The co-evolution of nuclear and mitochondrial genomes in vertebrates led to more than 100 specific interactions that are crucial for an optimized ATP generation. These interactions have been examined by introducing rat mtDNA into mouse cells devoid of mitochondrial DNA (mtDNA). When mtDNA-less cells derived from the common mouse (Mus musculus domesticus) were fused to cytoplasts prepared from Mus musculus, Mus spretus, or rat (Rattus norvegicus), a comparable number of respiring clones could be obtained. Mouse xenonitochondrial cybrids harboring rat mtDNA had a slower growth rate in medium containing galactose as the carbon source, suggesting a defect in oxidative phosphorylation. These clones respirated approximately 50% less than the parental mouse cells or xenonitochondrial cybrids harboring Mus spretus mtDNA. The activities of respiratory complexes I and IV were approximately 50% lower, but mitochondrial protein synthesis was unaffected. The defects in complexes I and IV were associated with decreased steady-state levels of respective subunits suggesting problems in assembly. We also showed that the presence of 10% mouse mtDNA co-existing with rat mtDNA was sufficient to restore respiration to normal levels. Our results suggest that evolutionary distance alone is not a precise predictor of nuclear-mitochondrial interactions as previously suggested for primates.

Nuclear-mitochondrial interactions play a fundamental role in cellular homeostasis. The nuclear genome encodes more than 95% of all proteins located in the mitochondria, whereas only 13 polypeptides (all subunits of the oxidative phosphorylation system, OXPHOS) are encoded by the mitochondrial genome. An optimal interaction between nuclear and mitochondrial encoded factors is essential for transcription and translation of mitochondrial DNA (mtDNA) and also for the correct assembly and function of the OXPHOS system (1).

Various attempts have been made to understand the interactions between the nuclear and mitochondrial genomes and their respective contributions to the expression of different phenotypes such as tumorigenicity (2, 3) and cell differentiation (4, 5). One approach that has facilitated the study of nuclear-mitochondrial interactions is the construction of interspecific hybrids and cybrids. Because the mtDNA sequence divergence is about 5–13 times more rapid than in nuclear DNA (nDNA), a general incompatibility between nuclear and mitochondrially coded gene products is expected between pairs of even recently diverged taxa (6).

Previously, Kenyon and Moraes (7) demonstrated that OXPHOS function of a human cell line lacking mtDNA (p0) could be restored by inserting mitochondria from other humanoid primates including common chimpanzee (Pan troglodytes), pigmy chimpanzee (Pan paniscus), or gorilla (Gorilla gorilla). These studies suggested that at least in primates, mitochondrial/nuclear compatibility has been retained over approximately 5–12 million years (Myr) period. On the other hand, mtDNA from orangutan (Pongo pygmaeus), a species that diverged from the other hominoids more than 12–18 Myr ago, could not functionally replace human mtDNA, suggesting an increase in failed nuclear-mitochondrial interactions. Further studies on human xenonitochondrial cybrids harboring chimpanzee or gorilla mtDNA revealed a 20–30% decrease in oxygen consumption rate and approximately 40% decrease in complex I activity (8). These deficiencies could be attributed to defective interactions between nDNA- and mtDNA-coded complex I subunits (9).

Interspecific hybrids between cells from different rodent species and rodent-human cells have been documented (10–13), and the maintenance of mtDNA has been shown to require an essentially complete set of cognate chromosomes. The retention of both species of mtDNA in mouse-rat and mouse-hamster cell hybrids also has been reported, although it has not been determined whether both mitochondrial genomes are expressed or if there is a selective repression of one mtDNA species (14). Moreover, the uniparental loss of mtDNA has been shown to occur in parallel with chromosomal loss (15–18).

In the present study, we attempted to transfer either rat (Rattus norvegicus) or Mus spretus mtDNA to a Mus musculus domesticus cell devoid of mtDNA (p0). As described under “Discussion,” the evolutionary distance between these species is controversial but is believed to be approximately 10–12 Myr ago between mouse and rat and 1 Myr ago between Mus musculus and M. spretus (19). We could obtain viable xenonitochondrial cybrids in both cases and report the characterization of these cells. We found reduced OXPHOS activity in cybrids harboring rat mtDNA, whereas the OXPHOS was not affected in cybrids harboring M. spretus mtDNA. These observations provide new insights into nuclear DNA-mtDNA interactions by
showing that predictions on the viability and phenotype of xenomitochondrial cells (and possibly interspecifically cloned mammals) do not correlate strictly with evolutionary divergence and will vary depending on the species used.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions**—Mouse (M. musculus domesticus) LM(TK⁺), NIH/3T3, and rat (R. norvegicus) NRK (normal rat kidney) cells were obtained from the American Type Culture Collection (ATCC CCL 1,3, CRL1658, and CRL6509, respectively). M. spretus skin fibroblasts were isolated in our laboratory. Cells were cultured in high glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS) and 100 μg/ml sodium pyruvate.

