

Cytoplasmic inheritance and its effects on development and performance

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In contrast to nuclear inheritance, cytoplasmic inheritance in mammals is derived mostly, if not exclusively, from the maternal line. Mitochondria, and their DNA molecules (mtDNA), are the genetic units of this method of inheritance. Mammalian mtDNA codes for 13 enzymes used in the mitochondrial energy-generating pathway, oxidative phosphorylation, 22 tRNAs and two rRNAs. Although all transcripts of mtDNA and their translational products remain in the mitochondria, most proteins used in mitochondria are from nuclear DNA and are imported after synthesis on cytoplasmic ribosomes. Spermatozoa introduce a small number of mitochondria into the cytoplasm of the egg at fertilization, which appear to be digested soon after penetration. Although the paternal contribution of mtDNA to the offspring is not believed to occur in mammals, some interspecific crosses have suggested that it does occur. Experiments with animals derived from reconstituted embryos, using nuclear or cytoplasmic transplantations, suggest that nuclear-mitochondrial interactions are important but not essential in the survival and replication of exogenous mitochondria introduced into the egg. As the levels of heteroplasmy varied in several tissues of animals derived from reconstituted embryos, it is suggested that differential partitioning of mitochondria occurs during embryogenesis. Mitochondrial morphology changes substantially during oogenesis and throughout early cleavage stages. Somatic morphology and normal replication patterns are regained at the blastocyst stage. In pig oocytes and embryos, mitochondria aggregate and are closely associated with endoplasmic reticulum, lipid granules and large vesicles. Although the direct correlation of mitochondrial genes with reproductive traits is still unclear, some human degenerative diseases and performance traits in cattle can be related directly to specific mtDNA polymorphisms. In pigs, reciprocal-cross comparisons have indicated greater offspring parent similarity with dam than sire for lean:fat ratio. A difference was also observed for oxygen consumption and oxidative phosphorylation, but not for anaerobic energy metabolism, in a pig reciprocal-cross experiment. Information on the transmission of mtDNA and its effects on performance will have many implications not only for our understanding of mitochondrial genetics but also for the increased productivity of animals. There are also potential ramifications to the animal cloning industry.

Introduction

Traditional genetics assumes that, at fertilization, male and female gametes contribute an equal amount of genetic material to the offspring. Although development and performance of the offspring is generally

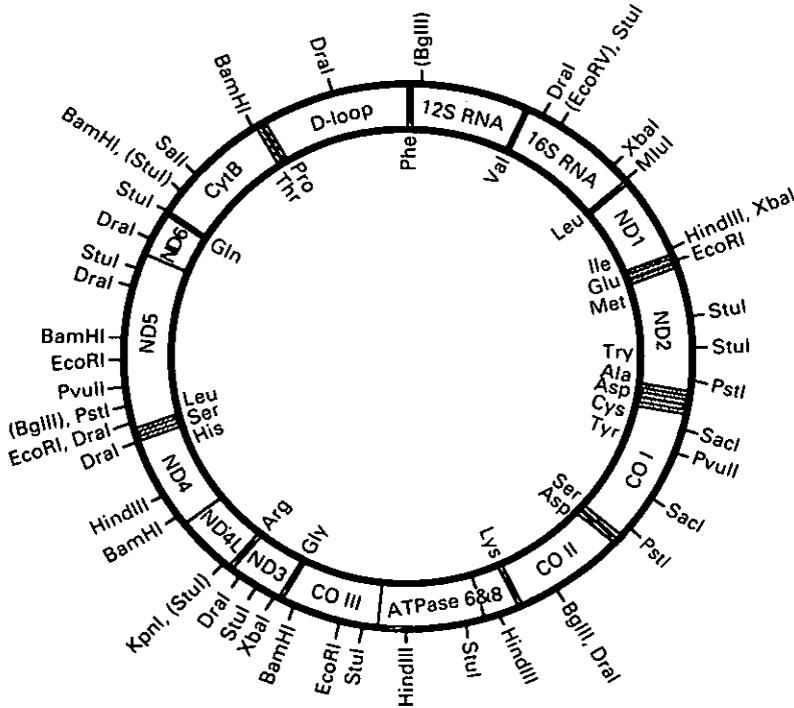


Fig. 1. Organization of pig mitochondrial DNA. mtDNA encodes seven subunits of the NADH-CoQ reductase complex (ND1, ND2, ND3, ND4L, ND4, ND5, ND6) ATPase subunits 6 and 8 (ATPase6 and 8), cytochrome *c* oxidase subunits 1, 2 and 3 (COI, COII, COIII), and cytochrome *b* (CytB). It also encodes for a small (12S RNA) and a large (16S RNA) ribosomal RNAs and 22 transfer RNAs (inner circle). No mammalian mtDNA genes contain introns. Restriction endonucleases in parentheses show the different polymorphic sites among breeds (based on data from Bibb *et al.*, 1981; restriction map adapted from Watanabe *et al.*, 1986).

believed to be due to additive, dominance or epistatic effects of genes contributed by both parents, other effects due to the differential imprinting of parental genes and the maternal effects on the pre- and perinatal environment in mammals further confound the contribution of each parent. These, however, are genetic effects derived from genes contained within the nucleus and transmitted via sexual reproduction. Mitochondria, cytoplasmic organelles which reproduce asexually, are the only known source of genetic material in the cytoplasm of mammalian cells and, therefore, provide an opportunity for an unbalanced contribution from each parent (maternal or cytoplasmic inheritance). We review herein the mechanisms of gene transmission by the mitochondrion and describe some of the experimental evidence for its effect on early development and performance in mammals. When available, specific references are made to work performed in pigs.

