Human Complex I Defects Can Be Resolved by Monoclonal Antibody Analysis into Distinct Subunit Assembly Patterns*

Received for publication, October 31, 2000, and in revised form, November 29, 2000
Published, JBC Papers in Press, December 8, 2000, DOI 10.1074/jbc.M009903200

Ralf H. Triepels‡, Bonnie J. Hanson§¶, Lambert P. van den Heuvel‡, Linda Sundell§, Michael F. Marusich§, Jan A. Smeitink§, and Roderick A. Capaldi§**

From the ‡Department of Pediatrics, Nijmegen Center for Mitochondrial Disorders, University Hospital Nijmegen St. Radboud, 6500 HB Nijmegen, the Netherlands and §Institute of Molecular Biology and ¶Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403

Complex I defects are one of the most frequent causes of mitochondrial respiratory chain disorders. Therefore, it is important to find new approaches for detecting and characterizing Complex I deficiencies. In this paper, we introduce a new set of monoclonal antibodies that react with 39-, 30-, 20-, 18-, 15-, and 8-kDa subunits of Complex I. These antibodies are shown to aid in diagnosis of Complex I deficiencies and add understanding to the genotype-phenotype relationships of different mutations. A total of 11 different patients were examined. Four patients had undefined Complex I defects, whereas the other patients had defects in NDUFS1, NDUFS2 (two patients), NDUFS4 (two patients), NDUFS7, and NDUFS8. We show here that Western blotting with these antibodies, particularly when used in conjunction with sucrose gradient studies and enzymatic activity measurements, helps distinguish catalytic versus assembly defects and further distinguishes between mutations in different subunits. Furthermore, different mutations in the same gene are shown to give very similar subunit profiles, and we show that one of the patients is a good candidate for having a defect in a Complex I assembly factor.

Disorders of mitochondrial energy metabolism occur in humans with a frequency of ~1 in 10,000 live births (1). Most are caused by the dysfunction of one or more of the enzyme complexes of oxidative phosphorylation (OXPHOS).1 Isolated enzymatic deficiency of the first OXPHOS complex, NADH:ubiquinone oxidoreductase (EC 1.6.99.3) or Complex I, is one of the most frequent causes of mitochondrial respiratory chain disorders (2). Complex I is the first multiprotein complex of the OXPHOS system (3) and participates in the formation of a proton gradient across the inner mitochondrial membrane coupled to transfer of electrons from NADH to ubiquinone. This proton gradient provides part of the proton-motive force used for ATP production. Complex I is the largest of the respiratory chain complexes, made up of seven different subunits encoded on mitochondrial DNA (mtDNA; ND1–6 and ND4L) and 35 or more different subunits encoded by nuclear genes (4, 5). Together, these subunits form a complex with an estimated molecular mass of 900,000 daltons (3). Mutations in both the mitochondrial and nuclear encoded genes are known to cause Complex I deficiencies (6). However, in addition to the structural genes, there may be additional genes encoding proteins required for the assembly of a functional Complex I. So-called “assembly factors” involved in assembly of Complex IV and the ATP synthase have already been reported (7–9). Mutations in SURF1, an assembly factor required for full assembly of Complex IV, has been shown to cause cytochrome c oxidase deficiency in many of the reported cases of Leigh’s disease (7, 10, 11).

The genes for all of the components of Complex I have now been identified (12), opening up the possibility of genetic approaches for diagnosis. However, such an analysis would not identify Complex I deficiencies caused by mutations in assembly factors until these factors are identified and even then would not yield sufficient information to understand the genotype-phenotype relationships of the various mutations that can occur. Therefore, in addition to genetic analysis, it is important to have protein-based approaches to detecting and characterizing Complex I deficiencies. Here, we introduce a monoclonal antibody set that will be useful in this regard. We show that Western blotting with these antibodies, particularly when used in conjunction with sucrose gradient studies and enzymatic activity measurements, distinguishes catalytic versus assembly defects. In the latter class, patient samples can be classified by their assembly profiles, which appear to be representative of which subunit is mutated.

