Biochimica et Biophysica Acta 1817 (2012) 851–862

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Review

Understanding mitochondrial complex I assembly in health and disease ☆

Masakazu Mimaki [a], Xiaonan Wang [a], Matthew McKenzie [b], David R. Thorburn [c,d], Michael T. Ryan [a,*]

[a] Department of Biochemistry, La Trobe University, Melbourne, VIC 3086, Australia
[b] Monash Institute for Medical Research, Melbourne, VIC 3168, Australia
[c] Murdoch Childrens Research Institute and Genetic Health Services Victoria, Royal Children's Hospital, University of Melbourne, Melbourne, VIC 3052, Australia
[d] Department of Paediatrics, University of Melbourne, Melbourne, VIC 3052, Australia

ARTICLE INFO

Article history:
Received 2 July 2011
Received in revised form 17 August 2011
Accepted 27 August 2011
Available online 2 September 2011

Keywords:
Mitochondria
Respiratory chain
Complex I
Complex I deficiency
Assembly factor

ABSTRACT

Complex I (NADH:ubiquinone oxidoreductase) is the largest multimeric enzyme complex of the mitochondrial respiratory chain, which is responsible for electron transport and the generation of a proton gradient across the mitochondrial inner membrane to drive ATP production. Eukaryotic complex I consists of 14 conserved subunits, which are homologous to the bacterial subunits, and more than 26 accessory subunits. In mammals, complex I consists of 45 subunits, which must be assembled correctly to form the properly functioning mature complex. Complex I dysfunction is the most common oxidative phosphorylation (OXPHOS) disorder in humans and defects in the complex I assembly process are often observed. This assembly process has been difficult to characterize because of its large size, the lack of a high resolution structure for complex I, and its dual control by nuclear and mitochondrial DNA. However, in recent years, some of the atomic structure of the complex has been resolved and new insights into complex I assembly have been generated. Furthermore, a number of proteins have been identified as assembly factors for complex I biogenesis and many patients carrying mutations in genes associated with complex I deficiency and mitochondrial diseases have been discovered. Here, we review the current knowledge of the eukaryotic complex I assembly process and new insights from the identification of novel assembly factors. This article is part of a Special Issue entitled: Biogenesis/Assembly of Respiratory Enzyme Complexes.

1. Introduction

1.1. OXPHOS and the mitochondrial respiratory chain

Mitochondrial ATP is produced by the oxidative phosphorylation (OXPHOS) machinery [1]. OXPHOS couples 2 sets of reactions, the phosphorylation of ADP and electron transfer through a chain of oxidoreductase reactions. In most eukaryotes, the process is carried out by the respiratory chain, which consists of 5 enzyme complexes embedded in the mitochondrial inner membrane: complex I (CI, NADH:ubiquinone oxidoreductase), complex II (CII, Succinate:ubiquinone oxidoreductase), complex III (CIII, ubiquinol:cytochrome *c* oxidoreductase), complex IV (CIV, cytochrome *c* oxidase) and complex V (CV, ATP synthase). OXPHOS begins with the entry of electrons into the respiratory chain through CI or CII. CI binds and oxidizes NADH and generates 2 electrons that are transferred through flavin mononucleotide (FMN) and 7 iron–sulfur (Fe–S) clusters to ubiquinone, the first electron acceptor [2]. CII is another electron entry point for the transfer of electrons from succinate to ubiquinone [3]. Electrons from CI or CII are subsequently transferred from reduced ubiquinone (ubiquinol) to CIII, then to cytochrome *c*, the second mobile electron carrier, and finally to complex IV. CIV is the terminal enzyme in the electron transfer chain, reducing O_2 to H_2O by using the delivered electrons [4]. Coupling electron transfer, CI also plays a role as a pump to transfer protons out of the matrix with a stoichiometry of 4 protons to 2 electrons [1,2,5]. The pathway through complexes I, III and IV pumps a total of 5 protons per electron that are translocated from the mitochondrial matrix to the intermembrane space, creating a membrane potential. The transmembrane electrochemical potential is then used to promote the conformational change of CV, resulting in the generation of ATP [6].

1.2. Complex I structure and function

Eukaryotic CI is located in the mitochondrial inner membrane and protrudes into the matrix to form an L-shaped structure [7]. This structure consists of a hydrophilic peripheral arm with a hydrophobic membrane arm lying perpendicular to it. This L-shaped structure is conserved from NDH-1 in *Escherichia coli*, which is a homolog of eukaryotic CI [8,9], to bovine heart CI [7].

☆ This article is part of a Special Issue entitled: Biogenesis/Assembly of Respiratory Enzyme Complexes.

* Corresponding author. Tel.: +61 3 9479 2156; fax: +61 3 9479 1266.
E-mail address: M.Ryan@latrobe.edu.au (M.T. Ryan).

0005-2728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2011.08.010

The crystal structure of the hydrophilic domain of CI in the bacteria *Thermus thermophilus* was solved and the relative positions of the 8 subunits that compose the peripheral arm of CI were defined [10]. The peripheral arm consists of 2 functional modules, an electron input module (N module) and an electron output module (Q module), and comprises all redox active cofactors [10] (Fig. 1). The N module contains an NADH oxidation site with an FMN molecule as the primary electron acceptor, while the Q module contains a ubiquinone reduction site. Electrons from the oxidation of NADH are transferred via FMN and a series of Fe–S clusters to ubiquinone. The membrane arm or the proton translocation module (P module) contains the 3 subunits, ND2, ND4 and ND5 [11] (Fig. 1). They are highly hydrophobic proteins, which contain around 15 transmembrane stretches, and are antiporter-like subunits presumably involved in proton-pumping activity [11]. However, how electron transfer is coupled to proton translocation, either by direct association through protein binding sites or indirectly through conformational changes of the enzyme, remained obscure because of the lack of a high quality 3-dimensional structure of CI [12,13]. Recently, the X-ray structures of the membrane domain of CI from *E. coli* at a resolution of 3.9 Å and of the entire CI from *T. thermophilus* at a resolution of 4.5 Å were solved [14]. These findings defined the positions of all of the subunits and revealed the long horizontal α-helical structure of the membrane domain of CI, suggesting that the conformational changes at the interface of the matrix and membrane domains may drive the long amphipathic α-helices in a piston-like motion, thereby leading to proton translocation. In addition, the low resolution X-ray structure of mitochondrial CI from the aerobic yeast *Yarrowia lipolytica* was also reported [15]. The arrangement of functional modules suggested the conformational coupling of redox chemistry with proton pumping. A long helical element in the NuoL/ND5 subunit stretches across the matrix face of the membrane domain of CI and is suggested to be critical for transducing conformational energy to proton-pumping elements in the distal module of the membrane arm.

Bovine (and human) mitochondrial CI consists of 45 different subunits with a total molecular weight of ~980 kDa [16,17]. In this review we will use the human nomenclature for complex I subunits, where nuclear encoded subunits contain the prefix "NDU" (NADH-dehydrogenase-ubiquinone) and mtDNA-encoded subunits are given the prefix "ND" (NADH-dehydrogenase). A number of complex I assembly factors have been given the prefix "NDUFAF" — for NADH-dehydrogenase (NDU), alpha subcomplex (F), assembly factor (AF), plus a number indicating when it was first named. The reference to the alpha subcomplex within the abbreviation does not appear to be substantiated. To limit confusion, the assembly factor proteins are in lower case within the text.

Seven subunits, ND1–ND6 and ND4L, are encoded by mitochondrial DNA (mtDNA), and are homologs of the 7 membrane subunits in NDH-1, forming the major part of the membrane domain [1,18]. The mtDNA-encoded subunits are thought to be involved in proton translocation and ubiquinone binding, as their bacterial homologs have these functions [1,7]. The remaining 38 subunits are encoded by nuclear DNA (nDNA) and imported into the mitochondria [18–20].