The Mitochondrial Genome

DNA is present in eukaryotes not only in the chromatin within the nucleus but also in some cytoplasmic organelles. Mammalian mitochondria, like the mitochondria of other kinds of eukaryotes, contain their own genome (Fig. 1). The mitochondrial DNA (mtDNA) is a closed circular molecule of approximately 16 500 nucleotides which is located within the inner mitochondrial membrane (Shoffner and Wallace, 1990). Most somatic cells have hundreds of mitochondria and each carries thousands of mtDNAs (Shuster *et al.*, 1988). The evolution of mitochondria has been the subject of continuing interest and considerable

speculation since they were first recognized as cellular entities. Current molecular data strongly support an endosymbiotic origin of the mitochondrial genome from a direct common ancestor with purple bacteria (Gray, 1992), because the mitochondrion has an independent replication, transcription and translation system, which combines the features of prokaryotic and eukaryotic cells.

The mitochondrial energy-generating pathway, oxidative phosphorylation is composed of five enzyme complexes (Complexes I to V) assembled from subunits derived from both mtDNA and nuclear DNA (nDNA). However, whereas most mtDNA genes encode subunits of the mitochondrial energy-generating pathway, the majority of these subunits are encoded by nDNA, translated in the cytosol and selectively imported into the mitochondrion (Shoffner and Wallace, 1990). Complex I of oxidative phosphorylation consists of approximately 39 polypeptides, seven (ND1, ND2, ND3, ND4L, ND4, ND5, ND6) encoded by mtDNA; complex II consists of four polypeptides, all nuclear; complex III consists of nine polypeptides, one (cytochrome *b*) encoded by the mtDNA; complex IV consists of 13 polypeptides, three (COI, COII and COIII) encoded by mtDNA; and complex V consists of 12 polypeptides, two (ATPase α and β) encoded by the mtDNA. Apart from these 13 polypeptides, mtDNA encodes for the 12S and 16S ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) involved in mitochondrial protein synthesis (Anderson *et al.*, 1981; Shoffner and Wallace, 1990). Complete mitochondrial nucleotide sequences and gene contents have been determined for several mammals including humans, cows, mice and rats (Anderson *et al.*, 1981, 1982a, b; Bibb *et al.*, 1981; Gadaleta *et al.*, 1989).

Partial sequence information has been obtained by restriction site mapping of mtDNA obtained from the kidney of several breeds of European and Asian pigs (Watanabe *et al.*, 1985; Watanabe *et al.*, 1986). Authors reported a restriction endonuclease cleavage map using 18 enzymes which recognize six nucleotides and one four-nucleotide-recognizing enzyme (Fig. 1). Detailed sequence information is also available for a 237 nucleotide pair (np) region enclosing part of the genes for ATPase β , ATPase α and COIII (Watanabe *et al.*, 1986). Within this region there was a 19.8%, 25.7%, 19.4% and 21.5% sequence difference with the same regions of cow, human, mouse and rat mtDNA, respectively. A comparison was also performed among several breeds of pig and Japanese wild boars based on their restriction endonuclease cleavage patterns (Watanabe *et al.*, 1985). Some mtDNA polymorphisms were observed in the cleavage patterns with BglIII, EcoRV, SacI, StuI and TaqI. Two distinct types of restriction pattern were observed. First, the restriction patterns of Landrace, Hampshire, Duroc, Pitman-Moore and one Large White group were similar and were classified as European type. Second, the patterns of another Large White group, the miniature pigs Ohmini and Gottingen, Taiwan native breeds, and Japanese wild boars were similar, and were grouped as the Asian type. The estimated percentage difference between the European and Asian breeds is 1.75%, which according to evolutionary theory translates into a separation of parent stocks of about 0.8–0.9 million years ago (Watanabe *et al.*, 1986). Analysis of mtDNA with HinfI and HaeIII showed that samples derived from pigs of the Hampshire breed exhibited patterns similar to the Asian (Gottingen minipig) type (Hecht, 1990). These results suggest that both the Gottingen minipig and the Large White breed represent maternal lineages both of European and Asian origin. Moreover, among the European pig breeds, two variable sites have been detected: one in a Duroc and another in the Belgian Landrace, using EcoRV and HincII enzymes, respectively (Hecht, 1990).

Mitochondrial Inheritance

For many years, it has been assumed that mitochondrial genes, in contrast to genes in the nucleus, have an exclusively maternal mode of inheritance in mammals (Hutchinson *et al.*, 1974; Giles *et al.*, 1980; Hayashi *et al.*, 1983; Gyllensten *et al.*, 1985). The predominance of mitochondria of maternal origin in the offspring can be partially explained by the fact that at fertilization the spermatozoon contains approximately 75 mitochondria (Bahr and Engler, 1970; DeMartino *et al.*, 1979; Hecht *et al.*, 1984) compared with approximately 100 000 mitochondria in the ooplasm in mice and cows (Piko and Matsumoto, 1976; Michaels *et al.*, 1982; Gyllensten *et al.*, 1985). Thus there is a 1:1000 ratio between paternal and maternal mtDNA molecules. However, use of a more sensitive test of paternal contribution, an interspecific backcross study between *Mus spretus* and *Mus domesticus* showed paternal transmission of mtDNA at a ratio of approximately 1:50 000 per generation (Gyllensten *et al.*, 1991) which is 50 times lower than expected if paternal and maternal mitochondria had equal abilities to survive and replicate throughout development. This

contrasts with a more recent finding involving intra- and interspecific crosses of the marine mussel *Mytilus*, showing a paternal mtDNA contribution of 1:10 (Zouros *et al.*, 1992). It is unclear whether paternal transmission of mtDNA occurs at any level in mammals, and the mechanism by which most or all sperm mitochondria become inactive is unknown.