EXPERIMENTAL PROCEDURES

Purification of Bovine Heart Complex I—Biochemically purified bovine heart Complex I as well as the flavoprotein, iron-sulfur protein, and hydrophobic protein subfractions of Complex I isolated as described previously (13–15) were kindly supplied by Dr. Youssef Hatefi (The Scripps Institute, La Jolla, CA). Immuno purified bovine heart Complex I was generated by solubilizing bovine heart mitochondria in 1% N-dodecyl-β-D-maltoside (LM; Calbiochem), centrifuging twice (10,000 × g, 12 min) to remove insoluble material, passing the supernatant over an immunoaffinity column generated as described previously (16) using the 15-kDa Complex I antibody created in this laboratory, washing with phosphate-buffered saline (PBS) containing 0.05% LM, and eluting with 100 mm glycine, pH 2.5.

Cell Lines—MRC5 fibroblasts were obtained from the American Type Culture Collection, and MRC5-Rho0 fibroblasts were derived from the MRC5 fibroblasts by culturing the cells in permissive medium supplemented with 50 ng/ml ethidium bromide as described previously (17). Patient fibroblasts were obtained from skin biopsies of young children in whom an isolated Complex I deficiency has been confirmed in

* This work was supported in part by Prinzes Beatrix Fonds Grant 97-0111 (to J. A. S. and L. P. v. d. H.), National Institutes of Health Grant HL24526 (to R. A. C.), and the Fonds Beoefening Wetenschap 97-0111 (to J. A. S. and L. P. v. d. H.), National Institutes of Health GM07759.
¶ Supported by National Institutes of Health Training Grant 5881; Fax: 541-346-5891; E-mail: rcapaldi@oregon.uoregon.edu.
† The abbreviations used are: OXPHOS, oxidative phosphorylation; mtDNA, mitochondrial DNA; LM, N-dodecyl-β-D-maltoside; PBS, phosphate-buffered saline; mAb, monoclonal antibody; CMF-PBS, Dulbecco’s phosphate-buffered saline.

** To whom correspondence should be addressed: Institute of Molecular Biology, University of Oregon, Eugene, OR 97403. Tel.: 541-346-5881; Fax: 541-346-5891; E-mail: rcapaldi@oregon.uoregon.edu.

Printed in U.S.A.

This paper is available online at http://www.jbc.org
muscle tissue as well as in cultured fibroblasts, using the slightly modified method of Fischer et al. (18). The phenotypes and genotypes of the patients included in this study have been extensively described by Loeffen et al. (2). Control fibroblasts were obtained from postcultural tissue from a child in the same age range in whom biochemical enzyme analyses revealed normal results.

**Geneic Characterization of Patient Cell Lines**—All patients included in this study were screened for the presence of DNA alterations in each of the known nuclear-encoded "structural" genes of Complex I as described previously (19, 20). Mutations were found in 7 of the 11 patients as listed in Table I. mtDNA was also screened for deletions and point mutations that have previously been shown to cause Complex I deficiency. These are T14484C, G14459A (mutations in the mitochondrial ATPase subunit 6) and T8993G/C (mutation in the mitochondrial complex I subunit ND6), G11778A (mutation in the mitochondrial complex I subunit ND1), T8993G/C (mutation in the mitochondrial complex I subunit ND6), G11778A (mutation in the mitochondrial complex I subunit ND1), and finally frozen at 80 °C. Protein amounts were estimated by A260 determination.

