Nuclear but Not Mitochondrial Genome Involvement in Human Age-related Mitochondrial Dysfunction

FUNCTIONAL INTEGRITY OF MITOCHONDRIAL DNA FROM AGED SUBJECTS*

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The role of mtDNA and nuclear genome in human aging was examined by their intercellular transfer using skin fibroblasts and mtDNA-less HeLa cells (p0-HeLa cells). We found in vivo age-related reductions in the activity of cytochrome c oxidase in human skin fibroblasts obtained from 16 donors of various ages (0–97 years). The abnormality in mitochondria of the aged donors was not attributable to either decrease in the copy number of mtDNA molecules or increase in the copy number of deletion mutant mtDNA molecules, but to significant decrease in overall polypeptide synthesis in the mitochondria. However, intercellular mtDNA transfer experiments showed that fibroblast mtDNA from elderly donors is functionally intact. By contrast, intercellular transfer of HeLa nuclei to fibroblasts from aged donors restored cytochrome c oxidase activity, suggesting that the age-related phenotype was nuclear recessive. However, during subsequent cultivation of these hybrids, the activity gradually reduced again, associated with gradual chromosome loss. These observations support the idea that accumulation of nuclear recessive somatic mutations, but not mtDNA mutations, is responsible for the in vivo age-related mitochondrial dysfunction observed in human skin fibroblasts.

Aging is a complex biological phenomenon associated with time-related degenerative processes. Recently, accumulation of various somatic mutations in mtDNA during a lifetime and the resultant decline of mitochondrial energy production have been proposed to be involved in aging processes (1–3) and in several degenerative diseases (1, 3). In fact, there are reports that the number of cytochrome c oxidase (COX)-negative fibers increases with age in human muscle (4, 5), and that mitochondrial respiratory function decreases with age in human muscle (6) and liver (7). This age-related decrease in energy production is proposed to be caused by the accumulation of a common mutant mtDNA with a 4977-base pair deletion (AmtDNA4977) in human brain (8), heart (9, 10), and liver (11). The rates of mtDNA transcription in rat brain (12) and translation in rat skeletal muscle (13) are also reported to decrease with age. However, there is as yet no convincing evidence that accumulated mtDNA mutations are causal genetic factors of age-related mitochondrial dysfunction. Moreover, this age-related phenotypic change may be under control of the nuclear genome, because nuclear genes encode most mitochondrial proteins including all the factors involved in expression of the mitochondrial genome (14, 15). This problem can be solved by examining whether mtDNA in cells of aged humans and age-related mitochondrial dysfunction are co-transferred to other cells. Such intercellular mtDNA transfer is technically possible when cultured cells from aged donors are available.

Since the discovery of the intrinsic limit of cell division, i.e. the limit of population doublings of human diploid fibroblasts grown in culture (16), these cells have been used extensively as a model system for studying in vitro cellular aging (17). Goldstein et al. (18) explored the relationship between their limited replicative life span and energy metabolism, using fibroblasts from the same donor at early and late passages, and found that there was no gross deficit in energy metabolism at increased population doubling levels (PDL), i.e. during in vitro aging of cultured human fibroblasts.

On the other hand, in the present study we observed in vivo age-related reduction of COX activity in cultured human skin fibroblasts from 16 different donors of various ages (0–97 years). The abnormality in mitochondria of the aged humans was not attributable to either decrease in the copy number of mtDNA molecules or increase in the copy number of deletion mutant mtDNA molecules, but to a significant decrease in overall polypeptide synthesis in the mitochondria. Intercellular transfer of mtDNA and nuclear genome using p0-HeLa cells, which completely lack mtDNA (19, 20), showed that nuclear genome, but not mtDNA, of elderly donors was responsible for the in vivo age-related down-regulation of mitochondrial energy production found in human skin fibroblasts.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—Normal human skin fibroblasts lines derived from 16 different donors of various ages (0–97 years) were obtained from the Tokyo Metropolitan Institute of Gerontology, Japanese Cancer Research Resources Bank, and Tohoku University School of Medicine (see Fig. 1). p0-HeLa cells, which are resistant to 20 μg/ml 6-thioguanine, and fibroblast lines were grown in glucose-rich medium RPMI 1640 supplemented with pyruvate (0.1 mM) and 10% fetal bovine serum (20).