Mitochondria from the sperm tail constituents have been shown by ultrastructural studies to be carried into the oocyte at fertilization (Szöllösi, 1965; Hiraoka and Hirao, 1988). One possible mechanism for maternal inheritance of mtDNA is the capacity of the oocyte to recognize paternal mitochondria and eliminate them as foreign organelles. This view is supported by studies in hamsters showing that sperm mitochondria are enclosed by multivesicular bodies after fertilization and later digested into small molecular elements during the two-cell stage of development (Hiraoka and Hirao, 1988). Other studies of sea urchin eggs and embryos have shown that the structure and metabolic activity of the paternal mitochondrion begins to degenerate soon after the first cell cycle division of the zygote (Anderson and Perotti, 1975).

Maternal inheritance of chloroplast DNA in higher plants is believed to be governed by methylation of specific DNA sites on the maternal organelle, which protects it from degradation in the zygote (Sager and Grabowy, 1983). Mitochondrial morphology varies during spermiogenesis (DeMartino *et al.*, 1979) and it has been proposed that mitochondria may also be altered and possibly debilitated during spermiogenesis (Vaughn *et al.*, 1980). However, an extensive study in CD1 mice showed no difference in the state of methylation of mtDNA among oocytes, spermatozoa and earlier testicular cell types (Hecht *et al.*, 1984) and between germinal and somatic cells of rats (Alcivar *et al.*, 1992). Moreover, no loss, amplification, or translocation of sizeable regions of the mitochondrial genome could be detected at different stages of spermiogenesis, suggesting that maternal inheritance is not mediated by alteration of the paternal mtDNA (Hecht *et al.*, 1984). The mtDNA present in spermatozoa is also known to transcribe mitochondrial ribosomal and messenger RNAs, suggesting that, at least at the time of fertilization, the paternal mitochondria are likely to be translationally competent (Alcivar *et al.*, 1989).

Mitochondria in Animals Derived from Reconstituted Embryos

Direct microinjection of mitochondria isolated from testis or liver into the cytoplasm of mouse zygotes has been performed to assess their ability to survive and proliferate in the developing embryo (Ebert *et al.*, 1989). Although microinjection caused no apparent detriment to the survival and viability of the embryos, no exogenous mtDNA could be detected in fetal samples or tissues from the mature progeny suggesting that the 'foreign' mitochondria have the same fate during early embryogenesis as the mitochondria of the spermatozoon. This result contrasts with the findings of low levels of paternal mtDNA in mouse interspecific crosses (Gyllensten *et al.*, 1991). Moreover, recent data indicate that there are high levels of mitochondrial heteroplasmy in progeny derived from reconstituted embryos of mice and cows (Smith *et al.*, 1991; Plante *et al.*, 1992). In mice, both karyoplast (nucleus and cytoplasm) and cytoplasm (cytoplasm) transplantations have been performed between strains carrying *Mus domesticus* (Md) and *Mus molossinus* (Mm) mitochondria. Embryos containing Mm mitochondria were obtained after four generations of backcrossing pure Mm females to C57Bl/6 (Md) males. Although 97% of the nuclear genes were of Md origin, they contained exclusively Mm mitochondria, as detected by our Southern and PCR protocols for mtDNA. A 1.6 kb segment of mtDNA containing restriction fragment length polymorphism (RFLP) for Md and Mm was amplified from total tail DNA samples of the reconstituted progeny. PCR primers were based on sequences reported by Bibb *et al.* (1981). Although only one kind of mtDNA was detected in all animals derived from embryos with reconstituted cytoplasm, animals derived from embryos with reconstituted karyoplast showed various degrees of mtDNA heteroplasmy (Fig. 2). Since at least twice the amount of 'foreign' mitochondria is transferred to the host zygote during cytoplasm reconstitution compared with that in karyoplasts, these results suggest that nuclei preferentially replicate mitochondria of their own species. This result can be interpreted as an explanation for the high levels of heteroplasmy in the karyoplast group.

Other experimental approaches have been used for introducing mitochondrial genes into mice. Teratocarcinoma stem cells were successfully used to introduce mtDNA mutants into mouse blastocysts to create mosaic animals (Watanabe *et al.*, 1978). Some degree of mtDNA sequence variation has been

