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# The NDUFS4 nuclear gene of complex I of mitochondria and the cAMP cascade

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**Abstract**

Results of studies on the role of the 18 kDa (IP) polypeptide subunit of complex I, encoded by the nuclear NDUFS4 gene, in isolated bovine heart mitochondria and human and murine cell cultures are presented.The mammalian 18 kDa subunit has in the carboxy-terminal sequence a conserved consensus site (RVS), which in isolated mitochondria is phosphorylated by cAMP-dependent protein kinase (PKA). The catalytic and regulatory subunits of PKA have been directly immunodetected in the inner membrane/matrix fraction of mammalian mitochondria. In the mitochondrial inner membrane a PP2Cγ-type phosphatase has also been immunodetected, which dephosphorylates the 18 kDa subunit, phosphorylated by PKA. This phosphatase is $Mg^{2+}$-dependent and inhibited by $Ca^{2+}$. In human and murine fibroblast and myoblast cultures "in vivo", elevation of intracellular cAMP level promotes phosphorylation of the 18 kDa subunit and stimulates the activity of complex I and NAD-linked mitochondrial respiration.Four families have been found with different mutations in the cDNA of the NDUFS4 gene. These mutations, transmitted by autosomal recessive inheritance, were associated in homozygous children with fatal neurological syndrome. All these mutations destroyed the phosphorylation consensus site in the C terminus of the 18 kDa subunit, abolished cAMP activation of complex I and impaired its normal assembly.


*Keywords:* Mitochondrion; Complex I; NDUFS4 gene; cAMP-cascade

## 1. Introduction

A key feature of cellular metabolism is represented by its complex regulation by a variety of factors. This can be effected by modulation of transcription, translation, post-translational modification, membrane traffic and assembly of enzymes and carrier proteins.

Ultimately the activity of mature proteins can be modulated "in situ" by covalent modification of the proteins or by conformational changes in response to specific ligands.

All this is of particular importance in mammalian tissues which have to adapt the rate of metabolic processes to continuous changes in their activity under different physiopathological conditions.

In mammalian tissues the respiratory chain complexes have a large number of supernumerary subunits, as compared to prokaryotes and lower eukaryotes, which if not necessary for the primary catalytic process can contribute to confer them large regulatory flexibility [1]. Studies on the relative activities and capacity reserves of the respiratory chain complexes, in isolated mitochondria and cell cultures, show these to vary considerably from tissue to tissue as well as in different cell lines [1,2]. Changes in the activities of redox complexes in the same tissue or cell lines in different physiopathological conditions and/or in response to biological effectors have been reported [1,3].

The present paper summarizes the results of studies from our group on the phosphorylation by cAMP-dependent protein kinase (PKA) of the 18 kDa (IP) subunit of complex I, encoded by the nuclear gene NDUFS4, the functional impact of this process and the involvement of this subunit in the assembly of complex I.

## 2. The 18 kDa (IP), NDUFS4-encoded subunit of complex I

In mammalian mitochondria, complex I catalyses the oxidation of NADH by ubiquinone and conserves the free energy so made available as transmembrane proton-motive

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Table 1
Relative activities and inhibition thresholds of respiratory chain complexes in isolated mitochondria and human and murine cell cultures

| | | Activities of complexes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | nequivalents·e$^{-}$·min$^{-1}$·mg prot$^{-1}$ | | | | | | |
| | | I–III | III | IV | III/I–III | IV/I–III | IV/III | Refs. |
| Human skeletal muscle mitochondria | Males | 228 | 420 | 739 | 1.84 | 3.24 | 1.76 | [6] |
| | Females | 223 | 586 | 729 | 2.63 | 3.27 | 1.24 | |
| | | fmoles (NADH) or fatoms O·min$^{-1}$·cell$^{-1}$ | | | | | | |
| | | I | | IV | | IV/I | | |
| Mouse fibroblasts | | 4.85 | | 9.40 | | 1.94 | | [7] |
| Human fibroblasts | | 2.39 | | 9.87 | | 4.13 | | [8] |
| Mouse myoblasts | | 7.75 | | 13.09 | | 1.68 | | [9] |
| | | Inhibition tresholds | | | | | | |
| | | I | | III | | IV | | |
| Synaptic Mitochondria | | 25% | | 80% | | 70% | | [10] |

force (PMF). The reaction mechanism of the complex, the functional role of its numerous constituent subunits [4] and the biogenesis are still poorly understood [5]. Complex I represents, at least under certain conditions, the rate-limiting step of the overall respiratory activity in mitochondria (Table 1). Complex I deficiency represents one of the most severe and frequent of the disorders in the mitochondrial energy metabolism associated with human diseases [11,12].