**Creation and Characterization of Mouse mtDNA-less (ρ⁻) Cell Line**—Mouse LM(TK⁺) cells were treated with 50 ng/ml ditercalcinium (20) for 6 weeks, then with 500 ng/ml ethidium bromide (Sigma) for 2 weeks, and finally 10 ng/ml ditercalcinium for 2 more weeks. Isolated clones were allowed to grow in complete medium for 15 days and then tested for the presence of mtDNA by Southern blot or PCR amplification of total DNA with mouse primers specific for the COXI gene or the D-loop (sequence of primers given below under “Mitochondrial and Nuclear DNA Analysis”). As expected, the isolated clones were also auxotrophic for uridine (21).

**Mitochondrial DNA Transfer in Somatic Cells**—Approximately 10⁶ mouse LM(TK⁺) cells were plated into a 75-cm² flask and treated with 3 μg/ml rhodamine 6G (R-6G) in complete medium for 7 days (23). The R-6G-treated LM(TK⁺) cells were fed with complete medium (without R-6G) 3 h before fusion. Approximately 3 × 10⁵ rat NRK cells were plated on 35-mm culture dish 1 day prior to fusion. Enculturation of NRK, NIH/3T3, and spretus cells and fusion with 1.5 × 10⁵ LM(TK⁺) cells, either ρ⁻ or R-6G treated, were performed as described previously (22). The fusion products were selected in DMEM containing high glucose and supplemented with 10% dialyzed FBS, 100 μg/ml pyruvate, and 100 μg/ml 5-bromo-2′-deoxyuridine (BrdUrd). Cells were fed every 3 days with selective medium. After approximately 2–3 weeks, 10–18 proliferating clones were isolated by ring cloning and cultivated for further characterization.

**Fusions with Rhodamine 6G-treated Cells**—Approximately 10⁶ mouse LM(TK⁺) cells were plated into a 75-cm² flask and treated with 3 μg/ml rhodamine 6G (R-6G) in complete medium for 7 days (23). The R-6G-treated LM(TK⁺) cells were fed with complete medium (without R-6G) 3 h before fusion. Approximately 3 × 10⁵ rat NRK cells were plated on 35-mm culture dish 1 day prior to fusion. Enculturation of NRK cells and fusion with 1.5 × 10⁵ LM(TK⁺) cells were performed as described previously (22). The fusion products were selected in DMEM containing high glucose and supplemented with 10% dialyzed FBS, 100 μg/ml pyruvate, and 100 μg/ml 5-bromo-2′-deoxyuridine (BrdUrd). Cells were fed every 3 days with selective medium. After approximately 2–3 weeks, 10–18 proliferating clones were isolated by ring cloning and cultivated for further characterization.

**Mitochondrial and Nuclear DNA Analyses**—After at least 30 days under selection followed by 20 days without, total DNA was extracted from cybrids following standard procedures (24). For the heteroplasmic line P12, genome DNA was extracted on microcarrier beads and amplification products were selected in DMEM containing high glucose and supplemented with 10% dialyzed FBS, 100 μg/ml pyruvate, and 100 μg/ml 5-bromo-2′-deoxyuridine (BrdUrd). Cells were fed every 3 days with selective medium. After approximately 2–3 weeks, 10–18 proliferating clones were isolated by ring cloning and cultivated for further characterization.

**Mitochondrial Functional Studies**—Mitochondria were isolated by N2 cavitation (27), and assays were performed spectrophotometrically (DU-640 spectrophotometer, Beckman Instruments Inc., Fullerton, CA) as described previously (8). The activities of NADH-decylubiquinone reductase (complex I), succinate decylubiquinone DCPIP reductase (complex II), succinate cytochrome c reductase (complex II + III), and cytochrome c oxidase (complex IV) were determined.