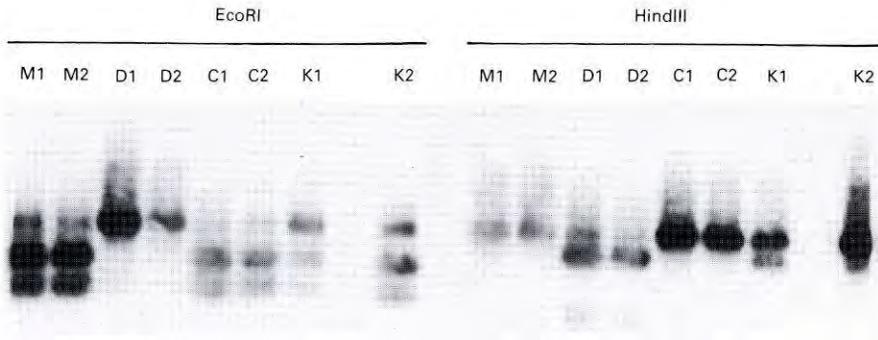


Fig. 2. Analysis of tail mtDNA patterns in control animals and animals derived from embryos with reconstituted cytoplasm and karyoplast. PCR products from the amplification of a 1.64 kb fragment of mtDNA contain the genes for ATPase6, COIII, tRNAGly and the first 80 bases at the 3' end of the ND3 gene. Oligonucleotide primers were designed on the basis of the sequence for *Mus domesticus* (Bibb *et al.*, 1981). Amplified fragments were digested with either EcoRI or HindIII. Digestion products were run on a 1% agarose gel and transferred by Southern blotting to a nylon membrane and hybridized with a clone containing part of the *Mus molossinus* mtDNA (Ebert *et al.*, 1989; Alcivar *et al.*, 1989). M1 and M2: *Mus molossinus*; D1 and D2: *Mus domesticus*; C1 and C2: animals derived from embryos with reconstituted cytoplasm; K1 and K2: animals derived from embryos with reconstituted karyoplast.

observed in bulls derived from a single embryo by nuclear transplantation (Plante *et al.*, 1992). Although it was not possible to trace the polymorphism to the host oocytes used in the reconstitution of embryos, it is likely that, as for mice, the mtDNA from the oocyte will be present in different proportions in many tissues of the cloned adult animals. As these were bulls, germ-line transmission of mtDNA to the progeny is unlikely. However, when cows are produced by cloning, the source of host oocytes should be considered to match beneficial nuclear and cytoplasmic genes. It should be noted, however, that mtDNA heteroplasmy has also been reported under normal conditions in many mammals including cattle (Hauswirth and Laipis, 1985; Ron *et al.*, 1992), rabbits (Hecht, 1990) and *Mus musculus* (Boursot *et al.*, 1987). In dairy cows, heteroplasmy was observed in the mitochondrial ND5 gene and D-loop region in individual animals of one lineage of Holstein cows, and mitochondrial DNA chimaerism was found in heart, liver and brain, suggesting that mtDNA segregation occurs early in embryogenesis (Hauswirth and Laipis, 1985). In Yorkshire pigs, however, hybridization analyses of the mtDNA from liver, testis, blood, brain, ovary, kidney and heart digested with EcoRI and other restriction endonucleases have shown no difference in the mtDNA patterns of the male and female tissues examined (A. A. Alcivar, unpublished results).

Further analysis of the mtDNA content of mice derived from karyoplast transplantations in several organs indicated different levels of heteroplasmy (Fig. 3) (Smith *et al.*, 1992). Using Southern blot analysis of SpeI digested total DNA hybridized with a whole mtDNA mouse probe cloned in our laboratory, all animals analysed contained, at least in a few tissues, mtDNA of both Mm and Md origin. One animal contained Mm mtDNA in all organs and tissues examined (brain, heart, muscle, kidney, liver, spleen, colon, pancreas, bladder and testis) plus a considerable amount of Md mtDNA in brain and spleen. Another animal contained Md mtDNA in all tissues and organs analysed (brain, lung, liver, spleen, colon and bladder) and a small amount of Mm mtDNA could be detected in brain, spleen and colon. Moreover, we determined that one out of four animals derived from cytoplasm reconstituted embryos was found to contain Mm and Md mtDNA in brain and heart, whereas other tissues and organs (muscle, kidney, lung, liver, spleen and stomach) contained only Mm mtDNA at our level of detection (Fig. 4). The results demonstrate that mtDNA from karyoplast and occasionally also from cytoplasm transplanted zygotes proliferates in the embryo throughout development and can be detected in several tissues of the adult animal. The degree of heteroplasmy varies among tissues in both karyoplasts and cytoplasm, which suggests differential partitioning of mitochondria during development. The brain was more commonly