Seven of the nDNA-encoded subunits, NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS7 and NDUFS8, represent the "core subunits" in the peripheral arm of CI, catalyzing the oxidation of NADH and electron transfer [1,21]. The N module, responsible for the oxidation of NADH, includes at a minimum the NDUFV1, NDUFV2 and NDUFS1 subunits. And the Q module, responsible for the electron transfer to ubiquinone, includes at a minimum the NDUFS2, NDUFS3, NDUFS7 and NDUFS8 subunits [22] (Fig. 1). The remaining 31 nDNA-encoded subunits are referred to as "supernumerary" subunits because they have no counterparts in NDH-1 [16]. Most of the supernumerary subunits are not involved in CI enzymatic activity, and their actual function is still unknown [18]. It has been proposed that the eukaryotic supernumerary subunits assist in CI biogenesis and support the structural stability of CI [7,17].

2. Complex I assembly

The assembly of subunits into CI has proved to be a very complicated process to characterize due to the large size and numerous subunits of the enzyme, lack of a detailed crystal structure, and its dual genomic control. The nDNA-encoded subunits must assemble in coordination with the hydrophobic mtDNA-encoded subunits to form the properly functioning mature complex; however, the assembly pathway is still not completely understood.

It has been suggested that the co-evolutionary structural relationship between CI subunits may be reflected by the order of assembly and composition of assembly intermediates [23]. Based on this concept, a number of model systems have been employed to study the assembly of eukaryotic CI, in various organisms such as the green alga *Chlamydomonas reinhardtii* [24–28], the fungus *Neurospora crassa* [29–36], the nematode *Caenorhabditis elegans* [37], and cultured mammalian cell lines [38–42].

2.1. Assembly of complex I in C. reinhardtii

In *C. reinhardtii*, the assembly of mtDNA-encoded subunits has been well characterized. The absence of ND4 or ND5 led to the accumulation of a 700-kDa subcomplex, i.e. partial assembly of an incomplete complex. In contrast, mutations in ND1 or ND6 subunit resulted in a failure to detect the mature 950-kDa holo-CI or the 700-kDa subcomplex [24,25]. Based on these results, it was proposed that the ND4 and ND5 subunits are incorporated at a late stage of assembly after the incorporation of the ND1 and ND6 subunits. Investigations of the role of ND3 and ND4L in assembly resulted in the generation of the first assembly model for *C. reinhardtii*, in which a 200-kDa nuclear-encoded subcomplex, containing the 76-kDa (NDUFS1) and 49-kDa (NDUFS2) subunits, is anchored to the membrane by combining with ND1, ND3, ND4L and ND6 forming the 700-kDa subcomplex and subsequently expanded by the addition of ND4 and ND5 to generate the holo-CI [26]. In subsequent investigation of the role of ND5, the lack of ND5 prevented the assembly of the holo-CI, but allowed the assembly in low amount of the 700-kDa subcomplex, which is loosely bound to the mitochondrial membrane [27]. The mass spectrometry analysis of the 700-kDa subcomplex identified eleven homologs to human CI subunits: nine (NDUFV1, NDUFV2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS7, NDUFS8 and NDUFA12) are associated with the matrix arm and two (ND3 and NDUFA9) are located at the junction between the matrix

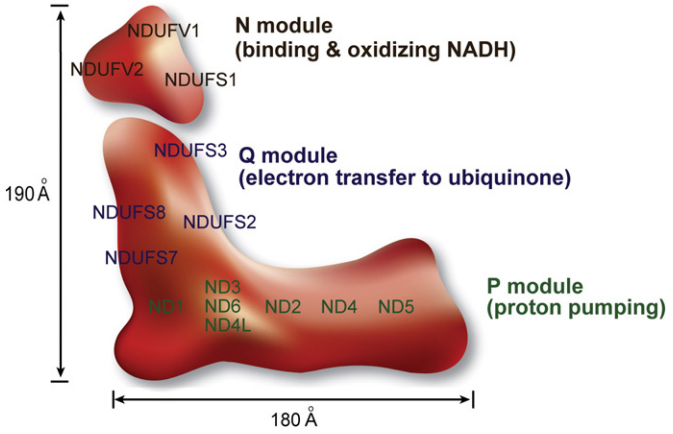


Fig. 1. Schematic graph of mammalian mitochondrial complex I structure. The matrix arm and the membrane arm form an L-shaped structure, with an angle of 100°. It is composed of three conserved functional modules: the NADH dehydrogenase module (N module), the electron transfer module (Q module) and the proton translocation module (P module). The positions of 14 core subunits are indicated, all of which are highly conserved from prokaryotes to eukaryotes.

and membrane arms of CI [27]. Together, it is proposed that the 700-kDa fragment is formed by the addition of hydrophilic and hydrophobic subunits to the 200-kDa fragment and then the 700-kDa complex is firmly anchored to the membrane by attachment of the 250-kDa hydrophobic module containing ND4 and ND5. These subunits ND4 and ND5 probably have a crucial role in the assembly of the distal part of the membrane arm of CI which is suggested to be critical for anchorage of the whole complex within the mitochondrial inner membrane [27,28].

2.2. Assembly of complex I in N. crassa

CI assembly in the aerobic fungus *N. crassa* has also been well studied through the characterization of mutant strains generated by systematic introduction of mutations in CI genes [31–36]. Mutants lacking subunits of the matrix arm could not assemble CI and accumulated the membrane arm of the complex [34]. Conversely, a mutant lacking a nuclear-encoded subunit of the membrane arm accumulated the matrix arm and 2 intermediates of the membrane arm [43]. From such studies, it was proposed that CI in *N. crassa* is assembled via evolutionarily conserved modules. In this model, the hydrophilic matrix arm is formed separately while the membrane arm is constructed from ~200-kDa and ~350-kDa intermediates. The small intermediate contains the ND2 and ND5 subunits, while the large intermediate contains ND1, 3, 4, 4L and 6 [30,32]. Although these findings provided the first detailed model of mitochondrial CI assembly in a eukaryote, the integration of subunits into different modules is not consistent with recent insights into the molecular architecture of CI [14,15]. Furthermore, its application to mammalian systems is limited since mammalian complex I contains additional subunits when compared to its fungal counterpart.

2.3. Complex I assembly in mammals

Assembly studies in rodent and human ND-subunit mutant cell lines have demonstrated that subassemblies of nDNA-encoded CI subunits could be formed in the absence of mtDNA-encoded subunits [44,45]. Cells lacking mtDNA, which lose all of the mtDNA-encoded subunits, maintain the levels of some nDNA-encoded subunits of the peripheral arm. They contain a subcomplex of the peripheral arm that consists of, at least, NDUFS2, NDUFS3 and NDUFS8 [44]; therefore, it has been suggested that the presence of the mtDNA-encoded subunits is not required for the formation of the peripheral arm subcomplex [45] (Fig. 2).

However, the entry points of the mtDNA-encoded subunits in the CI assembly process and their roles in the stability of CI had remained elusive. Recent research using several mouse cell lines deficient for ND4, ND6, and combination of ND6 and ND5 proposed five entry points of the mtDNA-encoded subunits in the CI assembly process [46]. This study defined a first entry point for ND1 in the ~400 kDa-subcomplex and a second entry point for ND2, ND3 and ND4L in the ~460 kDa-subcomplex. Subsequently, ND4, ND6 and ND5 appear to be incorporated into CI complex in order at a third, fourth and fifth entry point, respectively.

