FEBS Letters 585 (2011) 737–743

FEBS Letters

journal homepage: www.FEBSLetters.org

NDUFB7 and NDUFA8 are located at the intermembrane surface of complex I

Radek Szklarczyk [a,1], Bas F.J. Wanschers [a,b,1], Sander B. Nabuurs [a], Jessica Nouws [b], Leo G. Nijtmans [b], Martijn A. Huynen [a,*]

[a] Centre for Molecular and Biomolecular Informatics, Radboud University Nijmegen Medical Centre, 6500 HB, Nijmegen, The Netherlands
[b] Nijmegen Centre for Mitochondrial Disorders at the Department of Pediatrics, Radboud University Nijmegen Medical Centre, 6500 HB, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 24 December 2010
Revised 21 January 2011
Accepted 31 January 2011
Available online 22 February 2011

Edited by Peter Brzezinski

Keywords:
Mitochondria
Complex I
Sequence conservation
Disulfide bridge
Protein localization

ABSTRACT

Complex I (NADH:ubiquinone oxidoreductase) is the first and largest protein complex of the oxidative phosphorylation. Crystal structures have elucidated the positions of most subunits of bacterial evolutionary origin in the complex, but the positions of the eukaryotic subunits are unknown. Based on the analysis of sequence conservation we propose intra-molecular disulfide bridges and the inter-membrane space localization of three Cx_9C-containing subunits in human: NDUFS5, NDUFB7 and NDUFA8. We experimentally confirm the localization of the latter two, while our data are consistent with disulfide bridges in NDUFA8. We propose these subunits stabilize the membrane domain of complex I.

Structured summary: **NDUFA8** and **NDUFS3** physically interact by blue native page (View interaction)

1. Introduction

The NADH:ubiquinone oxidoreductase (complex I) catalyzes the first step of the oxidative phosphorylation and consists of 45 proteins in human. Embedded in the mitochondrial inner membrane, it couples the oxidation of NADH to the reduction of ubiquinone and the translocation of protons across the inner membrane. Given its size and role in energy conversion it is not surprising that complex I deficiencies are the most frequently encountered class of mitochondrial disorders [1,2].

The mitochondrial complex I originated from a 14–17 subunit bacterial complex [3]. In the eukaryotes the complex gained 25–30 subunits that are not present in bacteria (so-called "supernumerary subunits") [4]. The supernumerary subunits contribute 32% of the complex' molecular mass in human. In bacteria, the complex is active without the additional subunits, and the increased size and complexity of the enzyme as well as the function of supernumerary subunits in eukaryotes represent a puzzle. It has been hypothesized that the eukaryotic subunits assist in the biogenesis and support stability of the mitochondrial complex [5].

In the mitochondrial inter-membrane space (IMS) reside two families of short proteins that include repeated double cysteine motifs (Cx_9C or Cx_3C). The cysteines are oxidized in the IMS, forming disulfide bridges that stabilize a hairpin confirmation in which α-helices between the cysteines align in an anti-parallel manner [6–10] (Fig. 1). The oxidation of the cysteines is catalyzed by the MIA40/ERV1 disulfide bond relay that resides in the IMS [11–13]. The regularly spaced cysteines have been shown to be essential for the IMS localization [14]. Mature proteins become trapped in the IMS, with the disulfide bonds preventing membrane translocation [15]. Here we analyze three supernumerary subunits of complex I to show that they also contain Cx_9C domains and are in all likelihood located at the IMS surface of complex I. Experimental analyses of the localization of two of the subunits and the timing of assembly of one of the subunits are in agreement with this prediction.

2. Results

2.1. NDUFS5, NDUFB7 and NDUFA8 contain Cx_9C domains

Complex I consists of multiple proteins with conserved cysteines, a number of which are involved in Fe–S cluster binding. Nevertheless, for NDUFS5 (16kD subunit, PFFD), NDUFB7 (B18 subunit) and NDUFA8 (19kD subunit, PGIV) no Fe–S cluster binding has been observed [5,16]. Instead they exhibit a number of features typical of Cx_9C domains (see Table 1 and Fig. 1). The proteins are

* Corresponding author. Address: CMBI 260, NCMLS, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Fax: +31 24 3619395.
E-mail address: huynen@cmbi.ru.nl (M.A. Huynen).
[1] Contributed equally.

0014-5793/$36.00 © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2011.01.046