The complex consists of 43 subunits [4,13]. Seven subunits (ND1–ND6 and ND4L) are encoded by the mitochondrial genome; the others are encoded by nuclear genes. The cDNA of the seven mitochondrial subunits [14] and of 35 nuclear subunits [15,16] have been sequenced. The function of only few of the subunits of complex I has so far been identified [4]. Besides the six subunits which appear to be involved in redox functions, the SDAP 10 kDa subunit, sharing homology with acyl carrier proteins [17], appears to be involved in mitochondrial phospholipid metabolism. Evidence that the 24 kDa subunit is a G protein has also been presented [18].

The 18 kDa (IP) subunit [4] is encoded by the nuclear NDUFS4 gene [19]. It consists of 175 amino acids, which appear to be highly conserved in the known mammalian sequences [4,19,20]. The mature form of 133 aminoacids has at the carboxy-terminal position 129–131 a conserved cAMP-dependent protein kinase phosphorylation consensus site (RVS) [21]. The protein has a leader sequence, removed after import into mitochondria [4], that also contains a phosphorylation consensus site (RTS in the human protein, at position −7 −5). The leader sequence has a number of positively charged and hydroxylated residues which are characteristic of import signals of nuclear encoded subunits of oxidative phosphorylation complexes [22].

The human NDUFS4 gene, coding for the 18 kDa (IP) subunit of complex I, is localised at position 5q11.1 of chromosome 5 [19]. The Blast search reveals five exons with 100% identity with the cDNA of the gene, which are intercalated by four large introns [GENBANK] (Fig. 1). The first exon is preceded by a short 5′ UTR. The promoter region presents a 400-bp segment which shares 95% homology with the promoter of the mouse gene. The overall size of the human NDUFS4 gene amounts to 127 kb, showing this to be a relatively large gene. In the conserved segment of the promoter nucleotide sites for the transcription factors NRF2, myoD and YYI are localised. Investigations are being carried in collaboration with Richard Scarpulla of the North-

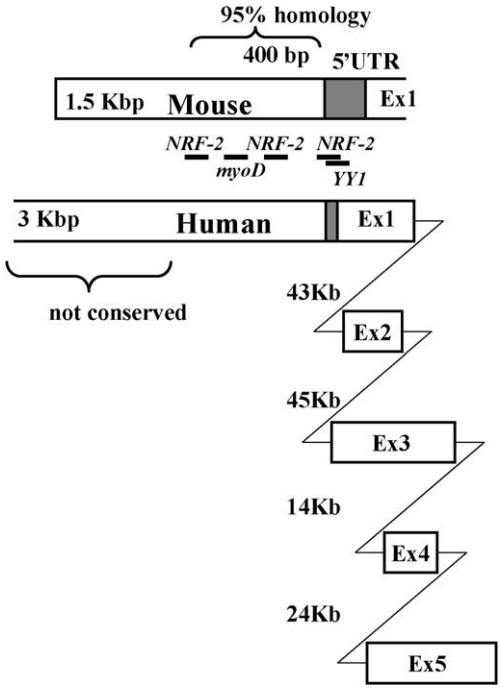


Fig. 1. Structure of the human NDUFS4 gene coding for the 18 kDa (IP) subunit of complex I (NADH-ubiquinone oxidoreductase) of the mitochondrial respiratory chain.