Intercellular Transfer of Fibroblast mtDNA—Intercellular transfer of mtDNA was carried out as described previously (20) by fusion of enucleated fibroblasts with p0-HeLa cells and hybrid clones were isolated in...
selective medium. Briefly, skin fibroblasts grown on round glass dishes were enucleated by centrifugation (23,000 × g, at 34 °C for 10 min) in the presence of cytochalasin B (Sigma; 10 μg/ml). The resulting cytoplasts were mixed with p²-HeLa cells, and fusion was carried out in the presence of 80% (v/w) polyethylene glycol 1500 (Boehringer Mannheim). The fusion mixture was cultivated in selective medium without glucose (DM170, Kyokuto Kagaku, Tokyo) supplemented with 20 μg 6-thioguanine and 10% fetal bovine serum (20). The residual non-nucleated parental fibroblasts and hybrids between non-nucleated fibroblasts and p²-HeLa cells were completely eliminated with 20 μg 6-thioguanine. Unfused parental p²-HeLa cells were removed by culture in DM170 medium, since they could not grow in the medium without glucose due to the complete absence of mtDNA (20). On day 14 after fusion, hybrid colonies grown in selective medium were harvested and cloned by the cylinder method (21). Cybrids were cultivated in normal medium (RPMI 1640 + pyruvate + 10% fetal bovine serum). As a control, HeLa mtDNA was introduced into p²-HeLa cells by the fusion of p²-HeLa cells with enucleated wild-type HeLa cells, which are sensitive to 6-thioguanine. The cybrids named HeEB cells, i.e. p²-HeLa cells containing mtDNA from wild-type HeLa cells, were isolated in selective medium DM170 with 20 μg 6-thioguanine, in which unfused p²-HeLa cells, wild-type HeLa cells, and their hybrids could not survive.

Intercellular Transfer of HeLa Nuclear Genome—Intercellular transfer of HeLa nuclei to TIG102 fibroblasts from a 97-year-old woman was attained by fusion of TIG102 fibroblasts with p²-HeLa cells using polyethylene glycol 1500 (21). The fusion mixture was selected with hypoxanthine/aminopterin/thymidine medium (Sigma), and colonies grown in the selection medium were cloned as described above.

Chromosome Analysis—The chromosome compositions of cybrid and the hybrid cytochalasin B-sensitive cells were analyzed immediately after cloning using air-dried chromosome preparations as described previously (22). At least 20 metaphase spreads of each clone isolated were counted.

Southern Blot Analysis—Total DNA (5 μg) extracted from 2 × 10⁵ cells was digested with the single-cut restriction enzyme PvuII to estimate the contents of mtDNA in fibroblasts from fetus and elderly donors, or digested with HhaI to determine whether mtDNA of the cybrid was derived from mtDNA of the donor cells. The fragments separated by agarose gel electrophoresis were then transferred to nitrocellulose membranes and hybridized with [α-32P]dCTP-labeled HeLa mtDNA. The membranes were washed and exposed to x-ray film for 1 h at −80 °C. To quantitate the contents of mtDNA of normal size in the fibroblasts, the membranes were exposed to imaging plates (Fuji Film, Tokyo) for 5 min, and radioactivity was measured with a bioimage analyzer, Fujix BAS 2000 (Fuji Film).

