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Investigation of the complex I assembly chaperones B17.2L and NDUFAF1 in a cohort of CI deficient patients

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Abstract

Dysfunction of complex I (NADH:ubiquinone oxidoreductase; CI), the largest enzyme of the oxidative phosphorylation (OXPHOS) system, often results in severe neuromuscular disorders and early childhood death. Mutations in its seven mitochondrial and 38 nuclear DNA-encoded structural components can only partly explain these deficiencies. Recently, CI assembly chaperones NDUFAF1 and B17.2L were linked to CI deficiency, but it is still unclear by which mechanism. To better understand their requirement during assembly we have studied their presence in CI subcomplexes in a cohort of CI deficient patients using one- and two-dimensional blue-native PAGE. This analysis revealed distinct differences between their associations to subcomplexes in different patients. B17.2L occurred in a 830 kDa subcomplex specifically in patients with mutations in subunits NDUFV1 and NDUFS4. Contrasting with this seemingly specific requirement, the previously described NDUFAF1 association to 500–850 kDa intermediates did not appear to be related to the nature and severity of the CI assembly defect. Surprisingly, even in the absence of assembly intermediates in a patient harboring a mutation in translation elongation factor G1 (EFG1), NDUFAF1 remained associated to the 500–850 kDa subcomplexes. These findings illustrate the difference in mechanism between B17.2L and NDUFAF1 and suggest that the involvement of NDUFAF1 in the assembly process could be indirect rather than direct via the binding to assembly intermediates.

Keywords: Complex I; NADH:ubiquinone oxidoreductase; Assembly; Chaperones; NDUFAF1; B17.2L; Mimitin

The importance of energy carrier ATP in each of our cells is inversely demonstrated in cases of mitochondrial disorders, which occur with an incidence of 1:5.000 newborns and display a broad clinical variety including Leigh syndrome, cardiomyopathy and encephalomyopathy [1]. The most frequent cause of such disorders is deficiency of complex I (CI, NADH:ubiquinone oxidoreductase, E.C.1.6.5.3) [2]. This complex is the first of five complexes (CI–CV) that constitute the oxidative phosphorylation (OXPHOS) system, responsible for the generation of mitochondrial ATP.

In all cases studied thus far, deficient CI activity was associated with a decrease in the amount of intact CI signifying that assembly or stability of this enzyme was affected. CI assembly is an intricate process, which involves 45 subunit proteins, one FMN and eight iron–sulphur clusters [3,4]. To date, two models have been published, both established on the basis of blue-native analysis [5,6]. The starting point in human CI assembly seems to be a core of highly conserved subunits that is anchored to the mitochondrial inner membrane and expanded with additional subunits [7]. This assembly mechanism at some points reflects the

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theoretical proposals that state that certain groups of subunits have co-evolved as distinct substructures [8–11]. The proposed modules are a dehydrogenase module, which oxidizes NADH, a hydrogenase module, which transports electrons to the proton translocation module, which transports protons across the mitochondrial inner membrane. Combination of (parts of) these substructures is proposed to result in the assembly of holo-CI [12,13].

By analogy to the other OXPHOS complexes, CI assembly must involve the action of assembly chaperones. The recent addition of B17.2L (previously termed mimitin [14]), a paralogue of a CI subunit which is not incorporated into CI but is vital for its assembly, to the previously described assembly factor NDUFAF1 brings the number of CI specific assembly factors to two [15–17]. Recently, both chaperones have been linked to CI deficiency. A *B17.2L* gene null mutation resulted in less than 20% CI activity and concomitant early-onset progressing encephalopathy with vanishing white matter [17]. Two novel heterozygous mutations in the *NDUFAF1* gene were described by Sugiana and colleagues, which resulted in a marked reduction in NDUFAF1 protein level in fibroblasts and EBV-lymphoblasts and clinically in cardiomyopathy, developmental delay and lactic acidosis [18].