**Fibroblast Culture and Mitochondrial Protein Isolation**—Human control and patient fibroblasts were grown in M199 (Life Technologies), 5 mg/liter Tween 20 medium with 10% fetal calf serum, 100 IU/ml penicillin, and 100 μM streptomycin. Approximately 30 × 106 cells were harvested at 95% confluence after mild trypsinization (3–5 min) with 2–3 ml 0.25% trypsin solution/175-cm2 (5 × 106 cells) cell culture. Cells were resuspended in 50 ml 10% fetal calf serum-PBS. Cells were rinsed three times with 1% fetal calf serum-PBS as well as with PBS and finally frozen at −80 °C. For mitochondrial pellets, cells were solubilized in 5 ml homogenization buffer (1 mM EDTA, 0.25 M sucrose, 10 mM Tris, pH 7.4) containing protease inhibitors (0.5 μg/ml leupeptin, 0.5 μg/ml pepstatin, and 1 mM phenylmethylsulfonyl fluoride). Cells were repeatedly (three times) homogenized with a motorized pestle (15–20 strokes), and the postnuclear supernatants were pooled after centrifugation (10 min, 15000 g). Mitochondrial pellets were obtained by centrifugation of the collected postnuclear supernatants (15 min, 10000 × g). The mitochondrial pellets were washed twice (15 min, 10000 × g) with 2 ml washing buffer (1 mM EDTA, 0.25 M sucrose, 10 mM Tris/HCl, pH 7.5) including protease inhibitors (0.5 μg/ml leupeptin, 0.5 μg/ml pepstatin, 1 mM phenylmethylsulfonyl fluoride). Finally, pellets were saved in 200 μl protease inhibitors/washing buffer and stored frozen at −80 °C. Protein amounts were estimated by A260 determination.

**Western Blot Analysis of Mitochondrial Proteins**—Approximately 5 μg/lane mitochondrial protein, dissolved in SDS-polyacrylamide gel electrophoresis-Tricine sample buffer (Bio-Rad) containing 2% β-mercaptoethanol (30 min, 37 °C), were separated on 10–20% gradient polyacrylamide gels in a Mini-Protein II Apparatus (Bio-Rad). After electrophoresis (100-V stacking gel and 150-V running gel), proteins were transferred electrophoretically (2 h, 0.10 A) to 0.45-μm polyvinylidene difluoride membranes in transfer buffer (10% methanol in 10 mM 3-[cyclohexylamin]-1-propanesulfonic acid, pH 11) on ice. The polyvinylidene difluoride membranes were blocked overnight with 5% nonfat dry milk powder in Dulbecco’s phosphate-buffered saline (CMF-PBS). Afterward the blots were treated with primary antibodies diluted in 5% milk CMF-PBS for 2 h. After rinsing the blot three times with CMF-PBS and 0.05% Tween 20, the blots were incubated for 2 h with horseradish peroxidase-conjugated goat anti-mouse IgG + M (heavy and light chain) at 0.2 μg/ml (Jackson ImmunoResearch) in CMF-PBS. Specific detection of the secondary antibody was obtained with the chemiluminescent Western blotting detection reagent ECL Plus (Amersham Pharmacia Biotech) after rinsing the blots with CMF-PBS three times. Fluorescence was quantified using a Storm 860 chemiluminescence imager and the accompanying Molecular Dynamics Imagequant software. Ratios of individual proteins were calculated in relation to the porin signal. The ratios of the control fibroblast cell line were set to 100%, and the ratios of other cell lines were reported in comparison with this. The obtained results represent average values of two to five independent experiments for each subunit.
**RESULTS**

**Antibody Characterization**—The antigen used to generate monoclonal antibodies was beef heart Complex I purified according to Hatefi (15). Screening involved an assay for binding to native Complex I, Western blotting, and/or immunohistochemistry. Biochemically purified and immunopurified bovine Complex I, 39-kDa subunit (CIII-Core 2), immunopurified bovine Complex I, and human mitochondria, and whole-cell extracts to purified bovine Complex I, immunopurified bovine Complex I, and human fibroblast cell lines from controls and patients were used in the screening. Unequivocal identification of two of the antibodies was also made by Western blotting after overexpressing the candidate human subunit antigens in E. coli; WB, Western blot with biochemically and immunopurified bovine Complex I and human mitochondria.

