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The Fate of Human Sperm-Derived mtDNA in Somatic Cells

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Summary

Inheritance of animal mtDNA is almost exclusively maternal, most likely because sperm-derived mitochondria are actively eliminated from the ovum, either at or soon after fertilization. How such elimination occurs is currently unknown. We asked whether similar behavior could be detected in somatic cells, by following the fate of mitochondria and mtDNAs after entry of human sperm into transformed cells containing mitochondria but lacking endogenous mtDNAs (ρ^0 cells). We found that a high proportion (10%–20%) of cells contained functioning sperm mitochondria soon after sperm entry. However, under selective conditions permitting only the survival of cells harboring functional mtDNAs, only $\sim 1/10^5$ cells containing sperm mitochondria survived and proliferated. These data imply that mitochondria in sperm can enter somatic cells relatively easily, but they also suggest that mechanisms exist to eliminate sperm-derived mtDNA from somatic cells, mechanisms perhaps similar to those presumed to operate in the fertilized oocyte.

Introduction

In most species in the animal kingdom, mtDNA is maternally inherited (Birky 1995). Morphological studies in rodents (Hiraoka and Hirao 1988; Shalgi et al. 1994) and humans (Ankel-Simons and Cummins 1996) have shown that sperm mitochondria enter the egg during fertilization but that they appear to be lost early in embryogenesis, soon after fertilization, between the two-cell and four-cell stages (Szollosi 1965; Hiraoka and Hirao 1988; Shalgi et al. 1994). In agreement with the morphological data, genetic analyses in several species have demonstrated that paternal mtDNA is completely absent in the offspring (Hutchison et al. 1974; Giles et al. 1980; Kaneda et al. 1995). The elimination of paternal mtDNA in mammals appears to occur only in matings between individuals of the same species, because studies in mice have demonstrated that interspecific hybrids retain PCR-detectable amounts of paternal mtDNA throughout development, from the pronucleus stage to the neonatal stage, whereas intraspecific hybrids lose the paternal mtDNA early in embryogenesis (Gyllensten et al. 1991; Kaneda et al. 1995). Birky (1995) has proposed different mechanisms—both active and stochastic—of uniparental inheritance of mtDNA, occurring either before fertilization, at fertilization, or after the formation of the zygote, but the exact mechanisms remain unidentified.

We were interested in determining whether the loss of sperm-derived mtDNA is an event that occurs not only in the fertilized oocyte but also in somatic cells into which sperm mitochondria had been introduced. Confirmation of the loss of sperm mtDNAs in such "transfected" somatic cells would suggest (but, of course, not prove) that mechanisms to eliminate paternal mtDNAs are not limited exclusively to oocytes. This information would not only be useful in the understanding of the process underlying uniparental mtDNA inheritance in humans but could also have practical implications for the design of genetic approaches to treat human mitochondrial diseases.

We chose, as the host, human cells containing mitochondria that had been completely depleted of their mtDNA by treatment with ethidium bromide (ρ^0 cells [Desjardins et al. 1986; King and Attardi 1989]). These cells lack a functional respiratory chain, thereby causing them to be dependent on uridine for survival (Morais et al. 1988; King and Attardi 1989). Importantly, ρ^0 cells can be repopulated with exogenous mitochondria (and mtDNAs); these cytoplasmic hybrids, or cybrids, can be selected for by growth in the absence of uridine. It had been reported elsewhere that live sperm penetrate somatic cells very efficiently, when the two cell types simply were mixed in vitro (Bendich et al. 1974; Higgins et al. 1975). We therefore coincubated live human sperm with ρ^0 cells and searched for penetration of host cells by functional sperm mitochondria. We also selected for

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hybrids repopulated by functional sperm mitochondria, by growing "transfected" cells in media lacking uridine.