A useful model system using Chinese hamster cells has also clarified the function of some CI subunits in the CI assembly process [40]. For example, NDUFA1 has been shown to be important for CI assembly [47]. The insertion and stabilization of NDUFA1 in the mitochondrial inner membrane were shown to require mtDNA-encoded subunits, in particular, ND4 and ND6 [48]. NDUFA1 is also unstable in the absence of other membrane domain subunits like NDUFB11 [40]. Chinese hamster fibroblasts also revealed that the stability of the peripheral arm subunits NDUFS1, 2, 3, 7, 8 and NDUFV1 and 2 were unaffected by the absence of NDUFA1, although holo-CI was not assembled [40]. These data suggest that the peripheral arm can be assembled in the absence of the membrane arm, similar to its assembly in *N. crassa* [29]. NDUFA1 was also suggested to form an assembly intermediate consisting of mtDNA- and nDNA-encoded subunits and to serve as a membrane anchor to which membrane subunits are attached during CI assembly [48]. Furthermore, recent bioinformatic analyses of the co-evolution of CI subunits coupled with yeast two-hybrid studies revealed the interaction of human NDUFA1 with ND1 and ND4, and the interaction of human NDUFC2 with ND4 [49]. The findings reinforce the important role of NDUFA1 in forming an assembly intermediate composed of mtDNA-and nDNA-encoded subunits. The direct physical interaction between NDUFC2 and ND4 indicates that these subunits may be incorporated into the membrane arm together. Since ND1, ND4 and NDUFA1 are essential for the assembly of the membrane arm of complex I, NDUFC2 may be also important for the assembly process.

Other supernumerary subunits have also been proposed to assist in CI biogenesis and support the structural stability of CI. The analysis of sequence conservation revealed intra-molecular disulfide bridges and the intermembrane space localization of three CX_9C-containing subunits in human: NDUFS5, NDUFB7 and NDUFA8 [50]. The presence of an intermembrane space targeting signal, in conjunction with a CX_9C domain (which is found in all known and predicted Mia40 substrates [51]), and the absence of a canonical mitochondrial targeting signal suggest the insertion of the three subunits into the complex directly from the intermembrane space. The predicted sizes of these CX_9C domain-containing subunits would fit within 3 intermembrane protrusions in the membrane arm in the recent bovine CI structure determined by 3D electron microscopy [52]. It was proposed that the stabilization of the membrane arm of CI by these subunits from the intermembrane space might be necessary for eukaryotic CI stability [50]. The import of the three subunits, NDUFS5, NDUFB7 and NDUFA8, to the intermembrane space via the Mia40 pathway was supported by sequence based analysis which predicts the sub-compartment localization of mitochondrial proteins [53]. In addition, the electron microscopic analysis of CI decorated with monoclonal antibody against NDUFA8 confirmed its predicted localization at the proximal end of the membrane arm on the intermembrane space side [53]. It is interesting to note that the CI assembly process would involve multiple import pathways that direct nDNA-encoded subunits to either the matrix or intermembrane space side.

In human cells, several assembly models have been proposed by conditional assembly systems where the assembly process is disturbed [39], by tracing assembly intermediates in wild-type cell lines using pulse-chase techniques, by tracking the assembly of individual nuclear-encoded subunits using an in vitro mitochondrial import and assembly assay [41], and by monitoring the assembly pattern of the green fluorescent protein-tagged NDUFS3 subunit and its progression into the mature holoenzyme [42].

Using a conditional assembly system by removing a block in mtDNA-encoded protein translation, it was proposed that the peripheral matrix arm (containing at least NDUFS2, 3 and 4, NDUFA9 and NDUFV2) and the membrane arm (containing at least ND1, ND6 and NDUFB6) are assembled separately [39], consistent with the modular assembly pathway in *N. crassa* [29]. A subsequent study by the same group monitored the assembly pattern of the green fluorescent protein-tagged NDUFS3 subunit and its progression into the mature holoenzyme [42]. The model proposed that an early peripheral arm assembly intermediate containing NDUFS2 and NDUFS3 is membrane anchored by ND1 prior to expansion with additional membrane arm and peripheral arm subunits. Interestingly, this proposed assembly model is different from the modular assembly pathway in *N. crassa* [29] (Fig. 2).

The model was supported by another study monitoring the assembly of subunits in the presence of endogenous CI using radiolabeling techniques [41]. In this study, a small peripheral complex is membrane anchored to a subcomplex containing membrane arm subunits. A smaller subcomplex containing at least ND1 is detected, to which ND2, 3 and 6 are presumably added. This finding is different from the recent proposed model in which ND6 appears to be incorporated at a later step than ND4 [46]. Because the recent crystal structures of the membrane arm of bacterial CI revealed that the ND6

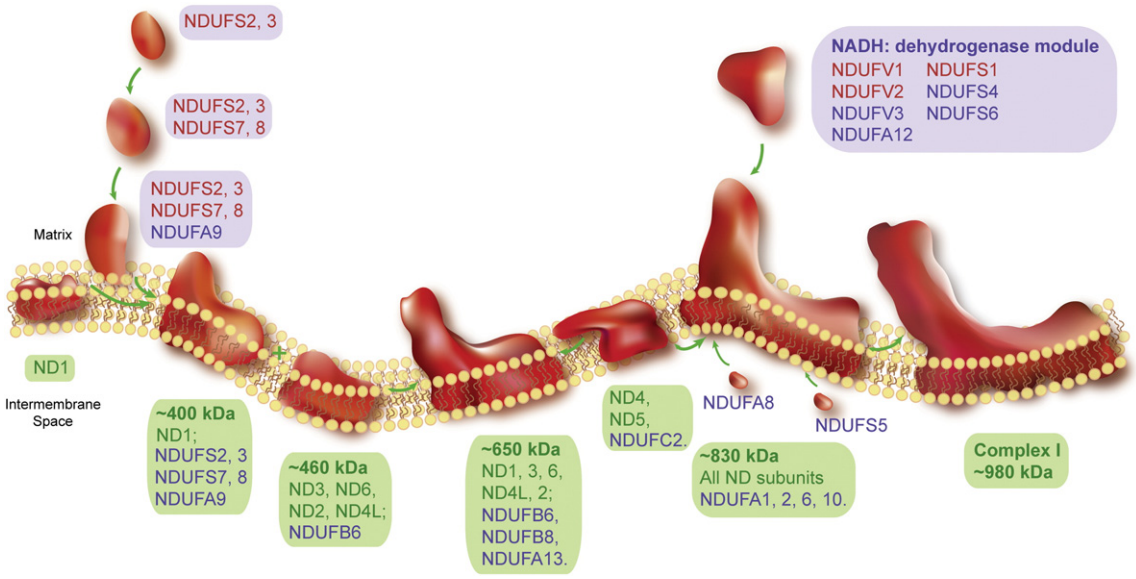


Fig. 2. The assembly model of human complex I biogenesis. In the early assembly stages, the core subunits NDUFS2 and NDUFS3 form a small hydrophilic assembly complex, which further expands by the incorporation of hydrophilic subunits such as NDUFS7, NDUFS8 and later NDUFA9. This peripheral complex, together with a small membrane complex containing mtDNA-encoded subunit ND1, forms a ~400 kDa assembly intermediate. This ~400 kDa complex incorporates with a ~460 kDa membrane complex containing ND3, ND6, ND2, ND4L and NDUFB6 to form a ~650 kDa complex. With the association of another membrane complex having ND4, ND5 and probably NDUFC2, an ~830 kDa assembly intermediate is formed. Meanwhile, a hydrophilic complex, NADH: dehydrogenase module (N module) is assembled by some nDNA-encoded subunits directly or indirectly involved in binding and oxidizing NADH. With the addition of the N module and remaining subunits (such as the intermembrane space subunits NDUFA8 and NDUFS5), mature complex I is assembled. The core subunits are colored with red, the rest of nDNA-encoded subunits are colored with blue. The mtDNA-encoded-subunits are in green.

subunit lies in a proximal portion of the membrane arm adjacent to ND3 and ND4L while ND5 and ND4 are located at the periphery of the membrane arm [14], ND6 is presumably assembled at an earlier step than ND5 and ND4. However, this entry point of ND6 in mammalian CI assembly requires further investigation due to evolutionary divergence. After assembly of peripheral and membrane intermediates, the subcomplex is expanded with membrane arm subunits including NDUFA9, NDUFA10 and NDUFB9. Subsequently, the N module containing NDUFV1, 2, 3, NDUFS4 and NDUFS6 is added (Fig. 2). Interestingly, nDNA-encoded subunits appeared to assemble directly into mature pre-existing CI much faster than mtDNA-encoded subunits. This finding leads to the proposal that newly imported nDNA-encoded subunits may be exchanged with pre-existing incorporated subunits [41].