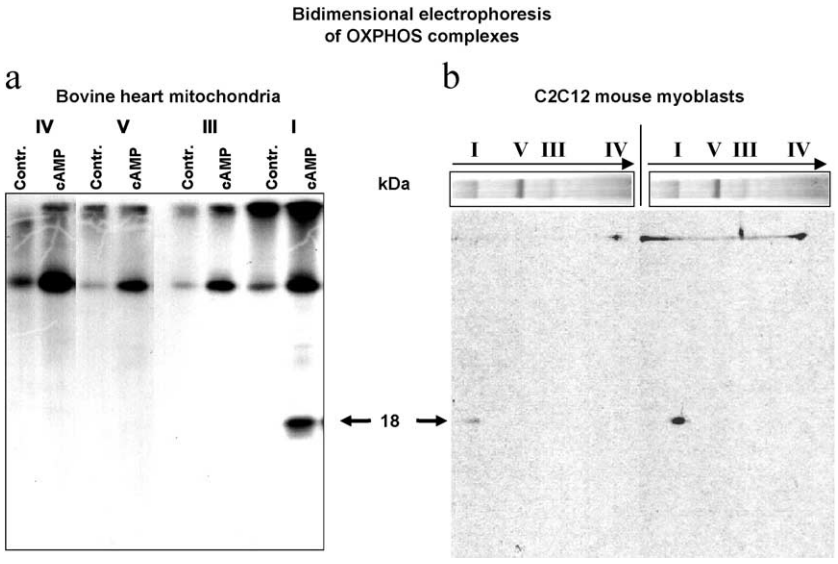


Fig. 2. Two dimensional electrophoretic pattern of protein labelling by [γ-$^{32}$P]ATP in oxidative phosphorylation complexes from bovine heart mitochondria separated by blue-native electrophoresis and resolved in their subunits by SDS-PAGE (reproduced from Ref. [24]). The 42-kDa $^{32}$P-labelled protein was loosely associated to the complexes [24] (panel a). Immunodetection of phosphoserine in oxidative phosphorylation complexes separated by two-dimensional Blu-native SDS-PAGE of mitoplasts from control and cholera-toxin-treated C2C12 mouse myoblasts to enhance the intracellular concentration of cAMP (reproduced from Ref. [9]).

western University in Chicago in order to better characterise the promoter region of the gene and its regulation.

## 3. cAMP-dependent phosphorylation and dephosphorylation of the 18 kDa subunit in mitochondria

In bovine heart mitochondria, the addition of cAMP or dibutyryl-cAMP results in the phosphorylation of protein bands of 29, 18 and 6.5 kDa, respectively [23]. In intact mitochondria, the cAMP-dependent phosphorylation of these proteins by [γ-$^{32}$P]ATP is inhibited by carboxyactractyloside [24], showing that both the catalytic site of PKA and the mitochondrial proteins phosphorylated are localized at the matrix side of the inner mitochondrial membrane.

Separation of the five complexes of oxidative phosphorylation by blue-native electrophoresis of the inner membrane fraction of bovine heart mitochondria and mouse myoblasts, incubated with [γ-$^{32}$P]ATP, followed by SDS-PAGE of the individual complexes, has shown that cAMP promotes $^{32}$P labelling of the 18 kDa protein band of complex I (Fig. 2) [25,9]. Furthermore, it was found that the purified catalytic subunit of PKA catalysed, in the presence of [γ-$^{32}$P]ATP, $^{32}$P labelling of the 18 kDa (AQDQ, $NH_2$-terminus) subunit of the purified complex I from bovine heart mitochondria [26].

The presence of PKA in mitochondria has been directly demonstrated by immunoblot analysis, which revealed the presence of both the regulatory R-II type and catalytic subunit in the mitochondrial inner-membrane matrix fraction of bovine heart and mouse myoblasts (Fig. 3A) [9]. In the inner membrane and matrix fraction, serine/threonine phosphatases of the PP2Cγ type were also immunodetected (Fig. 3B) [27]. Activity tests showed that the 18 kDa subunit of complex I, previously phosphorylated by PKA, was

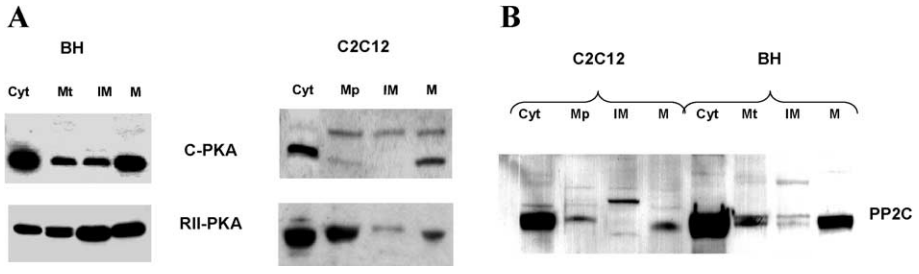


Fig. 3. Localization of PKA and PP2C type phosphatase in mitochondria. Localization of PKA (A) (from Ref. [9]) and PP2C (B) (from Ref. [27]) by immunoblot analysis with polyclonal antibodies against the regulatory (RII-PKA) and catalytic (C-PKA) subunits of PKA and with monoclonal antibody against PP2C in subcellular fractions of bovine heart (BH) and cultured C2C12 mouse myoblasts.