**Sucrose Gradient Centrifugation**—Mitochondria (1 mg) from control MRC5 fibroblasts and three patient cell lines (patients 1, 7, and 11) were pelleted (10,000 × g, 10 min, 4 °C) and resuspended at a protein concentration of 5 mg/ml in 100 mM Tris/HCl, 1 mM EDTA, pH 7.5, 1% LM, 1 mg/ml pepstatin, and 1 mg/ml leupeptin. After overexpressing the candidate human subunit antigens in E. coli, mitochondria were solubilized in this solution for 20 min on ice with stirring before any insoluble membranes were pelleted again by centrifugation (10,000 × g, 10 min, 4 °C). The supernatant was layered on top of a discontinuous sucrose gradient composed of 250 µl of 35% sucrose, 500 µl of 30% sucrose, 750 µl of 27.5% sucrose, 1 ml of 25% sucrose, 1 ml of 20% sucrose, and 1 ml of 15% sucrose. All sucrose solutions contained 100 mM Tris/HCl, pH 8.0, 0.05% LM, and 1 µg/ml pepstatin, 1 µg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride. The gradient was then centrifuged overnight at 4 °C (128,000 × g, 16.5 h, SW 50.1). The sucrose gradient was fractionated from the bottom of the tube into 500-µl fractions, which were frozen at −80 °C. For Western blotting, 20 µl of each fraction was loaded per lane.

**TABLE II**

Monoclonal antibodies used in this study

| mAb Name | Mol wt | Gene Name | Method of identification |
|----------|--------|-----------|--------------------------|
| 20C11B11B11 | 39 | NDUFA9 | OE, WB |
| 17D950C9 | 30 | NDUFS3 | OE, WB |
| 29ED9H10 | 20 | ND | WB |
| 22B5E8 | 18 | ND | WB |
| 17G3D9E12 | 15 | ND | WB |
| 17C3E4E11 | 8 | ND | WB |

* Molar weights based on SDS-polyacrylamide gel electrophoresis.
* OE, overexpression of the human form of the protein in E. coli; WB, Western blot with biochemically and immunopurified bovine Complex I and human mitochondria.
* ND, not determined.

**Patient Characterization**—Fibroblasts were cultured from 11 patients in whom an isolated enzymatic Complex I deficiency had been confirmed in muscle tissue as well as cultured fibroblasts. In seven of the patients, the pathogenic mutation was identified genetically, and for four patients the genetic defect was not identified. The residual Complex I activities of the 11 patient fibroblast cell lines ranged from 35 to 85%. Specifics of each patient are provided in Table I.

**Confirmation of Isolated Complex I Deficiency by Western Blotting**—Mitochondria were isolated from each of the patient fibroblast cell lines, a control skin fibroblast cell line, and normal and Rho0 MRC5 fibroblasts (a lung fibroblast cell line). Samples of each were examined by Western blotting with mixtures of antibodies, including ones specific to the 39-kDa subunit of Complex I, the 70-kDa subunit of succinate dehydrogenase (Complex II), core II protein of Complex III, subunit II of cytochrome c oxidase (Complex IV), subunit IV of Complex IV, the α subunit of F$_{1}$F$_{0}$ (Complex V), and porin (as a control for equal loading of lanes). Fig. 1 summarizes the data with a bar graph in which the levels of each complex are quantitated by determining the amount of the component subunit in each complex.
patient cell line in relation to that found in control skin fibroblasts. From the bar graph, it can be seen that the levels of the 39-kDa subunit of Complex I, but not that of any of the other OXPHOS subunit probed, are diminished in most of the patient cell lines. This is different from the MRC5-Rho0 mitochondria, in which loss of mtDNA leads to an absence of subunit II of Complex IV and lower levels of the core II protein of Complex III.

Variations in Complex I Assembly Identified by Western Blotting—The mitochondrial samples of the 11 patient fibroblasts were examined for levels of six different subunits of Complex I (referred to by their apparent molecular weights as listed in Table II). For the most part, mAbs to the 30-, 20-, 15-, and 8-kDa subunits were used as an antibody mixture along with porin, and the amounts of the 39- and the 18-kDa subunits were quantitated relative to porin separately. A representative Western blot and a bar graph of the levels of the six components of Complex I in the different samples are shown in Fig. 2. A significant reduction in the levels of one or more components of the complex was seen in all but one of the patient samples; that is, patient 7, with a mutation in NDUFV1. The patterns of subunit loss were similar in patients 3 and 4, each with a mutation in NDUFV1. The patterns of subunit loss were similar in patients 5 and 6, each with a different mutation in NDUF54. Similarly, the pattern of subunit loss was the same in patients 5 and 6, each with a different mutation in the same subunit, NDUF52. Patients 9 and 10, both of which have unidentified mutations, show remarkable similarity in the pattern of subunit loss. This pattern most closely resembles that of patients 3 and 4. In Rho0 cells, where there is an absence of the mitochondrially encoded subunits of Complex I, a different pattern from any of the patient samples is observed. In this case, the levels of the 20- and 18-kDa subunits are as low or lower than those of the 15- and 8-kDa subunits. Subunit amounts were lowest in patient 11, identifying this as a likely candidate for a mutation in an assembly factor (see below).