2.4. Complex I deficiency with CI subunit mutations

CI deficiency is the most common respiratory chain defect in human disorders [54–56]. It has a wide range of clinical presentations, from lethal infantile mitochondrial disease to isolated myopathy, or adult onset neurodegenerative disorders and it can be caused by mutations in both nuclear and mtDNA [54,57–59]. To date, genetic defects causing isolated CI deficiency have been reported for all of the 14 core subunits, including the 7 mtDNA-encoded subunits and the 7 nDNA-encoded subunits NDUFV1 [60], NDUFV2 [61], NDUFS1 [62,63], NDUFS2 [64,65], NDUFS3 [66], NDUFS7 [67] and NDUFS8 [68,69] (Table 1). In addition, pathogenic mutations in genes encoding supernumerary subunits such as NDUFS4 [70,71], NDUFS6 [72], NDUFA1 [73], NDUFA2 [74], NDUFA10 [75], NDUFA11 [76] and NDUFA12 [77] have also been reported (Table 1). Not much is known about the role of these supernumerary subunits, but the presence of disease-causing mutations in these genes indicates that at least some of them are important for proper CI function. Indeed, mutations in *NDUFS4*, *NDUFS6*, *NDUFA2* and *NDUFA10* lead to decreased levels of assembled holo-CI with the accumulation of CI subcomplexes, which indicates a disturbance in the assembly and/or stability of CI [70,72,74,75].

Roles of mtDNA-encoded CI subunits in CI assembly have been well studied in patients with mutations in these subunits. It appears that mutations in *MTND1* and *MTND6* cause gross deficiency of assembled CI [78,79], while ND4 and ND6 are essential for the integration of other mtDNA-encoded subunits into the complex [78,80]. Cells derived from a patient with a mutation in *MTND2* also showed the CI assembly defect with accumulation of subcomplexes [81]. On the contrary, mutations in *MTND3* and *MTND5* gave a relatively normal assembly profile [82,83]. As mentioned above, since ND5 is located at the periphery of the membrane, it may be the last of the ND subunits to assemble [14] (Fig. 2). However, the different effects of mtDNA mutations on CI assembly could also be partly attributed to the proportion of mutant mtDNAs and the nature of amino acid substitutions affecting protein function.

CI-deficient cells from patients have been utilized to determine how specific subunits including nDNA-encoded subunits are assembled into CI [38,84]. In these studies, various stalled assembly intermediates were identified. Based on the findings, a model for CI assembly in which the matrix and membrane arm subunits are found together as early-stage intermediates was proposed. This model for human CI assembly does not correspond to the modular, evolutionarily conserved model proposed for CI assembly in *N. crassa* in which the matrix and membrane arms are assembled via independent pathways [29]. However, this CI assembly process is generally similar to the models proposed by tracing assembly intermediates in wild-type cell lines [41,42] (Fig. 2).

3. Assembly factors

The assembly of such a large number of subunits into the mature holo-CI involves a number of assembly factors. These assembly factors are not part of the final structure of the holo-CI, but they are involved in CI biogenesis and are found in some CI intermediates, indicating their functions in CI assembly/stability. It is apparent that some assembly factors are involved in the biogenesis of specific subunits while others appear to stabilize assembly intermediates. There are also other factors that would be involved in the biogenesis of other proteins and their complexes. Here, we will concentrate on assembly factors which are specific for CI biogenesis.

3.1. C20orf7

Homozygosity mapping of a family with a lethal neonatal form of CI deficiency and the use of microcell-mediated chromosome transfer led to the identification of a novel assembly factor encoded by the *C20orf7* gene [85] (Table 2). A homozygous missense mutation in *C20orf7* segregated with the disease in the family and resulted in a marked isolated CI deficiency in skeletal muscle, liver and skin fibroblasts of the proband. Knockdown of C20orf7 expression in control cells using lentiviral-mediated RNA interference (RNAi) caused a marked decrease in CI activity. Analyses of mtDNA-encoded protein translation in mitochondria from the patient revealed the loss of the CI ND1 subunit, and a ~400-kDa membrane arm intermediate containing ND1 was not formed [85]. These observations suggested that C20orf7 is involved in the assembly or stability of an early CI assembly intermediate that contains the ND1 subunit (Table 2). Interestingly, C20orf7 possesses a predicted S-adenosyl-methionine (SAM)-dependent methyltransferase fold and it may methylate proteins, RNA, or DNA within mitochondria [86,87]. As far as protein methylation is concerned, only 2 methylated subunits have been detected in CI subunits [88]. One of them is NDUFS2, which harbors a methylated arginine, and the other is NDUFB3. At least 2 highly conserved histidines are methylated in NDUFB3 [89], and this subunit is located in the membrane arm of CI containing ND1. Recently, a second family carrying a homozygous *C20orf7* mutation with Leigh syndrome was reported [90]. This mutation affected the predicted SAM-dependent methyltransferase domain of C20orf7. Although the interaction of C20orf7 with NDUFB3 and its subsequent methylation remain to be investigated further, it is possible that this post-translational modification of the subunit plays a role in the assembly or stability of the mature CI. Recently, linkage analysis and DNA sequencing identified a new homozygous *C20orf7* mutation in five patients from two families [91]. Differing from the two previous reported C20orf7 defects associated with isolated CI deficiency, the patients showed a combined OXPHOS defect with not only CI but also CIV deficiency. Notably, decreased CIV was also observed by knockdown of C20orf7 expression in control cells using lentiviral-mediated RNAi [85]. These findings raise the possibility that C20orf7 may play a role in the post transcriptional modification of one or several proteins of importance for CI and CIV function and/or assembly [85,91]. Although the precise pathogenic mechanism is still unclear, a C20orf7 defect should be considered not only in isolated CI deficiency but also in combination with decreased CIV activity [91].

3.2. Ndufaf4 (C6orf66)

Homozygosity mapping of patients with infantile mitochondrial encephalopathy or antenatal cardiomyopathy attributed to isolated CI deficiency led to the identification of an assembly factor encoded by the *Ndufaf4* (*C6orf66*) gene [92] (Table 2). A missense mutation in a conserved residue of Ndufaf4 was associated with a reduction in its mRNA level in fibroblasts and a significant decrease of Ndufaf4 protein in muscle. Isolated mitochondria from this patient's muscle have only ~30% residual mature CI, with the accumulation of 2 complexes that were smaller than the CI holoenzyme, which may be stalled assembly intermediates. One of them resembled the ~830-kDa intermediate associated with the assembly factor B17.2L (see later). Dysfunction of CI was due to a direct consequence of this mutation, as demonstrated by the functional restoration of CI activity upon transfection of the patient's cells with wild-type Ndufaf4 cDNA.

3.3. Ndufaf3 (C3orf60)

Homozygosity mapping and gene sequencing of 5 CI-deficient patients from 3 different families identified mutations in the *Ndufaf3*

Table 1
Known complex I subunit defects which cause mitochondrial disease.