Material and Methods

Sperm Introduction and Cell Selection

Semen was collected immediately after ejaculation and was kept at room temperature until it was completely liquefied (~20 min), at which point 10 ml of Dulbecco's modified Eagle's medium (DMEM) containing 4.5 mg of glucose/ml and 110 mg of pyruvate/ml were added. The sample was centrifuged at 800 × g for 3 min, and the sperm pellet was washed three times in DMEM. The pellet was resuspended at a density of 6×10^6 cells/ml, in DMEM containing 5% FBS, and was incubated for 30 min at 37°C in 5% CO_2, to induce sperm capacitation. Sperm cells were counted and their motility was estimated by microscopic observation. The 143B ρ^+ parental cell line containing mtDNA and the 143B206 ρ^0 cell line devoid of mtDNA (King and Attardi 1989) were grown in DMEM supplemented with 5% FBS and 50 mg of uridine/ml. Approximately 1×10^6 cells were trypsinized and pelleted. One milliter of sperm suspension was thoroughly mixed with the cell pellet by pipetting, and the cell mixture was incubated for 4 h at 37°C in 5% CO_2. The cells were then plated in a 100-mm dish in DMEM supplemented with 5% FBS and 50 mg of uridine/ml and were incubated overnight at 37°C in 5% CO_2. Control 143B and 143B206 cells without added sperm were also plated. The following day the medium was completely removed and replaced with DMEM (without uridine supplementation) and 5% dialized FBS (i.e., selective medium).

When surviving colonies reached ~3 mm in diameter, independent clones of surviving cells (SC) were removed from the dishes by trypsinization in glass cloning cylinders. The clones were replated in selective media and were grown for histochemical and genetic studies.

For the secondary transfections, SC clones containing sperm-derived mtDNAs were made mtDNA free by exposure of the cells to 50 ng of ethidium bromide/ml (in the presence of uridine) for 7 wk (King 1996). Selected clones of cells were then grown in the presence of ethidium bromide and uridine for 2 wk and were tested for the presence of residual mtDNA by PCR (see below). Ethidium bromide was removed from the media, and seven of these clones, which were unable to survive in uridine-free media and which had no detectable mtDNA (i.e., ρ^0 cells), were employed for a second round of sperm mitochondrial transfer, by use of sperm from a donor different from the one used for the first transfer.

Biochemical Studies

To assay for the efficiency of sperm penetration, an aliquot of the cell/sperm mixture was stained with the vital stain JC-1 (Molecular Probes) ~4 h after incubation, as described elsewhere (Smiley et al. 1991). JC-1 is a mitochondrion-specific aggregation dye that fluoresces differentially on the basis of the mitochondrial-membrane potential (i.e., yellow-orange at the normal "high" potentials and green at abnormally "low" potentials; there is no fluorescence at zero potential [Smiley et al. 1991]).

In a second penetration assay, based on immunohistochemistry, cells were grown on coverslips at the bottom of a six-well dish in DMEM supplemented with 5% FBS and 50 mg of uridine/ml. The medium was removed, and 1 ml of a sperm suspension, prepared as described above, was overlaid on the coverslips, and the dishes were incubated for 4 h at 37°C in 5% CO_2. The sperm were then removed by rinsing the dishes three times with DMEM. Some coverslips were rinsed in PBS and then were fixed for 4 h in a 4% paraformaldehyde solution in PBS containing 0.1% Triton-X, pH 7.2. Cells on the remaining coverslips were grown for an additional 24 or 48 h in DMEM supplemented with uridine before being fixed as above. The cells were immunostained with antibodies to human cytochrome *c* oxidase (COX) subunit II (COX II), as described elsewhere (Tritschler et al. 1991). Control parental 143B and 143B206 cells, without added sperm, were also grown and immunostained under identical conditions.

A LIVE/DEAD™ assay (Molecular Probes) was performed on coverslips according to the manufacturer's protocol, after 4 h of coincubation of 143B206 cells with sperm. Control 143B206 cells without sperm were also tested. The test was repeated 8 and 24 h after the sperm had been rinsed off the dish.

For histochemical staining to detect COX activity, SC were grown on coverslips at the bottom of a six-well dish in selective medium. The coverslips were washed three times in PBS and were stained with diaminobenzidine, as described elsewhere (Seligman et al. 1969), but the procedure was modified by lengthening the incubation at 37°C to 6 h.