PCR Analysis—Total DNA (100 ng/ml) extracted from 2 × 10⁵ cells was digested with the single-cut restriction enzyme PvuII to estimate the contents of mtDNA in fibroblasts from fetus and elderly donors, or digested with HhaI to determine whether mtDNA of the cybrid was derived from mtDNA of the donor cells. The fragments separated by agarose gel electrophoresis were then transferred to nitrocellulose membranes and hybridized with [α-32P]dCTP-labeled HeLa mtDNA. The membranes were washed and exposed to x-ray film for 1 h at −80 °C. To quantitate the contents of mtDNA of normal size in the fibroblasts, the membranes were exposed to imaging plates (Fuji Film, Tokyo) for 5 min, and radioactivity was measured with a bioimage analyzer, Fujix BAS 2000 (Fuji Film).

Analysis of Mitochondrial Translation Products—Mitochondrial translation products were labeled with [35S]methionine as described elsewhere (23) with slight modifications. Briefly, semiconfluent cells in a dish were incubated in methionine-free medium containing 10% fetal bovine serum for 1 h at 37 °C. Then, the cells were labeled with [35S]methionine for 1 h in the presence of ethidium bromide (0.5 μg/ml). The mitochondrial fraction was obtained by homogenization in 0.25 M sucrose, 1 mM EGTA, 10 mM Hepes-NaOH, pH 7.4, followed by differential centrifugation. Proteins in the mitochondrial fraction (50 μg/lane) were separated by SDS-urea-polyacrylamide gel electrophoresis. The dried gel was exposed to an imaging plate for 6 h, and the labeled polypeptides were located with a bioimaging analyzer. mtDNA contents were measured by dot blot hybridization. The mitochondria were lysed in 0.5 M NaOH, 1.5 M NaCl and blotted directly onto a nylon membrane at serial dilutions. Purified human mtDNA was used as a standard. Incorporation of [35S]methionine into the total protein fraction was measured with a liquid scintillation counter.

Analysis of COX Activity—For biochemical analysis, log-phase cells were harvested, and COX activity was measured as the rate of cyanide-sensitive oxidation of reduced cytochrome c as previously described (24). For cytochemical analysis, cells grown on coverslips were fixed with freshly prepared 4% glutaraldehyde, 0.1 M phosphate buffer, pH 7.4, for 10 min, and stained with COX (25) for 2 h at 37 °C.

RESULTS

COX Activities of Normal Skin Fibroblasts Obtained from Donors of Various Ages—in this study, 16 lines of cultured normal human skin fibroblasts isolated from 16 persons of various ages (0–97 years) were used for studying "in vitro aging." First, we compared the COX activities of these fibroblasts and found that the activities decreased gradually as the fibroblast donors' age increased (Fig. 1a). Although the PDL, i.e. in vitro aging levels, of cultured human fibroblasts lines examined were not constant, they did not affect the COX activities (Fig. 1b), consistent with reports by Goldstein et al. (18) and Sun et al. (26). These results suggest that fibroblast lines from donors of various ages provide a good model system for studying in vitro aging.

The above data represent overall COX activities of the cells, not the activities of single cells. To determine whether a small number of fibroblasts, such as from muscle fibers of elderly donors (5), show normal COX activities, we carried out a cytochemical analysis of COX activities at the individual cell level using TIG102 fibroblasts from a 97-year-old woman and TIG3S fibroblasts from a fetus. As shown in Fig. 2, the COX activities in TIG102 fibroblasts were much lower than those in TIG3S fibroblasts, and the decrease was uniform in all the individual cells (Fig. 2b).

Comparison of Total Amounts of mtDNA and Mitochondrial Translation Products in Aged and Fetal Human Fibroblasts—Since three subunits of COX are encoded by mtDNA, the observed age-related reduction of COX activity could be due to reduction of the amount of normal mtDNA or to down-regulation of post-transcriptional activity in the mitochondria. To ex-
amino these possibilities, we first compared the total amounts of mtDNA per cell in TIG102 and TIG3S fibroblasts by Southern blot analysis after digestion of total DNA samples with a single-cut restriction enzyme, PvuII (Fig. 3a). The results showed that there were no large mtDNA deletions in the mtDNA of either the aged subject or the fetus, and that the amount of normal sized mtDNA was slightly increased in TIG102 fibroblasts from the aged donor, consistent with previous observations (27, 28). Thus the age-related decrease of COX activity in fibroblasts (Figs. 1 and 2) is not due to a decrease in the copy number of mtDNA or to the accumulation of large scale deletion mutations in the mtDNA.