	Human	Bovine homologue	Position in complex I	Clinical diagnosis	Ref.
Mitochondrial DNA encoded subunits	ND1	ND1	P module	LHON[a], MELAS[b]	[79,129]
	ND2	ND2	P module	L.S.[c]	[81]
	ND3	ND3	P module	L.S., LIMD[d]	[82,111,130–132]
	ND4	ND4	P module	LHON, L.S.	[80,133,134]
	ND4L	ND4L	P module	LHON	[135]
	ND5	ND5	P module	L.S., MELAS, LHON	[83,111,127,136]
	ND6	ND6	P module	L.S., LHON, dystonia	[127,137,138]
Nuclear DNA encoded subunits	NDUFA1	MWFE		L.S., mitochondrial encephalopathy	[73,139,140]
	NDUFA2	B8		L.S.	[74]
	NDUFA10	42 kDa		L.S.	[75]
	NDUFA11	B14.7		Mitochondrial encephalopathy, cardiomyopathy, LIMD	[76]
	NDUFA12	B17.2		L.S.	[77]
	NDUFS1	75 kDa	N module; Fe–S protein (N1b, N4, N5)	L.S., leukodystrophy	[62,63,141,142]
	NDUFS2	49 kDa	Q module	L.S., cardiomyopathy, mitochondrial encephalomyopathy, LIMD	[64,65]
	NDUFS3	30 kDa	Q module	L.S.	[66]
	NDUFS4	18 kDa	N module	L.S.	[70,71,107,111]
	NDUFS6	13 kDa	N module	LIMD	[72,143]
	NDUFS7	PSST	Q module; Fe–S protein (N2)	L.S.	[67,144]
	NDUFS8	TYKY	Q module; Fe–S protein (N6a, N6b)	Mitochondrial encephalopathy, cardiomyopathy, leukodystrophy, L.S.	[68,69,111]
	NDUFV1	51 kDa	N module; Fe–S protein (N3), FMN	LIMD, leukodystrophy, mitochondrial encephalopathy, L.S.	[60,62,107,111,127]
	NDUFV2	24 kDa	N module; Fe–S protein (N1a)	Cardiomyopathy, mitochondrial encephalopathy	[61,128]

[a] LHON, Leber Hereditary Optic Neuropathy.
[b] MELAS, Mitochondrial Encephalopathy, Lactic Acidosis, Stroke-like episodes.
[c] L.S., Leigh Syndrome or Leigh-like Syndrome.
[d] LIMD, Lethal Infantile Mitochondrial Disease.

Table 2
Pathogentic mutations in known complex I assembly factors.

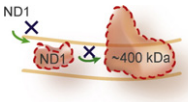
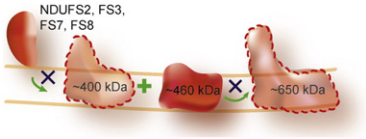
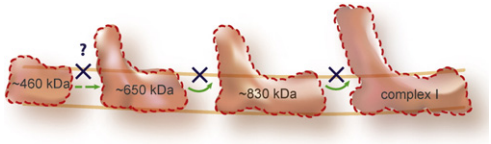
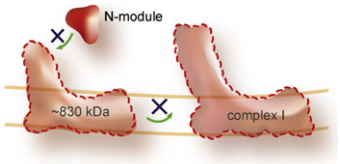
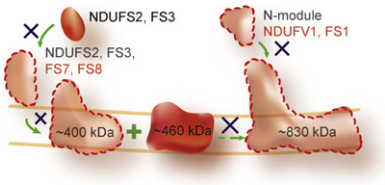
Assembly factor	Patient studies		Assembly defect description	Ref.
	Clinical diagnosis	Mutations		
C20orf7	LIMD[a] L.S.[b] L.S.	Homozygous: c.719T>C (p.L229P) Homozygous: c. 477A>C (p.L159F) Homozygous: c. 749G>T (p.G250V)	 The translation or stabilization of the ND1 subunit is impaired, which prevents the assembly of the ~400 kDa assembly intermediate containing ND1.[c]	[85,90,91]
Ndufaf3 (C3orf60) Ndufaf4 (C6orf66)	LIMD LIMD LIMD LIMD, antenatal cardiomyopathy	Homozygous: c.229G>C (p.G77R); Homozygous: c.365G>C (p.R122P) Compound heterozygous: c.2T>C (p.M1T); c.365G>C (p.R122P) Homozygous: c.194T>C (p.L65P)	 The peripheral subcomplex containing NDUFS2, FS3, FS7, and FS8 fails to be inserted into the membrane, resulting in the impaired assembly of the ~400 kDa assembly intermediate.	[92,93]
Ndufaf1 (CIA30)	Cardioencephalomyopathy	Compound heterozygous: c.1140A>G (p.K253R); c.1001A>C (p.T207P)	 The ~460 kDa assembly intermediate containing ND2, ND3 and ND4L is not formed, and the ~400 kDa assembly intermediate is accumulated initially, but quickly turned over.	[95,96]
ACAD9	Cardiomyopathy, hearing loss, short stature, exercise intolerance Encephalopathy, cardiomyopathy Exercise intolerance Exercise intolerance Cardiomyopathy, mitochondrial encephalopathy Cardiomyopathy, exercise intolerance Cardiomyopathy, mitochondrial encephalopathy Cardiomyopathy, mitochondrial encephalopathy	Homozygous: c.1553G>A (p.R518H) Compound heterozygous: c.187G>T (p.E63X); c.1237G>A (p.E413H) Homozygous: c. 1594C>T (p.R532W) Compound heterozygous: c.380G>A (p.R127Q); c.1405C>T (p.R469W). Compound heterozygous: c.130T>A (p.F441); c.797G>A (p.R266Q). Compound heterozygous: c. 130T>A (p.F441); c.797G>A (p.R266Q). Compound heterozygous: c.797G>A (p. R266Q); c.1249C>T (p.R417C) Compound heterozygous: c.976G>A (p. A326P); c.1594C>T (p. R532W)	 ACAD9 mutations cause decreased levels of NDUFAF1, Ecsit and mature CI.	[102–104]
Ndufaf2 (B17.2L or NDUFA12L)	Mitochondrial encephalopathy Mitochondrial encephalopathy L.S. L.S. L.S.	Homozygous: c.182C>T (p.R45X) Homozygous: c.1A>T (p.M1L) Homozygous: c.114C>G (p.Y38X) Homozygous: c.l03delA (p.I35SfsXI7) Homozygous: c.221G>A (p.W74X)	 The late stages of CI assembly are impaired.	[107,108,111,145]
NUBPL (Ind1)	Mitochondrial encephalopathy	Homozygous: c.166G>A (p.G56R)		[109,111]
C8orf38	L.S.	Homozygous: c.296A>G (p.Q99R)	Unknown.	[124]

Table 2 (*continued*)

Assembly factor	Patient studies		Assembly defect description	Ref.
	Clinical diagnosis	Mutations		
FOXRED1	L.S.	Compound heterozygous: c.694C>T (p. Q232X); c.1289A>G (p.N430S)	Unknown.	[111,123]
	L.S.	Homozygous: c.1054C>T (p.R352W)		

[a] LIMD, Lethal Infatile Mitochondrial Disease.
[b] L.S., Leigh Syndrome, or Leigh-like Syndrome.
[c] In the figures, the darkness represents levels of the assembly complexes.

(*C3orf60*) gene [93] (Table 2). These pathogenic missense mutations resulted in fatal neonatal mitochondrial disorders with severe CI deficiency. In mitochondria derived from one of the patients, CI assembly was disrupted without the accumulation of either peripheral or membrane arm assembly intermediates. These findings suggest that Ndufaf3 plays a role in the early stages of CI assembly. Furthermore, Ndufaf3 was shown to tightly interact with Ndufaf4 localizing in the membrane [93]. Their membrane localization also suggests that Ndufaf3 and Ndufaf4 may be involved in membrane anchoring of an early intermediate complex, which contains the CI subunits NDUFS2, 3, 7 and 8. Indeed, in BN-PAGE analyses, Ndufaf3 and Ndufaf4 accurately comigrate with a subcomplex containing NDUFS2, NDUFS3, NDUFS7, NDUFS8 and possibly NDUFA9, suggesting the interaction of these 2 proteins with this subcomplex [42,93]. At this stage, the early intermediate complex would be assembled with membrane arm intermediates which contain ND1. Then, this Ndufaf3 and Ndufaf4 intermediate would be assembled into the ~400 kDa-subcomplex and subsequently into the ~650 kDa- and ~830 kDa-complexes, close to finalization of the mature holo-CI [93] (Table 2).

