Development and Age-associated Differences in Electron Transport Potential and Consequences for Oxidant Generation*

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We determined the activities of NADH dehydrogenase (ND), succinate dehydrogenase, and cytochrome c oxidase (COX) in 29 skin fibroblast lines established from donors ranging in age from 12 gestational weeks to 94 years. The results of this study demonstrate that all three of the enzyme activities examined are greater in adult-derived fibroblasts than in the fetal cell lines. The ratio of enzyme activities that control electron entry into and exit from the electron transport chain varied directly with lucigenin-detected chemiluminescence (an indicator of \( \text{O}_2^\cdot \) generation) and inversely with \( \text{H}_2\text{O}_2 \) generation. These results indicate a clear difference in the predominant oxidant species generated during fetal and adult stages of life. We also examined the mRNA abundances of different components of the electron transport chain complexes. We observed higher abundances of mitochondrial encoded mRNAs (COX 1 and ND 4) in cell lines established from adults than in fetal cells. No differences in the mRNA abundances of the nuclear encoded sequences (COX 4 and ND 51) were observed in fetal and postnatal-derived lines. Succinate dehydrogenase mRNA abundance was greater in cell lines established from postnatal donors than in fetal cell lines. No significant differences between cell lines established from young and old adults were detected in any of the parameters examined.

Mitochondria are the primary site of aerobic energy production and are thus the major site of generation of reactive oxygen species (ROS), such as oxygen-centered free radicals and peroxides, which are by-products of metabolism (1–4). Estimates of the rate of ROS generation can differ greatly; however, it has been demonstrated repeatedly that the relative rate of oxidant generation increases with age and that this increase correlates strongly with age-associated changes in the cellular redox state (5–8). For example, the rates of superoxide (\( \text{O}_2^\cdot \)) (8–14) and \( \text{H}_2\text{O}_2 \) generation (7, 10, 14–17) increase in the cells of aging organisms, whereas the glutathione concentration declines progressively with advancing age (5, 6, 18, 19). Differences in ROS metabolism also exist between cells obtained from early developmental stages and from postnatal tissues (for review, see Refs. 20 and 21). The activities of enzymic antioxidant defenses tend to be lower in fetal cells than in cells derived from postnatal tissues (22–24); however, the GSH concentration is frequently greater in fetal cells than in cells from adults (20, 24).

Whether these observations reflect differences in the steady-state level of ROS in fetal and postnatal cells is not presently known (20, 21).

The rate of mitochondrial ROS generation in cells is largely dependent on the amounts of autoxidizable respiratory carriers and the redox state of electron carriers (1, 3, 25, 26). All electron carriers located prior to one of low abundance will exhibit a greater propensity to be chemically reduced because of the slowing of electron flux (electron stacking; 1, 16, 25, 27). Thus, either age or development-associated changes in the relative abundance of components of the electron transport chain (ETC) may exert significant effects on ROS generation. A second factor that can alter the rate of electron flow and the rate of cellular oxidant generation is the activity of the enzyme complexes that regulate electron entry and exit from the ETC. For example, an age-dependent decrease in cytochrome c oxidase (COX) activity has been implicated as one possible cause of the rise in ROS generation observed in aging insects (16) and in aging mammalian brains (28).

Changes in the relative proportions of electron carriers are not well studied in human cells. It has been reported that the activity of COX increases in cell cultures as they approach the mid phases of their proliferative lifespan (29). Furthermore, studies reported from this (30) and other laboratories (31) reveal that the mRNA abundance of a mitochondrial encoded subunit of NADH dehydrogenase (ND) increases in cell cultures that have reached advanced proliferative age (cellular senescence). Whether similar changes occur during aging in vivo is presently unknown. It is, however, noteworthy that various pathologies arising from deficiency of complex I (NADH dehydrogenase) have been reported to stimulate \( \text{O}_2^\cdot \) generation in human skin fibroblasts (32).

