Isolation of Mitochondrial DNA-less Mouse Cell Lines and Their Application for Trapping Mouse Synaptosomal Mitochondrial DNA with Deletion Mutations*

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For isolation of mouse mtDNA-less (ρ0) cell lines, we searched for various antimitochondrial drugs that were expected to decrease the mtDNA content and found that treatment with ditercalinium, an antitumor bis-intercalating agent, was extremely effective for completely excluding mtDNA in all the mouse cell lines we tested. The resulting ρ0 mouse cells were successfully used for trapping the mtDNA of living nerve cells into dividing cultured cells by fusion of the ρ0 cells with mouse brain synaptosomes, which represent synaptic endings isolated from nerve cells. With neuronal mtDNA obtained, all of the cybrid clones restored mitochondrial translational activity similarly regardless of whether the mtDNA was derived from young or aged mice, thus at least suggesting that defects in mitochondrial genomes are not involved in the age-associated mitochondrial dysfunction observed in the brain of aged mice. Furthermore, we could trap a very small amount of a common 5823-base pair deletion mutant mtDNA (ΔmtDNA5823) that was detectable by polymerase chain reaction in the cybrid clones. As the amount of mutant mtDNA with large scale deletions was expected to increase during prolonged cultivation of the cybrids, these cells should be available for establishment of mice containing the deletion mutant mtDNA.

Intercellular transfer of mtDNA between cultured mammalian cells has been used extensively for studying the contribution of mtDNA or cytoplasmic genetic factors to the expression of various phenotypes such as tumorigenicity (1, 2) and cell differentiation (3, 4). However, this mtDNA transfer system is problematic in that the mtDNA donor cells must be resistant to chloramphenicol for selective isolation of cells with exogenously transferred mtDNA (cybrids) (5, 6). Moreover, chloramphenicol selection and subsequent cultivation in the presence of chloramphenicol could not completely remove endogenous wild-type (chloramphenicol-sensitive) mtDNA in the host cells (7, 8). Therefore, the influence of the remaining host-cell mtDNA on the expression of the phenotypes cannot be excluded completely.

This problem could be overcome by using mtDNA-less (ρ0) cells as host cells. Recently, ρ0 cells were isolated from avian (9) and human (10, 11) cells by treating the cells with EtBr. Cytoplasmic transfer of mtDNA to these cells (particularly to ρ0 human cells) has been used extensively to provide unambiguous evidence of whether accumulation of the candidate mutant mtDNAs found in patients with mitochondrial diseases are responsible for the pathogenesis of the diseases (11–15), whether mtDNA is involved in the expression of tumorigenicity (16), and whether mitochondria fuse with one another and exchange contents (17, 18).

Furthermore, human ρ0 cells were used to study the correlation between aging and mtDNA mutations. Our previous study showed that the age-associated reductions in the activities of mitochondrial translation and cytochrome c oxidase (COX)1 (one of the mitochondrial respiratory chain complexes) observed in fibroblasts from aged subjects were not co-transferred to ρ0 HeLa cells together with their mtDNA, suggesting that mtDNA mutations are not involved in at least the age-associated mitochondrial dysfunction of human fibroblasts (19). On the other hand, it has been reported that mitochondrial dysfunction and accumulation of mtDNA mutations that have been shown to be pathogenic in mitochondrial diseases are associated with aging, particularly in human post-mitotic highly oxidative tissues such as brain and muscles (20–23). Since fresh human tissues with highly oxidative activities are difficult to obtain from healthy subjects, mouse tissues must be used for further investigations of whether the age-associated mitochondrial dysfunction in highly oxidative tissues is due to the accumulation of somatic mtDNA mutations.