**Mitochondrial Protein Synthesis**—Mitochondrial protein synthesis was determined by pulse-labeling cell cultures in the presence of emetine as described (28). Ninety percent confluent cells were treated with 100 μg/ml emetine for 4 min, followed by pulse-label with 350 μCi of [35S]methionine-[35S]cysteine (EXPRESS, NEN Life Science Products) for 30 min, washed twice with cold phosphate-buffered saline, and immediately collected in a minimum volume of 1% SDS. Approximately 10 g of resolved protein were then subjected to electrophoresis in a 15–20% gradient polyacrylamide gel (29). The gel was fixed in a 30% methanol, 10% acetic acid solution, treated with Fluoro-Enhance (Research Products International), dried, and exposed to an x-ray film at −80 °C for 7 days.

**Immunoblotting**—Mitochondria were isolated by N2 cavitation (27), and 40 μg of proteins were resolved by SDS-polyacrylamide gel electrophoresis on 10% gels and electroblotted onto polyvinylidene membranes (NEN Life Science Products). Blots were blocked with 5% milk and probed with different monoclonal antibodies, anti-complex I, 39-kDa subunit (1:2500 dilution), anti-complex III antibody (1:2000 dilution), anti-cytochrome c oxidase subunit IV (COX IV, 1:500 dilution), anti-flavoprotein of succinate dehydrogenase (1:1000 dilution), and anti-cytochrome c 1.5 μg/ml (PharMingen, San Diego, CA), followed by a secondary anti-mouse antibody conjugated to horseradish peroxidase. Primary antibodies were a gift from Dr. R. Capaldi, University of Oregon. The detection was done using Phototope-horseradish peroxidase Western blot Detection Kit (New England Biolabs, Inc., Beverly, MA). After scanning appropriately exposed autoradiograms, band signals were quantified using NIH Image 1.62 software package.

**Computer and Statistical Analysis**—Comparison between protein and RNA pairs was performed by “BLAST 2 sequences” (29). Experimental data were analyzed using Excell Statistical Package (Microsoft Co.). Results are expressed as mean ± S.D.

**RESULTS**

**Creation of a Mouse ρ⁻ Cell Line**—Mouse LM(TK⁺) cells were treated with ditercalcinium and ethidium bromide as described under “Experimental Procedures.” Selected clones were allowed to recover in medium without intercalating agents for 30 days. No mouse mtDNA could be detected in the house-specific primers were used to amplify either COXI I or D-loop DNA regions using DNA extracted from putative ρ⁻ clones (Fig. 1A). The absence of mtDNA was also confirmed by Southern blot analysis (not shown). In addition, treated cells became auxotrophic for uridine, which is characteristic of mtDNA-less (ρ⁻) cell lines.

**Introduction of Exogenous Mitochondria into M. musculus ρ⁻ Cells**—When the ρ⁻ derivative of LM(TK⁺) cells was fused with
cytoplasts from either rat (NRK), *M. spretus* (fibroblasts), or *M. musculus* (NIH/3T3) similar numbers of uridine-independent clones were observed in all instances (approximately 150–200 uridine-independent clones from 3 x 10^5 enucleated cytoplasts donors). Several clones were isolated from these experiments, and three from each group were characterized in detail as follows: MR1L, MR3L, and MR4L from the LM(TK^2) NRK fusion; MS5L, MS8L, and MS9L from the LM(TK^2) x *spretus* fibroblasts fusion; and MM1L, MM4L, and MM5L from the LM(TK^2) x NIH/3T3 fusion. When analyzed by PCR/RFLP, all clones were found to contain mtDNA from the cytoplast donors. Although, we could not identify any RFLP between LM(TK^2) and NIH/3T3 (both *M. musculus domesticus*), we considered uridine-independent clones from this fusion experiment bona fide cybrids. For detection of potential low levels of LM(TK^2) mtDNA in the cybrids, PCR fragments corresponding to part of COX I gene were labeled, digested with PvuII, and analyzed as described under “Experimental Procedures.” C shows a similar approach for the characterization of mouse xenomitochondrial cybrids harboring *R. norvegicus* mtDNA (MXC^Rn^) mtDNA. Amplons corresponding to a tr-loop region were labeled and digested with Sau3A1 and subjected to polyacrylamide gel electrophoresis and autoradiography. D exemplifies the study of nuclear markers (D5, D7, D8, D17, D19, and DX) by PCR and agarose gel electrophoresis. Primer pairs directed against rat specific markers were used to amplify DNA from mouse, rat, and MXC^Rn^ lines. Note the absence of rat nuclear markers in the MXC^Rn^ lines.