3.4. Ndufaf1 (CIA30)

Complex I assembly factors CIA30 and CIA84 were initially identified in *N. crassa* as important proteins that are specifically involved in the assembly of the membrane arm of CI [30]. While the CIA84 human homolog, PTCD1, is a negative regulator of mitochondrial leucine tRNA levels [94], the human homolog of CIA30 (Ndufaf1) has been established as a bona fide CI assembly factor. Knockdown of Ndufaf1 led to decreased levels of CI activity and assembly [95]. Ndufaf1 is present in two complexes of ~830 and ~460 kDa [96]. Screening of patient cells diagnosed with enzymatically deficient CI, revealed a patient with cardioencephalomyopathy, showing markedly reduced levels of Ndufaf1. Genetic analysis revealed that the patient had mutations in both alleles of the *NDUFAF1* gene [96] (Table 2). Steady-state CI levels were restored by complementing the deficiency in the patient's fibroblasts with normal Ndufaf1, which showed the pathogenicity of the mutations. In this patient, ND2 and the ~460-kDa CI intermediate were degraded and the ~830-kDa complex and the CI holoenzyme were not formed. This finding suggests that Ndufaf1 is an important factor for the assembly and/or stability of the ~460-kDa intermediate (Table 2). In normal cells, the ~830-kDa intermediate remains associated with Ndufaf1 and has been shown by co-immunoprecipitation to contain subunits ND1, ND2, ND3, ND6, NDUFB6, NDUFA6, NDUFA9, NDUFS3 and NDUFS7 [96]. Recently, the mRNA level and the protein expression of Ndufaf1 were found to decrease in muscles from mice lacking testicular nuclear receptor 4 (TR4) [97]. The TR4-knockout mice developed mitochondrial myopathy with CI deficiency and restoration of Ndufaf1 level in the myoblasts from the mice increased CI activity. A chromatin immunoprecipitation assay indicated that TR4 directly binds to *Ndufaf1* promoter. Since TR4 is a key transcriptional regulator of many signaling pathways, TR4 could modulate CI activity via transcriptionally regulating Ndufaf1 [97].

3.5. Ecsit

Evolutionary conserved signaling intermediate in Toll pathways (Ecsit) was originally identified as a cytosolic protein involved in the inflammatory response and embryonic development [98,99]; however, it has also been described as a mitochondrial protein that interacts with Ndufaf1 during CI assembly [100]. Ecsit is found in the ~460- and ~830-kDa intermediates with Ndufaf1. Furthermore, knockdown of Ecsit using RNAi reduced the levels of Ndufaf1 and led to impaired CI assembly. The levels of the intermediates associated with Ndufaf1 and Ecsit were also reduced following Ecsit knockdown and a ~500-kDa subcomplex accumulated [100] (Table 2). These findings suggest that the stability of Ndufaf1 and its associated intermediates is dependent on Ecsit. Conversely, Ndufaf1 knockdown results in a minor decrease in the amount of Ecsit in the ~500-kDa intermediate [100], indicating that Ecsit and Ndufaf1 may have different functions in CI assembly or stability.

3.6. ACAD9

Acyl-CoA dehydrogenase 9 (ACAD9) was initially cloned and identified as a member of the acyl-CoA dehydrogenase family [101]. Contrary to its previously proposed involvement in fatty acid oxidation, a new role for ACAD9 in oxidative phosphorylation was recently discovered. Tandem affinity purification demonstrated that ACAD9 was co-purified with the known CI assembly factors Ndufaf1 and Ecsit [100,102]. Subsequent 2-dimensional blue-native SDS-PAGE analysis showed that ACAD9 co-migrates with the previously described 500–850-kDa complexes that contain Ndufaf1 and Ecsit [42]. Knockdown of ACAD9 by RNAi led to decreased levels of CI along with Ndufaf1 and Ecsit, while Ndufaf1 knockdown reduced ACAD9 levels [102]. Moreover, pathogenic mutations in *ACAD9* that caused isolated CI deficiency in two patients with exercise intolerance, hypertrophic cardiomyopathy, lactic acidosis and failure to thrive were identified (Table 2). Consistent with the results of ACAD9 knockdown, fully assembled CI and Ecsit and Ndufaf1 protein levels were reduced in ACAD9 patient cell lines [102]. Whole-exome sequencing of a single individual with severe isolated CI deficiency identified other pathogenic *ACAD9* mutations and subsequent screening of additional patients led to the identification of two additional unrelated cases with *ACAD9* mutations [103]. Following this, homozygosity mapping using a large consanguineous family with an isolated CI deficiency led to the identification of a pathogenic homozygous mutation in *ACAD9* [104]. Importantly, riboflavin, the vitamin precursor of the flavin adenine dinucleotide (FAD) moiety, is known to be the catalytic cofactor of ACADs and enhances their assembly and stability [105]. Beneficial effects of riboflavin treatment were reported in some patients [103,104]; therefore, the identification of *ACAD9* mutations in a patient with CI deficiency is extremely important from a clinical point of view.

How ACAD9, a protein previously proposed to be involved in fatty acid oxidation, functions in CI maintenance remains unresolved. Interestingly, no biochemical evidence of disturbed fatty acid oxidation was detected in patients with *ACAD9* gene mutations [102–104], suggesting that the primary in vivo role of ACAD9 is in the assembly of CI.

3.7. Ndufaf2 (B17.2L)

B17.2L was originally identified as a mitochondrial protein of unknown function in a screen for transcriptional targets of c-myc [106]. However, whole genome subtraction identified B17.2L as a candidate factor for CI assembly [107]. This protein is a paralog of NDUFA12 (B17.2), a small structural subunit in the matrix arm of CI [1].

Null mutations in *B17.2L* in a patient with a progressive encephalopathy resulted in CI deficiency, and the associated CI assembly defect could be completely rescued by retroviral expression of B17.2L in the patient fibroblasts [107] (Table 2). An anti-Ndufaf2 antibody did not associate with the holoenzyme complex, but specifically recognized an 830-kDa subcomplex in several patients with CI assembly defects caused by pathogenic mutations in CI subunits NDUFV1 or NDUFS4, which form part of the N module (Fig. 2). Analyses of mitochondria from patients with mutations in *NDUFS6*, whose gene product is part of the N module, also revealed an accumulation of the ~830-kDa subcomplex [72]. However, this complex was not detected in controls, suggesting that it represents a stalled assembly intermediate, rather than a degradation product [41]. In another patient with a homozygous null mutation of *NDUFAF2*, the accumulation of subcomplexes containing the NDUFS2 and NDUFS3 subunits was also detected [108]. Furthermore, Ndufaf2 co-immunoprecipitated a subset of CI structural subunits including ND1, NDUFS2, NDUFS3, NDUFS4, NDUFV1, NDUFV2 and NDUFA13 [107]. These findings suggest that Ndufaf2 is associated with the ~830 kDa-complex and required in the late stage of CI assembly (Table 2).

3.8. Ind1 (NUBPL)

Fe–S protein required for NADH dehydrogenase (Ind1) or Nucleotide-binding protein-like protein (NUBPL), is an Fe–S protein that contains an N-terminal mitochondrial-targeting sequence, a highly conserved nucleotide-binding domain, and a putative Fe–S-binding signature (CXXC) [109,110]. CI contains 8 Fe–S clusters and Ind1 can bind to an Fe–S cluster via its CXXC motif [110]. Deletion of Ind1 in *Y. lipolytica* results in specifically decreased CI activity, whereas the activity of other mitochondrial Fe–S enzymes, e.g., aconitase and succinate dehydrogenase, is not affected [109]. Knockdown of human Ind1 in HeLa cells by RNAi strongly decreased CI protein and enzyme activity levels and massively decreased the levels of several subunits of the peripheral arm of CI, such as NDUFS1 and NDUFV1, which contain Fe–S clusters [1], plus NDUFS3 and NDUFA13 [110]. Furthermore, Ind1 depletion resulted in the appearance of a 450-kDa subcomplex representing part of the membrane arm. This subcomplex appears to be a stalled membrane arm assembly intermediate that accumulates due to the impaired assembly of the peripheral arm, including the Fe–S cluster-containing subunits. This 450-kDa subcomplex does not contain NDUFS1, NDUFV1, NDUFA13, or NDUFA9, but it does contain the membrane arm subunit NDUFB6 [110], suggesting that this subcomplex likely corresponds to the ~460 kDa-subcomplex (Fig. 2). As NDUFS7 and NDUFS8 also contain Fe–S clusters, Ind1 depletion might affect the ~400 kDa-subcomplex containing these subunits and may impair the assembly of the ~400 kDa-and ~460 kDa-subcomplexes into larger complexes, resulting in the accumulation of the 460-kDa subcomplex. Radiolabeling using ^{55}Fe-labeled transferrin, the physiological source of Fe for mammalian cells, revealed that the amount of Fe associated with CI reflects the dependence of this enzyme on Ind1 for its assembly. Together, these data identify Ind1 as an important factor for CI assembly, particularly in the assembly of N module and the subcomplex containing NDUFS7 and NDUFS8, with a possible role in the delivery of Fe–S clusters to CI subunits (Table 2).

