In Vivo Regulation of Oxidative Phosphorylation in Cells Harbor

The mechanisms that regulate oxidative phosphorylation in mammalian cells are largely unknown. To address this issue, cybrids were generated by fusing osteosarcoma cells devoid of mitochondrial DNA (mtDNA) with platelets from a patient with a stop-codon mutation in cytochrome c oxidase subunit I (COX I). The molecular and biochemical characteristics of cybrids harboring varying levels of mutated mitochondrial DNA were studied. We found a direct correlation between the levels of mutated COX I DNA and mutated COX I mRNA, whereas the levels of COX I total mRNA were unchanged. COX I polypeptide synthesis and steady-state levels were inversely proportional to mutation levels. Cytochrome c oxidase subunit II was reduced proportionally to COX I, indicating impairment in complex assembly. COX enzymatic activity was inversely proportional to the levels of mutated mtDNA. However, both cell respiration and ATP synthesis were preserved in cells with lower proportions of mutated genomes, with a threshold at 40%, and decreased linearly with increasing mutated mtDNA. These results indicate that COX levels in mutated cells were not regulated at the transcriptional, translational, and post-translational levels. Because of a small excess of COX capacity, the levels of expression of COX subunits exerted a relatively tight control on oxidative phosphorylation.

The peculiar characteristics of mammalian mitochondrial genetics, where each cell contains a large number of mtDNAs in the order of 10^3–10^5 copies (15) render the issues of "gene dosage" and "threshold" for phenotypic effect very compelling, especially in the case of mtDNA mutations. In fact, very little is known about the regulation of the expression of genes involved in OXPHOS in mammalian mitochondria in response to mtDNA abnormalities. In most cases, mtDNA harboring pathogenic mutations coexist with wild-type mtDNA, a condition called "heteroplasmacy." It is well known that for many tRNA point mutations a relatively high proportion of mutated mtDNAs is required to determine OXPHOS defects (10). This is probably because of the ability of the remaining normal tRNAs to complement the function of the defective ones, suggesting that tRNAs are normally synthesized in excess of the minimum required for normal mitochondrial protein synthesis (16, 17). On the contrary, despite the apparent genetic redundancy, mutations in mtDNA genes encoding COX subunits seem to cause enzymatic defects at low levels of heteroplasmly (i.e. low proportions of mutated mtDNA). Oftentimes, the level of heteroplasmacy corresponding to the threshold for COX deficiency in the muscle of patients harboring mutations in COX genes is ~40%, as demonstrated by genetic and biochemical analyses of COX deficient fibers (11, 12, 14).

Studies performed on intact human cultured cells treated with an excess of COX capacity, the levels of expression of COX subunits exerted a relatively tight control on oxidative phosphorylation.

This paper is available on line at http://www.jbc.org
with increasing doses of the COX inhibitor KCN demonstrate that COX capacity is in low excess compared with that required to support normal cell respiration (18, 19). Therefore, COX appears to operate a tight regulation on cell respiration in mammalian cells. To better understand how genetic defects in mtDNA-coded COX subunits affect the regulation of OXPHOS in vivo, we have analyzed a set of transmitochondrial cell lines (cybrids) containing various proportions (from 0 to 100%) of a G6930A stop-codon mutation in the COX I gene (13).

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**Cybrids were generated by fusion of platelets from a patient with the G6930A mtDNA mutation with human osteosarcoma 143B cells lacking mtDNA (p′ cells) as described elsewhere (13). Ethidium bromide was used to manipulate the mtDNA content in a cell line with high mtDNA mutation (20). Exponentially growing cells were cultured in DMEM supplemented with 5% fetal bovine serum, 50 ng/ml ethidium bromide, and 50 µg/ml uridine for 9 or 11 days. After ethidium bromide treatment cells were replated in 100-mm tissue culture dishes at the cloning dilution of 100 cells/plate. Subclones were isolated with cloning rings and expanded for genetic, molecular, and biochemical analyses.

**Growth of subclones in selective conditions** were selected by seeding 35-mm plastic Petri dishes with five 10⁵ cells in 3 ml of Dulbecco’s modified Eagle’s medium without glucose supplemented with 5% dialyzed fetal bovine serum, 110 mg/liter sodium pyruvate, 5 mM galactose, 50 µg/ml uridine. Cell counts were obtained daily for 6 days.