"Growth Features—We examined the growth properties of MXC. Cells with oxidative phosphorylation deficiencies are known to grow poorly in media where glucose is replaced by galactose (30, 31). The respiratory-competent parental cell lines grew exponentially in medium containing glucose as carbon source and they showed a slightly reduced growth rate in galactose-containing medium (Fig. 2). However, all the three MXC^Rn^ grew essentially at identical rates in glucose medium but had a lower doubling time in galactose medium as compared with the parental cells, suggesting an OXPHOS problem (Fig. 2). MXC^Mt^ clones grew similarly to the parental LM(TK^-) in galactose medium (not shown)."
Characterization of Mitochondrial Respiratory Function—
Compared with mouse LM(TK<sup>2</sup>) cells, rat NRK cells showed lower endogenous cell respiration (approximately 31% lower). When cell respiration was measured in MXC<sup>Rn</sup>, a significant decrease in oxygen consumption was observed in all the three clones as compared with the parental cell lines (Fig. 3A). Compared with the mouse parental cell line, clones MR1L, MR3L, and MR4L respired approximately 60, 55, and 50% less, respectively (<i>p</i>, 0.01), and when compared with the rat parental NRK the decrease in cell respiration was approximately 42, 34, and 27%, respectively (<i>p</i>, 0.5). We did not find any significant decrease in cell respiration in MXC<sup>Ms</sup> (Fig. 3B) or in intraspecific transmitochondrial cybrids obtained by fusing mouse r<sup>0</sup> with cytoplasts from NIH/3T3 cells (MTC<sup>Mm</sup>; Fig. 3C).

Analyses of Mitochondrial Protein Synthesis—To ensure that the rat mtDNA was expressed in the MXC<sup>Rn</sup> clones, we analyzed mitochondrial protein synthesis in these cells. Cells were pulse-labeled with [35S]methionine/[35S]cysteine in the presence of the cytoplasmic protein synthesis inhibitor emetine, and mitochondrial polypeptides were resolved by electrophoresis in an exponential gradient polyacrylamide gel (Fig. 5A). The three MXC<sup>Rn</sup> and the two parental cell lines contained comparable levels of bands representing both rat and mouse mitochondrialy synthesized proteins. The small differences in intensity were attributed to differences in the amount of protein loaded in the gel.

Steady-state Levels of Nuclear-coded OXPHOS Proteins—Because it would be difficult to make a direct comparison between mouse and rat immunodetectable mtDNA-coded prod-
ucts in MXC<sup>60</sup> and LM(TK<sup>2</sup>) cells, we studied the levels of polypeptides encoded by the common mouse nuclear genome. Purified mitochondrial fractions were analyzed by Western blots using several antibodies directed against OXPHOS components (Fig. 5, B and C). Steady-state levels of the flavoprotein subunit of complex II as well as core 1 of complex III were similar in all cell lines, with exception of the parental NRK cells that had reduced levels of Fp (Fig. 5, B and C). These reduced levels correlated with reduced SDH activity shown in Fig. 4. COX IV (a subunit of complex IV) and ND39 (a subunit of complex I) were reduced (albeit to a lesser degree) in MXC<sup>60</sup>. On the other hand, the levels of cytochrome c were greatly increased in MXC<sup>60</sup>. Fig. 5D summarizes the ratio of polypeptides that showed variability between clones (i.e., COX IV, ND39, and cytochrome c) to peptides that were essentially unaltered between clones (i.e., Fp of complex II and core 1 of complex III).

**Protective Effect of Small Percentages of Mouse mtDNA in Heteroplasmic MXC Harboruing Predominantly Rat mtDNA**—We fused rat NRK cytoplasts with the original *r* mouse LM(TK<sup>2</sup>) cells that were treated for 7 days with the mitochondrial poison rhodamine-6G (R-6G), a mitochondrial toxin that was previously shown to eliminate endogenous mtDNA during mitophagy. Small varitions in protein content in the different lanes correlated with the radioactive signal. B. Western blot analyses of mitochondri proteins prepared from the indicated cell lines. Antibodies against cytochrome c (cyt c), ND39 (a subunit of complex I), and core 1 (a subunit of complex III) were incubated with the same membrane after consecutive stripplings. C shows a similar experiment using antibodies against the flavoprotein (Fp) subunit of complex II and COX IV (a subunit of complex IV). D illustrates the ratios of the densitometric signals of COX IV, ND39, and cytochrome c to the signals corresponding to Fp or core 1 of complex III.