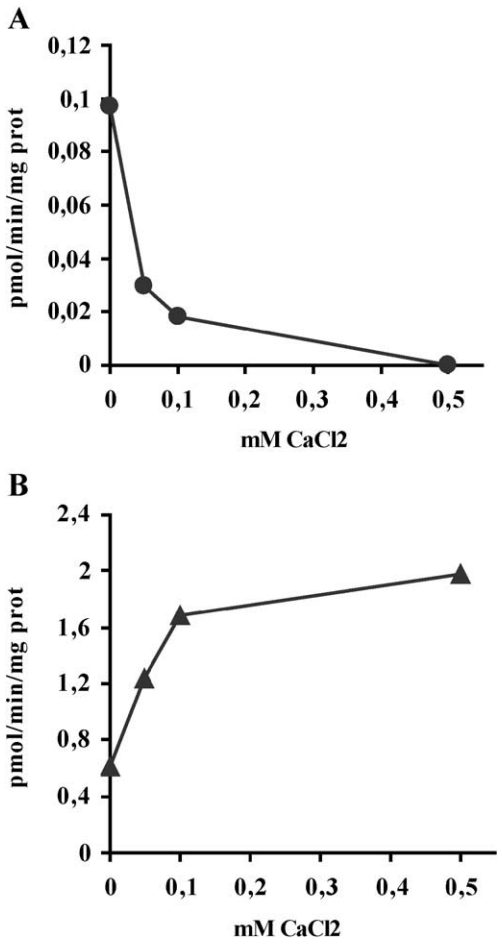


Fig. 4. Effect of $Ca^{2+}$ on dephosphorylation of mitochondrial phosphoproteins. (A) The 18 kDa subunit of purified complex I was phosphorylated by the catalytic subunit of PKA (cPKA) with [$\gamma-{}^{32}$P]ATP. Dephosphorylation was measured by incubation of the $^{32}$P-labelled subunit in complex I with cold inner-membrane fraction of bovine heart mitochondria in the presence of 4 mM $MgCl_2$ and $CaCl_2$ at the concentrations indicated in the figure. (B) For dephosphorylation of the 42 kDa $^{32}$P-labelled protein (α-subunit of pyruvate dehydrogenase) the inner-membrane fraction was preincubated with [$\gamma-{}^{32}$P]ATP and cPKA. Inhibitor of PKA was then added and dephosphorylation of the phosphoprotein was measured in the absence or presence of $CaCl_2$ (compiled from data in Ref. [27]).

dephosphorylated by both the membrane and matrix phosphatase. Dephosphorylation of the 18 kDa protein by the inner membrane phosphatase was activated by $Mg^{2+}$ and inhibited by $Ca^{2+}$, in the same concentration range at which this cation activated the pyruvate dehydrogenase phosphatase (Fig. 4) [27].

These observations clearly demonstrate the occurrence in the inner membrane matrix of cAMP-dependent reversible phosphorylation of mitochondrial proteins. Although the main pool of PKA is known to be localized in the cytosol, this enzyme is also found in other subcellular compartments [21]. An important breakthrough in understanding the intracellular distribution of PKA was provided by the discovery of a new family of proteins (protein kinase A anchor proteins, AKAP), which specifically anchor PKA to differ-

