Mitochondrial Genotype Segregation During Preimplantation Development in Mouse Heteroplasmic Embryos

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ABSTRACT

Mitochondrial DNA content remains constant between the mature egg and the blastocyst stage in mammals, making this the only period in development when genotypes segregate to daughter cells without the confounding effect of genotype replication. To analyze the segregation patterns of mitochondrial DNA during preimplantation development, we introduced polymorphic mitochondria either peripherally (cytoplast transplantation) or in the perinuclear vicinity (karyplast transplantation) into zygotes. Genotype ratios were significantly more variable among blastomeres from cytoplast (coefficient of variation = 83.8%) than karyoplast (coefficient of variation = 34.7%) reconstructed zygotes. These results suggest that heteroplasmy caused by polymorphic mitochondria positioned in the periphery of oocytes at the time of fertilization shows a more stringent segregation pattern than when the organelle is in the vicinity of the nucleus. Moreover, donor-to-host mitochondrial genotype ratios in karyoplast-derived groups increased significantly during development, particularly in the C57BL/6 group, where the ratio practically doubled between the four-cell (17.3%) and the blastocyst stage (29.6%). Although the mechanisms controlling this preferential replication of nuclear-type mitochondrial DNA are unknown, it is suggested that access to nuclear-derived transcription and replication factors could lead to the preferential replication of perinuclear mitochondrial genotypes during morula and blastocyst formation.

THE mechanisms controlling mitochondrial DNA (mtDNA) segregation and inheritance in mammals are controversial and poorly understood. Although mtDNA is known to mutate at rates 5–10 times faster than nuclear DNA (Brown *et al.* 1979), it is yet unclear why most individuals carry homogeneous mitochondrial genotypes. Researchers have relied mainly on the analysis of mitochondrial genotype segregation patterns in maternal lineages to demonstrate, for instance, that in cattle, one can observe complete switching of genotypes within a few generations or even a single generation (Ashley *et al.* 1989; Koehler *et al.* 1991). Although there has been a recent report of a rapid change in mitochondrial genotypes between generation in humans (Degoul *et al.* 1997), most analyses of human matrilineages indicate a less rapid segregation pattern in our species (Newman *et al.* 1991; Howell *et al.* 1992). The most accepted mechanism proposed is that of a genetic bottleneck in which only a small number of mtDNA molecules give rise to the entire genotype of an individual in the next generation (Hauswirth and Laipis 1985). It is suggested that a mutated genotype present in a mitochondrion of an oogonium could multiply and be compartmentalized in the cytoplasm during the exponential growth period of the oocyte (Hauswirth and Laipis 1985; Marchington *et al.* 1997). By chance, mitochondria containing the mutated genotype may segregate during cleavage and after mitotic divisions into the few cells that give origin to the fetus and its germ cells, thus enabling a complete switch within one or two generations. It remains unclear whether this segregation process occurs randomly, by drift, or whether it is further amplified by selective replication of a small proportion of mtDNA molecules.

Reconstructed mouse embryos have been used in some occasions to create heteroplasmic individuals carrying various ratios of polymorphic mitochondrial genotypes (Smith and Alcivar 1993; Laipis 1996; Jenuth *et al.* 1996; Meirelles and Smith 1997). These experimentally derived heteroplasmic animals contained highly variable levels of heteroplasmy in different organs and tissues, suggesting that mitochondrial genotypes are segregating substantially between the zygote and adult stages (Smith and Alcivar 1993; Meirelles and Smith 1997). Furthermore, heteroplasmy levels varied not only within but also among the progeny, with average percentages of donor type mtDNA ranging from zero to levels surpassing those found in any of the analyzed tissues from the mother. This suggests that a significant proportion of the segregation occurred

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before the establishment of the germ cells. Constant levels of mtDNA have been observed during development from the mature egg to blastocyst, leading to suggestions that mitochondria do not replicate their genomes during this period of development (Pikó and Taylor 1987; Ebert *et al.* 1988). This is a unique period in the mitochondrial life cycle, during which mitotic divisions are not accompanied by growth in the organelle population, and segregation is enabled during cleavage in the absence of the genetic drift caused by mtDNA replication. Moreover, replication of a mitochondria during oocyte development has been proposed to lead to regionalization of the cytoplasm in the mature egg (Hauswirth and Laipis 1985; Ashley *et al.* 1989). Our objective in this study, therefore, was to develop a model where polymorphic mitochondria were positioned either peripherally (cytoplast transplantation) or at a perinuclear position (karyoplast transplantation) to assess the segregation patterns among embryonic blastomeres during the initial stages of cleavage. We demonstrate that cleavage divisions lead to high variability in genotype ratios among daughter blastomeres of reconstructed embryos, suggesting that the preimplantation period of embryonic development plays an important role in the segregation of mitochondrial genotypes positioned in the periphery of the zygote. Moreover, increases in the ratio of karyoplast-derived mitochondrial genotypes positioned close to pronuclei supports recent findings suggesting that mtDNA replication first occurs in the perinuclear vicinity (Davis and Clayton 1996).

