Inherited complex I deficiency is associated with faster protein diffusion in the matrix of moving mitochondria

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Inherited complex I deficiency is associated with faster protein diffusion in the matrix of moving mitochondria. The electron transport chain system ATPase (complex V), constitutes the oxidative phosphorylation (OXPHOS) system. This potential is maintained by the electron transport chain system across the inner mitochondrial membrane (Δψ). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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We furthermore showed that chronic rotenone treatment increased matrix EYFP diffusion in both moving and stationary mitochondria. It was proposed that the observed increases in mitochondrial length and degree of branching and matrix protein diffusion may constitute part of an adaptive response to counterbalance the detrimental effects of the decrease in CI activity. In agreement with this idea, analysis of a cohort of 16 fibroblast lines from children with isolated CI deficiency caused by mutations in nuclear-encoded CI subunits showed that mitochondria were fragmented and/or less branched in fibroblasts with a severely reduced CI amount and activity (“class I”), whereas patient cells in which these latter parameters were only moderately reduced displayed a normal mitochondrial morphology (“class II”; 31). Class I cells (8 different patients) carried mutations in the NDUF5, NDUFS2, NDUFS4, and NDUF58 subunit of complex I, whereas class II cells (8 different patients) carried mutations in the NDUFS4, NDUFS7, NDUFS8, and NDUF5 V1 subunit of complex I.

In the present study, we aimed to establish the relationship between residual CI activity (maximal activity of CI determined in a homogenate of a mitochondrial enriched fraction), as a measure of mitochondrial OXPHOS capacity, and mitochondrial shape, mobility, and matrix protein diffusion in inherited CI deficiency. To this end, we applied confocal microscopy and FCS of mitoEYFP-expressing skin fibroblasts of a healthy subject and two genetically characterized CI-deficient patients, one with a R228Q mutation in the NDUF5 subunit and the other with a R95X/T423M mutation in the NDUF5 V1 subunit. These patients displayed either a large (61%; NDUFS2) or modest (27%; NDUFS V1) decrease in residual CI activity, paralleled by a decrease and increase in mitochondrial degree of branching, respectively (28).

MATERIALS AND METHODS

Fibroblast cell lines. Complex I (EC: 1.6.5.3)-deficient fibroblast lines were obtained from skin biopsies of two children in whom an isolated deficiency (OMIM 252010) was confirmed in both muscle tissue and cultured skin fibroblasts. Biopsies were performed following written informed parental consent and according to the relevant Institutional Review Boards. All procedures were reviewed and approved by the Institutional Review Boards. Both patients were clinically and genetically characterized previously (34, 45) and carried disease-causing mutations in either the NDUF5 (R228Q; no. 5170) or NDUF5 V1 (R95X/T423M; no. 5171) subunit. Both patients were negative with respect to mitochondrial DNA (mtDNA) mutations previously shown to cause CI deficiency. As a control, we used fibroblast lines of three healthy adult subjects (nos. 4996, 5118, and 5120). Fibroblasts were cultured in medium 199 with Earle’s salt supplemented with 10% (vol/vol) fetal calf serum, 100 IU/ml penicillin, and 100 IU/ml streptomycin in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Cell cycle analysis revealed no differences between the fibroblast lines (28). Moreover, cell cycle phase did not correlate with any of the parameters investigated in the present study. For analysis of mitochondrial morphology, cells were grown to 70% confluence on glass coverslips (22-mm diameter). For FCS recordings, Lab-Tek eight-well chambers (Nalgene Nunc International) were used. Prior to microscopy experiments, culture medium was replaced with a colorless HEPES-Tris solution (in mM: 132 NaCl, 4.2 KCl, 1 CaCl2, 1 MgCl2, 5.5 d-glucose, and 10 HEPES, pH 7.4).

Transient expression of mitochondria matrix-targeted EYFP. MitoEYFP was expressed using a modified baculovirus vector as described previously (30, 57). Measurements were performed three days after the onset of baculovirus transduction, when virtually all cells expressed the construct. The virus remained present in the culture medium during the entire incubation period.

Quantitative analysis of mitochondrial morphology. Quantitative analysis of mitochondrial morphology was carried out as described in detail elsewhere (27, 28, 29). Briefly, after correction for background fluorescence, images were subjected to linear contrast stretching followed by top-hat filtering, median filtering, and thresholding. This procedure yields binary images showing white mitochondria against a black background. These images were used to quantify the number of mitochondria per cell (N) and their form factor (F) using image analysis software (see below). F is calculated for each individual mitochondrion and equals perimeter^2/4π-area. The latter is a combined measure of mitochondrial length and degree of branching.