Recently, mtDNA molecules with large scale deletion mutations that were not detectable by Southern blot analysis were observed in human brain, muscle, and liver by the PCR technique. Of the large scale deletions, a 4977-base pair deletion of mtDNA was found to accumulate in these organs with increase in age (3). Using the PCR amplification conditions described by Ikebe et al. (8), we examined whether the \( \Delta \text{mtDNA}^{4977} \) was present in aged or fetal fibroblasts. However, no \( \Delta \text{mtDNA}^{4977} \) was detected in DNA samples of TIG102 fibroblasts from the aged donor or fetal TIG3S fibroblast, even under the conditions of PCR amplification that could detect 0.002% of \( \Delta \text{mtDNA}^{4977} \) molecules in control samples (Fig. 3b).

Second, to examine whether mitochondria of aged subjects show down-regulation of post-transcription, we compared the activities for protein synthesis of mitochondria in fibroblasts of the aged subject and the fetus. Mitochondrial translation products were analyzed by SDS-polyacrylamide gel electrophoresis after pulse-labeling the fibroblasts with \( ^{35} \text{S} \)methionine in the presence of emetine, a specific inhibitor of protein synthesis in the cytoplasm. In TIG102 fibroblasts, significant decreases of radioactivity were observed not only in three COX subunits (COI, COII, and COIII) but also in all other polypeptides encoded by mtDNA (Fig. 4). These results suggest that the reduction in \( ^{35} \text{S} \)methionine label incorporated into fibroblasts from aged subjects is the result of a decrease in overall mitochondrial protein synthesis, or the result of an increase in the turnover of mitochondrial translation products due to the lack of nuclear-encoded gene products with which to assemble into the inner membrane protein complexes. Inter cellular Transfers of Fibroblast mtDNA from Aged Subject and Fetus to \( p^0 \)-HeLa Cells—Since subunits of COX are encoded by nuclear and mitochondrial genomes and since mitochondrial protein synthesis is under the control of both genomes, it was necessary to determine which genome was responsible for the age-related down-regulation of mitochondrial protein synthesis and COX activity. For this purpose we used the fetal fibroblast line TIG3S and fibroblast line TIG102 from an old subject as mtDNA donors. \( p^0 \)-HeLa cells, which have no mtDNA and no COX activity (20), were used as mtDNA recipient cells. On transfer of mtDNA from these two types of fibroblasts to \( p^0 \)-HeLa cells, several cybrid clones were obtained, respectively. As a control experiment, we introduced HeLa cell mtDNA into \( p^0 \)-HeLa cells and isolated cybrids (named HeEB) with HeLa mtDNA. Karyotype analysis showed that all the cybrid clones had a modal chromosome number of 50 (range, 47-54), which was the same as that of \( p^0 \)-HeLa cells (50; range 47-52). On the other hand, the restriction patterns with HhaI, which can distinguish HeLa mtDNA from other human mtDNA (27, 28), showed that the mtDNA in all the cybrid clones were derived exclusively from those of the mtDNA donor cells.