MATERIALS AND METHODS

Source of mice and zygotes with different mtDNA background: Zygotes containing mtDNA of *Mus musculus domesticus* origin (C57BL/6) were obtained from F1 females derived from a cross between males of the C3H strain and C57BL/6 females (B6C3F1; Charles River Canada Inc., St-Constant, PQ, Canada). Zygotes containing mtDNA of New Zealand Black (NZB) origin were obtained from F1 females derived from females of a backcross line derived by mating NZB/BINJ strain females (Jackson Laboratories, Bar Harbor, ME) to C57BL/6 for four generations, mated with C3H males (Charles River Canada Inc.). The restriction fragment length polymorphism pattern of the NZB/BINJ mtDNA differs from C57BL/6 mtDNA (Yonekawa *et al.* 1982). Zygotes from the NZB/BINJ backcross line contained ~99.21% or more of their nuclear genome of C57BL/6 origin and, because of the predominant maternal inheritance of mitochondria (Gyllensten *et al.* 1985, 1991), mtDNA of NZB/BINJ origin. Zygotes containing both mtDNA genotypes were obtained from females derived from a persistent heteroplasmic lineage originated by the continuous mating of a founder heteroplasmic female originated from a karyoplast-derived zygote (Meirelles and Smith 1997).

To obtain zygotes, females averaging 4–8 wk of age were superovulated by intraperitoneal injection of 5 IU of pregnant mare's serum gonadotrophin (Folligon; Ayerst, Montreal, PQ, Canada) and 5 IU of human chorionic gonadotrophin (hCG; Ayerst) given 44–48 hr apart. After hCG injection, females were paired with C57BL/6 males and inspected the next morning for copulation plug. Pronuclear stage zygotes were flushed from the oviducts at 24 hr after hCG 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: Zygotes were placed in CZB medium with cytoskeleton inhibitors for microsurgery (1 μg cytochalasin D ml^{-1} and 0.3 μg nocodazole ml^{-1}; Sigma, St. Louis, MO). Karyoplast and cytoplast transplantations were carried out using a technique similar to that described previously in McGrath and Solter (1983). Briefly, this method involves removing a 50-pL membrane-bound structure containing either pronuclei and some cytoplasm (karyoplast), or a portion of the cytoplasm surrounded by the cytoplasm membrane (cytoplast), and subsequently introducing these into the periviteline space of another embryo on which the same manipulation had been performed. After microsurgery, manipulated zygotes were washed and placed in medium without cytoskeletal inhibitors. Approximately 2 hr after manipulation, at which time the cytoskeleton has reassembled, the karyoplast/cytoplast was fused to the recipient zygote by electrofusion in a 0.3-M mannitol solution using an Electro Cell Manipulator (ECM-200; BTX, San Diego, CA) using a 1 kV cm^{-1} pulse for 70 μsec. Successfully reconstructed zygotes were cultured for several hours to obtain embryos at the two-, four-, eight-cell, and blastocyst stage.

Blastomere mtDNA amplification: Embryonic blastomeres were individualized by removing the zona pellucida in acidified Tyrode's solution (pH 3.5) and placed in phosphate-buffered solution without calcium and magnesium before blastomere disaggregation using a fine-bore pipette. Isolated blastomeres were washed and placed in a PCR buffer before

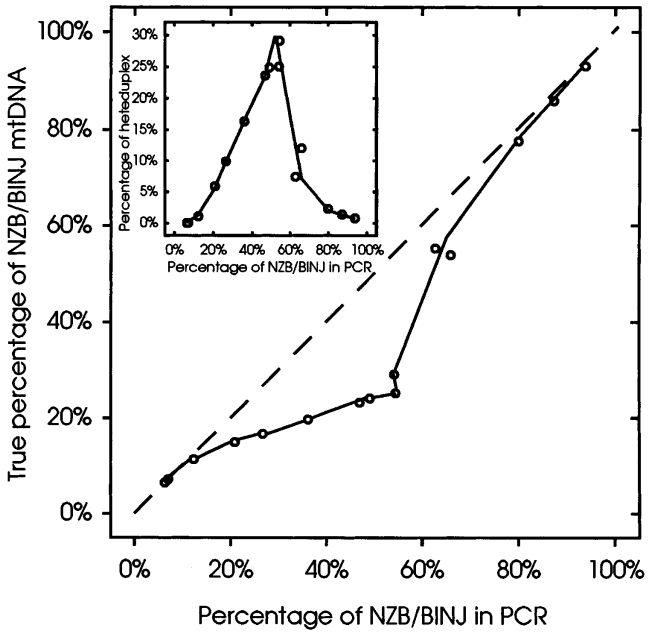

Figure 1—Comparison between true and artifactual NZB/BINJ mtDNA content (large panel) and heteroduplex formation (inset) in PCR reactions using single digestion with *Bam*HI. The true NZB/BINJ ratios were obtained by subtracting the heteroduplex band obtained from *Bam*HI-*Hin*fI double digestion from the PCR NZB/BINJ band obtained with *Bam*HI alone.