**Mutation Analysis—**Total DNA was extracted from exponentially growing cells by standard techniques. PCR/RFLP analysis was performed to detect the G6930A mutation and to quantify the proportion of mutated mtDNA as described elsewhere (13). Radiolabeled mtDNA fragments digested with AluI were separated on a 12% polyacrylamide gel and quantified with Cyclone phosphorimager using the OpiQuant software (Hewlett Packard).

**RNA Analyses—**Total RNA was extracted from a semi-confluent 100-mm dish (10⁷ cells) using a Totally RNA kit (Ambion) following the manufacturer’s protocol. RNA yield was measured by UV absorbance.

For Northern blot analyses, 16 µg of total RNA were loaded on a 1.5% agarose-formaldehyde gel following the described protocols (21). RNA was blotted on a Zeta- Probe membrane (Bio-Rad) according to the manufacturer’s recommendations. The membrane was probed sequentially with mtDNA fragments corresponding to COX I (nucleotides 6459–7315) and ND2 (nucleotides 7315–8095) genes, and β-actin DNA (CLONTECH) were labeled with ³²PdATP by a random primer labeling kit (Roche Molecular Biochemicals). Intensities of RNA bands were quantified with Cyclone phosphorimager using the OpiQuant software (Hewlett Packard).

**For RT-PCR/RFLP analyses of mtDNA,** 5–8 µg of total RNA were subjected to DNase I digestion for 1 h at 37°C. The reaction was arrested by incubation at 75°C for 15 min. One µg of DNase I-digested RNA was used for cDNA synthesis using a ThermoScript RT-PCR system (Life Technologies, Inc.) following the manufacturer’s protocol. Total cDNA was incubated with RNase A to digest any residual mtRNA for 1 h at 37°C. PCR amplification of COX I cDNA fragments encompassing the G6930A mutation was performed as described elsewhere (13). To exclude potential mtDNA contamination and unprocessed mtRNA, another PCR reaction was carried out using the primers 11F (3′ position at mtDNA nucleotide 7148 in the COX I gene) and 11R (3′ position at mtDNA nucleotide 8095 in the COX II gene). These primers could only amplify mtDNA and unprocessed mtRNA.

**RESULTS**

**Isolation and Genetic Characterization of Cybrid Cell Lines—**To obtain cybrid cell lines containing various different proportions of mtDNA G6930A point mutation, a previously characterized heteroplasmic cybrid clone with 65% mutated mtDNAs (13) was subjected to ethidium bromide treatment for 9 or 11 days. By decreasing the mtDNA copy number, ethidium bromide increases the likelihood of skewed mitotic segregation of the mtDNAs in dividing cells. After ethidium bromide treatment, cells were plated at low concentrations in medium containing uridine. mtDNA from single-cell-derived subclones was analyzed by PCR/RFLP. As expected, these subclones contained various amounts of mutated mtDNA ranging from 0 to 100% (Fig. 1A).

A genetic drift of mtDNA resulting in a variation of the proportion of mutated mtDNA over time has been described in heteroplasmic cell lines (29, 30). The content of mutated mtDNA was measured at 1 × 10⁶ cells/ml in buffer A containing our subclones for 90 days of continuous culture. We found that some heteroplasmic subclones had a tendency to drift toward higher levels of mutated mtDNA, although they always maintained their heteroplasmic state (Fig. 1B). There was no apparent correlation between the initial proportion of mutated mtDNA and its tendency to shift upward or remain stable. For
this reason, a portion of the cells was set aside and utilized for DNA extraction each time an experiment was performed to determine the exact proportion of mutated genomes at that particular time point.

**COX I mRNA**—To investigate whether the G6930A stop-codon mutation had any effect on COX I gene transcription, total RNA was extracted, and total cDNA was generated by RT. Samples were treated with DNase prior to RT to eliminate any residual mtDNA. To ensure that all residual mtDNA had been eliminated, cDNAs were subjected to PCR amplification with a forward primer corresponding to COX I gene and a reverse primer complementary to COX II gene. As expected, no PCR products were generated from the cDNA samples, whereas the positive mtDNA controls generated the 948-bp target product (not shown). cDNAs were subjected to PCR-RFLP to determine the relative content of mutated mtDNA (Fig. 2A). RNA from parental 143B\textsuperscript{a} cells was used as a negative control and did not yield any PCR product (not shown) excluding the presence of COX I-expressed nuclear pseudo-genes. There was a linear correlation between the proportion of mutated mtDNA and mutated mRNA (Fig. 2B), indicating that in heteroplasmic cells the rate of transcription of wild-type and mutated COX I genes was the same. We also looked at total COX I mRNA levels by Northern blot analysis (Fig. 2C). The COX I mRNA normalized to β-actin mRNA varied in different cell lines from 2.75 to 4.70 (Fig. 2D). However, there was no correlation between the proportion of mutated mtDNA and relative COX I mRNA content.