FCS. Combined FCS and confocal microscopy was performed on a Confocor II system (Carl Zeiss, Sliedrecht, The Netherlands) as described previously (30). Briefly, mitoEYFP was excited using the 514-nm line of an Ar-ion laser focused via a dichroic mirror and a Zeiss C-Apochromat objective (×40, 1.2 numerical aperture, water immersion) onto the sample. Laser output power was 20 kW/cm², and fluorescence emission light was guided via the dichroic mirror and a 545DF35 band-pass filter onto an avalanche photodiode. After selection of mitochondrial regions of interest in the confocal mode, autocorrelation curves were acquired during 10 s at 20°C.

The positions of the pinhole and the correction ring of the objective lens were optimized using an aqueous EYFP solution. Optimal settings were considered to be reached when the highest molecular brightness was observed. Fitting the autocorrelation curves of EYFP, of which the diffusion coefficient in water is known, to Eq. I revealed that the FCS detection volume had radii of 0.253 μm and 1.37 μm for the equatorial (r_0) and axial radius (r), respectively. Intensity signals were software-correlated and individual autocorrelations were displayed on-line. The autocorrelation function (ACF) describing j independent molecular species diffusing freely in a three-dimensional Gaussian-shaped observation volume (V_ort = πr_0^2r_1^2) is given by:

\[ G(\tau) = 1 + \sum_j F_j \left( 1 + \frac{\tau}{\tau_{dark,j}} \right) \left( 1 + \frac{\tau}{\tau_{0,j}} \right)^{-1} \]

with \( j = 1, 2, 3, \ldots \) and \( \sum_j F_j = 1 \) (59). Autofluorescent proteins like EYFP show additional fluorescence fluctuations due to conformational changes between fluorescent and dark states (46). The probability and relaxation time of the dark state are given by \( T \) and \( \tau_{dark} \), respectively. The lateral diffusion time \( \tau_{dark} \) describes the residence time of a particle in the observation volume, which is related to the translational diffusion coefficient:

\[ \tau_{dark} = r_0^2 / (4D_{trans}) \]

The amplitude of the ACF, \( G(0) \), represents the average number of molecules \( N \) found in the observation volume: \( G(0) = 1 - 1/N \). During fitting, a single-component model \( (j = 1) \) was evaluated first. This model was considered valid when the residuals did not exceed 0.05 (9, 21, 22, 26, 47). When the latter was not the case, the single-component model was rejected and a two-component model \( (j = 2) \) was evaluated using similar criteria. Recordings displaying excessive bleaching (>5% over the full duration of the recording) or high-amplitude spikes were omitted from the analysis.

To allow selection of the most appropriate model for fitting the ACF (17), we first assessed the mitochondrial diameter by calculating the width at half-maximal height of a 1 pixel wide intensity profile perpendicular to the long axis of the mitochondrial filament (as described in the supplement of Ref. 7). This analysis revealed no
difference between control (0.85 ± 0.05 μm, n = 35 mitochondria) and NDUFS2 cells (0.80 ± 0.03 μm, n = 33 mitochondria), and the values obtained agreed well with those determined by electron microscopy (EM; Ref. 1 and Fig. 3). Conversely, mitochondrial diameter was somewhat smaller in NDUFV1 cells (0.60 ± 0.03 μm, n = 24 mitochondria; P > 0.05). In analogy to our previous study (30), Eq. 1 was appropriate to fit all experimental ACFs. To determine diffusion times (τ_dif), ACF curves were fitted between τ = 0.01 ms and τ = 100 ms, a range compatible with translational diffusion (14). In aqueous solution, EYFP displayed a single diffusion time (τ) of 0.165 ± 0.04 ms (n = 4), which was equivalent to a translational diffusion constant (D) of 92.3 ± 0.22 μm²/s (30). In control and patient cells, no autocorrelation was detectable in the nucleoplasm and cytosol (30). This rules out interference of autofluorescence and demonstrates that mitoEYFP was exclusively present within the mitochondrial matrix.

EM. For EM analysis of mitochondria, cells were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and were postfixed for 1 h in 1% osmium tetroxide and 1% potassium ferrocyanide in 0.1 M cacodylate buffer. After being washed in buffer, cells were dehydrated in an ascending series of aqueous ethanol and were subsequently transferred via a mixture of propylene oxide and Epon to pure Epon 812 as embedding medium. Ultrathin gray sections (60–80 nm) were cut, contrasted with aqueous 3% uranyl acetate, rinsed, and counterstained with lead citrate, air dried, and examined in a JEOL JEM1010 electron microscope (JEOL, Welwyn Garden City, UK) operating at 80 kV.