Recently, high-throughput sequencing of over 100 candidate genes in more than 100 individuals with CI deficiency led to the identification of compound heterozygous mutations in the *Ind1* gene in a single patient [111] (Table 2). The patient presented with developmental delay accompanied by myopathy, nystagmus, ataxia, upper motor neuron signs and findings of leukodystrophy on brain magnetic resonance imaging. The transduction of the patient's fibroblasts with wild-type Ind1 restored CI activity, which confirmed the important role of Ind1 in CI biogenesis.

3.9. AIF

Apoptosis-inducing factor (AIF) was originally identified as a mitochondrial pro-apoptotic protein. It is an evolutionarily conserved, ubiquitously expressed flavoprotein with NADH oxidase activity that is normally located in the mitochondrial intermembrane space [112]. Upon apoptogenic stimuli, AIF is released from mitochondria into the cytosol and migrates to the nucleus where it mediates the nuclear features of apoptosis, e.g., chromatin condensation and large scale DNA degradation, in a caspase-independent manner [112–114].

Besides its apoptotic role, AIF has been shown to have a physiological role in sustaining CI-driven oxidative phosphorylation, independently of its pro-apoptotic properties [115–118]. AIF-depleted cells have reduced levels of CI subunits, decreased CI activity and impaired CI-driven mitochondrial respiration [116–118]. In mice with a partial AIF deficiency, Harlequin (Hq) mice, the levels of AIF are reduced by ~80% due to a fortuitous retroviral insertion in the first intron of the *AIF* gene encoding AIF, which is on the X chromosome. Brain mitochondria derived from Hq mice display reduced levels of CI and CI subunits along with defects in CI-driven mitochondrial respiration [118–120]. These animals exhibit a phenotype associated with mitochondrial respiratory chain diseases, including cerebellar ataxia and retinal degeneration [121], and have been established as a genetic model of human CI deficiency [118]. However, because AIF has not been found to be associated with any structural subunits of CI and the generation of incompletely assembled subcomplexes has not been detected in AIF-deficient mitochondria, the role of AIF in CI biogenesis remains elusive.

Recently, a pathological mutation in the human X-linked *AIFM1* gene encoding AIF was identified in 2 infant male patients with progressive mitochondrial encephalomyopathy [122]. These patients were born from monozygotic twin sisters and unrelated fathers, suggesting an X-linked trait, and single nucleotide polymorphism-based haplotype analysis of the X chromosome led to the identification of the mutation. Surprisingly, fibroblasts from the patients showed a reduction of respiratory chain complex III and complex IV activity, but not of CI activity. The mechanism underlying the discrepancy in the effect of AIF deficiency on CI activity between Hq mice and human patients is not clear. Approximately 75% of mutant cells from the patients showed mitochondrial fragmentation under galactose treatment compared with 23% of control cells, suggesting that cells containing the mutation are more sensitive to apoptotic stimuli than control cells. The *AIFM1* mutation might destabilize the inner mitochondrial membrane causing subsequent damage to the structure and activity of the respiratory chain, which is not a specific effect on CI assembly/stability. The findings in the patients' cells will require the reinterpretation of the role of AIF in CI biogenesis.

3.10. MidA

Mitochondrial dysfunction protein A (MidA) was originally identified and characterized in *Dictyostelium* [123], and reduced levels of ATP and various phenotypes, including slow growth and abnormal development, were observed in *Dictyostelium* lacking MidA. *Dictyostelium* and human MidA are highly homologous proteins, and yeast 2-hybrid screening and pull-down assays recently revealed that both proteins interact with the CI subunit NDUFS2 [88]. Consistent with this finding, *Dictyostelium midA* null cells showed decreased CI activity, while knockdown of human MidA in HEK293T cells resulted in reduced levels of assembled CI in BN-PAGE studies. Interestingly, structural bioinformatic analyses suggested that MidA has a methyltransferase domain, as does another

CI assembly factor, C20orf7 [88]. As previously mentioned, NDUFS2 can be methylated at an arginine residue. These facts raise the possibility that MidA methylates this subunit as a necessary step in the CI assembly process.

3.11. Complex I phylogenetic profile genes

Of note is the recent approach to elucidate CI function based on a comprehensive compendium of the mitochondrial proteome by phylogenetic profiling of 43 species containing or lacking CI [124]. Complex I phylogenetic profile (COPP) genes, which have coevolved with CI, were identified and 19 strong candidate proteins that might be involved in CI biogenesis were found. Among these proteins, knockdown of C8orf38, FOXRED1, LYRM5 and LACTB using lentiviral-mediated RNAi reduced the levels of CI activity [124]. Furthermore, 3 candidate genes, *C8orf38* [124], *C20orf7* [85] and *Ndufaf3* (*C3orf60*) [93], have been confirmed to be involved in CI biogenesis, as gene mutations have been found in patients with CI deficiency, which verified that the COPP genes are promising candidate CI assembly factors. In addition, pathogenic mutations in another COPP gene, FAD-dependent oxidoreductase domain-containing protein 1 (*FOXRED1*), were recently identified in patients with isolated CI deficiency; however, its exact function remains elusive [111,125].

4. Current model of human complex I assembly

The accumulation of research findings for human CI biogenesis allows us to propose a newly updated model for its assembly (Fig. 2, Table 2). The most recent consensus model proposes that an early assembly intermediate is anchored to the membrane prior to its extension with additional membrane and peripheral subunits [126].

In the early assembly stage, the core subunits NDUFS2 and NDUFS3 form a small hydrophilic assembly complex, which further expands by the incorporation of hydrophilic subunits, e.g., NDUFS7, NDUFS8, and later, possibly NDUFA9. This peripheral complex is anchored to the membrane by the assembly factors Ndufaf3 and Ndufaf4 [93]. The complex combines with a small membrane complex containing the mtDNA-encoded ND1 subunit, for which C20orf7 is involved in assembly or stability [85], to form a ~400-kDa assembly intermediate [41]. This ~400-kDa complex incorporates with a ~460-kDa membrane complex containing ND3, ND6, ND2, ND4L and NDUFB6 to form a ~650-kDa complex under the presence of the assembly factors of Ndufaf1, Ecsit and ACAD9 [85,102]. With the association of another membrane complex containing ND4, ND5 and possibly NDUFC2, an ~830 kDa assembly intermediate is formed [49]. The assembly factor Ndufaf2 is associated with this ~830 kDa-complex and would be required in the late stage of CI assembly [107]. Meanwhile, a hydrophilic complex, the NADH: dehydrogenase module, is built with some nDNA-encoded subunits that are directly or indirectly involved in binding and oxidizing NADH. With the addition of the NADH: dehydrogenase module and the remaining subunits, the mature holo-CI is assembled. In this complicated and elaborate assembly process, more assembly factors with unknown functions including Ind1, MidA, FOXRED1 and undiscovered proteins are involved [88,111].

5. Closing remarks

Recent remarkable advances in structural biology have given us new insights into the architecture and function of CI, and in the near future, they may elucidate the exact composition of CI intermediates and clarify the specific significance of the assembly factors in these complexes for CI assembly/stability.