**FIG. 5. Mitochondrial gene expression and stability of nuclear-coded OXPHOS proteins.** A, indicated cells were pulse-labeled with [35S]methionine/ [35S]cysteine in the presence of the cytoplasmic protein synthesis inhibitor, emetine, as described under “Experimental Procedures.” A human cell line (143B) was also analyzed for reference. The experiment showed that there were no major differences in mitochondrial protein synthesis between MXC<sup>60</sup> and the parental cell lines. Small variations in protein content in the different lanes correlated with the radioactive signal. B, Western blot analyses of mitochondrial proteins prepared from the indicated cell lines. Antibodies against cytochrome c (cyt c), ND39 (a subunit of complex I), and core 1 (a subunit of complex III) were incubated with the same membrane after consecutive stripplings. C shows a similar experiment using antibodies against the flavoprotein (Fp) subunit of complex II and COX IV (a subunit of complex IV). D illustrates the ratios of the densitometric signals of COX IV, ND39, and cytochrome c to the signals corresponding to Fp or core 1 of complex III.

and Southern blot analysis confirmed the presence of both rat and mouse mtDNA in the initial sample (Fig. 6A) and validated the PCR/RFLP “last cycle hot” quantitation shown in Fig. 6B. Cell respiration studies of clone RM44R6G at an early passage showed a significant reduction in oxygen consumption (Fig. 6C). Four weeks after the clone was isolated, the level of oxygen consumption was approximately 40% of that observed in the mouse parental cell line LM(TK<sup>2</sup>). After 8 weeks, cell respiration increased to 65%, and at 16 weeks they respired at indistinguishable levels from the LM(TK<sup>2</sup>) cells (97%). At this time point the activities of the respiratory complexes I, II + III, and IV were also indistinguishable from LM(TK<sup>2</sup>) cells (data not shown). These results show that the presence of approximately 10% mouse mtDNA in a xenoheteroplasmic environment is sufficient to confer a cell with mouse nuclear DNA with the capacity resprine efficiently. We have also attempted to identify recombinant molecules between mouse and rat mtDNA in clone RM44R6G at 16 weeks. The cleavage patterns of mtDNA obtained using four different restriction enzymes, EcoRI (not shown), PvuII, PvuII and XbaI (not shown), showed fragments specific to either parental cell line. No unique fragments differing from either LM(TK<sup>2</sup>) or NRK cells could be detected in clone RM44R6G, suggesting the absence of recombinant or rearranged molecules.

**DISCUSSION**

**Rat mtDNA Can Partially Replace mtDNA Function of Mouse**—The co-evolution of nuclear-mitochondrial DNA interactions and how these interactions optimized oxidative phosphorylation are poorly understood. In order to investigate the mechanism regulating the function of interspecific mtDNA in a mouse nuclear background, we attempted to produce xenochondriochondrial hybrids. Our results showed that *M. spreitus* and rat mtDNA were able to replace *M. musculus* mtDNA and restore (at least partially in the case of rat mtDNA) OXPHOS function. The maintenance and expression of rat mtDNA in a mouse cell implies that the replication (DNA polymerase γ, single-strand
binding proteins, RNases required for producing replication primers, etc.), transcription (RNA polymerase, transcription termination factors, RNA processing enzymes, etc.), and translation (initiation and elongation factors, aminoacyl tRNA synthetases, ribosomal proteins, etc.) machinery are not disturbed by the mtDNA nucleotide changes. On the other hand, the perfect assembly of functional OXPHOS complexes seems to be more sensitive to such changes. MXC<sup>RM4R6G</sup> showed defects in cell respiration and growth in galactose medium, which were caused by partial complex I and IV defects. Complex III, which contains a single mtDNA-coded factor (cytochrome b), was unaffected in MXC<sup>RM4R6G</sup>. This result is not surprising considering the relatively high homology between mouse and rat cytochrome b (see supplemental Fig. 7). Preliminary results from another laboratory (32) also found similar respiratory complex deficiencies in mouse cells with rat mtDNA. It is likely that the proper assembly of complexes I and IV was affected in MXC<sup>RM4R6G</sup>. The reduced steady-state levels of COX IV and ND39 in MXC<sup>RM4R6G</sup> corroborated this hypothesis. The increase in cytochrome c steady-state levels may be due to a compensatory mechanism. We have recently shown that a human cell line lacking mtDNA had a 3.5-fold increase in the steady-state levels of cytochrome c (33).