We reported previously that a common feature of age-associated changes in both human and mouse mitochondrial respiratory functions is the decrease in mitochondrial translational activity (24); therefore, mouse brain can be used as a model to understand the causes of age-associated mitochondrial dysfunction in non-dividing highly oxidative tissues. Since mtDNA cannot be transferred from any mouse cells or tissues to ρ0 human cells due to the incompatibility between mitochondrial

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The abbreviations used are: COX, cytochrome c oxidase; bp, base pair(s); PCR, polymerase chain reaction; DC, ditercalinium.

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and nuclear genomes of different species (25), ρ0 cell lines must be isolated from mouse cells for further studies. However, exposure of mouse cells to EtBr (which has been used effectively for isolation of ρ0 human cells) frequently induced EtBr-resistant mutant cell lines containing mtDNA as reported previously (26), and there have been no reports of successful isolation of ρ0 cell lines from mouse cells.

In this study, we tested various chemicals that could be expected to decrease the mtDNA content and finally succeeded in isolating ρ0 mouse cell lines. We then obtained cybrid clones with neuronal mtDNA by fusion with brain synaptosomes (which are equivalent to synaptic endings isolated from nerve cells). These cybrids all showed similar mitochondrial translation activity regardless of whether the neuronal mtDNA was imported from young or aged mice, which at least suggests that defects in mitochondrial genomes are not responsible for the age-associated mitochondrial dysfunction observed in the brain of aged mice. Furthermore, we trapped in the cybrid clones a very small amount of a 5823-bp deletion mutant mtDNA (ΔmtDNA8223) that was observed in mouse synaptosomes from all the individuals we tested. No effective system has been established thus far for transfecting artificially mutagenized mammalian mtDNA into mitochondria for isolation of cells with pathogenic mutant mtDNA, but we could trap mutant mtDNA accumulated in synaptosomes into ρ0 mouse cells, and these should be available for isolation of mtDNA-knockout mice.

**EXPERIMENTAL PROCEDURES**

**Cells and Cell Culture**—The mouse myoblast line C2C12 (C2 cells) and mouse fibroblast lines (5P cells derived from the B6mtJ strain and NIH3T3 cells) were grown in normal medium (RPMI 1640 (Nissui Seiyaku, Tokyo) containing 10% fetal calf serum, 50 μg/ml uridine, and 0.1 mg/ml pyruvate). A mouse pancreatic beta cell line (MIN6) was cultivated as described previously (27). The nuclear and mitochondrial genomes of all mouse cells except 5P cells are derived from Mus musculus domesticus. The mitochondrial genomes of 5P cells and the B6mtJ strain are derived from Mus musculus molossinus, whereas their nuclear genomes are from M. m. domesticus.

**Isolation of ρ0 Mouse Cells**—Cells were plated at 1 × 10^5−5 × 10^5 cells/dish, and beginning 24 h after plating they were treated with ditercalium (DC, an antitumor bis-intercalating agent) for 1 month. C2 and NIH3T3 cells were treated with 1.5 μg/ml DC, while MIN6 cells were treated with 56 ng/ml DC. The medium containing the drug was changed every 2 days. After 1 month, colonies growing in the medium were cloned out by the cylinder method. The cloned cells were then cultivated in normal medium without the drug.

**Introduction of Synaptosomal mtDNA into ρ0 Mouse Cells**—The brain of a B6mtJ or B6 strain mouse was washed in phosphate-buffered saline and homogenized in medium containing 0.25 M sucrose, 50 mM Hepes, pH 7.5, and 0.1 mM EDTA in a Teflon-glass Potter-Elvehjem homogenizer. The homogenate was centrifuged at 1000 × g for 10 min at 4 °C, and the resultant supernatant was centrifuged at 17,000 × g for 20 min at 4 °C. The pellet was mixed with 5 × 10^6 ρ2 C2 cells, and fusion was carried out in the presence of 50% (w/v) polyethylene glycol 1500 (Boehringer Mannheim). The fusion mixture was centrifuged and resuspended in selection medium (RPMI 1640 without pyruvate and uridine). On days 14–20 after fusion, the cybrid clones growing in the selection medium were clonally isolated by the cylinder method. Cybrid clones were cultivated in normal medium.