In this study we investigate the relationship of the activities of mitochondrial enzymes that regulate the rate of electron flow in the ETC and the rate of cellular oxidant generation in 29 human skin fibroblast lines established from donors of different ages. We also determined the mRNA abundances of representative sets of nuclear and mitochondrial...
drial encoded subunits of ND and COX in these lines. Additionally, we determined the mRNA abundance of succinate dehydrogenase (SD), which is encoded entirely by the nuclear genome (33, 34) for a comparison with the nuclear encoded subunits of the mosaic enzymes examined. We have determined previously that the skin fibroblast model, used in this study, maintains the development and age-dependent differences in antioxidant defenses known to exist in numerous organisms and tissues in vivo (22–24).

**EXPERIMENTAL PROCEDURES**

**Materials and Probes—**Unless otherwise stated chemicals used in this study were obtained from Sigma Chemical Co. and were of the highest purity. The probes for human COX subunit 1 (HHCJ–10), subunit 4 (pCOX4a–111), the 51-kDa subunit of NADH dehydrogenase (IB39), and succinate dehydrogenase (HH3F–42) were all obtained from ATCC. The probe for ND subunit 4 (ND4) was isolated previously in the Center for Gerontological Research (30).

**Cell Lines and Culture Procedures—**Human skin fibroblast cultures established from skin samples obtained from fetal (12–20 weeks gestational age), young (17–33 years), and old donors (78–92 years) were obtained from the National Institute on Aging cell repository at the Coriell Institute for Medical Research, Camden, N.J. Most of the cell lines were obtained when they had completed fewer than 10 population doublings. The lines were minimally expanded and harvested at the earliest possible population doubling level. The population doubling level of cultures at harvest has been published elsewhere (23).

When originally obtained none of the lines was known to exhibit genetic defects; however, several of the lines have since been found to exhibit karyotypic abnormalities. Chromosome preparation was present in 18% of the cells in AG04449; 6% of the cells in AG08387 exhibited random chromosome loss; 2% of cells in AG011016 exhibited random chromosome gain, and 2% were tetraploid; 8% of the cells in AG08433 exhibited random chromosome loss, 4% had a 47,XY + karyotype, and AG09602 exhibited a 26%74% mosaic karyotype of 45,X/46,XX. These lines retain the organelle membrane and exhibit limited protective lifespan. All other cell lines used in this study had a normal genotype, *i.e.* 46,XX or 46,XY. Cells were grown in minimum essential medium without antibiotics and supplemented with 2 mM L-glutamine and 10% (v/v) fetal bovine serum according to procedures described previously (35). Cultures were gassed with a mixture of 5% CO2 with the balance air and were grown at 37 °C. When the cultures had reached stationary phase, they were washed twice, refed with fresh medium, and cultured in the Gribbin Laboratories, Inc.), and then incubated at 37 °C for an additional 72 h. This treatment was used to ensure that the cells were in a state of growth arrest. Cultures used for detection of chemiluminescence and H2O2 were maintained in minimum essential medium with 10% fetal bovine serum until the time of assay.