Quantification of ND2 mRNA, another mtDNA-encoded gene unrelated to the G6930A mutation, also showed variability among subclones with no correlation with the proportion of mutated genomes (Figs. 2, C–D). These results show that the stop-codon mutation had no qualitative or quantitative effects on COX I mRNA expression and presumably did not affect the transcription of other mtDNA genes.

**COX I Protein Synthesis and Steady-state Levels**—Mitochondrial translation products were labeled for 20 min with \[^{35}\text{S}\]methionine in the presence of the cytosolic protein translation inhibitor emetine (Fig. 3A). Although equal amounts of proteins were loaded in each lane to correct for potential differences in protein loading, the intensity of the band corresponding to COX I was normalized to that of the ATPase subunit 6 (A6), and the amount of COX I polypeptide synthesized was expressed as the ratio COX I:A6. Although there was a gap between clones containing 8 and 58% mutated mtDNA, the trend of the data suggested a linear correlation between the proportion of mutated mtDNA and the decrease in synthesized COX I polypeptide (Fig. 3B).

Protein steady-state levels were assayed by Western blot using monoclonal antibodies against COX I and COX II (Fig. 4A). The intensities of the bands corresponding to the two COX subunits were quantified by densitometry. Again, we observed a direct correlation between the decrease in COX I steady state levels and the proportion of mutated mtDNA. Interestingly also, COX II was decreased in parallel with COX I (Fig. 4B). Because COX II synthesis was normal in mutated cells, the reduction in COX II steady-state levels was due to protein degradation, presumably caused by lack of complex IV assembly.

COX assembly was assayed in blue-native gel electrophoresis. As expected, the loss of COX I caused a deficit of COX assembly. Although we expected in cells with 65% mutated mtDNAs, assembled COX should have been 35% normal, using anti-COX I antibodies we found that as little as 65% mutated genomes caused complete disappearance of immunoreactive assembled COX IV (Fig. 4C). However, it has been shown that only ~80% of COX can be extracted under the mild conditions used (31). Moreover, it is possible, that the sample preparation for native gels did not allow for complete antigen presentation for the anti-COX I antibody, leading to an underestimation of assembled complex IV. In agreement with the results obtained by Western blot, immunostaining of the native blot with antibodies to anti-COX II also showed the absence of reactive material in cells with 65% mutated genomes (not shown). The apparently inverse linear correlation between the proportion of mutated mtDNA and the decrease in COX I protein suggested that the remaining wild-type mtRNAs were not able to compensate for the lack in COX I protein translation caused by the mutation. Moreover, COX I protein turnover and degradation were unchanged in mutated cells, again suggesting that any compensatory mechanisms at the protein level was either very small or not present at all.

**Respiratory Chain Activities**—COX activity measured in mitochondrial fractions was decreased proportionally to the mutation load (Fig. 5A). This correlation was maintained when COX activities were normalized by the activity of citrate synthase (Fig. 5B), a nuclear-encoded matrix enzyme of the Krebs cycle, to correct for the possible variations in mitochondrial content due to the isolation procedures. In addition, the activity...
of complex I + III (NADH-cytochrome c oxidoreductase) was unchanged in mutated cells (Fig. 5C). These results showed that there was no threshold for COX enzymatic activity and that there was no compensatory increase of the other mitochondrial enzymes that would have resulted in a steeper decrease of the COX:citrate synthase ratio and higher complex I + III activities in mutated cells.