The relationship between the loss of each subunit as detected by Western blot analysis and the residual Complex I activity is shown in Fig. 3. In each panel, the dashed line represents what would be expected if there were a perfect correlation between loss of subunit and loss of activity. As shown in Fig. 3, the levels of the 39- and 30-kDa subunits most closely track the loss of activity. However, in most cases the levels of the 20- and 18-kDa subunits are higher than predicted from the activity effects, whereas the levels of the 15- and 8-kDa subunits are lower than the residual levels of enzymatic activity.

Sucrose Gradient Analysis of Patient Cell Lines—Mitochondria from three cells lines, i.e. from patient 7 with a mutation in NDUFV1, patient 1 with a mutation in NDUF7, and patient 11, with an unknown mutation, were each dissolved in 1% LM and subjected to sucrose gradient centrifugation using a discontinuous gradient. In this gradient, the five complexes of OXPHOS are separated by size, and each can be identified by

---

**Fig. 3. Relationship between loss of Complex I subunits and residual Complex I activity.** For each patient and the control fibroblasts, the level of each of the indicated subunits as detected by Western blot was plotted in relation to the residual Complex I activity. In each panel, the dashed line represents what would be expected if there were a perfect correlation between loss of subunit and loss of activity.
Western blotting of the fractions with monoclonal antibodies. Densitometric scans of the Western blots can then be made, and, for convenience, the relative expression levels of each subunit in the various fractions can be expressed as a percentage of the highest intensity band in the gradient. Fig. 4 shows the distribution in the gradient of the Va subunit of cytochrome c oxidase as well as the 39- and 20-kDa subunits of Complex I for the three patient cell lines and a control of MRC5 fibroblasts. Complex I, with a molecular weight of close to 900,000, elutes before the other respiratory chain complexes after gradient separation. The 39- and 20-kDa subunits of patients 1 and 7 elute at a position similar to that of control Complex I, indicative of complete or near complete assembly. However, in patient 11, the 39- and 20-kDa subunits migrate in subcomplexes of ~200 and 500 kDa, respectively, and there is also a free subunit (eluting in fraction 9). Thus, in patient 11, assembly of Complex I is poor.

**DISCUSSION**

Monoclonal antibodies are finding widespread use in detecting and analyzing mitochondrial disorders (17, 24). In the case of cytochrome c oxidase, a panel of antibodies has been developed, which reveals different patterns of assembly of the enzyme complex (25), and this is providing a good indicator of which gene has been mutated, including not only structural components but assembly factors as well. Here, we show the utility of mAbs in the study of Complex I disorders. Six different mAbs were used to examine 11 different patients in which OXPHOS enzyme activity measurements had identified an isolated Complex I deficiency. In seven of these, the mutations had been determined by extensive gene sequencing. In the other four, a mutation has not yet been identified. Screening with an antibody mixture containing an mAb against at least one subunit of each of the five OXPHOS complexes supports the conclusion from enzymatic data that all are deficient in Complex I alone. It is important to note that the antibody screen to localize the defect by the complex or complexes involved is relatively rapid and easily performed and requires much less sample than enzymatic assays.

The reaction of the six mAbs against Complex I used here was variable, and several different patterns of steady-state levels of subunits could be distinguished. For two subunits, NDUF54 and NDUF52, two patient cell lines were available with different mutations in the same gene. In both cases, the subunit profiles resulting from the two different mutations were essentially the same.