In contrast to the assembly chaperones described for CIV, which play a role in the incorporation of prosthetic groups (e.g. COX10, COX11, Sco1, Sco2) or the maturation and membrane insertion of subunits encoded by mitochondrial DNA (e.g. Surf1 and Oxa1), the molecular role of the CI chaperones is still not clear [19–21]. Better understanding of the specific involvement of B17.2L and NDUFAF1 in the assembly process aids the identification of important stages in the assembly process and adds to the insight into their mechanism of action. For these purposes, we have investigated the presence of these chaperones in specific subcomplexes for a cohort of patients displaying various CI assembly disturbances.

Materials and methods

Patients and mutations

Table 1 lists the investigated control, cybrid and patient cell lines used in this study.

Cell culture

Fibroblast cells were cultured in M199 medium (Life Technologies) supplemented with 10% fetal calf serum (v/v) and penicillin/streptomycin. Cells were harvested by trypsinization, resuspended in PBS (Gibco) and solubilized using a final concentration of 2% digitonin (Calbiochem) for 10 min on ice. After centrifugation (10 min, 10,000*g*, 4 °C), the mitochondria enriched pellet was washed twice in PBS prior to its solubilization by 10 min of incubation on ice using 2% of *n*-dodecyl β-D-maltoside (DDM) (Sigma–Aldrich) in solubilization buffer (1.75 M 6-aminocaproic acid (Fluka), 75 mM bis–tris HCl (Fluka), pH 7.0). The protein concentration was determined using the MicroBCA protein assay kit (Pierce) and samples were processed for blue-native gel electrophoresis.

SDS–PAGE and BN–PAGE protein analysis and immunodetection

SDS–PAGE analysis was performed as described in [12], using 10% SDS and loading 40 μg of mitochondrial protein per lane. One- and two-dimensional BN–PAGE was performed as described in [22,23], using 5–15% gels and loading 80 μg of mitochondrial protein per lane. After Western blotting, proteins were detected using antibodies raised against NDUFAF1 [16], the NDUFS3 (Invitrogen), NDUFA6 (Invitrogen), NDUFA9 (Invitrogen) and ND1 (Dr. A. Lombes (Inserm, Paris, France)) subunits of CI and B17.2L (kindly provided by Professor M. Tsuneoka (Takasaki University of Health and Welfare, Japan)). Secondary antibodies used peroxidase-conjugated anti-mouse or anti-rabbit IgGs (Invitrogen). The signal was obtained using ECL® plus (Amersham Biosciences).

Results

CI activity and assembly

All studied patient cell lines exhibit a specifically impaired CI activity. Based on the structural relationship of the affected subunits during assembly, we have categorized the investigated cell lines into three "assembly groups". Cell

Table 1
Patients and mutations

Cell line	Assembly group	Affected gene	Mutation	Heteropl. (%)	CI activity (%)	Other activities (%)	Previously described in
Control (C)	—	—	—	—	—	—	—
Patient 1	Early matrix	NDUFS2	R228Q	—	39a	All normal	[26]
Patient 2	Early matrix	NDUFS7	V122M	—	68a	All normal	[27]
Patient 3	Early matrix	NDUFS8	R94C	—	18a	CIII 88a, CIV 229b	[28]
Patient 4	Late matrix	NDUFV1	R29X, T423M	—	64a	All normal	[28,29]
Patient 5	Late matrix	NDUFS4	K158fs (5-bp duplication)	—	75a	CIII 98a	[30]
Patient 6	Late matrix	NDUFS4	R106X	—	36a/30b	CIII 100a/88b	[31]
Patient 7	Late matrix	NDUFS4	VPEEHI67/VEKSIstop	—	53a	All normal	[28]
Patient 8	Membrane	ND3	S43P	75	54a	All normal	Manuscript in preparation
Cybrid control (CC)	—	—	—	—	—	All normal	This publication
ND1 cybrid (9)	Membrane	ND1	A52T	100	9b	All normal	This publication

a, COX normalization; b, citrate synthase normalization.
Listed are the investigated control, cybrid and patient cell lines used in this study. Indicated are which gene harbors which mutation, the amount of heteroplasmy in the case of mtDNA mutation, CI activity relative to either CIV (COX) or citrate synthase, the activities of the other OXPHOS complexes and the references in which the mutations were previously described.