**Cell Respiration and ATP Synthesis**—Oxygen consumption was assayed in intact cells respiring in Dulbecco’s modified Eagle’s medium containing pyruvate and l-glutamine as the sole energy sources. Cell respiration was almost completely inhibited by the addition of 1.5 mM KCN, a specific inhibitor of complex IV. Cell respiration plotted against the proportion of mutated genomes showed almost normal rates up to 30–40%, above which there was a steep decrease linearly correlated with the mutation load (Fig. 6A). To avoid the potential influence of proton cycling and OXPHOS on cell respiration, cells were completely uncoupled with 3 μM FCCP, a potent protonophore. The addition of FCCP increased the cellular respiration rate by ~100%. However, the correlation between respiration and mutation load did not change. Cells harboring 30–40% mutated genomes were still able to sustain almost normal cell respiration with a steep drop at higher levels of mutation (Fig. 6B).

ATP synthesis was measured by luminometry in permeabilized cells using malate and pyruvate as substrates with and without the addition of 15 μg/ml of the ATPase inhibitor oligomycin. In wild-type cells, more than 90% ATP synthesis was oligomycin-sensitive, indicating its mitochondrial origin. As for cell respiration, mitochondrial ATP synthesis plotted against the proportion of mutated genomes was normal up to 30–40%, above which there was a sharp decrease that correlated linearly with the mutation load (Fig. 6C).

**Cell Growth in Galactose Medium**—We investigated the impact of reduced respiratory chain functions on cell survival in culture conditions where ATP synthesis depends primarily on oxidative metabolism. Cells were grown in medium lacking glucose and containing pyruvate and galactose. Galactose can enter glycolysis via three consecutive reactions that convert galactose to glucose 6-phosphate. Because in cultured cells this...

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**Fig. 2. RNA analyses in mutated cybrids.** A, mitochondrial cDNA RT-PCR/RFLP autoradiogram. The sizes of AluI-digested products are as in Fig. 1. The corresponding percentages of mutated mtDNA for each subclone are indicated at the bottom. B, correlation curve between percentages of mutated mtDNA and mutated mtRNA. C, Northern blot analysis of cybrid subclones RNA. RNA was detected with COX I, ND2 (subunit 2 of complex I) probes, and with a β-actin probe to control for gel loading. Percentages of mutated mtDNA are indicated at the bottom. D, COX I and ND2 RNA band intensities normalized by the intensity of β-actin RNA in subclones harboring different percentages of mutated mtDNA.

**Fig. 3. Mitochondrial protein synthesis.** Autoradiogram of radio-labeled mitochondrial translation products in cybrid subclones with different percentages of mutated mtDNA. The bands corresponding to COX I and ATPase subunit 6 (A6) are indicated. B, correlation curve between the proportion of mutated mtDNA and the ratio of the band intensities of COX I and subunit 6.
reaction is much slower than the conversion of glucose to glucose 6-phosphate by hexokinase, the ATP produced by galactose-derived glycolysis alone is not sufficient for cell viability. Therefore, cells have to rely on mitochondrial OXPHOS as the main source of ATP (32). There was a rather high degree of variability in growth rates among clones also in medium containing glucose that was unrelated to the proportion of mutated genomes. However, in medium containing galactose and pyruvate the growth of cells harboring more than 50% mutated genomes was dramatically decreased, and cells containing more than 70% mutated genomes were unable to divide after only 2 days of culture (data not shown). Therefore, we estimated that the threshold of mutated genomes for defective growth in oxidative conditions was $\frac{1}{2}$.

**DISCUSSION**

In the present work, the metabolic effects of a stop-codon mutation in the mitochondrial gene coding for COX I were studied in cultured human cells. The G6930A mtDNA mutation creates a stop-codon resulting in a predicted loss of 170 amino acids, approximately $\frac{1}{3}$ of the polypeptide. A mitochondrial translation product corresponding to truncated COX I was not detected by gel electrophoresis of radiolabeled newly synthesized peptides or by immunoblotting even in cells containing 100% mutated genomes. This suggested that the loss of the C-terminal portion presumably prevented insertion in the inner mitochondrial membrane and assembly with the other components of COX, leading to rapid degradation of the truncated protein (13). For this reason, we speculated that a dominant negative effect of the mutation due to misincorporation of an inactive truncated peptide in COX holoenzyme was unlikely. To assess how cells respond to increasing levels of COX deficiency, we analyzed the molecular and biochemical characteristics in a series of subclones obtained by prolonged treatment with ethidium bromide of a heteroplasmic cybrid cell line that contained varying proportions of mutated genomes.