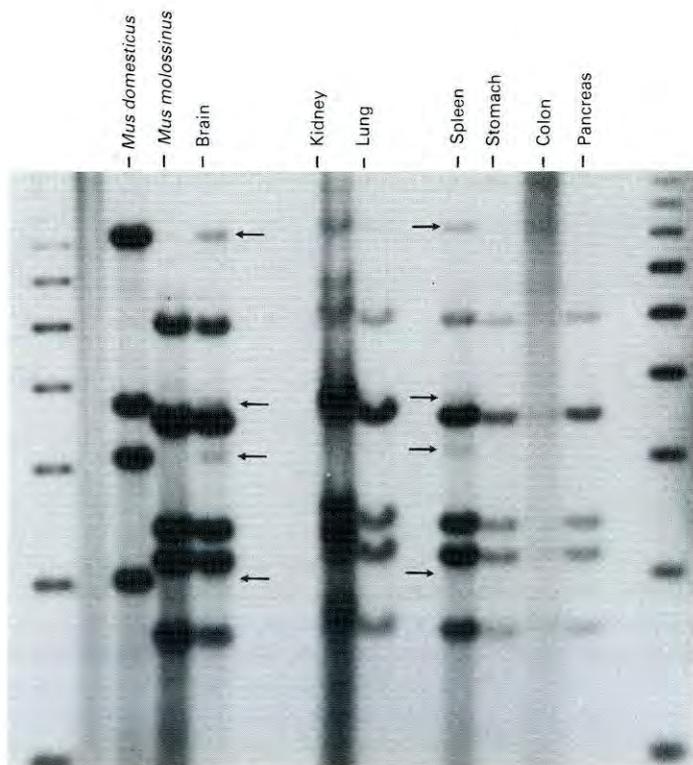


Fig. 3. Analysis of mtDNA content in several tissues from animals derived from embryos with reconstituted karyoplast. This animal was derived from an embryo produced by fusing a *Mus domesticus* karyoplast to an enucleated zygote containing *Mus molossinus* mitochondria. Total DNA was extracted from several tissues and digested overnight with SpeI at 37°C. Digestion products were run on a 1% agarose gel and transferred by Southern blotting to a nylon membrane. The mtDNA was detected by hybridization with a nick-translated probe containing the entire mitochondrial genome extracted from the liver of a New Zealand black mouse (*Mus domesticus*). The mtDNA was cut with BamHI into two fragments and cloned using the vector Bluescript (Promega, Madison, WI). One clone (NZB7) contained a fragment spanning the region from nucleotide 11068 to 3572, whereas a second (NZB10) consisted of nucleotide 3572 to 11068 (nucleotide position based on sequence by Bibb *et al.*, 1981). Arrows indicate RFLP characteristic of *Mus domesticus* mtDNA in a *Mus molossinus* dominated mtDNA RFLP pattern.

heteroplasmic than were other tissues analysed, followed by the heart and spleen which were heteroplasmic in two animals.

Mitochondria During Early Development

Ultrastructural studies have shown that large numbers of mitochondria of differing size and morphology appear during the period from oogenesis to early embryogenesis in mammals (Szöllösi, 1972; Piko and Matsumoto, 1976; Beerman and Hansmann, 1986). Meiotic maturation of the oocyte is accompanied by translocations of mitochondria to the perinuclear region during formation of the meiotic spindle. It has been suggested that this movement enables mitochondrial sequestration to areas that require high

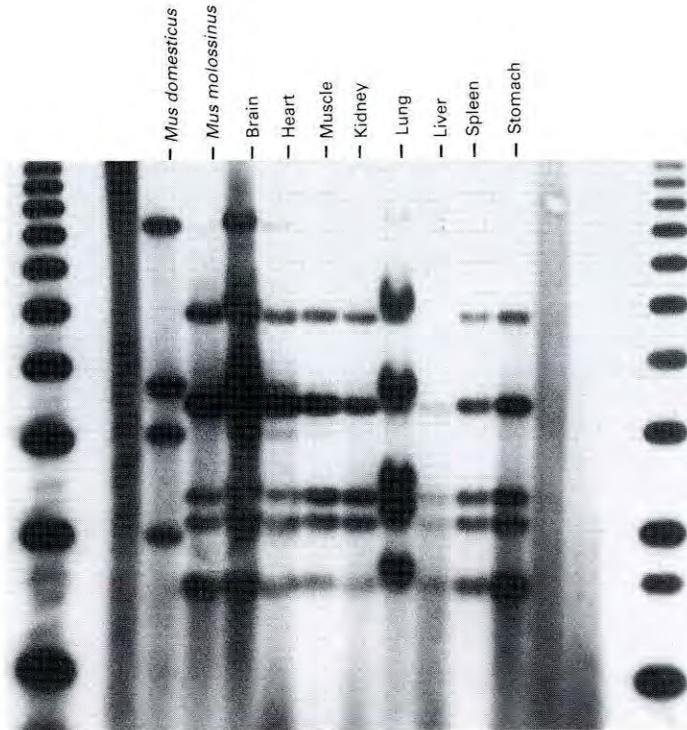


Fig. 4. Analysis of mtDNA patterns in several tissues from animals derived from embryos with reconstituted cytoplasm. This animal was derived from an embryo produced by fusing a *Mus domesticus* cytoplasm to a zygote containing *Mus molossinus* mitochondria. Total DNA was extracted from several tissues and digested overnight with *SpeI* at 37°C. Digestion products were run on a 1% agarose gel and transferred by Southern blotting to a nylon membrane. The mtDNA was detected by hybridization with a nick-translated probe containing the entire mitochondrial genome extracted from the liver of a New Zealand black mouse (*Mus domesticus*). Although *Mus molossinus* mtDNA is present in all tissues, *Mus domesticus* mtDNA sequences can be seen in brain and heart.

concentrations of ATP (Van Blerkom and Runner, 1984). During mouse preimplantation development, mitochondria undergo structural differentiation from a condensed, vacuolated appearance, with concentric cristae in late stage oocytes and early cleavage embryos, to become elongated with more numerous and predominantly transversely arranged cristae during development from the four- to eight-cell stage through the blastocyst stage (Stern *et al.*, 1971; Piko and Matsumoto, 1976; Wassarman and Josefowicz, 1978). In spite of these changes, however, mtDNA replication does not occur during early embryogenesis as the number of mtDNA molecules per embryo remains the same from the one-cell to blastocyst stage (Piko and Taylor, 1987).

In cattle, the number of mitochondria increases more than one hundred times during the period of oocyte growth to reach 136 000 mitochondria in the mature preovulatory oocyte (Hauswirth and Laipis, 1985). This value compares with an average of 92 500 mitochondria found in mouse eggs (Piko and Matsumoto, 1976). The increase in number of mitochondria is proportional to the increase in cytoplasmic volume, which may indicate that oocytes require a fixed amount of functional mitochondria per unit volume of cytoplasm to remain viable. Moreover, the mtDNA copy number appears to correlate with

oocyte volume since the amount of mtDNA per cell increased from about 0.1 pg in primordial cells to 4.5 pg (260 000 molecules) in bovine preovulatory eggs (Hauswirth and Laipis, 1985). The mitochondrial distribution in bovine oocytes has been associated with the hormonal patterns of both gonadotrophins and steroids (Kruip *et al.*, 1983; Hyttel *et al.*, 1986).