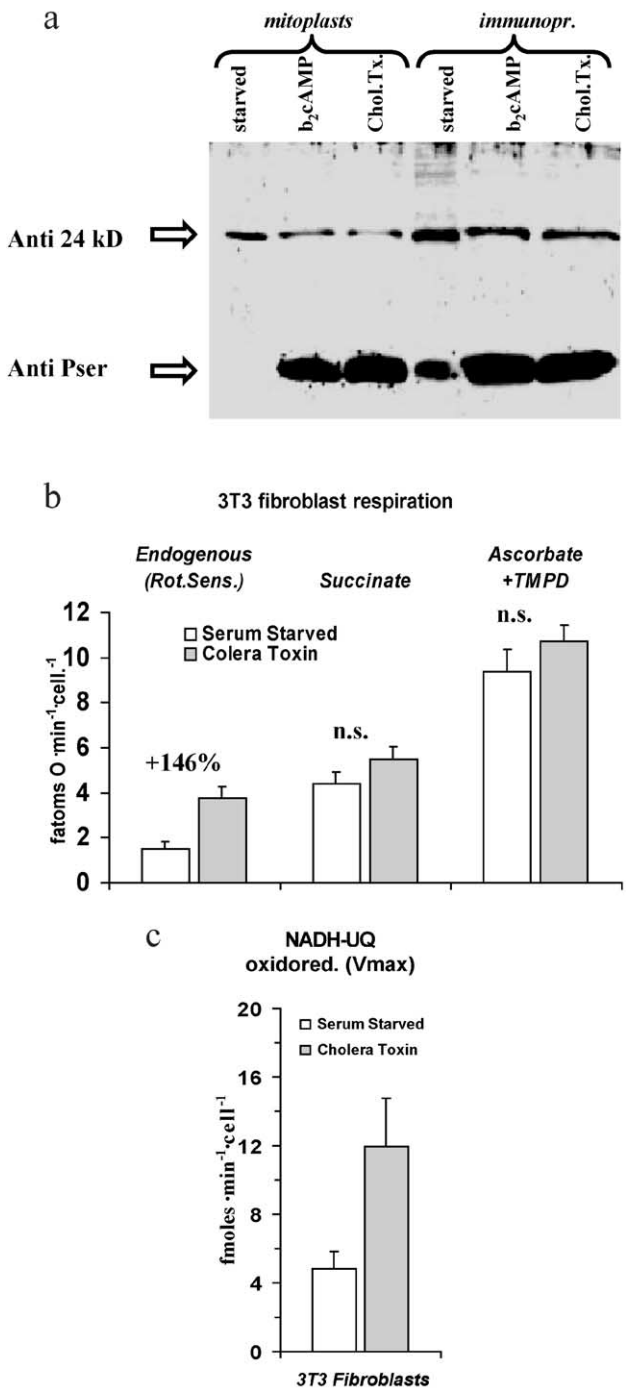


Fig. 5. cAMP-dependent phosphorylation of the 18 kDa subunit of complex I and its impact on the activity of the complex and mitochondrial respiration in mouse-fibroblast cultures (reproduced from Ref. [7]). 3T3 mouse fibroblasts were cultivated under serum starvation conditions in the absence or in the presence of cholera toxin. Panel a: immunodetection of phosphoserine-containing, proteins and of the 24 kDa subunit of complex I in mitoplasts from fibroblasts and in solubilized proteins immunoprecipitated from mitoplasts with an antibody against the 75 kDa subunit of complex I. Panel b: respiratory activities of serum starved and cholera-toxin treated fibroblasts. Panel c: Vmax of complex I in serum-starved and cholera-toxin-treated fibroblasts (for details see Ref. [7]).

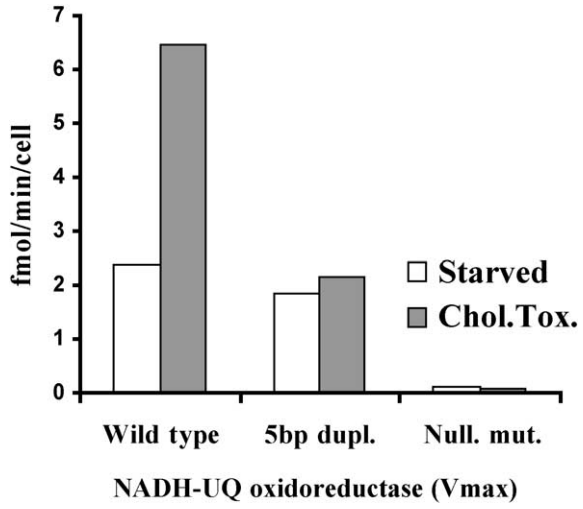


Fig. 6. Functional defects of complex I associated with homozygous mutations of the NDUFS4 gene (18 kDa subunit) in children, affected by fatal neurological syndrome. The specific activity (Vmax) of complex I was determined in the mitoplast fraction isolated from serum-starved and cholera-toxin-treated control and patient fibroblasts (compiled from Refs. [8,34]).

ent cellular structures [28,29]. Mammalian mitochondrial AKAPs which bind both RI and RII isoforms have been identified, D-AKAP1 [30] and D-AKAP2 [31]. Alternative N-terminus splice variants seem to control the direction of D-AKAP1 either to mitochondria or endoplasmic reticulum [30]. D-AKAP2 has a positively charged N terminus which might serve for its preferential localization in mitochondria [31]. It has been shown that AKAP protein can form supercomplexes containing, PKA, phosphatases and their substrates [32].