In general, the subunits behaved as three classes. The levels of the 39- and 30-kDa subunits varied in the same way, as did the 20- and 18-kDa subunits, whereas the 15- and 8-kDa subunits are a third class. In most patients, the levels of the 20- and 18-kDa subunits were higher than the levels of functional complex as measured by enzymatic activity, whereas the levels of the 15- and 8-kDa subunits were lower. Interestingly, the Rho0 cells seem to behave differently, because the 15- and 8-kDa subunits are present in higher amounts relative to the 20- and 18-kDa subunits.

The implication of the data in Fig. 3 is that the steady-state levels of fully assembled Complex I depend on expression levels of all of the subunits being examined here. When any one subunit is mutated, the levels of assembled Complex I are reduced. The lower levels of the 15- and 8-kDa subunits in relation to activity could indicate more lability of these subunits after assembly of the complex. One of the patient cell lines, patient 11, had very low levels of all of the subunits examined and values significantly lower than expected based on activity measurements. It could be that enzymatic activity was overestimated or that the complex is more labile to detergent solubilization with a resulting proteolysis of polypeptides because of the mutation. On the basis of the antibody data, patient 11 is the most likely to involve a mutation in an assembly factor for Complex I. The levels of subunits are low, and these subunits are not in a fully assembled complex based on the sucrose gradient data. The comparison of subunit profiles as shown here allows patients to be sorted as in genetic complementation studies, so that with wider screening of patients, a group of possible Complex I assembly factor mutants can be collected for chromosomal analysis and gene identification, as was done for the SURF1 mutations of cytochrome c oxidase (8).

Besides a role in diagnosis, the antibody studies here add to the understanding of genotype-phenotype relationships of mutations already specified genetically. Patient 7 was shown to carry a mutation, T423M, in NDUFV1, the flavin-containing subunit of Complex I. The subunit profile and, more definitively, the sucrose gradient experiments show that the ~25% loss of activity is due to altered catalytic function and not a failure to assemble the complex. This is different from other patients studied here, such as patients 5, 6, and 8, in whom the levels of all of the subunits probed were significantly decreased, suggesting a more profound defect.

In summary, we provide evidence of the utility of monoclonal antibody analysis in the characterization of Complex I deficiencies. It appears that different assembly profiles occur when different subunits are mutated. Antibodies to additional subunits of Complex I will be required to extend the work reported here, and this project is ongoing. Complex I patients have been reported from many centers studying mitochondrial disorders. A more comprehensive analysis of the range of assembly patterns and correlation with site of the mutation will require
collaboration and the sharing of the antibodies and cell lines, which should be possible.

Acknowledgments—We greatly appreciate the technicians of the Nijmegen Center for Mitochondrial Disorders biochemistry group for measurements of the OXPHOS activities in skeletal muscle and cultured skin fibroblasts. We also thank Frans Trijbe and Rob Sengers for continuous support and are grateful to all colleagues who provided the patient cell lines.