Comparison of Mitochondrial Functions of Cybrid Clones with mtDNA of Fibroblasts from Aged and Fetal Donors—The COX activities and mitochondrial protein syntheses of cybrid clones with mtDNA from TIG3S and TIG102 fibroblasts were compared. As shown in Figs. 4 and 5, irrespective of whether the mtDNA population was imported from fibroblasts of aged or fetal donors, all the cybrid clones showed similar activities of COX and mitochondrial protein synthesis to those of cybrid clone HeEB with HeLa mtDNA. Since COX activity was reduced similarly in individual TIG102 fibroblasts (Fig. 2b), these cybrid clones were unlikely to be restricted to those with mtDNA imported from TIG102 fibroblasts with normal COX activity. Accordingly, the phenotype of reduced mitochondrial function observed in fibroblasts of the aged subject was not co transferred to \( p^0 \)-HeLa cells with the mtDNA. Similar results were obtained when mtDNA of fibroblast line TIG106 from another aged subject was introduced into \( p^0 \)-HeLa cells. Moreover, on PCR analysis, no \( \Delta \text{mtDNA}^{4977} \) molecules were found in any cybrid clone (Fig. 3b). As all the cybrid clones share the same nuclear background as HeLa cells, these observations clearly suggest that mtDNA molecules in fibroblasts from the aged subject are functionally intact. Therefore, the age-related mitochondrial dysfunction observed in fibroblasts of old subjects is not due at least to the accumulation of various kinds of somatic mutations in mtDNA.
Functional Integrity of Human mtDNA from Aged Subjects

Fig. 3. **Southern blot and PCR analyses of mtDNA.** *a,* comparison of amounts of mtDNA in TIG3S and TIG102 fibroblasts by Southern blot analysis of *Pvu*II restriction patterns of mtDNA. ρ0, ρ0-HeLa cells; FT, TIG3S fibroblasts from a fetus; Ag, TIG102 fibroblasts from a 97-year-old woman. Total DNA (5 μg/lane) extracted from the cells was analyzed. *b,* screening of Δ- mtDNA in fibroblasts and cybrid clones by PCR amplification. The control DNA sample containing 20% Δ- mtDNA was prepared from a patient with Kearns-Sayre syndrome (kindly provided from Drs. R. Sakuta and I. Nonaka, National Center of Neurology and Psychiatry, Japan) was serially diluted with total DNA prepared from TIG3S fibroblasts to determine the minimal detectable content of Δ- mtDNA in our PCR amplification conditions (see "Experimental Procedures"). PO, 2, 3, 4, 5, and 6 are DNA samples of TIG3S and TIG102 and cybrid clones FT2, Ag1, Ag2, Ag3, and HeEB, respectively. FT2 is a cybrid clone with ρ0-HeLa cells; Control 1, 2, 3, 4, and 5 are DNA samples containing 2, 0.2, 0.02, 0.002, and 0.0002% Δ- mtDNA, respectively; Experimental 1, 2, 3, 4, 5, 6, and 7 are DNA samples of TIG3S and TIG102 and cybrid clones FT2, Ag1, Ag2, and HeEB, respectively. FT2 is a cybrid clone with TIG3S mtDNA; Ag1, Ag2, and Ag3 are cybrid clones with TIG102 mtDNA; HeEB is a cybrid clone with HeLa mtDNA. *Mw,* molecular weight standards (*X174/HincII* digests; Toyobo, Osaka). Fragments of 5750 and 773 base pairs are PCR products amplified from wild-type mtDNA and from Δ- mtDNA, respectively.

Fig. 4. **Analysis of mitochondrial protein synthesis in skin fibroblasts from a human fetus, an aged human subject, and cybrids.** 1, TIG3S fibroblasts; 2, TIG102 fibroblasts; 3, ρ0-HeLa cells; 4, cybrid clone FT2 (ρ0-HeLa cells imported mtDNA from TIG3S fibroblasts); 5, 6, and 7 are cybrid clones Ag1, Ag2, and Ag3 (ρ0-HeLa cells imported mtDNA from TIG102 fibroblasts), respectively; 8, cybrid clone HeEB (ρ0-HeLa cells imported mtDNA from HeLa cells); 9, HeLa cells. After specific [35S]methionine labeling of mitochondrial translation products in the presence of emetine, proteins of the mitochondrial fraction (50 μg/lane) were separated by SDS-urea-polyacrylamide gel electrophoresis. ND6, COI, ND4, Cytb, ND2, ND1, COII, COIII, ATP6, ND3, ATP8, and ND4L are polypeptides assigned to mtDNA genes.