Image analysis and statistical analysis. Confocal images were processed and analyzed using Image Pro Plus 5.1 software (Media Cybernetics, Silver Spring, MD). Numerical results were visualized using Origin Pro 7.5 (Originlabs, Northampton, MA) and are presented as means ± SE. Statistical differences were determined using Student’s t-test (Bonferroni corrected) and were considered significant when P < 0.05.

Chemicals. Culture materials were obtained from Invitrogen (Breda, The Netherlands). All other reagents were from Sigma (St. Louis, MO).

RESULTS

Changes in respiratory state of mitochondria can affect their shape, volume, and/or matrix configuration. Here, we address the possibility that mitochondrial motility and matrix protein diffusion may be altered in inherited CI deficiency. To this end, we transduced fibroblasts of two CI-deficient children displaying striking differences in residual CI activity and mitochondrial shape (27, 31) with a baculovirus vector encoding a fusion protein targeting mitochondria.
between EYFP and the targeting sequence of subunit VIII of cytochrome-c oxidase (COX-8), referred to as mitoEYFP (30, 57, 58), and determined its diffusion rate using FCS (14, 22, 24, 26, 30). For comparison, we used a fibroblast line derived from a healthy adult individual (no. 5120). Previous functional and biochemical assays showed that this healthy cell line was representative of five others obtained from adults and age/sex-matched children (28, 31, 49, 52, 54, 55, 57, 58, 63).

Quantification of mitochondrial shape in healthy and patient fibroblasts. Confocal imaging of mitoEYFP-expressing healthy control (CT; Fig. 1A) and CI-deficient (NDUFS2, Fig. 1B, and NDUFV1, Fig. 1C) patient fibroblasts revealed numerous threadlike structures, previously identified as mitochondria (27–31). The shape of these mitochondrial structures was quantified by calculating the mitochondrial form factor (F), a combined measure of mitochondrial length and degree of branching, using a previously described protocol (27–29). This analysis revealed that, compared with healthy control cells, F was lower in NDUFS2 cells and higher in NDUFV1 cells (Fig. 1D and Table 1). In both patient cell lines, the number of mitochondria per cell (Nc) was identical to control. To describe mitochondrial morphology, we calculated the operational parameter “mitochondrial complexity,” given by the ratio of F to Nc (28, 31). Relative to control cells, mitochondrial complexity was significantly smaller in NDUFS2 cells and significantly larger in NDUFV1 cells (Fig. 1D). The change in mitochondrial shape as determined in the present study with mitoEYFP was quantitatively identical to that reported previously (28, 31) using mitoEYFP (NDUFS2: F = 138 ± 8%, Nc = 103 ± 2%, n = 30; NDUFV1: F = 64 ± 2%, Nc = 103 ± 2%, n = 27) and the mitochondria-specific cation rhodamine 123 (NDUFS2: F = 133 ± 6%, Nc = 107 ± 5%, n = 79; NDUFV1: F = 65 ± 2%, Nc = 95 ± 5%, n = 336).

MitoEYFP diffusion in healthy and patient fibroblasts as revealed by FCS. To determine the translational diffusion coefficient of EYFP within the mitochondrial matrix, mitoEYFP-expressing fibroblasts were analyzed by FCS, as recently described in detail (30). Cells were visualized by confocal microscopy for random selection of mitochondrial regions of interest (Fig. 1; crosses). Next, fluorescence intensity fluctuations were acquired during 10 s, after which the experimental ACF of the photodetector output was calculated for each individual region of interest. Finally, translational diffusion times (τd) were computed from the ACF curve (see MATERIALS AND METHODS). In both healthy and patient fibroblasts, ~20% of the mitochondria displayed one single (τfast1) diffusion time, and the remainder displayed two separate (τslow2 and τfast2) diffusion times (Fig. 2A). Importantly, both “types” of mitochondria were present within one and the same cell. When cells were treated with nocodazole (Noc; 10 μg/ml; 2 h), an established inhibitor of mitochondrial movement (36, 37, 42, 63), τslow2 was abolished and only a single fast translational diffusion time remained (τfast2; Fig. 2A). These results were in accordance with previous findings (30), which demonstrated that the value of τslow2 reflects mitochondrial velocity and τfast1 and τfast2 reflect matrix protein diffusion in stationary and moving mitochondria, respectively.