Genetic Studies

For DNA analyses, total DNA was extracted from $\sim 1 \times 10^6$ each of both SC and 143B parental cells and from 10×10^6 sperm, as described elsewhere (Zeviani et al. 1988). Total DNA was subjected to PCR amplification using primers spanning the human D-loop mtDNA (5′→3′) at nt 15803 to 15826 and nt 175 to 153 (nomenclature is according to Anderson et al. [1981]), which generated a 942-bp DNA fragment. Direct sequencing of a portion of the PCR product, using a primer located at nt 16000 to 16026 (yielding readable sequence from nt 16110 to nt 16390), was performed by use of an fmol™ DNA sequencing kit (Promega) according to the manufacturer's recommendations. In

order to look for residual mtDNA in ethidium bromide–treated cells, a second region of mtDNA was amplified, by use of primers from nt 3316 to 3334 and nt 3353 to 3333.

For RFLP analyses, the PCR products were submitted to an additional amplification cycle in the presence of [α-^{32}P]dATP. The radiolabeled products were digested with the restriction enzyme *Hae*III and were electrophoresed through a 10% nondenaturing polyacrylamide gel. The gel was transferred to Whatman 3 MM paper, dried, and subjected to autoradiography.

Quantification of mtDNA on Southern blots was performed by hybridization of *Pvu*II-digested total DNA with ^{32}P-labeled probes of both a nuclear-encoded 18S ribosomal DNA fragment and of purified whole human mtDNA, as described elsewhere (Moraes et al. 1991). The intensities of the *Pvu*II-digested mtDNA fragments were quantitated by scanning the membrane in a Phosphorimager (model GS-363; Bio-Rad).

Analysis of chromosomal microsatellite markers by use of simple tandem-repeat elements was performed as described elsewhere (Weber and May 1989). We used primer sets DYS389, DYS391, DXS1003, D2S1326, D4S1625, D11S1985, and D19S247, obtained from the human screening set, version 5 (Research Genetics).

Results

Sperm from human donors were incubated with human 143B206 ρ^0 cells devoid of mtDNA and were plated in selective media (i.e., without uridine). Five separate experiments with sperm from four different donors were performed. In three of these experiments, we identified one SC clone; two of these three clones were obtained with sperm from the same donor. Colonies appeared after 21–30 d of selection. In the other two experiments no colonies appeared. All the cells on the control plates (i.e., ρ^0 cells alone) died in media lacking uridine.

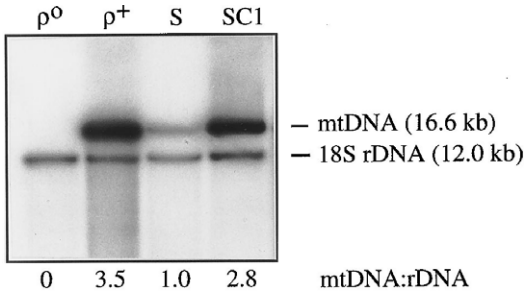

Figure 1 Southern blot analysis. Total DNA isolated from 143B (ρ^+) and 143B206 (ρ^0) parental cell lines, from donor sperm (S), and from clone SC1 were digested with *Pvu*II, electrophoresed through a 0.8% agarose gel, transferred to a nylon membrane, and probed with whole human mtDNA and with a fragment of nuclear 18S rDNA (sizes of hybridizing bands are indicated to the right of the gel). The ratios of the intensities of the two bands, as measured by phosphorimager, are indicated below each lane. Note the absence of mtDNA in the ρ^0 cells.