freezing. The following were amplified by PCR in 100 μl of reaction mixture: an mtDNA region consisting of a 1126-bp fragment encoding part of ND1 gene; the entire tRNA genes for isoleucine, glycine, and methionine; and part of ND2 gene (positions 3401–4527; according to Bibb *et al.* 1981). The PCR reaction mixture consisted of 10 mm Tris-HCl (pH 8.3); 50 mm KCl; 1.5 mm $MgCl_2$; 0.01% gelatin; 200 μM each dGTP, dATP, dTTP, an dCTP (dNTP; Pharmacia, Piscataway, NJ); 100 pmol of each oligonucleotyde primer; and 2.5 units 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). Oligonucleotyde primer sequences were as follows: mtNZB.F2, 5′ CGGCCCATTCGCGTTATTC 3′ (forward), and mtNZB.R2, 5′ AGGTTGAGTAGAGTGAGGGA 3′ (reverse). A second 995-bp fragment (positions 3476–4471) was obtained by amplification of the previous reaction (nested PCR) using the following oligonucleotides: mtNZB.F3, 5′ CCTAGGAC CCCTATAC 3′ (forward), and mtNZB.R3 5′ GTGGGCAAT TGATGAAT AGGC 3′ (reverse). mtDNA from most *M. musculus domesticus* strains, including C57BL/6, can be differentiated from NZB/BINJ by digestion with *Bam*HI (Meirelles and Smith 1997), since amplified mtDNA fragments contain one or two *Bam*HI restriction sites in NZB/BINJ and C57BL/6 mtDNA, respectively. The amplification product of some blastomeres was double digested with *Bam*HI (Promega, Madison, WI) and *Hin*fI (New England Biolabs, Mississauga, ON, Canada) to assess the formation of heteroduplex complexes. The ratio between C57BL/6 and NZB/BINJ mtDNA was determined by densitometry of the respective strain-specific restriction fragments and was corrected for fragment size and heteroduplexes. Data were analyzed using analysis of variance, and groups were compared among each other with Student's *t*-test at 5% level of significance.

RESULTS

An initial experiment was undertaken to determine the amount of heteroduplex molecule formation in our nested PCR protocol. This was performed by double digestion of samples using *Bam*HI and *Hin*fI with a large range of proportions of NZB/BINJ and C57BL/6 mtDNA to establish a curve for correcting for the true mtDNA content (as described in Meirelles and Smith 1997). Heteroduplexes were present at a maximum when the NZB/BINJ PCR recording approached 55% (Figure 1). At this ratio, NZB/BINJ genotypes were 30% overestimated, whereas negligible amounts of heteroduplex molecules were observed at concentrations <15% of either donor genotype. Thereafter, all NZB/BINJ proportions obtained through densitometric

TABLE 1

Percentage and range of mitochondrial genotype content and CV in C57BL/6 and NZB/BINJ cytoplast- and karyoplast-derived reconstructed embryos during the first six-cell cycles of preimplantation development

	Cell cycle	Donor mtDNA	Number	Mean percentage	Range	Mean CV	Range
Cytoplast	1	C57BL/6	07	10.6	5.8–15.0		
		NZB/BINJ	10	10.9	7.0–16.0		
	2	C57BL/6	11	12.0	12.2–27.2	82.0	11.2–139.9
		NZB/BINJ	12	11.5	7.6–15.4	60.1	23.1–121.8
	3	C57BL/6	13	16.1	4.6–40.6	96.3	2.9–141.4
		NZB/BINJ	13	11.0	4.6–28.2	78.0	43.3–130.3
	4	C57BL/6	12	14.1	5.9–24.6	101.0	44.9–140.0
		NZB/BINJ	12	11.4	4.6–19.2	84.3	16.3–125.5
	6	C57BL/6	11	15.2	8.7–23.3		
		NZB/BINJ	13	10.7	6.7–15.0		
Karyoplast	1	C57BL/6	13	18.7	12.1–24.6		
		NZB/BINJ	10	14.0	11.7–15.7		
	2	C57BL/6	13	18.1	14.0–22.4	55.4	11.8–90.9
		NZB/BINJ	13	13.7	11.8–17.3	19.7	4.8–55.8
	3	C57BL/6	12	17.3	7.7–23.4	47.4	15.1–72.8
		NZB/BINJ	12	13.4	11.1–16.1	21.5	5.4–30.0
	4	C57BL/6	12	22.3	18.5–27.0	46.6	28.8–87.6
		NZB/BINJ	12	15.5	11.6–17.5	17.2	5.2–45.1
	6	C57BL/6	14	29.5	13.9–43.0		
		NZB/BINJ	15	16.5	12.9–20.1		
Persistent lineage[a]	1	LP22	5	22.6	19.6–23.8		
	2	LP22	6	22.0	19.3–23.8	5.1	0–10.5
	3	LP22	6	22.4	20.4–23.5	5.1	1.3–14.7
	4	LP22	4	21.7	21.0–22.2	9.9	8.0–11.1
	6	LP22	5	23.5	23.1–23.8		