In pigs, during the period immediately following germinal vesicle breakdown, the number of mitochondria is reduced by two-thirds with a concomitant and proportional 300% increase in individual mean mitochondrial volume (Cran, 1985). Whereas some degeneration takes place during the period, it has been suggested that this decline in the number of mitochondria is due to fusion between mitochondria. Although mitochondria are highly concentrated in the area close to the plasma membrane before germinal vesicle breakdown, no such concentrations are observed immediately before ovulation. However, mitochondria of oocytes, zygotes and early embryos tend to occur in small groups or aggregates, which are not seen in cells of the trophoblast or of the inner cell mass in blastocysts. These mitochondrial aggregates are closely associated with endoplasmic reticulum, lipid granules and large vesicles. A recent report using a three-dimensional reconstruction of mitochondria with the aid of a computer graphics program indicates that most aggregates consist of two or three mitochondria rather than several, and that most are irregular in shape (Krause *et al.*, 1992). It is interesting to note that both biochemical and structural differences have been observed between ovarian mitochondria from follicular and luteal stages of pigs (Campbell *et al.*, 1980). The drastic changes in mitochondria observed during growth and differentiation of pig Graafian follicles were accompanied by differential expression of mitochondrial genes, and were influenced by LH (Campbell *et al.*, 1980; Rowe *et al.*, 1981; Rowe and Bremm, 1989).

In other species, however, mitochondria are dispersed throughout the cells during early development. In mice, mitochondria in normal cleaving embryos *in vitro* and *in vivo* were found to be homogeneously distributed throughout the cytoplasm of the blastomeres during interphase. In embryos in which development is arrested *in vitro*, the mitochondria become progressively aggregated and localized in the perinuclear region and the area of the cytocortex immediately adjacent to the plasma membrane. Specific translocations to the mitotic spindle were observed during cleavage suggesting that mitochondrial migration is a prerequisite for normal cleavage (Muggleton-Harris and Brown, 1988). Similar results were found when we used *Mus domesticus* zygotes; the mitochondria were found closely associated with pronuclei and the surrounding cytoskeleton (Fig. 5b), but addition of cytoskeletal inhibitors gave a pattern of mitochondrial dispersal (Fig. 5d) (L. C. Smith, unpublished data).

These early nucleo-mitochondrial interactions may also be responsible for the incompatibility found in interspecific nuclear transplantations with mouse zygotes (Smith *et al.*, 1991). Zygote-derived exogenous mitochondria injected into zygotes through cytoplasm transplantation did not interfere with the development of embryos to the blastocyst stage. This is not the case with karyoplast-transplanted zygotes. As expected, the transplantation of karyoplasts from the Mm zygotes, which contain a 97% Md nucleus due to four generation backcross to Md males, to Md-enucleated zygotes did not interfere with development. However, Md karyoplasts transplanted to enucleated zygotes containing mitochondria of Mm origin failed to develop normally in many cases suggesting nuclear-mitochondrial incompatibility. Although fewer blastocysts were obtained from this reconstituted group, no apparent difference was found in their ability to undergo successful gestation (L. C. Smith, unpublished). Similar incompatibility has been observed after reconstitutions between *Mus musculus* and *Mus caroli* (Solter *et al.*, 1985). As in the latter case, the inability of these interspecific nuclear transplanted embryos to develop did not result from the simultaneous transfer of membrane or small amount of cytoplasm in the pronuclear karyoplast, as control embryos that received only interspecific membrane-bound cytoplasm did develop to term. Taken together, these results suggest that a threshold proportion of exogenous mitochondria is required to cause this interspecific nuclear-cytoplasmic incompatibility for early embryonic development. It should be noted, however, that in mice and rats, under normal breeding or embryo transfer conditions, differences in mtDNA between donors and recipients do not affect pregnancy rates (Fisher-Lindahl and Burki, 1982; Alcivar and Pineda, 1990). The percentage survival to weaning, however, was significantly affected by the mtDNA composition of the embryo under embryo transfer conditions only, and may be related to differences in the levels of mitochondrial COII and ATPase6 mRNA between type A and B rats (Alcivar and Pineda, 1990; Alcivar *et al.*, 1992).

Cytoplasmic Inheritance, Mitochondria and Performance

The genetic component of performance of domestic animals is generally assumed to be under the control of many additive direct genes each of small effect, and rarely includes other aspects such as dominance, epistatic, and maternal genetic effects. Maternal genetic influences on performance may originate from specific imprinting of maternal nuclear genes or from genes stored in the cytoplasm of the oocyte, that is mtDNA. However, maternal genetic effects may not be transmitted exclusively through DNA but also through cytoplasmic determinants stored in the oocyte that interact with the embryo later in development, that is the *o* allele in amphibians (Brothers, 1976), or environmental effects brought about either by the uterus during gestation or by the postnatal environment, that is milk, which may modify gene expression in the progeny.