In healthy fibroblasts, the translational diffusion coefficient of mitoEYFP appeared to be similar for stationary (Dfast1) and moving (Dfast2) mitochondria (Table 1). This finding revealed that mitochondrial movement and matrix protein diffusion were not related in healthy fibroblasts. Neither Dfast1 (Fig. 2B) nor Dfast2 (Fig. 2C) differed between the two patient fibroblast lines. On the other hand, Dfast2 was larger than Dfast1 in these cells, indicating that, in patient cells, matrix protein diffusion was fastest in moving mitochondria. In patient cells compared with healthy cells, Dfast1 (Fig. 2B) and Dfast2 (Fig. 2C) were slightly decreased and markedly increased, respectively. The marked increase in Dfast2 demonstrated that matrix protein diffusion in moving mitochondria was fastest in patient fibroblasts. Dslow2 was not different between the two patient cell lines but considerably higher than control, revealing that the velocity of mitochondrial movement was ~2.5-fold higher in patient cells.

In healthy fibroblasts treated with Noc, the translational diffusion coefficient (Dnoc) was identical to Dfast1 and Dfast2 in untreated fibroblasts (Fig. 2E and Ref. 30), indicating that, in these cells, blocking mitochondrial movement with Noc did not affect matrix protein diffusion. Dnoc was not different

Table 1. Mitochondrial morphology, motility, and mitoEYFP diffusion in control and patient cell lines

<table>
<thead>
<tr>
<th>Cell line number</th>
<th>Control</th>
<th>NDUFS2 Patient</th>
<th>NDUFV1 Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>NA</td>
<td>R228Q</td>
<td>R59X/T423M</td>
</tr>
<tr>
<td>Complex I activity, %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>39</td>
<td>73</td>
</tr>
<tr>
<td>Mitochondrial morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length and degree of branching (F), % (n)</td>
<td>100±1 (62)</td>
<td>63±1 (27)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>140±9 (30)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nc, %</td>
<td>100±2</td>
<td>102±2</td>
<td>105±2</td>
</tr>
<tr>
<td>Mitochondrial motility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moving mitochondria, % (n)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79 (58)</td>
<td>76 (76)</td>
<td>73 (44)</td>
</tr>
<tr>
<td>Speed of mitochondria (D&lt;sub&gt;slow2&lt;/sub&gt;), μm&lt;sup&gt;2&lt;/sup&gt;/s (n)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.06±0.01 (46)</td>
<td>0.16±0.04 (58)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.16±0.06 (32)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>MitoEYFP diffusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stationary mitochondria (D-fast), μm&lt;sup&gt;2&lt;/sup&gt;/s (n)</td>
<td>22±2 (12)</td>
<td>15±2 (18)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>16±4 (12)</td>
</tr>
<tr>
<td>Moving mitochondria (D-fast), μm&lt;sup&gt;2&lt;/sup&gt;/s (n)</td>
<td>18±5 (46)</td>
<td>38±4 (58)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>46±8 (32)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of cells (morphology) or mitochondria (motility, mitoEYFP diffusion) analyzed. NDUF, NADH dehydrogenase ubiquinone flavoprotein; NA, not appropriate; F, form factor; Nc, number of mitochondria per cell; mitoEYFP, enhanced yellow fluorescent protein targeted to the mitochondrial matrix. *Cell line number as designated by the Nijmegen Center of Mitochondrial Disorders. Mutations are given at the protein level. Activity is measured in mitochondria-enriched fractions and is expressed as % of the lowest control value (28, 30, 34, 45, 49). Percentage represents the fraction of mitochondria displaying a double mitoEYFP mobility during fluorescence correlation spectroscopy analysis (FCS). <sup>a</sup>Value represents the apparent diffusion coefficient D<sub>slow2</sub> of mitoEYFP determined by FCS. <sup>b</sup>f<sup>s</sup>Significant (P < 0.05) differences with mitochondria in <sup>*untreated control, #NDUF2, and ^NDUF1.</sup>
between the two patient cell lines (Fig. 2E; NDUFS2: \(D_{\text{Noc}} = 30 \pm 2 \mu m^2/s, n = 30 \) mitochondria; NDUVF1: \(D_{\text{Noc}} = 28 \pm 2 \mu m^2/s, n = 45 \) mitochondria). Calculation of the weighed average of \(D_{\text{fast1}}\) and \(D_{\text{fast2}}\) in untreated patient fibroblasts yielded a value similar to \(D_{\text{Noc}}\), suggesting that Noc immobilized moving mitochondria without altering matrix protein diffusion.