Intercellular Transfer of HeLa Nuclei to Fibroblasts of Aged Subject—Then, to examine whether the reduced COX activity in cultured fibroblasts is inherited in a nuclear-dominant way or a nuclear-recessive way, HeLa nuclei were introduced to TIG102 fibroblasts. Since ρ0-HeLa cells were completely without mtDNA, introduction of HeLa nuclei to the aged fibroblasts was attained simply by the fusion of TIG102 fibroblasts with ρ0-HeLa cells. We subsequently isolated their hybrid clones in the selective medium (cf. "Experimental Procedures") and examined their COX activity. The results showed that the reduced COX activity in the aged fibroblasts recovered completely with the introduction of HeLa nuclei (Fig. 6), suggesting that human age-related mitochondrial dysfunction was nuclear-recessive. Since HeLa nuclei-donor cells, i.e. ρ0-HeLa cells, showed no COX activity (Fig. 6), restoration of COX activity in the hybrids must be the result of cooperation between the imported HeLa nuclear genome and mtDNA of host fibroblasts of the aged subject.

During their subsequent cultivation, however, the hybrids gradually lost COX activity (Fig. 6) in a similar way to the gradual age-related loss of COX activity observed in human skin fibroblasts (Fig. 1). Moreover, the gradual loss of COX...
activity in the hybrids was paralleled by gradual chromosome loss (Fig. 6). Therefore, these observations suggest that nuclear genes of HeLa cells responsible for the restoration of COX activity are located separately on several chromosomes.

DISCUSSION

In this work, we found an in vivo age-related decrease of mitochondrial energy production in cultured human skin fibroblasts obtained from 16 normal donors of various ages (0–97 years) and showed that this phenotype was due to accumulation of nuclear-recessive somatic mutations, and not due to a decrease in the copy number of mtDNA molecules or the accumulation of various kinds of somatic mutations in mtDNA in fibroblasts of aged persons.

A similar decrease of mitochondrial respiratory activity related to donor age has been observed in human muscle (4–6) and liver (7). The amount of the common large scale deletion mutant ΔmtDNA4977, which was first found to accumulate predominantly in skeletal muscles of patients of mitochondrial myopathy (29, 30), was shown to increase with age in human skeletal muscle, liver, and brain (8–11). However, its content in old subjects was very small (0.005–0.1%) compared with that in patients with mitochondrial myopathy (20–90%). Since reduction of COX activity was heterogeneous in muscle fibers of aged subjects (4, 5) and accumulation of ΔmtDNA4977 was also heterogeneous in brain tissues (31, 32), focal accumulation of a small amount of the mutant mtDNA possibly induced age-related mitochondrial dysfunction in limited regions of these organs. It is also possible that, in addition to this common deletion, the accumulation of various other unidentified somatic mutations in the mtDNA population causes the age-related mitochondrial dysfunction (33, 34).

On in vivo aging of normal human skin fibroblasts, however, donor age-related reduction of COX activity was not heterogeneous in individual fibroblasts from the same donors, and transfer of their mtDNA to ρ0-HeLa cells showed that the fibroblast mtDNA in aged donors was functionally intact. Moreover, ΔmtDNA4977 was not detected in any cybrid clones or in mtDNA donor fibroblasts by PCR, which can detect 0.002% of ΔmtDNA4977. Large scale deletion mutant mtDNA molecules presumably do not accumulate in dividing tissues due to selection against the survival of cells containing these deletion mutants (29), but they could be propagated predominantly in blood cells of cases of Pearson syndrome (35) and in cybrid clones isolated by fusion of ρ0-HeLa cells with enucleated fibroblasts from a patient with Kearns-Sayre syndrome (20). Accordingly, it is also unlikely that deletion mutations in mtDNA of fibroblasts from aged subjects were selectively eliminated during the enucleation, cell fusion, and cloning processes. These considerations indicate that accumulation of various kinds of mtDNA somatic mutations, even if it occurs as supposed in aged brain and muscle, is not involved in the donor age-related decrease in mitochondrial respiratory function observed in human skin fibroblasts (Fig. 1a).