Although recent evidence points to the effects of maternal lineages (i.e. cytoplasmic inheritance) on production traits of economic significance in livestock animals (Bell *et al.*, 1985; Hiendleder *et al.*, 1991; Schultz *et al.*, 1992), the effects of cytoplasmic inheritance are not well understood. Bell *et al.* (1985) used 4461 cows in 102 cytoplasmic lineages to show that 2% of the variation in milk yield, 1.8% of the variation in fat yield, and 3.5% of the variation in percentage of fat could be explained by cytoplasmic lineages. In another study, 10% of the variation in milk, fat and protein production and 13% of the variation in economic returns of milk have been attributed to cytoplasmic components (Huizinga *et al.*, 1986). In a recent study of 38 different maternal lineages, variations in the mtDNA were found (Schultz *et al.*, 1992). The effects of the maternal lineages on production and reproduction traits were examined by statistically partitioning nuclear and cytoplasmic effects using 17 of the most common bovine mtDNA polymorphic lineages (Schultz *et al.*, 1992). Cytoplasmic effects also accounted for significant differences in reproductive traits, such as time to conception, which accounted for additional reproductive and health costs (Freeman and Beitz, 1992). Most of the mtDNA changes in cows within different maternal lineages are due to mutations in the D-loop – a non-coding region where transcriptional promoters of both the light and heavy strands of mtDNA are located (Upholt and Dawid, 1977; Hauswirth and Laipis, 1985). In sheep, mtDNA sequence variability also occurs in the D-loop, but changes in other regions of the mtDNA such as those coding for cytochrome *b*, COIII, ATPase6 and ND5 genes also occur (Hiendleder *et al.*, 1991). The types of mtDNA were shown to influence the birth weight and weight of lamb per ewe per birth significantly, but mtDNA affected neither the activity of some mitochondrial enzymes nor oxygen consumption in these animals (Hiendleder *et al.*, 1991).

As mentioned earlier, heterogeneity of mtDNA sequences has been shown among many mammals and there are occasional variations within maternal lineages. The direct effect of the mitochondrial locus on economic traits (mtETL) can be determined by comparing the mean trait values of maternal lineages with differing alleles for a mitochondrial RFLP (mtRFLP) (Ron *et al.*, 1992). Since progeny of a single maternal lineage are expected to have the same mitochondrial allele unless an mtDNA mutation has occurred, it is sufficient to determine the genotype of only a few individuals per lineage and to use the records between lineages to determine the effect of the mtRFLP on the mtETL. A study of dairy cattle using the mtRFLP of 67 maternal lineages divided into four milk production groups demonstrated that the distribution of rare alleles was not random across groups and among lineages, indicating a possible relationship between these polymorphisms and milk production (Ron *et al.*, 1992). The authors concluded that by choosing individuals with extreme phenotypes for genotyping (selective genotyping) and at least ten recorded animals per lineage, an mtDNA locus with an effect of 0.25 standard deviations could be detected by genotyping two to three individuals of each of 120 maternal lineages.

In contrast to the molecular approach, variation in mitochondrial genes affecting productivity may be measured in terms of their effects on cellular energy metabolism. A crossbreeding experiment between German Landrace (GL) and German Yorkshire (GY) pigs was carried out to verify the effects of the cytoplasm on heterosis. A significant positive heterosis and reciprocal-cross difference for lean:fat ratio with greater offspring–parent similarity to the dam than to the sire was observed (Dzapo *et al.*, 1983). This was taken to indicate a probable effect of extrachromosomal inheritance on fat deposition. Whereas a strong heterotic effect was found for reproductive performance (12% more piglets per year), litter sizes were approximately equal between reciprocal crosses. The relationship of the heterotic effect with mitochondria was assessed by metabolic measurements in heart, liver and scrotal tissue at different ages