[a] The NZB/BINJ mtDNA content in embryos obtained from a nonsegregating, persistent heteroplasmic mouse lineage (LP22) originated from a C57BL/6 karyoplast-derived founder female.

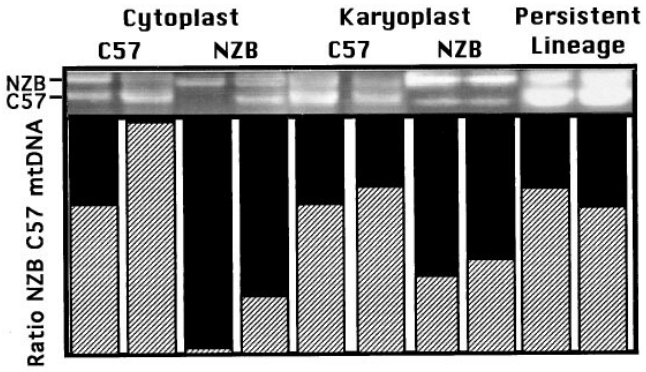

Figure 2—Comparison of mitochondrial genotype ratios among blastomeres from single two-cell embryos. Bars represent mitochondrial DNA patterns among blastomeres from representative two-cell stage embryos derived by karyoplast reconstruction (large variation), from cytoplast reconstruction (medium variation), and from a persistent heteroplasmic lineage (small variation). Column patterns represent the heteroduplex-corrected proportion of C57Bl/6 mtDNA (hatched) and NZB/BINJ mtDNA (black). The inset shows ethidium bromide-stained NZB/BINJ- (top) and C57BL/6-specific (lower) bands after *Bam*HI digestion of the PCR product derived from a single two-cell blastomere.

readings of *Bam*HI digestions alone were corrected following a curve obtained with samples digested with both enzymes.

Changes in ratios among blastomeres during cleavage: To determine the mitochondrial genotype segregation levels during early development, we compared the proportion of the donor mtDNA genotype among blastomeres at the two-, four-, and eight-cell stages, and we calculated the coefficient of variation (CV) for each embryo analyzed (Table 1; Figure 2). Embryo CVs are directly related to segregation patterns, and they were used for comparisons among different developmental stages and groups (Figure 3). In general, cytoplast-reconstructed embryos (CV = 83.8%) segregated significantly more ($P < 0.01$) than either karyoplast-derived embryos (CV = 34.7%) or embryos derived from the persistent heteroplasmic lineage (CV = 6.3%). Zygotes derived from the persistent lineage underwent little segregation, an indication of either homogeneous distribution of the mitochondrial population or of intramitochondrial heteroplasmy, as suggested previously (Meirelles and Smith 1997). Segregation was higher in C57BL/6 (CV = 49.9%) karyoplast-reconstructed embryos than in the NZB/BINJ (CV = 19.5%) karyoplast group. Moreover, no group showed significant changes in CVs during the three cell cycles analyzed, indicating that segregation levels did not alter during cleavage.

Changes in embryo mtDNA ratios during development: Although microsurgery was identical among reciprocal groups, zygotes reconstituted with C57BL/6 karyoplasts had significantly more donor-type mtDNA than the NZB/BINJ karyoplast group (18.8% *vs.* 14.1%, $P < 0.05$), indicating that mitochondria may be more closely associated with the pronuclei in zygotes containing C57BL/6 mtDNA (Table 1). During devel-