**Southern Blot Analyses of mtDNA**—Total cellular DNA (1–2 μg) extracted from 2 × 10^5 cells was digested with the restriction enzyme XhoI or BamHI (Nippon Gene, Tokyo, Japan), and restriction fragments were separated in 0.6% agarose gel, transferred to a nylon membrane, and hybridized with [α-32P]dATP-labeled mouse mtDNA. The membrane was washed and exposed to an imaging plate for 2 h, and radioactivities of fragments were measured with a bioimaging analyzer (Fuji BAS 2000, Fuji Photo Film, Tokyo, Japan).

**PCR Analyses**—For detection of a small amount of normal mtDNA in ρ0 C2 cells, total cellular DNA (0.5 μg) extracted from 2 × 10^5 ρ2 C2 cells was used as a template. The nucleotide sequences from position 15495 to 15511 and from 15713 to 15697 were used as oligonucleotide primers. The cycle times were 60 s of denaturation at 94 °C, 60 s of annealing at 45 °C, and 120 s of extension at 72 °C for 30 cycles. The products were separated in 4% agarose gel. For detection of large scale deletion mutant mtDNA in synaptosomes and cybrids, PCR was carried out using 0.5 μg of total cellular DNA. The nucleotide sequences from position 7558 to 7581 and from 13666 to 13642 were used as oligonucleotide primers. The cycle times were 30 s of denaturation at 94 °C, 30 s of annealing at 65 °C, and 120 s of extension at 72 °C for 30 cycles. This amplification was repeated using an 0.02 volume of this mixture as a template. The products were separated in 2% agarose gel. In these conditions, only deleted mtDNA was amplified.

**DNA Sequencing of PCR Products**—PCR products purified using a QIAEX II gel extraction kit (Qiagen, Hilden, Germany) were directly sequenced using a [35S]methionine-labeled polypeptides, dried gel was exposed to an imaging plate for 12 h, and the radioactivities of polypeptides were measured with a bioimaging analyzer.

**RESULTS**

**Influence of Antimitochondrial Drugs on the mtDNA Contents of Mouse Cell Lines**—C2 cells of a mouse myoblast cell line were treated with various antimitochondrial drugs, and the mtDNA contents of the cells were then examined by Southern blot analysis. EtBr is effective for isolating ρ0 lines from avian (9) and human (10, 11) cells, but we failed to isolate them from EtBr-treated mouse cells (the copy number of mtDNA in C2 cells did not change substantially (Fig. 1A), whereas its transcription was completely inhibited by EtBr treatment (data not shown), consistent with our previous observations (26)). Similar results were obtained on treatment of C2 cells with rhodamine 6G (29), dideoxycytidine (30), and streptozotocin (31), even though these compounds were shown to be toxic to mito-

**FIG. 1. Screening of antimitochondrial drugs by Southern blot analysis of XhoI fragments for isolation of ρ0 mouse cell lines. A, effect of antimitochondrial drugs on the mtDNA content in the mouse myoblast cell line C2. Mouse C2 cells were treated with EtBr (EB, 100 ng/ml), Rh6G (R6G, 50 μg/ml), dideoxycytidine (ddC, 422 ng/ml), or streptozotocin (STZ, 400 μg/ml) for 1 week; –, C2 cells not treated with the drugs. B, effect of DC on the mtDNA contents in various mouse cell lines. Mouse C2, NIH3T3, and MIN6 cells were cultivated in the presence (DC) or absence (−) of DC for 1 week. C2 and NIH3T3 cells were treated with 1.5 μg/ml DC, whereas MIN6 cells were treated with 56 ng/ml DC.**
For isolation of $\rho^0$ mouse cells, these mouse cell lines were cultivated in the presence of DC for 1 month, and growing colonies were isolated clonally. Southern blot analysis showed no detectable mtDNA in any of these clones. We picked up one clone, named it $\rho^0$ C2, and found that it did not recover mtDNA even after 3 months of cultivation in the absence of DC (Fig. 2A). Then, using the PCR technique, we examined whether a mitochondrial genome derived from $\rho^0$ C2 cells was diluted as much as 1/104 (Fig. 2B). These observations suggest that $\rho^0$ C2 cells are entirely devoid of mtDNA; thus, we had isolated $\rho^0$ cells from the mouse cell line. Moreover, these $\rho^0$ C2 cells required pyruvate and uridine in the medium for growth as do $\rho^0$ avian (9) and $\rho^0$ human cells (10, 11).