REFERENCES
1. Bougeron, T., Rustin, P., Chretien, D., Birch-Machin, M., Bourgeois, M., Viegas-Pequignot, E., Munnich, A., and Rotig, A. (1995) Nat. Genet. 11, 144–149
2. Loeffen, J. L., Smeitink, J. A., Trijbels, J. M., Janssen, A. J., Triepels, R. H., Sengers, R. C., and van den Heuvel, L. P. (2000) Hum. Mut. 15, 123–134
3. Walker, J. E. (1992) Q. Rev. Biophys. 25, 253–324
4. Grigorieff, N. (1999) Curr. Opin. Struct. Biol. 9, 476–483
5. Skehel, J. M., Fearnley, I. M., and Walker, J. E. (1998) FEBS Lett. 438, 301–305
6. Smeitink, J., and van den Heuvel, L. (1999) Am. J. Hum. Genet. 64, 1505–1510
7. Ackerman, S. H., and Tragou, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 87, 4986–4990
8. Tiranti, V., Hoernagel, K., Carrozzo, R., Galimberti, C., Munaro, M., Granatiero, M., Zelante, L., Gasparini, P., Marzella, R., Rocchi, M., Bayona-Bafaluy, M. P., Enriquez, J. A., Uziel, G., Bertini, E., Dionisi-Vici, C., Franco, B., Meitinger, T., and Zeviani, M. (1998) Am. J. Hum. Genet. 63, 1609–1621
9. Papadopoulou, L. C., Sue, C. M., Davidson, M. M., Tanji, K., Nishino, I., Sadlock, J. E., Krishna, S., Walker, W., Selby, J., Glumom, D. M., Coster, R. V., Lyon, G., Seilais, E., Lebel, R., Kaplan, P., Shanske, S., De Vivo, D. C., Bonilla, E., Hirano, M., DiMauro, S., and Schon, E. A. (1999) Nat. Genet. 23, 333–337
10. Teraoka, M., Yokoyama, Y., Ninomiya, S., Inoue, C., Yamashita, S., and Seino, Y. (1999) Hum. Genet. 103, 560–563
11. Poyau, A., Buchet, K., Bouzidi, M. F., Zahot, M. T., Echenne, B., Yao, J., Shoubridge, E. A., and Godinot, C. (2000) Hum. Genet. 106, 194–205
12. Walker, J. E., Skehel, J. M., and Buchanan, S. K. (1995) Methods Enzymol.
13. Hatef, Y. (1978) Methods Enzymol. 53, 11–14
14. Galante, Y. M., and Hatef, Y. (1978) Methods Enzymol. 53, 15–21
15. Galante, Y. M., and Hatef, Y. (1979) Arch. Biochem. Biophys. 192, 559–568
16. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Marse, M., Robinson, R. H., Taanman, J. W., Kim, S. J., Schillace, R., Smith, J. L., and Capaldi, R. A. (1997) Biochim. Biophys. Acts 1362, 145–159
18. Fischer, J. C., Ruitenbeek, W., Trijbe, J. M., Veerkamp, J. H., Stadhouders, A. M., Sengers, R. C., and Janssen, A. J. (1986) Clin. Chim. Acta 155, 263–273
19. Tribels, R., Smeitink, J., Loeffen, J., Smeets, R., Trijbels, J. M., and van den Heuvel, L. (2000) Hum. Genet. 106, 385–391
20. Smeitink, J. A. M., Loeffen, J. L. C. M., Trijbels, R. H. S., Smeets, R. J. P., Tribels, J. M. F., and van den Heuvel, L. P. (1998) Hum. Mol. Genet. 7, 1573–1579
21. Li, Y. Y., Hengstenberg, C., and Mainsch, B. (1995) Biochem. Biophys. Res. Commun. 210, 211–218
22. Marusich, M. F. (1988) J. Immunol. Methods 114, 155–159
23. Capaldi, R. A., Marusich, M. F., and Taanman, J. W. (1995) Methods Enzymol.
24. Garcia, J. J., Ogilvie, I., Robinson, B. H., and Capaldi, R. A. (2000) J. Biol. Chem. 275, 11073–11081
25. von Kleist-Jetzow, J. C., Vial, E., Chattrel-Grousard, K., Rötig, A., Munnich, A., Rustin, P., and Taanman, J. W. (1999) Biochim. Biophys. Acts 1453, 35–44
26. Trijbels, R. H., van den Heuvel, L. P., Loeffen, J. L., Buskens, C. A., Smeets, R. J. R., Rubio-Goralbo, M. E., Budde, S. M., Mariman, E. C., Wijburg, F. A., Barth, P. G., Trijbels, J. M., and Smeitink, J. A. (1999) Ann. Neurol. 45, 797–799
27. Loeffen, J., Smeitink, J., Trijbels, R., Smeets, R., Schuclke, M., Sengers, R., Trijbels, P., Hame, N., Pillaart, R., and van den Heuvel, L. (1999) Am. J. Hum. Genet. 65, 1508–1608
28. van den Heuvel, L., Ruitenbeek, W., Smeets, R., Gelman-Kohan, Z., Elpeleg, O., Loeffen, J., Trijbels, F., Mariman, E. D., and Smeitink, J. (1999) Am. J. Hum. Genet. 65, 262–268
29. Schuclke, M., Smeitink, J., Mariman, E., Loeffen, J., Plecek, B., Trijbels, F., Stockler-Ipsiroglu, S., and van den Heuvel, L. (1999) Nat. Genet. 21, 260–261