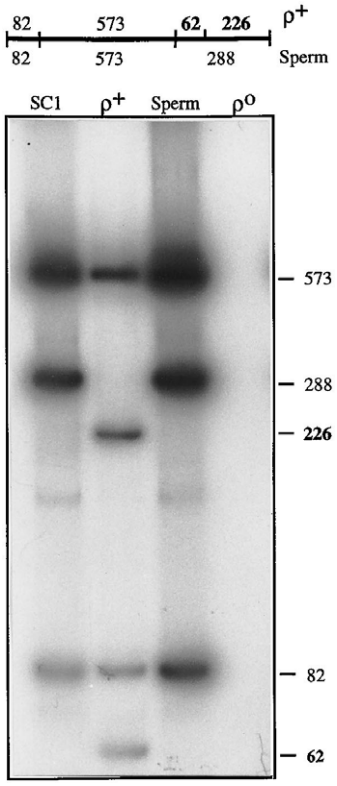

Figure 2 Genotyping of mtDNAs by PCR/RFLP analysis. Total DNA was isolated from 143B (ρ^+), 143B206 (ρ^0), donor sperm, and SC1 cells, and PCR products representing 942 bp of the mtDNA D-loop region (from nt 15803 to nt 175) were amplified and digested with *Hae*III. The *Hae*III cleavage pattern of the 143B and sperm mtDNA is represented schematically, above the gel. Fragment sizes (in bp) are indicated to the right of the gel. The restriction pattern is identical in SC1 and the donor's sperm, whereas it is different in 143B cells, which have an additional *Hae*III restriction site (due to a G→C mutation at nt 16519 [King et al. 1992]) that allows *Hae*III to cleave the 288-bp fragment into two smaller fragments (shown in boldface). As expected, no PCR products were obtained from the ρ^0 cells.

Genetic Tests

We demonstrated that SC contained mtDNA, by Southern blot analysis (fig. 1). Furthermore, the mtDNA copy number (i.e., the amount of mtDNA normalized to the amount of nuclear-encoded 18S rDNA [Moraes et al. 1991]) in SC1 cells (mtDNA:rDNA ratio 2.8) was similar to the amount in 143B cells (ratio 3.5). The content of mtDNA in the sperm (ratio 1.0) was approximately sixfold lower than that in 143B and SC, when the fact that sperm cells are haploid is taken into account. Because there are ~9,000 mtDNA molecules in 143B cells (King and Attardi 1989), we estimated that each sperm contained ~1,500 molecules of mtDNA. The number of mitochondria in human sperm is not known,

Table 1

Nucleotide Polymorphisms in D-Loop (nt 16110–16390)

mtDNA Position	Cambridge Sequence	143B	SC1	SC3	SC4
16240	A	A	T	T	A
16294	C	C	C	C	T
16362	T	T	C	C	T

but the number of mitochondria in bull sperm has been estimated to be ~75 (Bahr and Engler 1970). Use of this value for humans (Ankel-Simons and Cummins 1996) yields a figure of ~20 molecules of mtDNA in each human sperm mitochondrion. This number, although slightly higher than that found in mammalian somatic cells (Robin and Wong 1988; Veltri et al. 1990), is of a comparable order of magnitude.

We performed genetic analyses to determine the origin of the mtDNA and nDNA genotypes in the SC. Genotyping of mtDNA was performed by DNA sequence analyses of a portion of the mtDNA D-loop amplified from SC, 143B parental cells, and sperm. We found three independent polymorphisms that demonstrated that the mtDNA D-loop polymorphism in the SC was identical to that of the donor sperm and was different from that of the 143B parental cells used to generate the 143B206 ρ^0 cells (table 1). Digestion of PCR-amplified D-loop mtDNA with *Hae*III confirmed that the RFLP patterns from SC and sperm were identical, that both were different from that from the 143B parental cells, and that the mtDNA populations in all three cell types were homoplasmic (fig. 2).

Genotyping of nuclear DNA from sperm, 143B206, and SC was performed by detection of microsatellite DNA length polymorphisms, by use of markers for chromosomes 2, 4, 11, 19, X, and Y. These markers showed identical band patterns in 143B206 and SC and showed a different pattern in sperm (fig. 3), demonstrating that 143B206 and SC had the same nuclear genetic background, as expected. The analysis also showed that the 143B cells were "female," since we were able to amplify X-specific, but not Y-specific, micosatellite markers from these cells. Although these data imply that the SC were not a cell line derived from the donor but were derived from 143B206 cells, we cannot exclude the formal possibility that there were chromosomal rearrangements between 143B206 and sperm, since only a small portion of the nuclear genome was investigated. Nevertheless, both the mtDNA and nDNA analyses were consistent with fact that the mtDNA in the SC was derived from the donor sperm and that the nuclear DNA was derived from the 143B206 ρ^0 host cells.

Functional and Biochemical Tests

The presence of a functioning respiratory chain in SC was implied by their growth in medium lacking uridine. Corroboration of this fact was obtained by positive histochemical staining for COX activity in SC1 cells; the intensity of the stain was comparable to that observed in 143B cells (fig. 4).