Mitochondrial ultrastructure in healthy and patient fibroblasts. Evidence in the literature demonstrated that alterations in mitochondrial metabolism can be paralleled by changes in mitochondrial ultrastructure (18, 19, 32, 35, 41, 44). As a consequence, protein diffusion in the mitochondrial matrix may be affected (39, 56). These observations suggest that the difference in matrix protein diffusion between control and
mitochondria. In both control and patient fibroblasts. Visual inspection of a large number of EM images (n > 30 cells) revealed no differences in mitochondrial cristae morphology and matrix density between control and patient fibroblasts (Fig. 3). EM analysis of two additional control cell lines [nos. 4996 and 5118 (28, 31, 54, 55, 58, 63)] also revealed no differences (data not shown). These findings suggest that alterations in matrix protein diffusion observed by FCS are not caused by gross alterations in mitochondrial ultrastructure.

**DISCUSSION**

Disease-causing mutations in nuclear-encoded subunits of complex I (CI), the first and largest complex of the mitochondrial OXPHOS system, are associated with multisystem disorders affecting brain, skeletal muscle, and the heart (48). Here we assessed whether inherited CI deficiency alters motility and/or matrix protein diffusion in mitochondria of patient skin fibroblasts and, if so, whether these changes are related to residual cellular CI activity and/or mitochondrial shape. The large reduction in CI activity in NDUFS2 cells was paralleled by a reduced mitochondrial length and degree of branching (F) without alterations in the number of mitochondria per cell (Nc), suggesting a decrease in mitochondrial mass. On the other hand, the moderately reduced CI activity in NDUFV1 cells was associated with an increase in F and normal Nc, suggesting an increase in mitochondrial mass (31).

Using FCS, we found here that a similar fraction of mitochondria (∼80%) was moving in both healthy and patient fibroblasts. However, patient mitochondria moved 2.5-times faster. Intramatrix protein diffusion was similar for moving and stationary mitochondria in healthy fibroblasts. In contrast, patient mitochondria displayed either a 20% slower (stationary organelles) or 2.5-times increased intramatrix diffusion (moving organelles). These results clearly demonstrate that the major difference between healthy and patient fibroblasts involves the velocity and matrix protein diffusion rate of moving mitochondria.

Blocking mitochondrial movement with Noc did not affect the rate of intramatrix protein diffusion in both healthy and patient fibroblasts. This shows that mitochondrial velocity and intramatrix protein diffusion are mutually independent. Although the two patient fibroblast lines analyzed in the present study represent opposite extremes in terms of residual CI activity and F (28), no differences in fraction and velocity of moving mitochondria and/or intramatrix protein diffusion were observed. This finding suggests the absence of a quantitative relationship between these latter parameters and either residual CI activity or mitochondrial shape for the patient fibroblasts.

**Mitochondrial velocity is increased in patient fibroblasts.** On average, ∼80% of the mitochondria present in healthy and patient fibroblasts displayed two EYFP translational diffusion coefficients (D_fast2 and D_slow2), whereas the remainder exhibited only a single EYFP translational diffusion coefficient (D_fast1). Treatment with Noc abolished mitochondrial movement and caused complete disappearance of the slow diffusion component (D_slow2). The same result was obtained before with healthy fibroblasts and led to the conclusion that D_slow2 is a quantitative measure of microtubule-mediated mitochondrial movement (30). At first sight, our finding that the fraction of moving mitochondria is not altered in patient fibroblasts seems to contradict our observation that chronic CI inhibition by rotenone (100 nM, 72 h) decreased this fraction in healthy fibroblasts (30). However, the residual CI activity was lower in rotenone-treated healthy fibroblasts (20% of control) in comparison with the patient fibroblasts used in the present study (Table 1). This may suggest that, in living fibroblasts, mitochondria stop to move when their CI activity decreases to below a critical level. In accordance with this idea, the velocity of moving mitochondria was lower in rotenone-treated healthy fibroblasts (30). Alternatively, in view of recent work showing that mitochondrial movement is inhibited at high cellular ADP levels (36), our FCS data may be interpreted as an indication that CI-deficient patient fibroblasts have metabolically adapted to the CI-deficient state, whereas, in contrast, healthy fibroblasts chronically treated with rotenone have not. Such an adaptation may also explain the unexpected finding that mitochondrial velocity was two times higher in patient compared with healthy fibroblasts. Our data furthermore show the lack of any correlation between fraction and velocity of moving mi-
Intra-matrix protein diffusion is faster in moving mitochondria of patient fibroblasts. EYFP diffusion within the mitochondrial matrix was quantified by $D_{fast1}$ in stationary mitochondria, $D_{fast2}$ in moving mitochondria and $D_{noc}$ in mitochondria of cells treated with Noc. In healthy fibroblasts, $D_{fast1}$, $D_{fast2}$, and $D_{noc}$ were equal, demonstrating the absence of any relation between intra-matrix protein diffusion and mitochondrial movement. Similarly, $D_{fast1}$, $D_{fast2}$, and $D_{noc}$ did not differ between the two patient fibroblast lines, suggesting that intra-matrix protein diffusion is not quantitatively related to residual CI activity and/or mitochondrial shape in CI-deficient patient fibroblasts. In both patient fibroblast lines, however, $D_{fast2}$ was three times larger than $D_{fast1}$, showing that intra-matrix protein diffusion is faster in moving compared with stationary mitochondria. Together, these results are compatible with the idea that, in patient fibroblasts, primarily mitochondrial velocity and intra-matrix protein diffusion are increased but that these increases are mutually independent.

