# NDUFA4 (renamed COXFA4) is a cytochrome-*c* oxidase subunit

Robert D.S. Pitceathly[1] and Jan-Willem Taanman[2,*]

[1]MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery, London, UK

[2]Department of Clinical Neurosciences, UCL Institute of Neurology, London, UK

*Correspondence: j.taanman@ucl.ac.uk (J.-W. Taanman)

Keywords: Cytochrome-*c* oxidase, COXFA4, Detergent, Electron transport chain, Mitochondria, NDUFA4

**Abstract**

Groundbreaking work by Kadenbach and colleagues in the 1980's revealed the presence of 13 subunits in the mammalian mitochondrial cytochrome-*c* oxidase (complex IV). This observation stood the test of time until 2012 when it was demonstrated that NDUFA4, a polypeptide previously attributed to mitochondrial complex I, was a 14$^{th}$ subunit of cytochrome-*c* oxidase. In his recent Opinion article, Kadenbach argued that NDUFA4 is not a subunit of cytochrome-*c* oxidase. However, based on the findings that NDUFA4 deficiency results in a severe loss of cytochrome-*c* oxidase activity and that NDUFA4 represents a stoichiometric component of the individual cytochrome-*c* oxidase complex, we reason that NDUFA4 is a *bona-fide* cytochrome-*c* oxidase subunit and propose renaming it as COXFA4.

As the terminal member of the mitochondrial electron transport chain (ETC), cytochrome-*c* oxidase (COX) has a crucial role in aerobic metabolism. The catalytic core of the enzyme is composed of three mitochondrial DNA-encoded subunits and their prosthetic groups. They are surrounded by a number of nuclear-encoded subunits, many of which are expressed as tissue-specific isoforms (Table 1). The nuclear-encoded subunits are postulated to perform an insulating or regulatory role [1]. Determining the precise subunit composition of COX has proven challenging because of the loss of individual subunits during detergent extraction and later purification steps. Pioneering work undertaken by Kadenbach and colleagues in the 1980's showed that the mammalian enzyme consisted of 13 subunits when extracted using sodium cholate or immunoprecipitated in the presence of Triton X-100 [2,3]. In a more recent structural characterization of the mammalian ETC, Balsa *et al.* solubilized the enzyme complexes with the milder detergent digitonin, followed by resolution on blue-native gels [4]. Their experiments indicated that NDUFA4, a protein previously considered to be a subunit of ETC complex I, was a subunit of COX. In a recent issue of *Trends in Endocrinology and Metabolism*, Kadenbach argues against NDUFA4 representing the 14$^{th}$ subunit of mammalian COX [5]. However, we believe that there is compelling evidence that NDUFA4 is a subunit of COX and we propose renaming NDUFA4 as COX subunit FA4 (COXFA4) and its gene to *COXFA4*.

To recommend reassigning an ETC subunit, the criteria for assigning a protein as a structural subunit must first be considered. We propose as criteria that the protein is specifically associated with the mature enzyme complex at stoichiometric amounts and has a role in activity, and/or stability, and/or assembly. COXFA4 follows these principles. We previously showed that patients harboring homozygous loss-of-protein *COXFA4* mutations exhibit a

severe, but isolated, reduction in COX activity in both skeletal muscle tissue and cultured fibroblasts [6], and a selective COX deficiency was evident in HeLa cells after downregulation of the gene by Balsa *et al*. [4]; facts omitted in Kadenbach's Opinion article. Furthermore, in contrast to Kadenbach's interpretation, our experiments and those of Balsa *et al.* confirmed that COXFA4 is part of the individual holo-COX complex on one-dimensional blue-native and two-dimensional blue-native/SDS-denaturing gels [4,6]. Importantly, Balsa *et al.* demonstrated that COXFA4 exists at stoichiometric amounts, as judged from native gels. Our titration experiments with *n*-dodecyl β-D-maltoside revealed that COXFA4 dissociates from the COX complex at >0.08% of the detergent. This low concentration implies that COXFA4 is loosely associated and could explain why it is not present in COX preparations isolated with relatively high concentrations of harsher detergents, such as cholate and Triton X-100. The loose attachment of COXFA4 parallels that of COX VIa and VIb, both undisputed subunits of COX. Yeast COX VIa, removed when the enzyme is isolated using cholate and Triton X-100, is not required for COX assembly, but modulates its activity [7,8]. Likewise, yeast and mammalian COX VIb are readily dissociated following COX assembly and removal of mammalian COX VIb has also suggested an activity modifying role for this subunit [7,9]. Mutations in the human genes encoding COX VIa and VIb result in a severe, but isolated, reduction in COX activity, as was observed with *COXFA4* [6,10,11].

Kadenbach argues that a wide range of proteins, which are not considered COX subunits, have been shown to interact with the enzyme and modify its activity. However, unlike COXFA4, these proteins are not required to establish and/or maintain full activity of COX. Kadenbach claims that COXFA4 is predominantly expressed in tumor cells or proliferating cell cultures. However, the *COXFA4* gene was ubiquitously transcribed at high levels in

human postmitotic tissues [12] and protein expression was confirmed in skeletal muscle [6]. Originally, COXFA4 was only detected in a minority of bovine heart complex I preparations and its presence correlated with contamination by COX VIb [4]. As suggested by Balsa *et al.*, there is strong evolutionary evidence to support a fundamental role for COXFA4 in COX rather than complex I biogenesis, as fungal species without complex I still retain their *COXFA4* homolog [4].