Low Levels of Self-mtDNA Can Compensate for OXPHOS Defects in MXC—We found a strong correlation between the percentage of mouse mtDNA and oxygen consumption in clone RM44R6G, which had a mouse nuclear background and predominantly rat mtDNA. Initially, when the levels of mouse mtDNA were less than 2%, the cell respiration was approximately 40% of the parental mouse LM(TK<sup>-</sup>) cell line. With time in culture, there was an increase in the levels of mouse mtDNA with a simultaneous increase in the level of cell respiration. If the mouse mtDNA was restricted to only a fraction of cells, the respiration of the total cell population would not return to 100% of controls, indicating that the mouse mtDNA was homogeneously distributed. The activity of the individual complexes of the oxidative phosphorylation system was also normal. It is likely that the small percentage of mouse mtDNA in clone RM44R6G could complement a functional impairment caused by the rat mtDNA, as reported for complementation of human mtDNAs with pathogenic mutations. The percent of mouse mtDNA necessary for restoration of normal respiration (10%) is remarkably similar to the percentage of wild-type mtDNA necessary to complement some pathogenic mtDNA mutations (6–15% (34–36)). Induction of shifts in heteroplasmy by different selective pressure in cells has been described previously (37, 38). We believe that the increase in mouse mtDNA in clone RM44R6G occurred by a similar mechanism (i.e., an increase in respiration accompanied by a small improvement in growth performance of cells harboring higher levels of mouse mtDNA, in a competing environment). However, the mouse mtDNA did not replace completely the rat mtDNA, a somehow surprising finding in a cell containing exclusively mouse chromosomes. It seems likely that the selection for increased amounts of mouse mtDNA came to a halt once heteroplasmic cells achieved normal respiratory function. This phenomenon has been described for heteroplasmic human cells harboring a heteroplasmic ATP6 gene mutation (38).

It has been suggested that the co-existence of different species of mtDNA is detrimental to the survival of the cells (12, 39). Studies with mouse-hamster hybrids showed that in the hybrids that harbored mtDNA from both parents, the synthesis of hamster mtDNA-coded proteins was greatly diminished (40). Our results showed that mouse and rat mitochondrially encoded proteins do not seem to interfere with each other in cells containing exclusively mouse nuclei and predominantly rat mtDNA. This is in agreement with the findings of Hayashi et al. (41) showing that the presence of both mouse and rat mitochondria within a nuclear hybrid cell did not affect their growth properties. However, this feature cannot be generalized to other xenomitochondrial cybrids as it depends on specific alterations. In other words, a single dominant negative mtDNA alteration could cause a functional interference. If the defect in complexes I and IV observed in MXC<sup>RM4R6G</sup> was due to problems with dominant negative protein-protein interactions, it is difficult to envision how 10% mouse mtDNA could compensate the defect, as many complexes would still have a mixture of rat and mouse subunits. It is more likely that mouse mtDNA-encoded polypeptides would assemble preferentially with the nuclear
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coded (mouse) subunits, providing more fully active complexes per cell. Although recombination has been reported between mammalian mtDNA molecules (42, 43), it is thought to be a rare event. Endonuclease digestion patterns with four different enzymes showed that mtDNA of clone RM44R6G was a simple mixture of the two parental mtDNAs indicating that mtDNA recombination did not occur, at least in the region including all the restriction sites tested. Our results are in agreement with other work showing that inter- and intra- species mtDNA recombination does not occur frequently in mammalian cells (44, 45). We still believe that the two types of genomes are in physical proximity because if only 10% of the organelles had mouse mtDNA, respiration probably would not be restored to normal.