Assays for Entry of Sperm Mitochondria into Host Cells

In order to estimate the proportion of 143B206 cells penetrated by sperm and to determine whether the mitochondria in those sperm were still functional after entry, we stained the sperm with the aggregation dye JC-1 prior to incubating them with the host ρ^0 cells (fig. 5, *upper panels*). JC-1 stains mitochondria differentially on the basis of their membrane potential (Smiley et al. 1991): mitochondria with a normal (i.e., high) transmembrane potential (e.g., mitochondria in 143B ρ^+ cells) fluoresce orange, whereas mitochondria with an abnormally low membrane potential (e.g., mitochondria in 143B206 ρ^0 cells) fluoresce green. After 4 h of coincubation with JC-1–stained sperm, 10%–20% of 143B206 cells had orange-staining mitochondria within the cytoplasm (fig. 5, *upper right panel*), demonstrating (1) that the mitochondria from the sperm had entered the 143B206 cells and (2) that these mitochondria were functioning, at least by the criterion of high membrane potential.

We followed the fate of sperm-derived mitochondria in ρ^0 cells by immunostaining the population of cells with an antibody to COX II, one of the three mtDNA-encoded subunits of complex IV of the mitochondrial respiratory chain (fig. 5, *lower panels*). 143B ρ^+ cells, but not 143B206 ρ^0 cells, stain positively with this antibody. After 4 h of coincubation with sperm, ~10%–

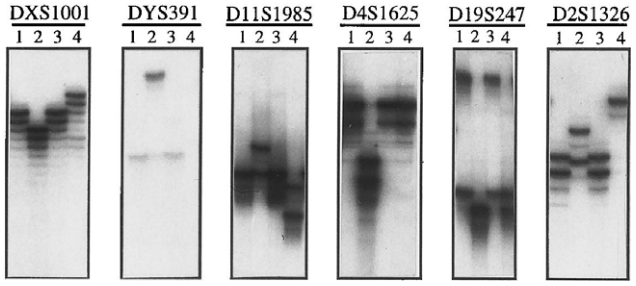

Figure 3 Genotyping of nuclear DNAs by detection of length polymorphisms in chromosomal microsatellite markers. Each set was loaded in the following order: 143B (lane 1), donor sperm (lane 2), SC1 (lane 3), and an unrelated control DNA (blood DNA from a normal control; lane 4). The banding patterns show that the indicated nuclear DNA microsatellite markers in 143B and SC1 were identical and differed from those in donor sperm and control DNA. Note that Y-chromosome marker bands were absent in 143B and SC1, indicating that these cell lines have a "female" karyotype (minimally XO).

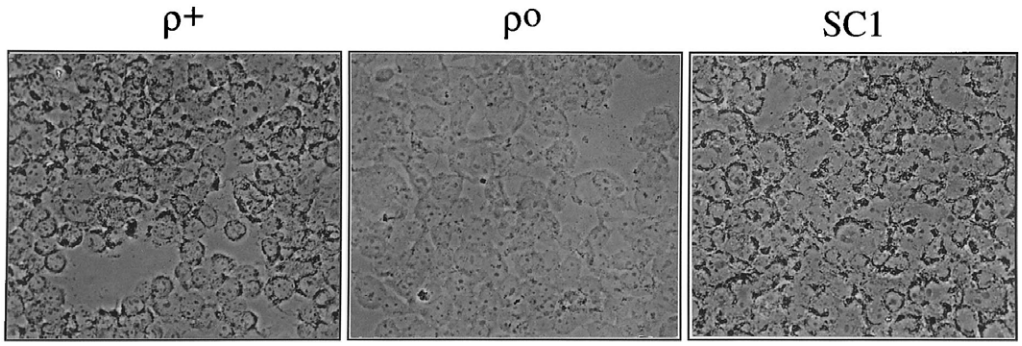

Figure 4 Histochemical staining for cytochrome *c* oxidase (COX) activity. SC1 cells show levels of COX activity similar to those in 143B (ρ^+) cells, indicating repopulation of the cells by functional mtDNA. As expected, 143B206 (ρ^0) cells showed no staining.