The absence of mitochondrial translation activity in $\rho^0$ C2 cells was confirmed by the absence of [35S]methionine incorporation into mitochondrially synthesized polypeptides (Fig. 3B). Moreover, the activity of COX, a mitochondrial respiratory chain complex, was completely lost in $\rho^0$ C2 cells (Fig. 3C). These observations suggest that complete depletion of mtDNA resulted in complete absence of mitochondrial translation, leading to loss of the mitochondrial enzyme activities involved in oxidative phosphorylation.

Characterization of $\rho^0$ C2 Cells with Respect to Acceptance of Synaptosomal mtDNA—Before carrying out the transfer of synaptosomal mtDNA from an aged mouse to $\rho^0$ C2 cells, it was necessary to confirm that the $\rho^0$ C2 cells maintain their abilities to receive and allow replication of exogenously imported mouse mtDNA. Moreover, it was essential to exclude the possibility that mtDNA-repopulated $\rho^0$ C2 cells were not revertant $\rho^0$ C2 cells containing recovered internal C2 mtDNA, which might have remained in such a small amount that it could not be detected by PCR (Fig. 2B). These possibilities were examined by fusion of $\rho^0$ C2 cells with synaptosomes prepared from the brain of a B6mtJ congenic strain; this strain has the nuclear background of $M. m. domesticus$ but has mtDNA derived from a different subspecies, $M. m. molossinus$, whereas C2 cells and most mouse cells established from old inbred strains possess nuclear and mitochondrial genomes derived from $M. m. domesticus$. Fig. 3A shows that $M. m. molossinus$ mtDNA in 5P cells derived from the B6mtJ strain and $M. m. domesticus$ mtDNA in C2 cells can be distinguished by their cleavages with the restriction endonuclease BamHI.

Therefore, synaptosomes were prepared from a 6-week-old B6mtJ congenic mouse, and after their fusion with $\rho^0$ C2 cells in the presence of polyethylene glycol, colonies growing in the selective medium without uridine were isolated as cybrid clones Mol6-1, -2, and -3, cybrid clones isolated by fusion of $\rho^0$ C2 cells with synaptosomes prepared from B6mtJ strain mice (Fig. 3A). These observations suggest that synaptosomal mtDNA from mice of different ages can be used as a template. $M. m. molossinus$, molecular weight standard, 100-bp DNA ladder (Life Technologies, Inc.).

**Fig. 2. Depletion of mtDNA in $\rho^0$ C2 cells.** A, Southern blot analysis of XhoI restriction patterns of mtDNA in C2 and $\rho^0$ C2 cells. PCR amplification of mouse mtDNA in DNA samples prepared from C2 and $\rho^0$ C2 cells. PCR was carried out using oligonucleotide primers with the nucleotide sequences from positions 15495 to 15511 and 15713 to 15697, giving a 218-bp fragment when mouse mtDNA is present in the cells. C, determination of the minimal detectable content of mouse mtDNA in the PCR amplification conditions. DNA prepared from C2 cells was serially diluted with DNA prepared from HeLa cells. 1, $10^{-4}$, $10^{-3}$, and $10^{-2}$ are DNA samples containing 100, 10, 1, 0.1, 0.01, and 0.001% C2 DNA, respectively. For PCR amplification, 0.5 $\mu$g of DNA was used as a template. M, molecular weight standard, 100-bp DNA ladder (Life Technologies, Inc.).