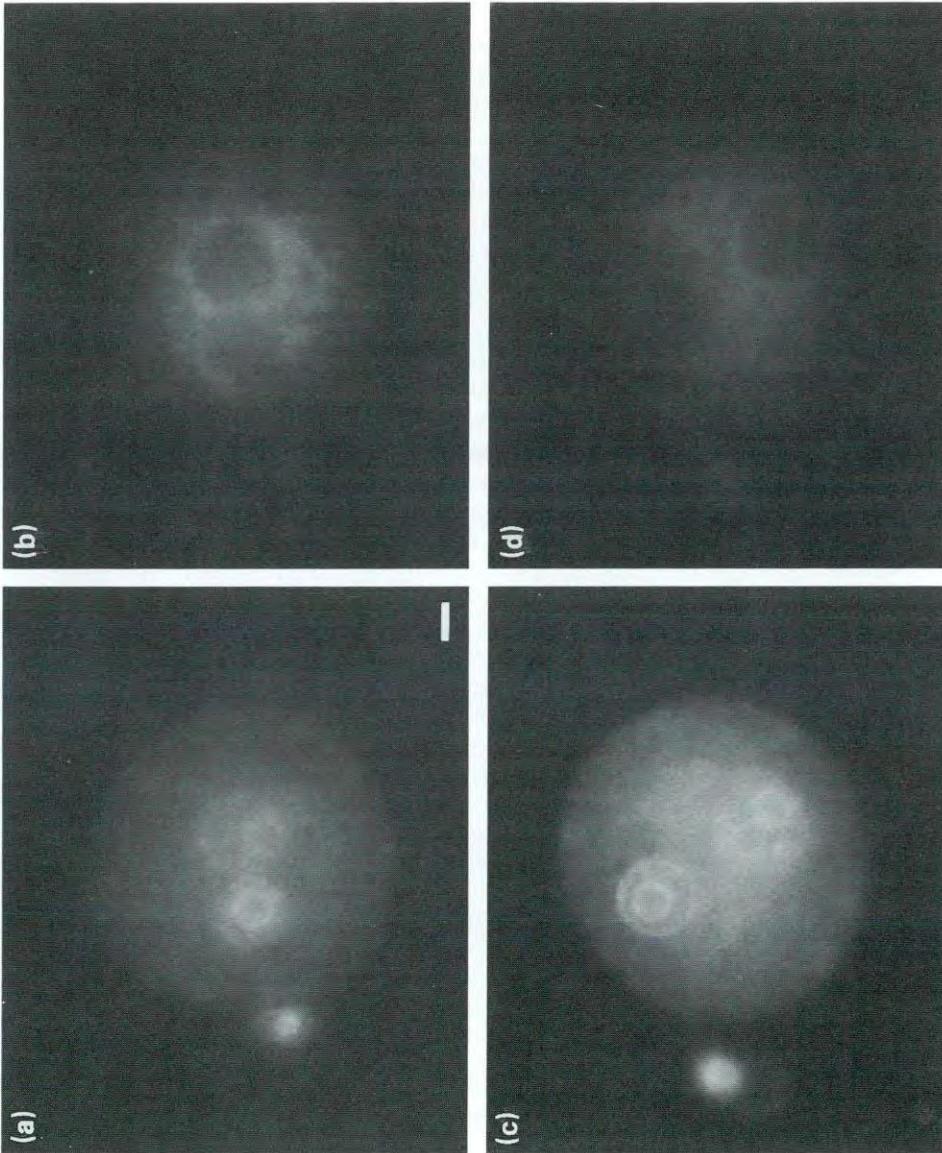


Fig. 5. *Mus domesticus* zygotes stained both with the bisbenzimidazole dye Hoechst 33342 (DNA) and the laser dye Rhodamine 123 (mitochondria) and excited, respectively, at 365 nm and 546 nm wavelength to ascertain the localization of pronuclei and mitochondria, respectively, before and after exposure to cytochalasin D. A control zygote is shown in (a) and (b), whereas (c) and (d) show a zygote exposed to cytochalasin D ($1 \mu\text{g ml}^{-1}$) for 30 min before exposure to these dyes. Mitochondria are closely attached to pronuclei in (b) and dispersed in the cytochalasin-D treated zygote (d). Scale bar represents 10 μm .

(Dzapo and Wassmuth, 1983). Oxygen consumption and oxidative phosphorylation differed significantly between reciprocal crossbreeds in some cases, tending towards the dam breed rather than the sire breed. It was suggested that mitochondrial energy metabolism can act as a metabolic indicator of the interaction of nuclear and mitochondrial genes which result from the crossbreeding and its consequent heterosis. It is proposed that heterosis in mitochondrial energy metabolism may stimulate additional heterosis for carcass fat and reproductive traits (Dzapo and Wassmuth, 1983). Finally, reciprocal crosses showed small to medium heterosis for aerobic enzyme activity in liver mitochondria and large heterosis in enzyme activity in scrotal mitochondria. No heterosis was found for anaerobic energy metabolism. The results clearly showed that oxidative energy metabolism in the mitochondria is one of the most important manifestations of heterosis in cell physiology and that interactions between chromosomal and mitochondrial genes play an important role in the expression of this heterosis (Dzapo and Wassmuth, 1984).

Since the mtDNA genes are either subunits of oxidative phosphorylation enzymes or the structural RNAs necessary for their expression, deleterious mtDNA mutations invariably result in deficiencies in mitochondrial energy metabolism. Several mtDNA mutations that cause human degenerative diseases have been identified in recent years. The mtDNA disease mutations that have been described to date fall into four groups: missense mutations, protein synthesis mutations, insertion-deletion mutations and copy number mutations (Wallace, 1992). These mutations are associated with a broad spectrum of clinical manifestations, including blindness, deafness, dementia, movement disorders, weakness, cardiac failure, diabetes, renal dysfunction and liver disease. Organs and tissues rely differently on oxidative phosphorylation and, therefore, as mitochondrial ATP production declines, it successively falls below the minimum energetic levels necessary for each organ to function normally. This results in a progressive increase in the number and severity of clinical symptoms, and the visual pathway, the central nervous system, muscle, heart, pancreatic islets, kidney and liver are preferentially affected.

More research is needed to determine clearly the role of cytoplasmic genetic systems in humans and domestic animals. With regard to oxidative phosphorylation, detailed molecular studies are needed to understand the regulation of expression of both nuclear and mitochondrial genes involved in this pathway. Specifically, research should be aimed at understanding mitochondrial biogenesis, the fate of paternal mtDNA in early embryos and the segregation, replication and expression of mtDNA during development and growth of livestock animals. This information will have important implications not only for our understanding of mitochondrial genetics and diseases in humans (Shoffner and Wallace, 1990) but for the increased productivity of animals (Schultz *et al.*, 1993). There will also be ramifications to the animal cloning industry. In the latter case, those undertaking the production of female cloned families should be particularly attentive to the source of oocytes used in the protocol, since mtDNA of the oocyte will be permanently incorporated and transmitted to all female progeny. This may be used as a tool to create 'new' breeds of livestock carrying the best of both nuclear and mitochondrial genes or, as a result of inappropriate crossing, the introduction of mutations or undesirable traits. Moreover, after the identification of mtDNA polymorphisms correlated with performance traits, the appropriate sequences could be transfected into mitochondria. Introduction of these mitochondria into embryos will result in transgenic animals at the mtDNA level which would be useful for pharmaceutical or production purposes.

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