Evolutionary Constraints of Nuclear-Mitochondrial Interactions—Genetic divergence between species is the main determinant of the probability of obtaining viable somatic cybrids, presumably because with increased genetic distance there is the potential for an impairment in functional interactions between factors coded by divergent mitochondrial and nuclear genomes. This concept has been elegantly illustrated recently in populations of the aquatic crustacean Tigriopus californicus (46). By performing isofemale backcrossings of genetic isolates from different California coastal regions (i.e. male B × female A = F1 × female A = F2 × female A = F3), they found that the higher the “n” the higher was the activity of cytochrome c oxidase, indicating that COX activity was reduced proportionally to the nuclear contribution of the isolate B. These findings correlated with the relatively high divergence in the mitochondrial coded COX I and the nuclear coded cytochrome c genes observed between the isolates. In primates, functional replacement (although not perfect, see below) is possible in cybrids containing human nuclear DNA and either chimpanzee or gorilla mtDNA, but it is lost with orangutan mtDNA (7). Therefore, beyond 8–12 Myr (47) of evolutionary divergence in primates, interactions between interspecific nuclear and mitochondrial genomes became too inefficient for the restoration of OXPHOS function.

Nuclear-Xenomitochondrial Incompatibilities Cannot Be Predicted Based Solely on Evolutionary Distance—Although one could argue that the evolutionary distance described above (i.e. 8–12 Myr) would be a cut-off for nuclear-mitochondrial minimal functional interactions in different interspecific systems, the present work suggests that functional incompatibilities are species-specific. There are variable rates of evolutionary changes depending on the mitochondrial gene, and these variabilities in substitution rates can also accelerate at any point during evolution (48, 49). For instance, it has been shown that the substitution rate of mitochondrially encoded proteins in mammals is almost an order of magnitude higher than in fish (50). Our previous work with primate xenomitochondrial cybrids showed that human xenomitochondrial cybrids harboring mtDNA from chimpanzee or gorilla had a partial complex I defect (8). Considering nucleotide sequences, the variation between rat and mouse mtDNA is higher than the variation observed between human and gorilla. Although the evolutionary distance between mouse and rat is controversial (51, 52), Chaline and colleagues (53) found that considering the divergence between Rattus and Mus as 10 Myr ago, mitochondrial DNA variation rates of 4.8–9.7% per Myr are observed, which are at least three times more than those in primates (2% per Myr) or other mammal groups. In fact, both nuclear and mitochondrial genes seem to evolve faster in rodents than in most other mammals. Holmes (48) showed that using an uniform molecular clock rate, nuclear coded proteins give an average time of divergence between mouse and rat of 27 Myr ago, whereas the variation between mtDNA coded proteins sets the divergence at 42 Myr ago. Both these estimates are outside reasonable boundaries of 10–15 Myr ago corresponding to fossil dating (52).

In order to parallel and better understand the primate results and the rodent observations, we analyzed the similarities between different mtDNA coded factors in human-gorilla (mtDNA replaceable; complex I deficiency), human-orangutan (mtDNA not replaceable), and mouse-rat (mtDNA replaceable; complexes I and IV deficiencies) pairs (Fig. 7, published as supplementary material on the JBC web site). It is not known why orangutan mtDNA cannot restore OXPHOS function to a human ρ0 cell, but we have preliminary evidence suggesting problems in respiratory complexes assembly. This pairwise comparison showed that overall, mtDNA-coded subunits of complexes III and IV are more similar in the mouse-rat pair than in the human-orangutan and even human-gorilla pair, suggesting that these specific cross-species interactions in the rodent system would probably be functional. Nevertheless, there was a marked decrease in complex IV activity in MXCρ0 that was not observed in the primate system. The fact that COX I is relatively more divergent in the rodent pair and that a single specific evolutionary-related change may affect the efficiency of complex assembly or catalysis may explain this discrepancy. On the other hand, differences between complex I subunits, rRNA and tRNAs are as extensive (or more) in the mouse-rat pair as in the human-orangutan pair. Variations in complex I subunits probably underlie the complex I defect observed. Because translation does not seem to be affected in MXCρ0, it is likely that variations in rRNAs and tRNAs are not responsible for the OXPHOS defect in MXCρ0. In summary, our data showed that functional incompatibilities are not strictly associated with either the evolutionary distance or the overall number of amino acid or nucleotide differences.

Implications for the Creation of Animal Models and Interspecific Cloning—The cellular system described here suggests that a mouse model with complex I and IV deficiencies could be generated. Such a model would be useful by mimicking combined OXPHOS defects commonly seen in patients with mitochondrial disorders (54). Future studies utilizing these cellular models should help identify nuclear-mitochondrial interactions most vulnerable to evolutionary constraints. Results derived from these studies will become increasingly important as interspecific cloning of mammals becomes a reality. Examples of such approaches include the potential production of human stem cells using bovine eggs or rescuing endangered species by interspecific nuclear transfer (55).

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