**Fig. 3. Characterization of $\rho^0$ C2 cells with respect to acceptance of synaptosomal mtDNA.** A, C2, C2 cells; 5P, a fibroblast cell line derived from the B6mtJ strain; Mol6-1, -2, and -3, cybrid clones isolated by fusion of $\rho^0$ C2 cells with synaptosomes prepared from B6mtJ strain mice. A, Southern blot analysis of BamHI restriction patterns of mtDNA in the cybrids. The BamHI fragments of 10.24 and 4.9 kilobase pairs are specific to mtDNA of $M. m. molossinus$, whereas those of 8.35 and 6.9 kilobase pairs are specific to mtDNA of $M. m. domesticus$ (2). B, analysis of mitochondrial protein synthesis in the cybrids. After specific [35S]methionine labeling of mitochondrial translation products, proteins in the mitochondrial fraction (15 $\mu$g/lane) were separated by SDS-urea-polyacrylamide gel electrophoresis. C, biochemical analysis of COX activity in the cybrids.
sively for 21 weeks after birth and then gradually decrease with aging (24). Therefore, mtDNAs from synaptosomes of 4-, 22-, and 75-week-old mice, respectively, were introduced into \( \rho^0 \) C2 cells by polyethylene glycol fusion (Table I). The colonies growing in selective medium without uridine were isolated as cybrid clones for determination of the genomes that were responsible for the age-associated decline of mitochondrial translation activity observed in synaptosomes. It is unlikely that we preferentially selected only cybrid clones expressing normal mitochondrial translation function, because cybrid clones with very low mitochondrial translation activity can grow in the same selective medium as used in this study (15).

Southern blot analysis of the XhoI restriction fragment showed that the all cybrid clones contained mtDNA (Fig. 4A), suggesting that synaptosomal mtDNA of non-dividing tissues could be recovered in dividing culture cell lines by the use of \( \rho^0 \) C2 cells. First, we compared the mitochondrial translation activities of cybrid clones with imported mtDNA from synaptosomes of young and aged mice by analyzing \([^{[35S]}\text{methionine}} integration into mitochondrialy synthesized polypeptides. Fig. 4B shows that the amounts of newly synthesized polypeptides encoded by mtDNA were similar when synaptosomal mtDNA was introduced into \( \rho^0 \) C2 cells. Therefore, the abnormalities of mitochondrial respiratory function in the brain of aged mice (24) are not co-transferred with the synaptosomal mtDNA to \( \rho^0 \) C2 cells. This suggests that mtDNA is not involved in the observed age-related dysfunction of mouse brain mitochondria.

Next, we searched for large scale deletion mutations of mtDNA in synaptosomes and in the cybrid clones using PCR techniques. All samples of synaptosomes and some cybrid cells, respectively, was amplified using oligonucleotide primers of native mitochondrial DNA sequences around the region of the deletion break point of mtDNA (Fig. 5A). Sequence analysis of the amplified fragment from the cybrid clone Dom75-4 showed that the common deletion was 5823 bp long with a break point from nucleotide positions 7819 within the \( \text{ATP8} \) gene to 13641 within the \( \text{ND6} \) gene and that the deletion was flanked by a 5-bp direct repeat (TACCC) (Fig. 5B). Therefore, we can trap the mouse somatic mutant mtDNA with a common 5823-bp deletion (\( \Delta \text{mtDNA}^{5823} \)) into the cybrid clones, and its amount can be expected to increase during prolonged cultivation.