COXFA4 is not essential for assembly of the other COX subunits. Fully assembled COX without abnormal subassemblies was evident after blue-native gel electrophoresis of mitochondrial membrane proteins isolated from patient muscle tissue, despite the absence of COXFA4 [6]. In contrast, gel analyses of mitochondrial membrane proteins from controls demonstrated that COXFA4 is part of the individual COX complex and is present at stoichiometric amounts [4,6]. The finding that active COX lacking COXFA4 can be isolated indicates that COXFA4 is not directly required for catalysis *per se*. However, the severe COX deficiency in the patients implies that COXFA4 plays a critical role in the regulation of COX activity. Collectively, these data strongly support reassignment of COXFA4 and affirm its role as a *bona-fide* subunit of COX.

**Acknowledgements**

R.D.S.P. is supported by a Medical Research Council Centre grant (G0601943). J.-W.T. is supported by the Royal Free Charity.

**Disclaimer Statement**

The authors declare no conflict of interest.

**References**

1. Sinkler, C.A. *et al.* (2017) Tissue- and condition-specific isoforms of mammalian cytochrome *c* oxidase subunits: from function to human disease. *Oxid. Med. Cell. Longev.* 2017, 1534056

2. Kadenbach, B. *et al.* (1983) Separation of mammalian cytochrome *c* oxidase into 13 polypeptides by a sodium dodecyl sulfate-gel electrophoretic procedure. *Anal. Biochem.* 129, 517-521

3. Merle, P. *et al.* (1981) Immunological and chemical characterization of rat liver cytochrome *c* oxidase. *Biochim. Biophys. Acta* 669, 222-230

4. Balsa, E. *et al.* (2012) NDUFA4 is a subunit of complex IV of the mammalian electron transport chain. *Cell Metab.* 16, 378-386

5. Kadenbach, B. (2017) Regulation of mammalian 13-subunit cytochrome c oxidase and binding of other proteins: role of NDUFA4. *Trends Endocrinol. Metab.* 28, 761-770

6. Pitceathly, R.D.S. *et al.* (2013) *NDUFA4* mutations underlie dysfunction of a cytochrome *c* oxidase subunit linked to human neurological disease. *Cell Rep.* 3, 1795-1805

7. Taanman, J.-W. and Capaldi, R.A. (1992) Purification of yeast cytochrome *c* oxidase with a subunit composition resembling the mammalian enzyme. *J. Biol. Chem.* 267, 22481-22485

8. Taanman, J.-W. and Capaldi, R.A. (1993) Subunit VIa of yeast cytochrome *c* oxidase is not necessary for assembly of the enzyme complex but modulates the enzyme activity. Isolation and characterization of the nuclear-coded gene. *J. Biol. Chem.* 268, 18754-18761

9. Weishaupt, A. and Kadenbach, B. (1992) Selective removal of subunit VIb increases the activity of cytochrome *c* oxidase. *Biochemistry* 31, 11477-11481

10. Massa, V. *et al.* (2008) Severe Infantile encephalomyopathy caused by a mutation in COX6B1, a nucleus-encoded subunit of cytochrome c oxidase. *Am. J. Hum. Genet.* 82, 1281-1289

11. Tamiya, G. *et al.* (2014) A mutation of *COX6A1* causes a recessive axonal or mixed form of Charcot-Marie-Tooth disease. *Am. J. Hum. Genet.* 95, 294-300

12. Garbian,Y. *et al.* (2010) Gene expression patterns of oxidative phosphorylation complex I subunits are organized in clusters. *PLoS One* 5, e9985

Table 1. Human COX Subunits, Their Estimated Molecular Weight and Their Expression in Tissues[a]

| Subunit | Isoform | MW (kDa) | Tissue expression | Gene name |
|---|---|---|---|---|
| I | | 57.0 | Ubiquitous | *MTCO1* |
| II | | 25.6 | Ubiquitous | *MTCO2* |
| III | | 30.0 | Ubiquitous | *MTCO3* |
| IV | IV-1 | 17.2 | Ubiquitous | *COX4I1* |
| | IV-2 | 17.6 | Primarily lung | *COX4I2* |
| Va | | 12.5 | Ubiquitous | *COX5A* |
| Vb | | 10.6 | Ubiquitous | *COX5B* |
| VIa | VIa-H | 9.5 | Skeletal and cardiac muscle | *COX6A2* |
| | VIa-L | 9.6 | Ubiquitous but not skeletal muscle | *COX6A1* |
| VIb | VIb-1 | 10.0 | Ubiquitous | *COX6B1* |
| | VIb-2 | 10.4 | Testes | *COX6B2* |
| VIc | | 8.7 | Ubiquitous | *COX6C* |
| VIIa | VIIa-H | 6.7 | Skeletal, cardiac and smooth muscle | *COX7A1* |
| | VIIa-L | 6.7 | Non-muscle tissues | *COX7A2* |
| | VIIa-R | 6.6 | Ubiquitous | *COX7A2L* |
| VIIb | VIIb-1 | 6.4 | Ubiquitous | *COX7B* |
| | VIIb-2 | 6.3 | Testes | *COX7B2* |
| VIIc | | 5.4 | Ubiquitous | *COX7C* |
| VIII | VIII-L | 4.9 | Ubiquitous | *COX8A* |
| | VIII-3 | 4.8 | Primarily testes | *COX8C* |

| | | | | |
|---|---|---|---|---|
| FA4 | | 9.4 | Ubiquitous | *COXFA4* (*NDUFA4*) |

[a]For further information on the subunits, see [1].