20% of the sperm-incubated 143B206 cells showed positive immunofluorescence in the cytoplasm (fig. 5, *lower right panel*), which was in good agreement with the JC-1 data. However, 24 h later, <1% of the cells showed anti–COX II immunostaining, and, after 48 h, only one COX II–positive cell was observed on the coverslip (~1/10^5 cells; data not shown). These results indicate that sperm-derived mitochondria were lost rapidly after entry into cells.

To exclude the possibility that the rapid disappearance of sperm-derived mitochondria in host cells was due to death of those cells that contained sperm, we performed a LIVE/DEAD™ assay at 4, 8, and 24 h after coincubation. We found no evidence that the ρ^0 cells with sperm had a higher incidence of cell death than did control ρ^0 cells without sperm (data not shown).

Secondary Transfers

We performed a secondary transfer of these SC clones with sperm, in order to see whether the frequency of mtDNA repopulation was somehow altered in these rare SC. We therefore treated the SC clones with ethidium bromide, in order to render them ρ^0 once again. Seven such clones devoid of mtDNA, as demonstrated by the absence of an observable mtDNA-derived PCR product, were picked (one clone was from SC1, two were from SC2, and four were from SC4). These clones were then used for a second round of coincubation with sperm. Importantly, the donor sperm used for each secondary transfer were different than those used in the initial transfer. Of the seven secondary transfers, five yielded no surviving clones at all, and the other two (SC1-1 and SC4-1) yielded only a few survivors. As in the first round of transfers, we demonstrated that the mtDNA that repopulated these two SC clones was derived from the donors' sperm (data not shown).

Discussion

We have demonstrated here that the repopulation in vitro of somatic cells by sperm-derived mtDNAs can occur. However, this event was extremely rare, occurring in only $1/10^5$ cells penetrated by sperm mitochondria and grown under the selection conditions specific for intact, functioning mitochondria. COX II immunostaining showed that the sperm mitochondria disappeared rapidly from most of the penetrated cells over a period of 48 h (two cell divisions, on average). These results are similar to those reported by others regarding the fate of sperm organelles after fertilization of oocytes in rodents and cows (Hiraoka and Hirao 1988; Shalgi et al. 1994; Sutovsky et al. 1996).

The mechanism of entry of sperm into oocytes is still not fully understood, and very little is known about the penetration of sperm into somatic cells in vitro. Although it cannot be ruled out that sperm entry into human ρ^0 osteosarcoma cells was via the endocytic-phagocytic pathway, a mechanism involving cell binding and membrane fusion appears to be more likely, for a number of reasons. First, the time scale of endocytic processes (including phagosome-lysosome fusion and digestion) is on the order of minutes (Zucker-Franklin and Hirsch 1964), not hours or days. Second, sperm pentration of human lymphocytes was apparently mediated by cell-surface receptors, since penetration was blocked by preincubation of the cells with antibodies to specific histocompatibility complexes (Ashida and Scofield 1987). In addition, electron-microscopy studies after in vitro penetration of sperm into rodent and human somatic cells indicated that many of the sperm heads and midpieces (containing all the mitochondria) were present free in the cell cytoplasm and were not contained within phagocytic vacuoles (Bendich et al. 1974; Ashida and Scofield 1987). Finally, we observed functionally intact sperm mitochondria in different areas of the cytoplasm after penetration into the ρ^0 cells, suggesting that unwinding of the mitochondrial sheath had occurred, followed by release of the organelles in the cytoplasm (fig. 5); this would be an unlikely event if the sperm were contained within phagosomes.