**Correlation between mtDNA Mutation, COX I mRNA, Protein Synthesis, and Enzyme Activity**—The direct correlation between levels of COX I-mutated mtDNA and mutated mRNA observed in our cellular model strongly suggested the absolute lack of compensatory up-regulation and stabilization of wild-type mRNA as compared with mutated mRNA. Our results are
the proportion of mutated mtDNA and the levels of COX I polypeptide was supported by the observation that COX activity decreased linearly with increasing mtDNA mutation, even at relatively low levels of mutated genomes (i.e. 28%). Because the stop-codon mutation most likely caused COX deficiency by decreasing the levels of COX I polypeptide, any compensatory effect at the protein level was presumably either very small or absent.

On the other hand, Bai et al. (33) report that in ND5-mutated cells the proportion of mutated mtDNA above which ND5 protein levels and complex I activity were decreased was ~40%. The reason for this difference could partially depend on the turnover rate of the polypeptide affected by the mutation, and at this point, we don’t know if COX I and ND5 turnover rates in mammalian cells are different. It should also be taken into account that the ND5 mutation was studied in mouse lung carcinoma cells, whereas our COX I mutation was studied in human osteosarcoma cells. Again, we don’t know if differences exist between mitochondrial peptide turnover rates in different cell types and in different mammalian species. Alternatively, there could be an effect of mitochondrial translation factors that modulate the synthesis of polypeptides, since this has been observed in isolated rat brain synaptosomes, where the synthesis of ND5 was differently regulated than that of other mitochondrial polypeptides (34). In any case, both our observations and those of Bai et al. (33) agree in demonstrating that in the case of protein-coding gene mutations the threshold for protein synthesis defects is either low or absent. On the contrary, mutations of the tRNA gene, which are expressed severalfold more than the minimum required for normal protein synthesis, have very high thresholds (16, 17). It will be important to identify and analyze cellular models with mtDNA nonsense or frameshift mutations in different genes to determine whether the threshold values found in COX I and in ND5 mutants can be extended to other mitochondrial protein-coding genes.

Complex IV Stability—It has been proposed that in mammalian cells complex IV is assembled in a process that entails several steps and proceeds through the formation of different intermediates. COX I together with subunit IV seems to be part of the first intermediate complex that is formed (27), suggesting that COX I has a primary role in the assembly of complex IV. It has been previously reported that cells partially depleted of COX I also lack COX II (13). Accordingly, we have found a profound reduction of fully assembled complex IV in cells containing high levels of COX I-mutated mtDNA (i.e. >65%). We have also shown that the steady-state levels of another mtDNA-coded complex IV subunit, COX II, were reduced proportionally to those of COX I despite being normally synthesized. Similarly, a lack of COX II has been reported in the muscle of a patient with a frameshift micro-deletion in COX I (35). Moreover, Tiranti et al. (6) demonstrate that in cells harboring a COX III frameshift mutation, there was a reduction of COX I and COX II steady-state levels even though they were normally synthesized (6). Also, in cells harboring virtually homoplasmic levels of a 15-bp deletion in COX III, there was a decrease of COX I and COX II steady-state levels (36). These observations strongly support the concept that in mammalian cells, COX subunits that are not properly assembled due to a lack of assembly intermediates are rapidly degraded. Also, in mutants of the yeast Saccharomyces cerevisiae lacking individual COX subunits there is a reduction in the steady-state levels of the other constituents of the complex, particularly those encoded by mtDNA (37). Those polypeptides are lost as a result of proteolysis, and it seems that only the fully assembled complexes are protected against the action of endogenous proteases (37). Therefore, mitochondrial polypeptide steady-state levels
**OXPHOS Regulation in Heteroplasmic COX I Mutant Cells**

*in vivo* appear to depend more on their turnover rate than on transcriptional or translational regulation.

**COX Threshold and Maximum COX Capacity for Cell Respiration and ATP Synthesis**—We correlated residual COX activity with cell respiration and ATP synthesis rates in cybrid subclones containing varying proportions of mutated mtDNA. Percentages of residual respiration and ATP synthesis plotted against the percentage of COX deficiency were unchanged up to a threshold point, where they started decreasing in a linear correlation with increasing COX deficiency (Figs. 7, A–C). Substituting the value 100 (corresponding to 100% respiration or ATP synthesis) to the y value in the equation that defines the best-fit linear regression lines in each plot (Figs. 7, A–C), we derived the x values corresponding to the COX deficiency threshold points. The estimated threshold values were 37% for coupled cell respiration, 44% for uncoupled cell respiration, and 46% for ATP synthesis. Similarly, in cells with more than 50% mutated genomes, we found markedly decreased growth rates in galactose-containing medium, which requires oxidative metabolism to maintain adequate ATP synthesis. Interestingly, also Porteous et al. (38) have shown that in a set of cybrids containing various levels of the mtDNA 4997-bp “common” deletion, the threshold for ATP synthesis defect was around 50% deleted genomes.