**Northern Blots and Hybridization—**All materials used in preparation of Northern blots were made RNase-free either by baking for 6 h at 170 °C or by pretreatment with 0.2% diethyl pyrocarbonate for 24 h followed by autoclaving. RNA samples were denatured by mixing 2 volumes (v/v) of a denaturing mixture and incubating at 50 °C for 15 min. The denaturing mixture was made by mixing 32.4 μl of glyoxal (Fluka, highest grade available), 3 μl of 1 mM sodium phosphate, pH 6.5, 3 μl of 10% SDS, 10 μl of H2O, and 150 μl of dimethyl sulfoxide. The samples were resolved on 1.5% agarose gels. After electrophoresis, gels were treated with 50 mM NaOH for 30 min and then neutralized with 100 mM Tris, pH 7.5. Materials used beyond this point were not treated with diethyl pyrocarbonate; instead high quality water was used. Gels were transferred to Nytran Plus nylon membrane (prewettet in H2O and soaked in TAE (40 mM Tris acetate, 1 mM EDTA), pH 7.8) using a model TE-42 electrophoretic transfer apparatus (Hoefer Scientific Instruments) in 1 x TAE, 1.5 h at 25 volts. After transfer, the membrane was rinsed in 1 x TAE and allowed to air dry. It was then exposed to a UV transilluminator (Fotoprep I; Fotodyne Inc.) at the high setting for 8 min. The membrane was baked for 1 h at 80 °C in vacuo. Blots were prehybridized for at least 18 h at 45 °C in 50% formamide, 4 x SSPE (150 mM sodium chloride, 10 mM sodium phosphate, 1 mM EDTA), 0.5% BLOTTO, 1% SDS, 300 μg/ml sheared herring sperm DNA, and then hybridized in 50% formamide, 3 x SSPE, 0.5% BLOTTO, 1% SDS, 300 μg/ml sheared denatured herring sperm DNA, and 10% dextran sulfate with 1–4 x 10^6 cpm/ml 32P random primer-labeled (Boehringer Mannheim) probe at 45 °C overnight. Blots were washed in three changes of wash buffer (0.1 x SSC, 0.5% SDS) for 15 min each wash. The blots were then exposed to Kodak XAR film using DuPont Lightning Plus intensifying screens.

**Homogenates—**Postnuclear fractions were prepared by a modification of the method of Sun et al. (29). Cultures were washed twice with PBS, once with 1 x isolation buffer (70 mM sucrose, 220 mM mannitol, 1 mM Tris, pH 7.4). The isolation buffer used for SD activity also contained 7 mM sucrose. Cells were incubated for 4 °C for 5 min in a hypotonic buffer (5 mM Tris, pH 7.4) and then homogenized with seven strokes of a Dounce homogenizer. After homogenization, an equal volume of 2 x isolation buffer was added, and the homogenate was centrifuged at 600 x g for 2 min. The supernatant was collected, and the pellet was resuspended in 0.5 ml of 1 x isolation buffer. The resuspended pellet was centrifuged at 600 x g for 1 min. The supernatants from both fractions were pooled, lyophilized, and debris removed by centrifugation at 3,000 x g.

**Cytchrome c Oxidase Activity—**COX activity was determined by a modification of the method of Sun et al. (29). A 50 μl cytochrome c solution (in 100 mM Tris, pH 7) was reduced with enough 0.1 M dithionite to yield an A600/A550 ratio that was between 6 and 9. The rate of change in A600 of 100 μl of homogenate and 900 μl of reduced cytochrome c at 37 °C was monitored as a measure of COX activity. Specific activity was calculated as nmol/min/mg of protein using an extinction coefficient of ε = 19.2 mm−1 cm−1. Although several groups have reported improved results when detergents were included in the homogenate preparation (28, 36, 37), we observed no benefit from the addition of detergents to our reaction mixture and therefore included none.

**NADH Dehydrogenase Activity—**ND activity was determined by a slight modification of the method of Galante and Hatefi (38). Postnuclear homogenates were mixed with a solution containing 55 mM Tris, pH 8.0, 130 μM 2,6-dichlorodihydrofluorescein, and 750 μM NADH, and the rate of change in A600 was monitored at 37 °C. ND activity was expressed as nmol/min/mg of protein based on the extinction coefficient ε = 21 mm−1 cm−1 for 2,6-dichlorodihydrofluorescein.

**Succinate Dehydrogenase Activity—**SD activity was determined by a modification of the method described by Hatefi and Stiggall (39). Solution I contained 55 mM potassium phosphate buffer, pH 7.4, 0.1 mM EDTA, 0.1% bovine serum albumin, and 40 mM sodium succinate (from a stock that was brought to a pH of 7.3–7.4 with 2 M KOH), and 288 μM 2,6-dichlorodihydrofluorescein. Solution II contained 55 mM potassium phosphate buffer, pH 7.4, 0.1 mM EDTA, 0.1% bovine serum albumin, and 3.25 mM phenazine methosulfate. 200 μl of homogenate was mixed with 0.4 ml of solution I, and 0.4 ml of solution II was then added to start the reaction. After allowing the assay mixture and homogenate to react for 10 min at 37 °C, the change in A600 was monitored at 37 °C. ND activity was calculated as nmol/min/mg of protein using extinction coefficient ε = 21 mm−1 cm−1 for 2,6-dichlorodihydrofluorescein.