King and Attardi (1989) found that somatic-cell mito-

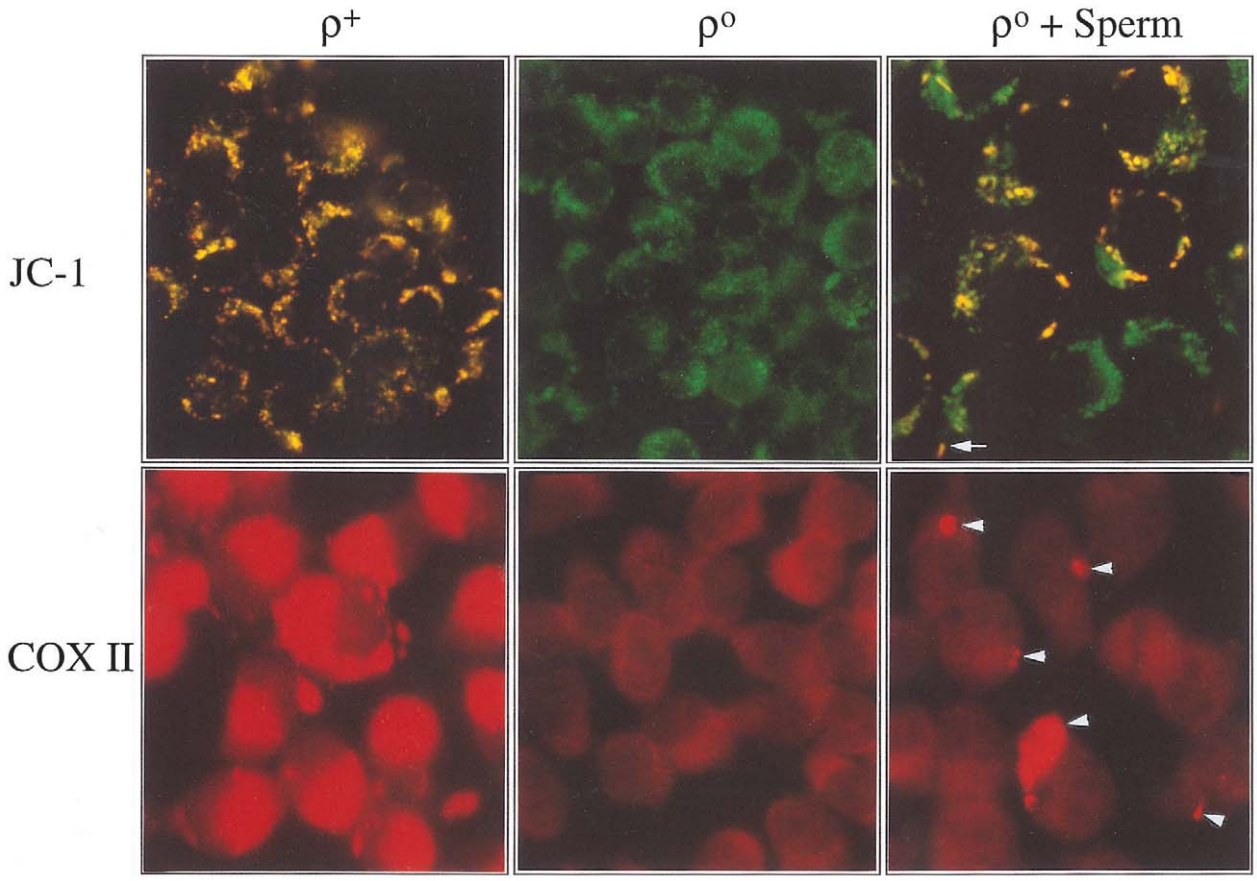

Figure 5 Morphological analyses. *Upper panels,* JC-1 staining. Mitochondria in 143B (ρ^+) cells containing a normal (i.e., high) mitochondrial transmembrane potential, fluoresce orange (*left* [×400]), whereas mitochondria in 143B206 (ρ^0) cells, containing a low mitochondrial transmembrane potential (due to absence of a functioning respiratory chain), fluoresce green (*middle* [×400]). In 143B206 cells 4 h after incubation with sperm, some cells show orange mitochondria within the cytoplasm, indicating that functional sperm mitochondria have entered the cells (*right* [×630]). A free-swimming sperm with orange mitochondria concentrated in the midpiece can be seen (*arrow*). *Lower panels* (×630), Immunohistochemistry with antibody to COX II. 143B cells show positive staining of mitochondria (*left*), whereas 143B206 cells show no staining (*middle*). After 4 h incubation with sperm, some 143B206 cells show positive immunostaining in the cytoplasm (*arrowheads*), indicating that sperm mitochondria have entered the cells (*right*).