The accumulation of somatic mutations in mtDNA is supposed to play a significant role in carcinogenesis (36–39) and aging (1–3) for the following reasons. Mitochondria are highly oxygenic organelles due to their energy production function; mtDNA lacks histones, which protect DNA from mutagenic damage; and mtDNA repair systems are limited. Moreover, this possibility is supported by recent findings that the hydroxyl radical adduct, 8-OH-dG (40), very small amounts of deletion mutations (8–11, 31, 32, 41, 42), and a point mutation (43) accumulate in mtDNA during aging. In fact, mammalian mtDNA evolved 10 times faster than single-copy nuclear DNA (44). On the other hand, many studies have failed to demonstrate accumulation of somatic mutations in mtDNA populations of human individuals. For example, the mtDNA mutation levels within single individuals have been shown to be extremely limited (45, 46), and thus about 1016 mtDNA molecules within an individual are considered to be almost identical to one another (47). Even in the case of the heteroplasmic mtDNA molecules in a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, the mutation was not somatic, and no other mutations were observed among them (48). Bodenteich et al. (49) detected no age-related accumulation of somatic mutations in mtDNA of human retina, even though this tissue is postmitotic and is constantly exposed to UV light in life. Moreover, Monnat and Realy (50) observed no accumulation of somatic mutations in the mtDNA of different tissues within one individual. Furthermore, no somatic mutations were induced in the mtDNA of HeLa cells by treatment with chemical carcinogens (51). However, approaches using mtDNA sequence analysis could not provide direct evidence of whether accumulation of somatic mutations in mtDNA plays a causal role in aging and/or carcinogenesis.

Cytoplasmic mtDNA transfer techniques should resolve this issue by testing whether mtDNA genotypes and the phenotypes related to carcinogenesis or aging can be co-transferred to other cells. These techniques have been used successfully for demonstrating that accumulation of mtDNA mutations is responsible for mitochondrial dysfunction observed in mitochondrial diseases (20, 52–54). These techniques have also shown that mtDNA mutations are not involved in the expression of tumorigenicity in HeLa (27, 55) or rat glioma (56) cells and that the phenotype of carcinogen-induced tumorigenicity expressed in mouse skin fibroblasts is transmitted through the nuclear genome only, not through the mitochondrial genome (57). In this work, intercellular transfer of the mitochondrial genome from fibroblasts of aged subjects to ρ0-HeLa cells provided convincing evidence that the in vivo age-related mitochondrial dysfunction found in human skin fibroblasts is not controlled by the mitochondrial genome.

In contrast, intercellular transfer of HeLa nuclear genome to fibroblasts from an aged donor clearly showed that the reduced COX activity in the aged fibroblasts can be restored by introduction of HeLa nuclear genome, suggesting that human age-
related mitochondrial dysfunction was inherited in a nuclear-recessive way. Moreover, these cells subsequently began to lose the restored COX activity during cultivation in association with gradual chromosome loss. Accordingly, nuclear genes of HeLa cells, which were responsible for the restoration of COX activity, appeared to be localized separately on several chromosomes (cf. Fig. 1). This age-related phenotype appears to be quite different from the age-related mortality phenotype observed in human diploid fibroblasts, where mortality is inherited in a nuclear-dominant way, because hybrids obtained from fusion of normal human fibroblasts with various transformed human cell lines exhibited limited potential for cell division (17, 58).

To extend our conclusion to postmitotic, highly oxidative organs such as the brain and heart, we are now investigating whether these organs also show the age-related mitochondrial dysfunction and whether mtDNA and the phenotype in these organs can be co-transferred to p0-HeLa cells.

REFERENCES