## 4. cAMP-dependent phosphorylation of the 18 kDa subunit of complex I and activation of the complex in cell cultures in vivo

Whilst mitochondrially encoded proteins can only be phosphorylated by mtPKA, nuclear encoded proteins can first be phosphorylated in the cytosol, where the major pool of PKA is localized, and then imported in mitochondria where they can also be phosphorylated. These aspects were investigated in primary cultures of various mammalian cell lines. Intracellular "in vivo" elevation of the level of cAMP in cultures of mouse fibroblasts, effected by the addition of cholera toxin, which induces permanent activation of adenylate kinase, promoted phosphorylation of the 18 kDa subunit of complex I (Fig. 5) [7]. This was accompanied by stimulation of the rotenone-sensitive activity of complex I and activation of the overall NAD-linked mitochondrial respiratory activity (Fig. 5) [7].

A short-term stimulatory effect on complex I activity and NAD-linked respiration, although smaller than that observed in intact cells, was also produce by incubation with dibutyryl-cAMP of mitoplasts isolated from starved fibroblasts [7].

The same observations have been made on human fibroblasts [8] and mouse myoblast cultures [9]. Thus, phosphorylation of the 18 kDa subunit by PKA regulates the activity and possibly also the biogenesis of complex I. Through this effect, a rise in the cellular level of cAMP can result in activation of mitochondrial respiration.

## 5. NDUFS4 mutations associated with pathological deficiency of complex I

Recently, four families have been found with different mutations in the cDNA of the NDUFS4 gene [19,33–35]. These mutations were transmitted by autosomal recessive inheritance and children homozygous for the mutations were affected by a fatal neurological syndrome. The first of these mutations, identified by van den Heuvel et al. [19], consisted of a 5-bp duplication in the cDNA at po-

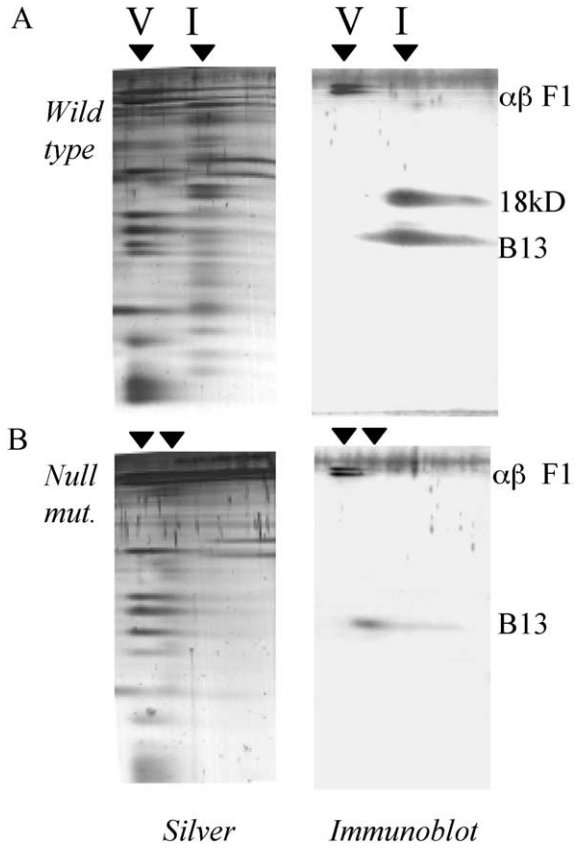


Fig. 7. Blue-native SDS-PAGE two-dimensional gel separating the constituent subunits of oxidative phosphorylation complexes of the mitoplast fraction (inner-membrane plus matrix) of human fibroblasts from a control subject and a child with homozygous non-sense G → A transition at nucleotide +44 of cDNA of the NDUFS4 gene. (A) Silver staining of the gel. (B) Immunodetection with specific polyclonal antibodies for the α/β subunit of complex V–F1, serine-phosphorylated carboxy-terminus of the 18 kDa subunit and B13 subunit of complex I (for details see Refs. [8,34]).

sition 466–470 (AAGTC), with destruction of the phosphorylation consensus site and elongation of the C terminus of the 18 kDa subunit of complex I. The other two, consisting of point mutations, caused in the homozygous patients premature translation stop, resulting in truncated proteins with destruction of the phosphorylation site in the C-terminal region [33]. A fourth mutation, identified by our group, consisted of a G-to-A transition, changing a tryptophan codon (TGG) to a stop codon (TAG) with premature termination of the protein after only 14 amino acids of the putative mitochondrial targeting sequence [34]. Results from investigations on skin fibroblast cultures from the patient showed that the 5-bp duplication abolished cAMP-dependent phosphorylation of the 18 kDa subunit of complex I, cAMP activation of the normal rotenone-sensitive NADH-UQ oxidoreductase activity of the complex (Fig. 6) and of NAD-linked respiratory activity [8].