chondria microinjected into ρ^0 cells were able to repopulate individual host cells completely with mtDNA, with an efficiency of $\sim 1-3 \times 10^{-3}$, after selection in uridine-lacking medium. Furthermore, repopulation occurred on microinjection of even a single organelle (King and Attardi 1988). In our experiments, using much higher numbers of sperm-derived mitochondria, the frequency of repopulation was $\geq$100-fold lower. Since the time taken for the colonies to appear (10–30 d) was similar to that reported by King and Attardi (1989), the extraordinarily low efficiency of repopulation that we observed is not attributable to a greater time requirement for the establishment of a stable mtDNA population in the host ρ^0 cells when sperm mitochondria are used. On the other hand, the difference in the efficiencies of repopulation might be attributable to the two different routes of entry employed—microinjection versus sperm penetration. In any event, the fate of mtDNAs (and, presumably, of mitochondria as well) derived from sperm is apparently different from the fate of mtDNAs and mitochondria derived from somatic cells, when those mitochondria are introduced into somatic ρ^0 cells. This could be due either to destruction of sperm mitochondria or to impaired replication of sperm mtDNA in the host cells. Our experiments do not allow us to distinguish these possibilities, although we favor the former one.

There is electron-microscopic (Hiraoka and Hirao 1988; Shalgi et al. 1994; Sutovsky et al. 1996) and immunocytochemical (Szollosi 1965; Shalgi et al. 1994; Sutovsky et al. 1996) evidence in rodents and cows that sperm mitochondria are degraded in oocytes very soon after fertilization. In fact, bovine sperm mitochondria are not redistributed between cells in the zygote during the first mitosis; rather, the mitochondrial sheath is passed to only one of the blastomeres, in an essentially intact form, and it disappears by the late four-cell stage

(Sutovsky et al. 1996). If a similar process occurs in somatic cells, the combination of organellar segregation followed by destruction could explain the extremely low number of repopulated clones in our experiments. Destruction of exogenous mitochondria suggests the existence of intracellular mechanisms of self/nonself recognition, leading to elimination of foreign organelles. The persistence of paternal mtDNA in interspecific mouse hybrids might be due to lack of recognition of sperm mitochondria as being foreign, because of the large genetic differences between the parents (Gyllensten et al. 1991), and this notion has been supported by data on the survival of paternal mtDNAs in interspecific, but not in intraspecific, mouse crosses (Kaneda et al. 1995). It has been suggested that proteins specific to sperm mitochondria, such as the cysteine-rich structural proteins of the sperm mitochondrial surface (Hecht and Kennington 1983), might be tagged by ubiquitin in the oocyte cytoplasm and might be eventually destroyed (Sutovsky et al. 1996).

We do not know why there were rare cases of sperm mitochondria surviving after entry into ρ^0 cells. It may be that sperm mitochondria are destroyed only in the context of the spermatozoon itself but that, if an organelle escapes the confines of the sperm prior to destruction, repopulation of the cell by that organelle can occur. Alternatively, perhaps in rare instances a sperm mitochondrion fuses with a ρ^0 cell mitochondrion and transfers its mtDNA, followed by normal mtDNA replication and organellar expansion of the host organelle.

Yet another possibility is that the putative selective mechanisms of organellar destruction or inhibited replication were themselves lost (e.g., via a nuclear gene mutation) in the rare clones in which mtDNA repopulation took place. We tested for this possibility, by secondary transfer of mitochondria: the three repopulated cell lines were treated with ethidium bromide, to completely deplete them of their mtDNA, and the resulting ρ^0 cells were coincubated with sperm from donors different than those used for the first transfers. We expected that, if these putative mechanisms were lost because of one or more nuclear mutations, the number of repopulated clones would be significantly higher in the secondary transfection, approaching the number of cells penetrated (i.e., 10%–20% of the ρ^0 cells used in the experiment). We found, however, that the rate of repopulation of mtDNAs in the secondary transfers was essentially similar to that in the primary transfers.

In conclusion, our data indicate that, at least in the great majority of cases, somatic cells are inimical to the survival of sperm-derived mtDNAs. This process may be analogous to that observed in the early stages of embryonal development, suggesting that the mechanisms responsible for this event are not specific to germline cells.

Acknowledgments

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