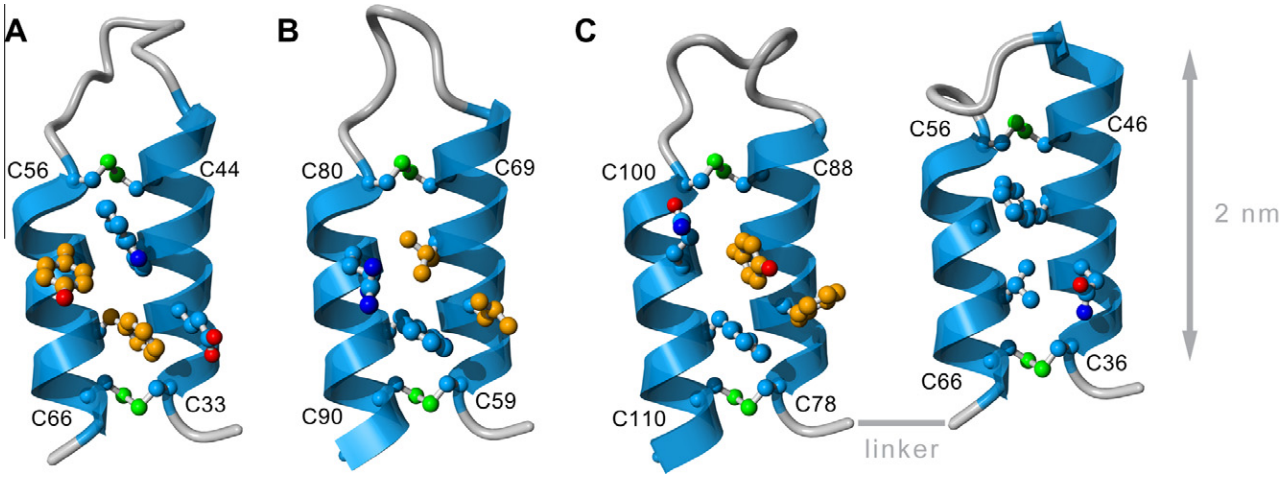

Fig. 1. Molecular models of the Cx_9C domains of NDUFS5, NDUFB7 and NDUFA8. Homology models are shown for the helical hairpins of (A) NDUFS5, (B) NDUFB7 and (C) NDUFA8. The position of the linker between the two Cx_9C domains of NDUFA8 is indicated. For all models, the bridged cysteine side chains involved in stabilizing the helical hairpin structure are shown and labeled. Additionally, the amino acids located at the 4th and 7th position of each Cx_9C motif are also shown. Amino acid side chains at these positions matching the IMS targeting signal consensus sequence [17] are depicted (orange).

Table 1
Features characteristic to NDUFS5, NDUFB7 and NDIFA8 and to the Fe–S binding central subunit NDUFV2. Columns denote: the size of proteins in amino acids in human; the form of the Cx_9C domain (conserved in all eukaryotes); distance between the motifs that corresponds to the loop of the hairpin; overlap between the motifs and (predicted) α helices; presence of a cleaved N-terminal import signal; conservation pattern for the domain, 4th and 7th positions of constrained variation are marked with a star.[1]) See the Supplementary data for the list of human Cx_9C domain proteins.

	Size	Cys domain	Distance between Cys motifs	CxC in α-helices	Cleaved targeting presequence	Localization	Conservation pattern
NDUFS5	106	Twin Cx_9C	12	Yes	No	Membrane arm of CI	
NDUFB7	137	Twin Cx_9C	10	Yes	No	Membrane arm of CI	
NDUFA8	172	Quadruple Cx_9C	9–11, 10–11	Yes	No	Membrane arm of CI	
Other human IMS Cx_9C proteins[1]	63–227	Twin Cx_9C; Twin Cx_3C	5–22	Yes	No	IMS	(COX17)
NDUFV2 (Fe-S)	250	Cx_3C, Cx_4C	35	No	Yes	Matrix	

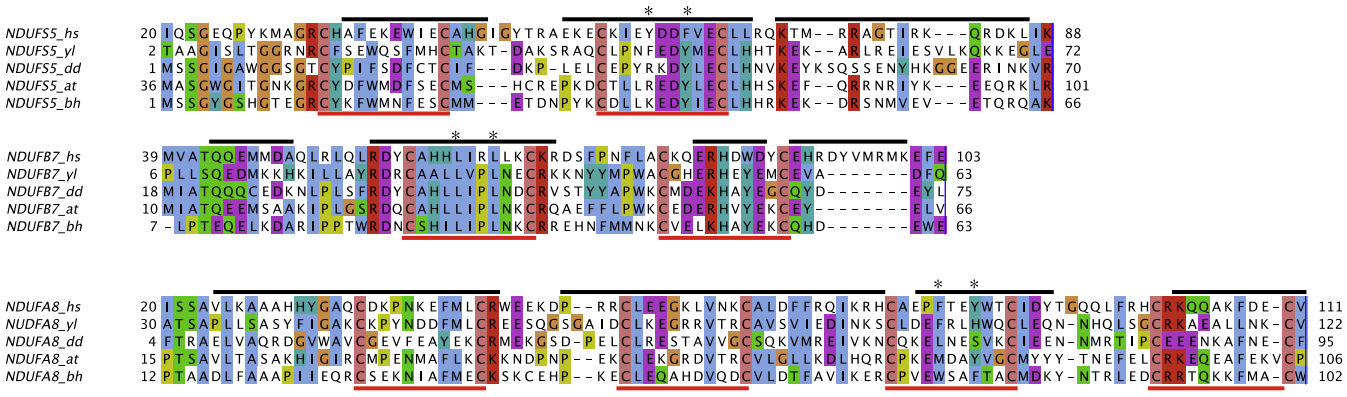

Fig. 2. Alignments of Cx_9C domain-containing NDUFS5, NDUFB7, NDUFA8. Twin Cx_9C motifs are indicated under the alignment (red line) and predicted (Jpred3, [45]) α-helices (black) are indicated above the sequences. Stars mark the localization of the IMS targeting signal in the human sequences. The NDUFA8 gene for *B. hominis* was predicted from the DNA (see Supplementary data). Abbreviations: hs, *Homo sapiens*; yl, *Y. lipolytica*; dd, *Dictyostelium discoideum*; at, *Arabidopsis thaliana*; bh, *B. hominis*.