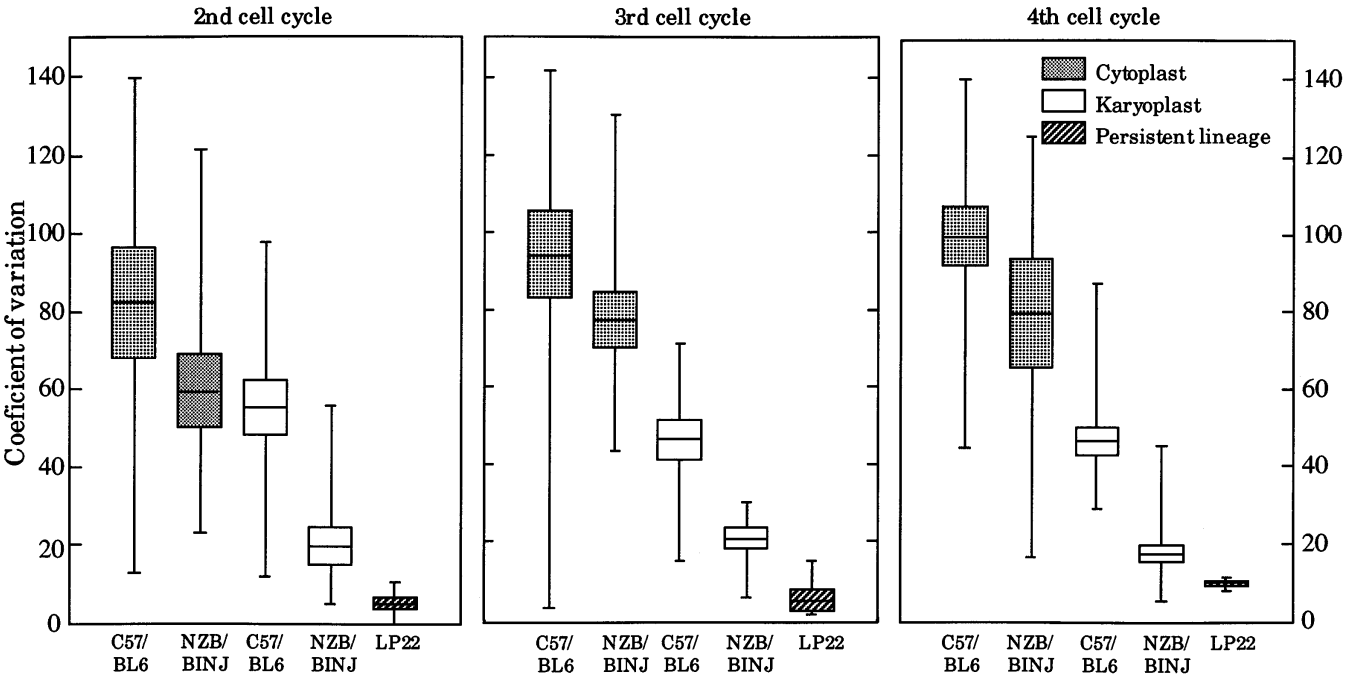

Figure 3—Distribution of CVs of donor-type mtDNA in cytoplast (gray) and karyoplast-derived (white) zygotes in the second, third, and fourth cell cycles. CVs are also shown for embryos derived from a persistent (hatched) heteroplasmic lineage (LP22). Bars depict tissue averages (central bar) plus (top bar) and minus (lower bar) 1 SD. Vertical bars depict maximal (top) and minimal (bottom) values for each group.

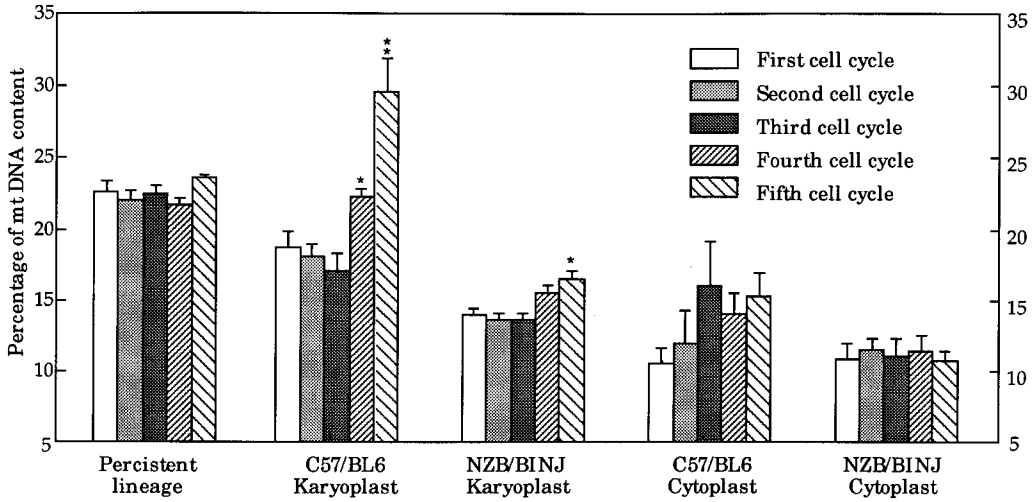

Figure 4—Percentage of donor mtDNA content in karyoplast- and cytoplast-derived reconstructed embryos from zygote (first cell cycle) to blastocyst stage (sixth cell cycle). NZB/BINJ mtDNA content in embryos derived from a persistent heteroplasmic lineage (LP22) are also shown. Asterisks indicate that the mtDNA content was significantly different from other stages within the same group.

opment of the reconstructed zygotes, the proportion of mtDNA molecules originated from the donor zygote increased consistently in embryos derived by karyoplast transplantation (Figure 4). This increase was observed both in embryos reconstructed using karyoplast of C57BL/6 and NZB/BINJ mtDNA origin, and it occurred consistently from the eight-cell stage onwards, so that blastocysts contained a significantly higher proportion of mtDNA of karyoplast origin than embryos at the one-, two-, and four-cell stages ($P < 0.05$). Zygotes derived by cytoplast reconstruction and those derived from the persistent lineage, however, did not show any significant change in mtDNA ratios during cleavage. Together, these results indicate that karyoplast-derived mitochondria are somehow better able to multiply their genotypes at around the stage of compaction and blastocyst development.