**DISCUSSION**

There are no previous reports on successful isolation of \( \rho^0 \) lines from mouse cells, probably because exposure of mouse cells to EtBr, which has been effectively used for isolation of \( \rho^0 \) lines from yeast, avian, and human cells (33), induced EtBr-resistant mutant cell lines containing mtDNA instead of isolating \( \rho^0 \) lines. In this study, by screening drugs that were expected to decrease the mtDNA content, we found that only

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**TABLE I**

<table>
<thead>
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<th>mtDNA recipient</th>
<th>Combination by fusion</th>
<th>mtDNA type</th>
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</thead>
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<tr>
<td>( \rho^0 )C2 cells</td>
<td>M. m. molossinus</td>
<td>mtDNA-deficient</td>
</tr>
<tr>
<td>M. m. domesticus</td>
<td>M. m. domesticus</td>
<td>mtDNA-deficient</td>
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<tr>
<td>M. m. domesticus</td>
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<td>M. m. domesticus</td>
<td>M. m. domesticus</td>
<td>mtDNA-deficient</td>
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<table>
<thead>
<tr>
<th>mtDNA donors (strain, age)</th>
<th>mtDNA type</th>
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</thead>
<tbody>
<tr>
<td>B6 (6-week-old)</td>
<td>M. m. molossinus</td>
</tr>
<tr>
<td>B6 (2-week-old)</td>
<td>M. m. molossinus</td>
</tr>
<tr>
<td>B6 (4-week-old)</td>
<td>M. m. molossinus</td>
</tr>
<tr>
<td>B6 (75-week-old)</td>
<td>M. m. molossinus</td>
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</table>

**FIG. 4.** Characterization of cybrids with synaptosomal mtDNA from young and aged mice. Dom4-1 and -2 are cybrid clones with mtDNA from a 4-week-old B6 strain mouse; Dom22-1 and -2 are those with mtDNA from a 22-week-old B6 strain mouse; Dom75-1, -2, -3, and -4 are those with mtDNA from a 75-week-old B6 strain mouse (cf. Table I). A, Southern blot analysis of XhoI restriction patterns of mtDNA in the cybrids. B, analysis of mitochondrial protein synthesis in the cybrids.

**FIG. 5.** Isolation and characterization of \( \Delta \text{mtDNA}^{5823} \). A, screening of mutant mtDNA with large scale deletion mutations in synaptosomes and their cybrid clones by PCR amplification. Total DNA (0.5 \( \mu \)g) prepared from synaptosomes of 4-, 22-, and 75-week-old mice, cybrid clones of Dom4-1, -2, Dom22-1, -2, Dom75-1, -2, -3, -4, C2, and \( \rho^0 \) C2 cells, respectively, was amplified using oligonucleotide primers of nucleotide positions 7558 to 7581 and 13666 to 13642. M, molecular weight standard, 1-kilobase DNA ladder (Life Technologies, Inc.). B, mtDNA sequences around the region of the deletion break point of \( \Delta \text{mtDNA}^{5823} \). Brackets indicate the deletion break point, and the interrupted genes and numbers of nucleotides are shown below the sequences. Underlines and capital letters denote direct repeats.
DC is effective for elimination of mouse mtDNA and isolation of \( \rho^0 \) lines from various mouse cell lines.

Mouse \( \rho^0 \) cells isolated from myoblast C2 cells (\( \rho^0 \) C2 cells) were effective for investigation of the correlation between age-associated mitochondrial dysfunction and accumulation of somatic mtDNA mutations in highly oxidative tissues. Mouse \( \rho^0 \) cells are necessary for study of this problem, since human \( \rho^0 \) cells cannot be used for the following two reasons. One is that fresh human tissues with highly oxidative activities are very difficult to obtain from healthy subjects. The other is that \( \rho^0 \) human cells do not accept mouse mtDNA from any tissues due to incompatibility between mitochondrial and nuclear genomes of different species (25). In this study, neuronal mtDNA was transferred to \( \rho^0 \) C2 cells by their fusion with synaptosomes prepared from the brains of young and aged mice. We obtained several mtDNA-repopulated cybrid clones and examined whether the reduced mitochondrial translation property observed in brain mitochondria of aged mice was co-transferred with mtDNA. Results showed that all cybrid clones with impaired mitochondrial translation activity (Fig. 4B), suggesting that defects in the mitochondrial genome, even if they are present and have progressed with aging, are not responsible for the age-associated mitochondrial dysfunction observed in synaptosomes of aged mice.