Analysis of skin fibroblasts from the patient with nonsense mutation of the NDUFS4 gene showed this to result in complete suppression of the rotenone-sensitive NADH-UQ oxidoreductase activity of the complex both in the absence and presence of cholera-toxin induced cAMP production (Fig. 6) [34]. Two-dimensional native/SDS-PAGE electrophoresis showed that the nonsense mutation was associated with defective assembly of the complex, with appearance of a nonfunctional lower molecular weight subcomplex [34] (Fig. 7). More recent work in our laboratory has shown that also the other reported mutations of the NDUFS4 gene prevented the normal assembly of the mature complex, with appearance of immature lower molecular weight subcomplex.

## 6. Conclusions

cAMP-dependent phosphorylation of the 18 kDa subunit is associated with potent stimulation of complex I. The 18-kDa phosphoprotein is dephosphorylated by a $Mg^{2+}$-dependent phosphatase, which is inhibited by $Ca^{2+}$ at the same concentrations at which this cation stimulates the pyruvate dehydrogenase phosphatase (Fig. 4) and activates the dehydrogenase [36]. In this way, $Ca^{2+}$ can exert two convergent stimulatory effects on NAD-linked respiration in mitochondria. It is worth noting that mitochondrial $Ca^{2+}$ concentrations of $10^{-5}$–$10^{-4}$ M, effective on both phosphatases, have been directly measured "in vivo" with a fluorescent recombinant probe [37]. The cAMP activation of complex I was missing in fibroblasts of patients affected by fatal neurological syndrome, in which mutation in the NDUFS4 gene destroying the phosphorylation consensus site in the 18-kDa protein had been identified. All the above shows that the cAMP- and $Ca^{2+}$-dependent phosphorylation-state of the protein encoded by the NDUFS4 gene regulates the activity of complex I and NAD-linked mitochondrial respiration. Since the cAMP cascade mediates the action of a variety of cellular effectors, regulation of mitochondrial respiration by cAMP appears to be a major control factor in the bioenergetic function of mammalian tissues. All the mutations so far identified in the NDUFS4 gene were associated with impaired formation of a normal complex I. This gene thus appears essential not only for regulation of the activity of the complex by the cAMP cascade but also for its assembly in the inner mitochondrial membrane. Further investigations should elucidate the regulation of the expression of the NDUFS4, the mechanism by which the NDUFS4 is involved in the assembly of the complex in mammalian cells and the mechanism of regulation of complex I by the cAMP-dependent phosphorylation of the 18 kDa subunit.

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## References

[1] S. Papa, F. Guerrieri, J.M. Tager (Eds.), Frontiers of Cellular Bioenergetics, Molecular Biology, Biochemistry and Physiopathology, Kluwer Academic/Plenum Publishers, New York, 1999.
[2] G. Villani, G. Attardi, Free Radic. Biol. Med. 29 (2000) 202–210.
[3] S. Papa, Biochim. Biophys. Acta 1276 (1996) 87–105.
[4] J.E. Walker, Q. Rev. Biophys. 25 (1992) 253–324.
[5] A.D. Vinogradov, V.G. Grivennikova, IUBMB Life 52 (2001) 129–134.
[6] M.N. Gadaleta, B. Kadenbach, A.M.S. Lezza, A. Reith, P. Cantatore, D. Boffoli, S. Papa, in: S. Papa, et al. (Eds.), Frontiers of Cellular Bioenergetics, Molecular Biology, Biochemistry and Physiophathology, Kluwer Academic/Plenum Publishers, New York, 1999, pp. 693–727.
[7] S. Scacco, R. Vergari, R.C. Scarpulla, Z. Technikova-Dobrova, A.M. Sardanelli, R. Lambo, V. Lorusso, S. Papa, J. Biol. Chem. 275 (2000) 17578–17582.
[8] S. Papa, S. Scacco, A.M. Sardanelli, S. Vergari, F. Papa, S. Budde, L. van den Heuvel, J. Smeitink, FEBS Lett. 489 (2001) 259–262.
[9] Z. Technikova-Dobrova, A.M. Sardanelli, F. Speranza, S. Scacco, A. Signorile, V. Lorusso, S. Papa, Biochemistry 40 (2001) 13941–13947.
[10] G.P. Davey, S. Peuchen, J.B. Clark, J. Biol. Chem. 273 (1998) 12753–12757.
[11] J. Smeitink, L. van den Heuvel, Am. J. Hum. Genet. 64 (1999) 1505–1510.
[12] J. Smeitink, L. van den Heuvel, S. Di Mauro, Nat. Rev., Genet. 2 (2001) 342–353.
[13] J.M. Skehel, I.M. Fearnley, J.E. Walker, FEBS Lett. 438 (1998) 301–305.
[14] A. Chomyn, M.W. Cleeter, C.I. Ragan, M. Riley, R.F. Doolittle, G. Attardi, Science 234 (1986) 614–618.
[15] J.L.C.M. Loeffen, R.H. Triepels, L.P. van den Heuvel, M. Schuelke, C.A.F. Buskens, R.J.P. Smeets, J.M.F. Trijbels, J.A.M. Smeitink, Biochem. Biophys. Res. Commun. 253 (1998) 415–422.
[16] J.A. Smeitink, J.L. Loeffen, R.H. Triepels, R.J. Smeets, J.M. Trijbels, L.P. van den Heuvel, Hum. Mol. Genet. 7 (1998) 1573–1579.
[17] M.J. Runswick, I.M. Fearnley, J.M. Skehel, J.E. Walzer, FEBS Lett. 286 (1991) 121–124.