**Calculated Chemiluminescent Intensity (CCI)—**For this study, lucigenin (bis-N-methylacridinium nitrate) was used as an indicator of O2. Cells exposed to lucigenin tend to sequester it in their mitochondrial, which should increase specificity. Furthermore, changes in chemiluminescence stimulated by various metabolic inhibitors are consistent with the cytochrome c method of O2 determination (40). It must be noted that lucigenin is capable of redox cycling reactions that produce O2 (41–43), which can potentially skew the results of quantitative assays of O2. Hence, although the assay has limited usefulness for quantitative analysis of O2 concentration, it does provide a qualitative estimate of mitochondrial O2 generation which is rapid and far more sensitive than other methods available.

Lucigenin-dependent chemiluminescence was determined in intact cells by a modification of the O2 assay suggested by Atiken et al. (44). Confluent monolayers were released from plastic tissue culture vessels with 0.25% trypsin. The trypsin was neutralized using soybean trypsin inhibitor (1 mg/ml). Cells were pelleted by centrifugation at 500 x g for 7 min, washed once with reaction buffer (PBS, 1 g of glucose/liter, and 2 mM MgCl2). After pelleting the cells were resuspended in fresh reaction buffer. Typically 1.5 x 106 cells were suspended in 0.5 ml of buffer. 50 μl of suspended cells was mixed with 50 μl of 2 mM lucigenin (dissolved in reaction buffer). The reaction was placed in the dark for 10 min and then read using a Turner model 20e luminometer. The instrument was used to determine the integration of light emitted during a 15-s period. The luminometer was calibrated using an uranyl acetate light-emitting herring sperm DNA (Turner Instrument).

After the measurements were collected, CCI was determined as (1/uranyl acetate standard × chemiluminescent intensity)/mg of protein.

**H2O2 Generation—**The rate of H2O2 generation was determined using 2′,7′-dichlorofluorescin diacetate (DCFH-DA). Because it is nonpo-
A compound is deacetylated to 2',7'-dichlorofluorescin (DCFH). DCFH is polar and is thus trapped in cells. DCFH is oxidized by H$_2$O$_2$ to the highly fluorescent 2',7'-dichlorofluorescein (DCF; 45). The oxidation of DCFH to DCF has been used widely to determine H$_2$O$_2$ generation by flow cytometry and direct visualization techniques (46–48). In this study, we used DCFH oxidation as a measure of H$_2$O$_2$ generation by entire cultures. Confluent T25 flasks were washed twice with PBS and then incubated 45 min at 37 °C with 3 ml of PBS containing 1 g
glucose/liter, 2 mM MgCl₂, and 5 μM DCFH-DA. The cultures were then washed twice with cold PBS and incubated for 1 min in cold hypotonic solution (10% PBS). The flasks were then scraped with a rubber policeman to remove any remaining material. The suspension was sonicated for 20 s using a Branson sonicator with a stepped microtip and a setting of 4. 0.5 ml of the homogenate was diluted by adding 1.5 PBS and then read using a Hitachi F2000 fluorometer (excitation = 499, emission = 524). The amount of H₂O₂ in the samples was calculated using known concentrations of DCF (ACS grade; Sigma).

RESULTS

Cytochrome c Oxidase—COX is the terminal protein of electron transport and is the sole determinant of the rate of electron exit from the ETC when mitochondria are coupled. The control of COX by cells requires coordinate regulation of both nuclear and mitochondrial encoded subunits (49, 50). Fig. 1 shows that the mRNA abundance of COX 4 was relatively uniform, whereas the abundance of COX 1 was significantly greater in cultures established from either young or older adults than in cultures derived from fetal skin (Table I). COX activity was also at a significantly lower level in fetal fibroblasts than in postnatal cultures.