It is unlikely that we selected cybrids with normal mitochondrial translation preferentially, because human cybrid clones with very low mitochondrial translation activities due to the predominance of pathogenic mtDNA mutations have been shown to grow in the same selective medium as that used in this study (15). Although our synaptosomal fraction might be contaminated with glial mitochondria, the possibility that we introduced glial mtDNA into \( \rho^0 \) C2 cells was also excluded by the fact that mitochondrial transfer was limited solely to the situation when they were surrounded by an intact cell membrane, as with mitochondria in synaptosomes or in cytoplasts, and mitochondria alone were not imported into cells by the cell fusion techniques. It was also unlikely that nuclear genomes of non-enucleated neuronal cells were introduced into the cybrid clones because the modal chromosome number of the cybrid clones (71) was comparable to that of the recipient \( \rho^0 \) C2 cells.

It has been generally thought that somatic mutations are more likely to accumulate in mtDNA than in nuclear DNA because mtDNA is a target of most mutagens and is always exposed to oxygen-free radicals produced in mitochondria but is not protected by proteins like histones (34). Moreover, accumulation of various somatic mutations in mtDNA and the resultant decline of mitochondrial respiratory function during a lifetime has been proposed to be involved in aging processes. For example, mitochondrial respiratory functions were reported to decrease with aging in highly oxidative tissues, and these processes appeared to be associated with accumulation of pathogenic mtDNA mutations during aging (35–37). However, it was possible that the age-associated accumulation of mtDNA mutations are not necessarily responsible for age-associated mitochondrial dysfunction, since mitochondrial respiratory functions are controlled by both mitochondrial and nuclear genomes and the nuclear genome encodes most mitochondrial proteins including factors necessary for replication and expression of the mitochondrial genome (33, 38, 39). Our study using \( \rho^0 \) C2 cells solved this problem, showing that defects in the mitochondrial genome, if present, are not involved in age-associated mitochondrial dysfunction.

These observations are consistent with our previous observations that the phenotypes of reduced activities of COX and mitochondrial translation observed in fibroblasts from aged subjects were not co-transferred to \( \rho^0 \) HeLa cells together with their mtDNA (19). Therefore, the conclusions from results on human fibroblasts could be extended to non-dividing, highly oxidative tissues.

In this study, we also found that \( \Delta m t D N A \) (commonly observed in mouse synaptosomes, was introduced into some cybrid clones. The trapping of mtDNA with a large scale deletion mutation in synaptosomes into dividing cultured cells will enable us to produce mtDNA-knockout mice. Even though the amount of \( \Delta m t D N A \) in the cybrid clones was very small, it would proliferate faster than the wild-type mtDNA (40) and eventually accumulate during prolonged cultivation as does human deletion mutant mtDNA in the cybrids (11). We are now trying to establish mtDNA-knockout mice using the cybrid clone with \( \Delta m t D N A \); when mitochondria containing mouse \( \Delta m t D N A \) are introduced into fertilized mouse eggs by microinjection, mtDNA-knockout mice should be obtained. These should be useful as models of human mitochondrial diseases, aging, diabetes, and age-related neurological diseases and for studies on the mechanisms of transmission of mutant mtDNA and expression of its pathogenic effects in various tissues.

Mouse \( \rho^0 \) lines were also available for studying the functional consequences of mtDNA depletion in differentiated cells. For example, a \( \rho^0 \) line isolated from the mouse pancreatic beta cell line MIN6 was used for studying the influence of mtDNA depletion and its repopulation on phenotypic expression of glucose-stimulated insulin secretion (27).

REFERENCES

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