[18] R. Hegde, Biochem. Biophys. Res. Commun. 27, 244 (3) (1998) 620–629.
[19] L. van den Heuvel, W. Ruitenbeek, R. Smeets, Z. Gelman-Kohan, O. Elpeleg, J. Loeffen, F. Trijbels, E. Mariman, D. de Bruijn, J. Smeitink, Am. J. Hum. Genet. 62 (1998) 262–268.
[20] A. Bairoch, P. Bucher, K. Hofmann, Nucleic Acids Res. 25 (1997) 217–221.
[21] S. Papa, A.M. Sardanelli, S. Scacco, Z. Technikova-Dobrova, FEBS Lett. 444 (1999) 245–249.
[22] F.U. Hartl, W. Neupert, Science 247 (1990) 930–938.
[23] Z. Technikova-Dobrova, A.M. Sardanelli, M.R. Stanca, S. Papa, FEBS Lett. 350 (1994) 187–191.
[24] A.M. Sardanelli, Z. Technikova-Dobrova, F. Speranza, A. Mazzocca, S. Scacco, S. Papa, FEBS Lett. 396 (1996) 276–278.
[25] A.M. Sardanelli, Z. Technikova-Dobrova, S.C. Scacco, F. Speranza, S. Papa, FEBS Lett. 377 (1995) 470–474.
[26] S. Papa, A.M. Sardanelli, T. Cocco, F. Speranza, S.C. Scacco, Z. Technikova-Dobrova, FEBS Lett. 379 (1996) 299–301.
[27] A. Signorile, A.M. Sardanelli, R. Nuzzi, S. Papa, FEBS Lett. 25715 (2002) 1–4.
[28] C.S. Rubin, Biochim. Biophys. Acta 1224 (1994) 467–479.
[29] T. Pawson, J.D. Scott, Science 278 (1997) 2075–2080.
[30] L.J. Huang, K. Durick, J.A. Weiner, J. Chun, S.S. Taylor, J. Biol. Chem. 272 (1997) 8057–8064.
[31] L. Wang, R.K. Sunahara, A. Krumins, G. Perkins, M.L. Crochiere, M. Mackey, S. Bell, M.H. Ellisman, S.S. Taylor, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3220–3225.
[32] A. Feliciello, M.E. Gottesmaan, E.V. Avvedimento, J. Mol. Biol. 308 (2001) 99–114.
[33] S.M. Budde, L.P. van den Heuvel, A.J. Janssen, R.J. Smeets, C.A. Buskens, L. DeMeirleir, R. Van Coster, M. Baethmann, T. Voit, J.M. Trijbels, J.A. Smeitink, Biochem. Biophys. Res. Commun. 275 (2000) 63–68.
[34] V. Petruzzella, R. Vergari, I. Puzziferri, D. Boffoli, E. Lamantea, M. Zeviani, S. Papa, Hum. Mol. Genet. 10 (5) (2001) 529–535.
[35] V. Petruzzella, S. Papa, Gene 286 (2002) 149–154.
[36] W.M. Teague, F.H. Pettit, T.L. Wu, S.R. Sibelman, L.J. Reed, Biochemistry 21 (1982) 5585–5592.
[37] A. Chiesa, E. Rapizzi, V. Tosello, P. Pintoni, M. De Virgilio, K.E. Fogarty, R. Rizzuto, Biochem. J. 355 (2001) 1–12.