the adult tissue should be high. However, with the exception of the nearly homoplasmic lung (94% NZB/BINJ), most other tissues derived from the resulting female contained similar amounts of both mtDNA, suggesting that replicative segregation during embryonic

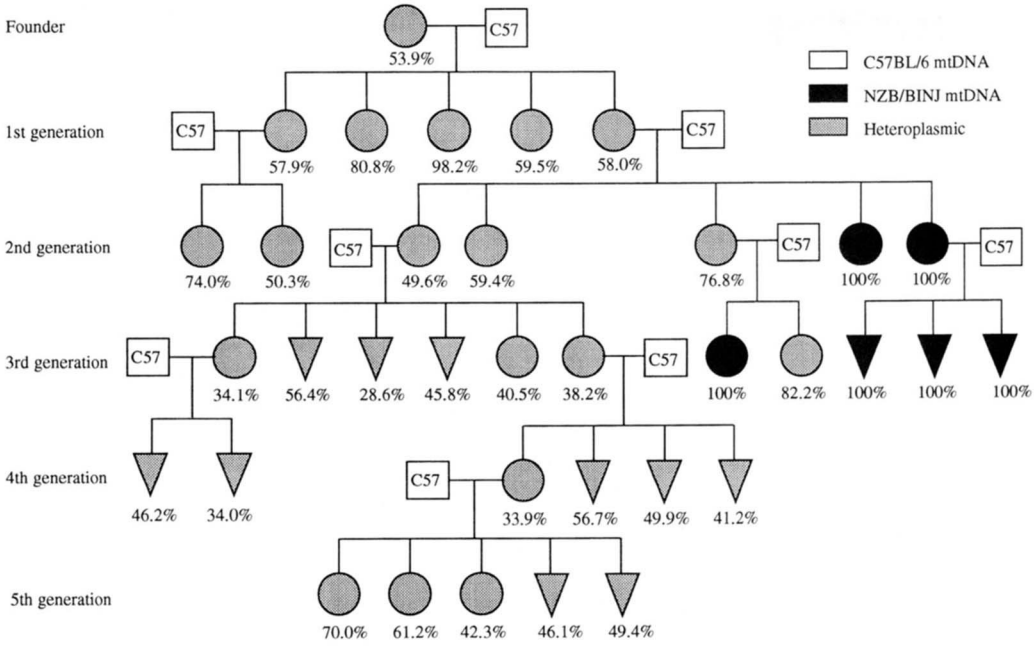

FIGURE 4.—Matrilineal pedigree of heteroplasmic female obtained from NZB-C57BL/6 reconstituted zygote. Average NZB/BINJ mtDNA percentages values for several tissues are given beneath gray (heteroplasmic) and black (homoplasmic for NZB/BINJ mtDNA) circles. Females were always mated to pure C57BL/6 (homoplasmic C57BL/6 mtDNA) males (squares).

and somatic mitotic divisions did not lead to a rapid drift to homoplasmy. Interestingly, there was higher than expected mitochondria of C57BL/6 origin, suggesting a drift toward karyoplast mitochondria. These findings contrast with the other progeny obtained through this technique and our previous interspecific *molossinus* model where, using a less sensitive Southern-blot technique, several tissues in the adult derived from a zygote reconstructed with a C57BL/6 karyoplast showed no evidence of C57BL/6 mtDNA and none of the heteroplasmic tissues had higher than expected karyoplast mtDNA (SMITH and ALCIVAR 1993). This difference could be explained either by random sampling of mtDNA molecules for replication or a slight incompatibility between NZB/BINJ and C57BL/6 mitochondria. Support for these mechanisms may be found in somatic cell culture studies in which 10 of 11 clones produced through fusion between BALB/c myeloma cells, which contain mtDNA similar to C57BL/6 strain used in our study and most other *M. m. domesticus* strains (YONEKAWA *et al.* 1982), and NZB/BINJ spleen cells segregated toward homoplasmy to BALB/c mtDNA (SMITH III *et al.* 1983; HUSTON *et al.* 1985).

Mitochondrial heteroplasmy in the progeny of first and following matrilineal generations is affected not only by mitotic but also by meiotic replicative segregation events. Although homoplasmic individuals were not produced in first generation progeny, a large variation in ratios among tissues was observed, including one offspring containing five out of seven NZB/BINJ homoplasmic tissues. Segregation may have arisen during the formation of germ cells in the heteroplasmic founder female and/or during the replication of embryonic and somatic tissue during fetal development