NADH Dehydrogenase—ND is the primary component of complex I and the initial step in the ETC. ND is composed of multiple peptides that are encoded by both the nuclear and mitochondrial genomes (49–51). As in the case of COX, the mitochondrial encoded subunit (ND 4) was significantly greater in adults than in fetal cultures, whereas the nuclear encoded subunit (ND 51) was not (Fig. 2 and Table I). ND activity was significantly greater in adult fibroblasts than in fetal cultures (Fig. 2 and Table I).

Succinate Dehydrogenase—SD is about 50% of the protein component of complex II and provides a second point of entry for electrons into the ETC (52). Although it is bound to the inner mitochondrial membrane, it is encoded entirely in the nucleus (34). An examination of fibroblasts established from donors of different ages revealed that fetal lines exhibited a significantly lower mRNA abundance than adults (Fig. 3 and
The rate of H\textsubscript{2}O\textsubscript{2} generation also correlated inversely with the ratio of enzyme activities controlling electron entry to exit described above \((r = -0.59; p = 0.0008)\).

**DISCUSSION**

In this study, we determined the activity of mitochondrial enzymes that regulate the rate of electron flow through the ETC and examined their relationship to the rate of cellular oxidant generation. The activities of all of the enzymes examined were lower in fetal skin fibroblasts than in cultures established from adult skin. A strong positive correlation was observed between the ratio of enzyme activities that control electron entry and exit in the ETC and chemiluminescence. This ratio of electron entry to exit correlated inversely with H\textsubscript{2}O\textsubscript{2} generation. The results also revealed a difference in the predominant oxidant species generated during fetal and adult stages of life with H\textsubscript{2}O\textsubscript{2} generation being greater in adults. For COX and ND, we observed a greater abundance of the mitochondrial encoded mRNAs (COX 1 and ND 4) in cell lines established from adults than in fetal cells, but we found no differences in fetal and adult mRNA abundances of the nuclear encoded sequences (COX 4 and ND 51). Conversely, for SD, a complex encoded entirely by the nuclear genome, mRNA abundance was greater in adult than in fetal cell lines. Young and old adults did not vary in any of the parameters examined.

All of the enzyme activities examined were lower in fetal
fibroblasts than in adult fibroblasts; however, COX activity was disproportionately lower compared with ND and SD as clearly indicated by taking the ratio of these enzyme activities. Because components of the ETC preceding a point of decreased electron flux exhibit a greater propensity toward being chemically reduced, the relatively low COX activity observed in fetal fibroblasts would be expected to increase the likelihood that the cytochromes will be chemically reduced, which would be expected to increase the probability of univalent electron transferers to oxygen (1, 3, 16, 25, 26). Hence, it would also be predicted that cells exhibiting low COX activity would also tend to produce more oxidants because their cytochromes are in a reduced state. The relatively greater chemiluminescence observed in fetal fibroblasts would appear to support this hypothesis, and we infer that the ETC of fetal cells is maintained at a higher reduction potential than in adults.

It is important to recall that the calculated ratio of electron entry to exit ((ND + SD)/COX) is based on enzyme activities that were estimated using zero order kinetics, i.e. under conditions where the enzyme is saturated by the substrate. The components of the ETC are predominately in an oxidized form in the active mitochondria (state 3 respiration) of normal adult tissues and are actually more likely to be chemically reduced in resting mitochondria (state 4 respiration) because of electron stacking (1, 3, 25, 27). The relative proportions of active and inactive mitochondria in cells would be expected to depend on both environmental stimuli and cell type. The extent to which our calculated ratio of electron entry to exit modulates the rate of ROS generation in fetal tissues in vivo remains unclear. Nevertheless, our results do illustrate that this is a potential factor in determining the rate of cellular oxidant production. A second factor that undoubtedly contributed to the greater chemiluminescence observed in fetal fibroblasts is their relatively low level of enzymic antioxidant defense, particularly superoxide dismutase (SOD).