From the plots in Fig. 7, we have determined the theoretical maximum COX capacity corresponding to the intersection of the best-fit linear regression lines with the y axis, expressed as a ratio to the actual values obtained in cells with 0% COX deficiency. The maximum COX capacity values were 1.5 for coupled respiration, 1.7 for uncoupled respiration, and 1.9 for ATP synthesis.

The equations for endogenous respiration and ATP synthesis rates conformed to the general equation proposed for endogenous respiration by Villani and Attardi (18), \( \text{COXR}_i/\text{H}_{1005} \text{COXR}_{\text{max}}(100 - x) \), where \( x \) represents the percent inhibition of COX activity at \( i \) concentration of the inhibitor KCN, \( \text{COXR}_i \) represents the percentage of endogenous respiration rate at \( i \) concentration of the inhibitor KCN, and \( \text{COXR}_{\text{max}} \) represents the ratio of the maximum COX capacity to the maximum endogenous respiration rate. In their experiments, Villani and Attardi (18) used, among other lines, osteosarcoma 143B cells, the same parental cells from which our cybrids were derived, treated with different concentrations of the COX inhibitor KCN. They found a 28% threshold for endogenous respiration and a maximum COX capacity 1.4 times higher than the endogenous respiration rate. The difference in the threshold values of COX deficiency observed by us and Villani and Attardi (18) could be attributed to several factors. First, instead of a single cell line, we looked at a number of cybrid subclones that might have differed slightly in their metabolic regulation. Second, the KCN inhibition system used by Villani and Attardi (18, 19) might not exactly compare biochemically to our genetic model of COX deficiency. Last, these authors measured both COX activity and endogenous respiration in whole cells by polarographic methods, whereas we measured COX activity on mitochondrial fractions by spectrophotometry and cell respiration by polarography. However, both Villani and Attardi (18) and our results show that the reserve of COX capacity in living cells is limited, and the threshold for respiratory and ATP synthesis defects could be reached at values of residual COX activity of ~60–70%. This could have important implications in the relationship between mtDNA mutation in COX genes and their consequences on OXPHOS functions in patients with mitochondrial disorders. Although it is not known how OXPHOS regulation differs in human tissues from cultured cells,

**Fig. 7.** COX threshold and maximum COX capacity for cell respiration and ATP synthesis. A, percentage of cell respiration as a function of percentage of COX deficiency. B, percentage of cell respiration in FCCP-uncoupled cells as a function of percentage of COX deficiency. C, percentage of ATP synthesis as a function of percentage of COX deficiency. The least-squares regression lines through the filled symbols were extended to zero COX deficiency. The equations describing these extrapolated lines were used to determine the COX maximum capacity. COX threshold was defined as the x value corresponding to the interception point between the least-squares regression lines and the equation line \( y = 100 \).
the excess COX capacity was also estimated to be ~1.4 in isolated permeabilized muscle fibers (39). These data are consistent with the finding that the threshold for COX deficiency in the muscle of patients harboring mutations in COX genes is usually around 40%, as estimated by single fiber PCR/RFLP analyses of COX-deficient fibers (12, 14, 40). Thus, it is likely that a mild (i.e. less than 30%) COX deficiency will not cause impairment of respiration and ATP synthesis in muscle fibers. Although there might be differences in the way OXPHOS is regulated in different tissues in vivo, this caveat should be considered when assessing the physiological significance of low levels of mtDNA mutations and mitochondrial enzyme defects found in patients affected by chronic degenerative disorders such as Alzheimer's and Parkinson's diseases.

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REFERENCES

In Vivo Regulation of Oxidative Phosphorylation in Cells Harboring a Stop-codon Mutation in Mitochondrial DNA-encoded Cytochrome c Oxidase Subunit I
Marilena D'Aurelio, Francesco Pallotti, Antoni Barrientos, Carl D. Gajewski, Jennifer Q. Kwong, Claudio Bruno, M. Flint Beal and Giovanni Manfredi


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