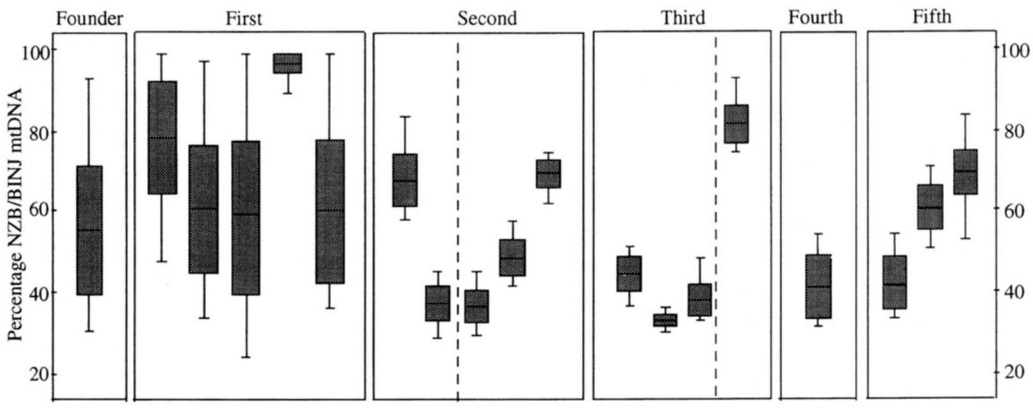

FIGURE 5.—Distribution of tissue NZB/BINJ mtDNA levels among heteroplasmic individuals from founder and five consecutive generations. Tissue variability is reduced beyond the second generation whereas litter homogeneity is observed only in third generation progeny. Hatched vertical line separates individuals into litters from second and third generations. Gray bars depict tissue averages (central bar) plus (top bar) and minus (lower bar) 1 SD. Vertical bars depict maximal (top) and minimal (bottom) values for each heteroplasmic animal.

and after birth. Approximately 30 cell divisions are believed to occur for the establishment of the germline at each generation (UPHOLT and DAWID 1977; WASSARMAN and ALBERTINI 1994) and at least another 50 cell divisions to adult tissues, depending on the organ and age at assessment. When considering passage to the next generation, a meiotic step is imposed between these two replicative segregation stages. Because mitochondria with few or a single genome replicate exponentially within the growing oocyte (PIKO and TAYLOR 1987), it is assumed that subpopulations of identical mitochondria will be regionalized during cell division (BIRKY 1994), enabling further segregation during embryogenesis and the establishment of the germline. Since our founder-reconstructed zygote had a regionalized population of mitochondria, it is likely that a highly heterogeneous population of heteroplasmic oocytes were developed that gave origin to heteroplasmic progeny with high tissue heterogeneity. These animals are likely to have established a less heterogeneous heteroplasmic oocyte pool that gave origin to second generation progeny with either homogeneous heteroplasmy or homoplasmic tissues. Two NZB/BINJ homoplasmic animals were obtained in the second generation from a heteroplasmic mother containing 58% NZB/BINJ mtDNA where both spleen and liver were practically homoplasmic (98% and 95%). It is unknown whether her homoplasmic progeny were derived from homoplasmic oocytes or from a heteroplasmic zygote that drifted uniformly to homoplasmy by somatic segregation during development to adulthood. However, oocyte homoplasmy seems more likely, since tissue heterogeneity was substantially reduced at this generation, suggesting limited replicative segregation during mitosis.

A central observation reported herein is the gradual loss of heterogeneity in the heteroplasmic levels of tissues and litters in consecutive generations. Decreasing variability in mtDNA heteroplasmy among hair follicles has been observed in polymorphic human lineages (HAO *et al.* 1995). There was considerable variability of heteroplasmy in different tissues not only within our founder female but also within and among her progeny. However, tissue heterogeneity within animals was reduced beginning from the second generation progeny, followed by loss of heterogeneity among litter in the third generation. We postulate that litter homogeneity of heteroplasmy is indicative of persistency of the heteroplasmic state, a condition that conflicts the narrow bottleneck hypothesis for mitochondrial genetics (KOEHLER *et al.* 1991). It is possible that intraorganellar heteroplasmy caused by fusions among NZB/BINJ and C57BL/6 mitochondria is the cause of stable levels of heteroplasmy among and within siblings. This intraorganellar heteroplasmy has been suggested as cause for persistent heteroplasmy in slowly segregating human lineages and in cell culture models using transformants obtained from fusions between mtDNA-less (ρ^0) cells and human heteroplasmic cells (YONEDA *et al.* 1994, 1995; ATTARDI *et al.* 1995; BENDALL and SYKES 1995). However, "mitochondrial meiosis" may occasionally cause reversion to the stable heteroplasmic condition, thereby explaining why a heteroplasmic line produced a homoplasmic individual in the third generation and also the increased variability within and among individuals of forth and fifth generations. Nonetheless, it is unclear why only animals homoplasmic for NZB/BINJ mtDNA were obtained in our model. We have recently shown that transfers of small amounts of NZB/BINJ cytoplasm to C57BL/6 zygotes can significantly affect early embryonic development (unpublished observations). It is possible that heteroplasmic embryos or cells with high proportions of C57BL/6 mtDNA are less viable, leading to a developmental barrier of homoplasmic C57BL/6 mtDNA. These aspects of mitochondrial incompatibility are currently being addressed in a zygote microinjection model using purified organelles.

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