The protein concentration in the mitochondrial matrix is estimated to be 300 mg/ml (23), which makes it the most crowded compartment of the cell. It has been demonstrated that the mitochondrial matrix can assume two configurations, the orthodox state, which is characterized by a large volume, a low protein concentration, and fast metabolite diffusion, and the condensed state, with a small volume, a high protein concentration, and slower metabolite diffusion (18). EM analysis of mitochondrial ultrastructure revealed that the density of the mitochondrial matrix was not detectably different between control and patient fibroblasts. Similarly, cristae morphology, previously predicted to affect matrix protein diffusion (39, 56), was unaltered in patient cells. Although 80% of all mitochondria were moving in our cell FCS experiments, we do not know how quickly matrix protein diffusion increases when stationary mitochondria start moving or decreases when moving mitochondria become stationary. Obviously, mitochondrial movement will immediately seize during fixation prior to EM, and this may explain why no difference in matrix density and/or cristae morphology between control and patient cells was observed. Alternatively, the change in matrix density toward a less dense state may only be relatively small and therefore not detectable by EM analysis.

In light of the above findings, the marked increase in matrix protein diffusion in moving patient fibroblast mitochondria is compatible with the idea that, in these mitochondria, the matrix has adopted a less dense configuration. It has been demonstrated that mitochondria with a more orthodox matrix have a lower metabolic activity than mitochondria with a condensed matrix (44). Accordingly, the mitochondrial metabolic activity may be decreased in moving mitochondria of patient fibroblasts. This conclusion is in agreement with the CI-deficient state of the above mitochondria and is supported by our previous finding that intramatrix EYFP diffusion is markedly increased in mitochondria of healthy fibroblasts chronically treated with rotenone (30). Recent work revealed that the matrix configuration of cancer cell mitochondria changed from condensed to orthodox upon replacement of galactose with glucose in the culture medium, thereby forcing the cells to change their mode of energy production from oxidative into glycolytic (41). Extrapolation of this result to the present study supports our idea that patient fibroblasts have also become more glycolytic. This is supported by previous findings revealing an increased lactate-to-pyruvate ratio in cultured skin fibroblasts harboring respiratory chain defects (61). In agreement with this idea, cells with impaired OXPHOS function were found to generate ATP via the glycolytic pathway when grown in a medium containing both d-glucose and pyruvate (16). Similarly, it was demonstrated that glycolysis was increased in 143B hybrid cells with pathogenic mtDNA point mutations (60). Finally, mouse hearts depleted of mtDNA by ablation of the tfam gene displayed a global switch from oxidative to glycolytic metabolism (20).

Conclusions. Taken together, our findings are compatible with a model in which inherited CI deficiency causes two mutually independent changes, first, a change toward a less dense configuration of the mitochondrial matrix, and second, a change toward a more glycolytic mode of energy production associated with an increase in mitochondrial velocity. According to this model, the latter change is not (yet) achieved in healthy fibroblasts treated with rotenone for 3 days. Most likely, the observed change in matrix protein diffusion reflects an adaptation to the CI-deficient state of the mitochondria in that it may serve to reduce diffusion bottlenecks, thus promoting exchange of metabolites across the inner mitochondrial membrane and diffusion of matrix constituents.

GRANTS

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REFERENCES