Effects on preimplantation development: Reconstructed zygotes had their developmental competence affected differentially in karyoplast and cytoplast groups (Figure 5). As expected, karyoplast-derived zygotes had lower blastocyst formation ability than did cytoplast groups, possibly because of a sensitivity of pronuclei to trauma induced by the more complex microsurgical technique required to remove and transfer karyoplasts. A difference in development was observed between the karyoplast and cytoplast reciprocal groups, however. NZB/BINJ karyoplast and cytoplast groups had 15–16% reduction in blastocyst development relative to their C57BL/6 counterparts.

DISCUSSION

Our results clearly demonstrate that mitochondrial genotypes in cytoplast- and karyoplast-reconstructed embryos are segregated during the preimplantation period, which is comprised of the development of the zygote to the formation a blastocyst. The lower segregation levels observed in the karyoplast group may reflect the position of the donor mitochondria at the time of reconstruction. A large proportion of mitochondria are attached to cytoskeletal elements positioned in a perinuclear position within the cell. Since the cytoskeletal elements reassemble mitochondria around the nucleus, at the time of fusion, most mitochondria transplanted in karyoplasts are transported with the pronuclei to the center of the zygote, and, at mitosis, equal amounts of the donor mitochondria would be distributed to daughter blastomeres. On the other hand, cytoplast-derived mitochondria remain most likely localized at the periphery of the reconstructed zygote, enabling stringent segregation at cleavage.

Although mature oocytes have been shown to con-

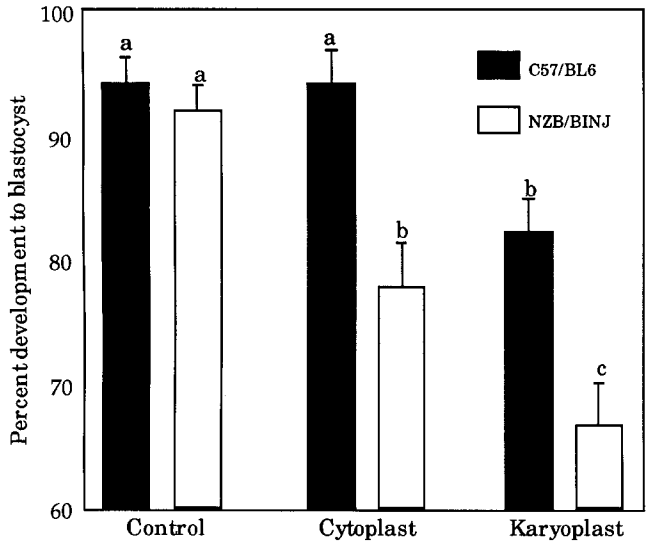

Figure 5—Preimplantation developmental rates of cytoplast- and karyoplast-reconstructed zygotes and control zygotes. Bars represent the percentage of the reconstructed and control zygotes that developed *in vitro* to the blastocyst stage after 4 days of culture. Reconstructed groups are identified as to the source of mtDNA in the zygote from which cytoplasts or karyoplasts were obtained for reconstructions (donor zygotes). Different letters denote significant differences at the 5% level.

tain regionalized cytoplasm (Calarco 1995), it is unclear whether in nature one would encounter a partitioning of mtDNA genotypes as extreme as that obtained using this model. Nonetheless, these data indicate that blastomeres resulting from a heteroplasmic oocyte could carry highly heterogeneous mtDNA genotypes. Because approximately three to four cells (19–25%) within a 16-cell stage embryo will become the inner cell mass (Balakier and Pedersen 1982), one may assume that very diverse individuals could arise from a homogeneous group of heteroplasmic eggs. This has been confirmed by several studies where individuals derived from cytoplast- and karyoplast-reconstructed zygotes had both lower and higher than the expected amount of donor mtDNA introduced (Smith and Alcivar 1993; Jenuth *et al.* 1996; Meirelles and Smith 1997). Indeed, tissue heterogeneity has also been consistently observed in the progeny, suggesting that segregation occurring before, during, and after the establishment of the three primary layers is an important component in the heteroplasmic levels found in adult animals (Lertrit *et al.* 1992). This same postimplantation replicative segregation may further increase heterogeneity in the primordial germ cells and originating oogonia and oocytes with various degrees of heteroplasmy.