The relatively low superoxide dismutase activity present in fetal fibroblasts may also have affected the equilibrium between \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \) in the fetal cell lines. The product of the dismutation of \( \text{O}_2 \) by SOD is \( \text{H}_2\text{O}_2 \), and we have demonstrated previously that the SOD activity of adult cells is dramatically higher than in fetal cells (22, 23). In this study, significant relationships observed between our previously reported activities for the copper-zinc form of SOD (\( r = 0.44; p = 0.018 \)) and Mn-SOD (\( r = 0.54; p = 0.003 \)) and the rate of \( \text{H}_2\text{O}_2 \) generation. In other studies, the rate of \( \text{H}_2\text{O}_2 \) generation is observed to increase during aging even when SOD activity declines; however, the magnitude of the loss of SOD activity associated with aging is only a small fraction of the increase in SOD activity associated with development (23, 55, 56). This is also true of the change in COX, which decreases moderately in mammals during aging in vivo (28) but, as we demonstrate, increases dramatically during development.

Although the physiological relevance of the developmental increase in SOD activity remains unclear there is evidence that it affects the subsequent course of developmental pathways. Augmentation of SOD activity via liposomes stimulates differentiation in a nondifferentiating strain of Physarum polycephalum (57) and in Friend cell leukemia (58). Overexpression of a the Mn-SOD gene stimulates changes in the state differentiation in human melanoma (59) and mouse C10HT1/2 cells (60). Interestingly, other antioxidants fail to stimulate differentiation, but some oxidants do (57, 58). Shifts in the redox environment resulting from the production of \( \text{H}_2\text{O}_2 \) by SOD may account for many SOD effects. It is noteworthy that the redox-modulating protein Ref-1 was recently found to be essential to normal early embryonic development (61). Furthermore, both \( \text{O}_2 \) (54) and \( \text{H}_2\text{O}_2 \) (62) have recently been implicated as second messengers in various signal transduction pathways. The fact that the predominant oxidant differs in fetal and postnatal lines may exert a significant physiological impact in the cells.

In contrast to previous reports of age-associated increases in generation of both \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \) (9, 14), we observed no aging-associated increase in oxidation. In vivo, age-associated decreases in the activities of COX, ND, and to a lesser extent SD activities have been reported in a wide variety of mammalian species (28, 63) including humans (64–66). These changes are believed to play an important role in aging-dependent increases in oxidation in vivo (16, 28). The aging-associated decreases in COX which occur in vivo appear to result from age-dependent changes in lipid-protein interactions (13, 67–71). Furthermore, restoring young levels of mitochondrial membrane cardiolipin in rats by treatment with acetyl-L-carnitine restores COX activity to the level seen in young animals (70, 71). The results of this study revealed no aging-dependent change in any of the parameters examined; however, cell cultures can usually obtain the lipids they require directly from the serum added to the growth medium. It is probable that the highly controlled lipid content of the culture environment will limit or reverse changes in lipid composition which occur during aging in vivo.

Regulation of the activities of the ETC components is extremely complex because aside from complex II (SD), which is encoded entirely by the nuclear genome, the various ETC components are protein mosaics of subunits encoded by both the nuclear and mitochondrial genomes. During development and regeneration, which are both periods of high rates of organelle neogenesis, components of the ETC encoded by the nuclear and mitochondrial genomes must be up-regulated in a coordinate manner (72–75). The various mechanisms by

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**Table II**

Analysis of the ratio of electron entry and exit, chemiluminescence, and \( \text{H}_2\text{O}_2 \) generation in fibroblasts from people of different ages

| Group | Mean | Total ANOVA<sup>a</sup> | Groups compared | LSD<sup>b</sup> p value |
|-------|------|------------------------|----------------|------------------------|
| (ND + SD)/COX |     |                        |                |                        |
| Fetal | 9.4  | 0.000001               | Fetal/young    | 0.000002               |
| Young | 4.0  | 0.000001               | Fetal/old      | 0.000001               |
| Old   | 3.9  |                        | Young/old      | 0.9                    |
| C10HT1/2 | 15.5 | 0.003                  | Fetal/young    | 0.001                  |
| Young | 8.4  |                        | Fetal/old      | 0.003                  |
| Old   | 9.0  |                        | Young/old      | 0.6                    |
| \( \text{H}_2\text{O}_2 \) |       |                        |                |                        |
| Fetal | 1.1  | 0.00004                | Fetal/young    | 0.00009                |
| Young | 2.3  |                        | Fetal/old      | 0.00003                |
| Old   | 2.5  |                        | Young/old      | 0.7                    |

<sup>a</sup> All effects.