A somewhat surprising finding in this study was that mitochondrial genotype ratios varied significantly during early development in karyoplast-derived zygotes. Previous reports have indicated that mtDNA content does not change during preimplantation, suggesting that no replication occurs during this period of development (Pikó and Taylor 1987; Ebert *et al.* 1988). The nuclear genetic background of the recipient cell has previously been shown to influence the segregation of mitochondrial genomes in cell cybrids (Dunbar *et al.* 1995). One possible explanation for the shift in ratio observed is that mitochondria contained within karyoplasts are preferentially replicated or initiate replication at an earlier stage than those placed further away from the nuclei. Perinuclear mitochondria from avian and amphibian oocytes replicate more actively and appear to segregate to the somatic cells of the fetus, while another subcortical group appears to become localized in the primordial germ cells (D'Herde *et al.* 1995).

In mammalian cells, mtDNA synthesis consistently radiates outward from the perinuclear position, suggesting that replication first occurs in the vicinity of nuclear-provided materials (Davis and Clayton 1996). Although detailed mtDNA replication studies have not yet been performed with mammalian embryos, mitochondria from mouse oocytes and embryos have also been shown to be mostly localized in a perinuclear position and to translocate occasionally to form cytoplasmic aggregates (Tokura *et al.* 1993; Calarco 1995). Mitochondrial and nuclear genes involved in energy biogenesis are transcribed actively and in a coordinated fashion during cleavage from the two- to four-cell stage onwards (Taylor and Pikó 1995). This interaction between nuclear-derived mtDNA transcription factors and mitochondria may not function properly across these strains, particularly between C57BL/6 nuclear genotype and mitochondria of the NZB/BINJ genotype, which explains why the C57BL/6 mtDNA karyoplast appears to have had a more significant effect both on the time and amount of the ratio change. The reciprocal reconstructed group is expected to be less affected because the NZB/BINJ karyoplast carries nuclear genes that are 95% C57BL/6 and, therefore, more compatible with C57BL/6 mtDNA. The stress imposed by the limited number of functional mitochondria may induce an earlier replication of these karyoplast-derived organelles, leading to the observed increase in donor mtDNA ratios. On the other hand, cytoplast-reconstructed zygotes may not impose similar interactive stress on the reconstructed embryo, leading to the usual initiation of mtDNA replication at the blastocyst stage with no consequent alteration in mitochondrial genotype ratios.

The effect of mitochondrial genotypes on development into blastocysts is less clear. A recent report has indicated that the developmental rate and speed to develop into blastocysts was significantly reduced in C57BL/6-reconstructed zygotes containing mitochondria from the *Mus spretus* species (Nagao *et al.* 1997). Our previous studies using karyoplast- and cytoplast-reconstructed embryos with *Mus musculus molossinus* backcross embryos are also indicative of mitochondrial developmental effect during cleavage and blastocyst stages (Smith and Alcivar 1993). Whereas the two latter studies involved reconstructions between different species and subspecies of mice, NZB/BINJ and C57BL/6 are both *M. musculus domesticus* strains. Since reconstructions were within species, one would expect less incompatibility than the cross-species or subspecies transfers described above. The NZB/BINJ mtDNA genotype, however, seems to have originated from the *Mus brevirostris* (Yonekawa *et al.* 1982), leading to cross-species incompatibilities between the *M. musculus domesticus* nuclei and NZB/BINJ mitochondria, which may explain why small amounts of NZB/BINJ mitochondria affected significantly the development of the cytoplast-reconstructed embryo.

LITERATURE CITED

Ashley, M. V., P. J. Laipis and W. W. Hauswirth, 1989 Rapid segregation of heteroplasmic bovine mitochondria. Nucleic Acids Res. **17:** 7325–7331.

Balakier, H., and R. A. Pedersen, 1982 Allocation of cells to inner cell mass and trophectoderm lineages in preimplantation mouse embryos. Dev. Biol. **90:** 352–362.

Bibb, M. J., R. A. van Etten, C. T. Wright, M. W. Walberg and D. A. Clayton, 1981 Sequence and gene organization of mouse mitochondrial DNA. Cell **26:** 167–180.

Brown, W. M., M. George and A. C. Wilson, 1979 Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA **76:** 1967–1971.

Calarco, P. G., 1995 Polarization of mitochondria in the unfertilized mouse oocyte. Dev. Genet. **16:** 36–43.

Chatot, C. L., C. A. Ziomek, B. D. Bavister, J. L. Lewis and I. Torres, 1989 An improved culture medium supports development of random-bred one-cell mouse embryos in vitro. J. Reprod. Fert. **86:** 679–688.

Davis A. F., and D. A. Clayton, 1996 In situ localization of mitochondrial DNA replication in intact mammalian cells. J. Cell Biol. **135:** 883–893.

Degoul, F., D. Francois, M. Diry, G. Ponsot, I. Desguerre *et al.*, 1997 A near homoplasmic T8993G mtDNA mutation in a patient with atypic Leigh syndrome not present in the mother's tissues. J. Inher. Metabol. Dis. **20:** 49–53.

D'Herde, K., M. Callebaut, F. Roels, B. De Prest and L. van Nassauw, 1995 Homology between mitochondriogenesis in the avian and amphibian oocyte. Reprod. Nutr. Dev. **35:** 305–311.

Dunbar, D. R., P. A. Moonie, H. T. Jacobs and I. J. Holt, 1995 Different cellular backgrounds confer a marked advantage to either mutant or wild-type mitochondrial genomes. Proc. Natl. Acad. Sci. USA **92:** 6562–6566.

Ebert, K. M., H. Liem and N. B. Hecht, 1988 Mitochondrial DNA in the mouse preimplantation embryo. J. Reprod. Fert. **82:** 145–149.

Gyllensten, U. B., D. Wharton and A. C. Wilson, 1985 Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. J. Heredity **76:** 321–324.

Gyllensten, U. B., D. Wharton, A. Josefsson and A. C. Wilson, 1991 Paternal inheritance of mitochondrial DNA in mice. Nature **352:** 255–257.

Hauswirth, W. W., and P. J. Laipis, 1985 Transmission genetics of mammalian mitochondria: a molecular model and experimental evidence, pp. 49–59 in *Achievements and Perspectives of Mitochondrial Research*, edited by E. Quagliriero. Elsevier Science Publishers, Milan, Italy.

Howell, N., S. Halvorson, I. Kubacka, D. A. McCullough, L. A. Bindoff *et al.*, 1992 Mitochondrial gene segregation in mammals: is the bottleneck always narrow? Hum. Genet. **90:** 117–120.

Jenuth, J. P., A. C. Peterson, K. Fu and E. A. Shoubridge, 1996 Random genetic drift in the female germ line explains the rapid segregation of mammalian mtDNA. Nature Genet. **14:** 146–151.

Koehler, C. M., G. L. Lindberg, D. R. Brown, D. C. Beitz, A. E. Freeman *et al.*, 1991 Replacement of bovine mitochondrial DNA by sequence variant within one generation. Genetics **129:** 247–255.

Laipis, P. J., 1996 Construction of heteroplasmic mice containing two mitochondrial DNA genotypes by micromanipulation of single-cell embryos, pp. 345–357 in *Mitochondrial Bigenesis and Genetics (Part B)*, edited by G. M. Attardi and A. Chomyn. Academic Press, San Diego.

Lertrit, P., A. S. Noer, E. Byrne and S. Marzuki, 1992 Tissue segregation of a heteroplasmic mtDNA mutation in MERRF (myoclonic epilepsy with ragged red fibers) encephalomyopathy. Hum. Genet. **90:** 251–254.

Marchington, D. R., G. M. Hartshorne, D. Barlow and J. Poulton, 1997 Homopolymeric tract heteroplasmy in mtDNA from tissues and single oocytes: support for a genetic bottleneck. Am. J. Hum. Genet. **60:** 408–416.

McGrath, J., and D. Solter, 1983 Nuclear transplantation in the mouse embryo by microsurgery and cell fusion. Science **220:** 1301–1302.

Meirelles, F. V., and L. C. Smith, 1997 Mitochondrial genotype segregation in a mouse heteroplasmic lineage produced by embryonic karyoplast transplantation. Genetics **145:** 445–451.

Nagao, Y., Y. Totsuka, Y. Atomi, H. Kaneda, H. Yonekawa *et al.*, 1997 Heterogeneous mitochondrial DNA introduced by nuclear transfer influences the developmental ability of mouse embryos in vitro (Abstr.). Theriogenology **47:** 233.

Newman, N. J., M. T. Lott and D. C. Wallace, 1991 The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation. Am. J. Ophthalmol. **111:** 750–762.

Pikó, L., and K. D. Taylor, 1987 Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early mouse embryos. Dev. Biol. **123:** 364–374.

Smith, L. C., and A. A. Alcivar, 1993 Cytoplasmic inheritance and its effects on development and performance. J. Reprod. Fert. Supplement **48:** 31–43.

Taylor, K. D., and L. Pikó, 1995 Mitochondrial biogenesis in early mouse embryos: expression of the mRNAs for subunits IV, Vb, and VIIc of cytochrome *c* oxidase and subunit 9 (P1) of H^+-ATP synthase. Mol. Reprod. Dev. **40:** 29–35.

Tokura, T., Y. Noda, Y. Goto and T. Mori, 1993 Sequential observation of mitochondrial distribution in mouse oocytes and embryos. J. Assis. Reprod. Genet. **10:** 417–426.

Yonekawa, H., K. Moriwaki, O. Gotoh, N. Miyashita, S. Migita *et al.*, 1982 Origins of inbred mice deduced from restriction patterns of mitochondrial DNA. Differentiation **22:** 222–226.

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