<sup>b</sup> Post hoc analysis; LSD, least significant difference.
which cells coordinate expression of the mitochondrial and nuclear genomes are presently unknown. In this study, the abundances of several subunits of the mosaic enzyme complexes (ND and COX) were determined. We observed that the mitochondrial encoded subunits (COX 1 and ND 4) differed between fetal and postnatal groups, whereas those encoded by the nuclear genome (COX 4 and ND 51) did not. Although COX 1 mRNA abundance and COX activity were both lower in fetal than in adult lines, we observed no significant correlation between these two parameters. However, it is known that factors other than COX 1 mRNA abundance determine COX activity. In fact, control of mitochondrial protein levels is often at a translational or post-translational rather than a transcriptional level (76, 77). For example, the peptide encoded by COX 1 is rate-limiting to complex IV assembly (78–80). On the other hand, we did observe that ND 4 abundance correlated with ND activity, which is consistent with the hypothesis that ND 4 mRNA abundance is rate-limiting to complex IV assembly. Paradoxically, the mRNA abundance of both of the mitochondrial encoded subunits examined mosaic enzyme complexes. The mRNA abundance of both the mitochondrial encoded subunits examined correlated significantly with the mRNA abundance of succinate dehydrogenase (COX 1, r = 0.5, p = 0.006; ND 4, r = 0.70, p = 0.00002). Hence, it is possible that the same stimulus acts to signal up-regulation of all of these components. Further information about the development-associated changes in the other components of each of these complexes must, of course, be obtained before a more definitive assessment can be made.

Hayashi et al. (66) observed an age-associated decline in COX activity in skin fibroblast cultures established from donors of different ages; they also inferred that this decrease was dependent on an accumulation of mutations in nuclear encoded components of complex IV. In our study, low levels of expression of nuclear encoded subunits of COX and ND were observed in some of the lines established from older individuals; however, we also observed similar low abundances of these mRNAs in several young individuals. In no case did we observe a clear relationship between the abundance of nuclear encoded mRNA transcripts and enzyme activities. The primary reason for the differences in our results and those reported by Hayashi et al. (66) may stem from our use of a larger population of old individuals, which will limit statistical drift. An examination of the results presented by Hayashi et al. (66) reveals that their claim of an age-associated decline is actually based on data from only two individuals compared with the 11 used in our study. Furthermore, it must also be noted that the fusion method used by Hayashi et al. (66) does not eliminate the possibility of interference by non-genetic causes.

In summary, we have investigated the relationship between mitochondrial enzyme activities that regulate electron flow in the ETC and the rates of oxidant generation. The results of this study are consistent with the hypothesis that fetal cells maintain a greater steady-state level of $O_2$ and lower steady-state level of $H_2O_2$ because of imbalances in the enzymes that control electron flow and antioxidant defense levels. The study also probed possible relationships that existed in coordination of the expression of nuclear and mitochondrial encoded components of the same complexes. The results indicate that the activities of the mosaic enzyme complexes are independent of the transcript abundance of nuclear encoded subunits, whereas the activity of SD is not. Our results are consistent with the hypothesis that the rate of transcription of the mitochondrial genome is greater in adult cells than in fetal fibroblasts. The results presented here indicated a clear difference in the capacity of fetal and adult fibroblasts to generate oxidants; they also revealed a difference in the predominant oxidant species generated during fetal and adult stages of life. In view of the recently discovered role of oxidizing equivalents as second messengers, the differences we report here can potentially exert a profound impact on signal transduction pathways during different stages of human life.
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