Furthermore, analyses of patients with deficits in CI subunits or assembly factors have provided a better understanding of the CI assembly process. At present, the genotype–phenotype correlation in patients with CI deficiency is not clear (Tables 1 and 2) [127], so we need to establish an exhaustive diagnostic system to screen routinely for mutations in all of the CI subunits, and both known and candidate factors that play a role in CI assembly/stability [128]. Recent powerful technologies such as next generation sequencing or tiling arrays combined with functional validation such as assembly analysis are facilitating the identification of patients with mutations causing CI deficiency. Continuing concerted efforts to expand knowledge of CI assembly and to identify all factors involved in this assembly process are also needed. These achievements are clearly important for the future diagnosis and treatment of these patients.

Acknowledgements

The authors' work is supported by grants from the Australian Research Council, Australian National Health and Medical Research Council (NHMRC), the Victorian Government's Operational Infrastructure Support Program and DRT is supported by an NHMRC Principal Research Fellowship and MM by an NHMRC Career Development Award.

References

[1] R.J. Janssen, L.G. Nijtmans, L.P. van den Heuvel, J.A. Smeitink, Mitochondrial complex I: structure, function and pathology, J. Inherit. Metab. Dis. 29 (2006) 499–515.
[2] V. Zickermann, S. Kerscher, K. Zwicker, M.A. Tocilescu, M. Radermacher, U. Brandt, Architecture of complex I and its implications for electron transfer and proton pumping, Biochim. Biophys. Acta 1787 (2009) 574–583.
[3] N.V. Dudkina, S. Sunderhaus, E.J. Boekema, H.P. Braun, The higher level of organization of the oxidative phosphorylation system: mitochondrial supercomplexes, J. Bioenerg. Biomembr. 40 (2008) 419–424.
[4] A.R. Crofts, The cytochrome bc1 complex: function in the context of structure, Annu. Rev. Physiol. 66 (2004) 689–733.
[5] T. Yano, The energy-transducing NADH: quinone oxidoreductase, complex I, Mol. Aspects Med. 23 (2002) 345–368.
[6] B.E. Schultz, S.I. Chan, Structures and proton-pumping strategies of mitochondrial respiratory enzymes, Annu. Rev. Biophys. Biomol. Struct. 30 (2001) 23–65.
[7] T. Friedrich, B. Bottcher, The gross structure of the respiratory complex I: a Lego system, Biochim. Biophys. Acta 1608 (2004) 1–9.
[8] V. Guenebaut, A. Schlitt, H. Weiss, K. Leonard, T. Friedrich, Consistent structure between bacterial and mitochondrial NADH:ubiquinone oxidoreductase (complex I), J. Mol. Biol. 276 (1998) 105–112.
[9] L.A. Sazanov, J. Carroll, P. Holt, L. Toime, I.M. Fearnley, A role for native lipids in the stabilization and two-dimensional crystallization of the *Escherichia coli* NADH-ubiquinone oxidoreductase (complex I), J. Biol. Chem. 278 (2003) 19483–19491.
[10] L.A. Sazanov, P. Hinchliffe, Structure of the hydrophilic domain of respiratory complex I from *Thermus thermophilus*, Science 311 (2006) 1430–1436.
[11] C. Mathiesen, C. Hagerhall, Transmembrane topology of the NuoL, M and N subunits of NADH:quinone oxidoreductase and their homologues among membrane-bound hydrogenases and bona fide antiporters, Biochim. Biophys. Acta 1556 (2002) 121–132.
[12] I. Belevich, M.I. Verkhovsky, M. Wikstrom, Proton-coupled electron transfer drives the proton pump of cytochrome c oxidase, Nature 440 (2006) 829–832.
[13] K. Faxen, G. Gilderson, P. Adelroth, P. Brzezinski, A mechanistic principle for proton pumping by cytochrome c oxidase, Nature 437 (2005) 286–289.
[14] R.G. Efremov, R. Baradaran, L.A. Sazanov, The architecture of respiratory complex I, Nature 465 (2010) 441–445.
[15] C. Hunte, V. Zickermann, U. Brandt, Functional modules and structural basis of conformational coupling in mitochondrial complex I, Science 329 (2010) 448–451.
[16] J. Carroll, I.M. Fearnley, J.M. Skehel, R.J. Shannon, J. Hirst, J.E. Walker, Bovine complex I is a complex of 45 different subunits, J. Biol. Chem. 281 (2006) 32724–32727.
[17] J. Carroll, I.M. Fearnley, R.J. Shannon, J. Hirst, J.E. Walker, Analysis of the subunit composition of complex I from bovine heart mitochondria, Mol. Cell. Proteomics 2 (2003) 117–126.
[18] J. Hirst, J. Carroll, I.M. Fearnley, R.J. Shannon, J.E. Walker, The nuclear encoded subunits of complex I from bovine heart mitochondria, Biochim. Biophys. Acta 1604 (2003) 135–150.
[19] D. Stojanovski, A.J. Johnston, I. Streimann, N.J. Hoogenraad, M.T. Ryan, Import of nuclear-encoded proteins into mitochondria, Exp. Physiol. 88 (2003) 57–64.
[20] N.J. Hoogenraad, L.A. Ward, M.T. Ryan, Import and assembly of proteins into mitochondria of mammalian cells, Biochim. Biophys. Acta 1592 (2002) 97–105.
[21] M. Lazarou, D.R. Thorburn, M.T. Ryan, M. McKenzie, Assembly of mitochondrial complex I and defects in disease, Biochim. Biophys. Acta 1793 (2009) 78–88.
[22] R.O. Vogel, J.A. Smeitink, L.G. Nijtmans, Human mitochondrial complex I assembly: a dynamic and versatile process, Biochim. Biophys. Acta 1767 (2007) 1215–1227.
[23] R. Vogel, L. Nijtmans, C. Ugalde, L. van den Heuvel, J. Smeitink, Complex I assembly: a puzzling problem, Curr. Opin. Neurol. 17 (2004) 179–186.
[24] C. Remacle, F. Duby, P. Cardol, R.F. Matagne, Mutations inactivating mitochondrial genes in *Chlamydomonas reinhardtii*, Biochem. Soc. Trans. 29 (2001) 442–446.

[25] P. Cardol, R.F. Matagne, C. Remacle, Impact of mutations affecting ND mitochondria-encoded subunits on the activity and assembly of complex I in Chlamydomonas. Implication for the structural organization of the enzyme, J. Mol. Biol. 319 (2002) 1211–1221.
[26] P. Cardol, M. Lapaille, P. Minet, F. Franck, R.F. Matagne, C. Remacle, ND3 and ND4L subunits of mitochondrial complex I, both nucleus encoded in *Chlamydomonas reinhardtii*, are required for activity and assembly of the enzyme, Eukaryot. Cell 5 (2006) 1460–1467.
[27] P. Cardol, L. Boutaffala, S. Memmi, B. Devreese, R.F. Matagne, C. Remacle, In Chlamydomonas, the loss of ND5 subunit prevents the assembly of whole mitochondrial complex I and leads to the formation of a low abundant 700 kDa subcomplex, Biochim. Biophys. Acta 1777 (2008) 388–396.
[28] C. Remacle, M.R. Barbieri, P. Cardol, P.P. Hamel, Eukaryotic complex I: functional diversity and experimental systems to unravel the assembly process, Mol. Genet. Genomics 280 (2008) 93–110.
[29] G. Tuschen, U. Sackmann, U. Nehls, H. Haiker, G. Buse, H. Weiss, Assembly of NADH:ubiquinone reductase (complex I) in Neurospora mitochondria. Independent pathways of nuclear-encoded and mitochondrially encoded subunits, J. Mol. Biol. 213 (1990) 845–857.
[30] R. Kuffner, A. Rohr, A. Schmiede, C. Krull, U. Schulte, Involvement of two novel chaperones in the assembly of mitochondrial NADH:ubiquinone oxidoreductase (complex I), J. Mol. Biol. 283 (1998) 409–417.
[31] U. Schulte, Biogenesis of respiratory complex I, J. Bioenerg. Biomembr. 33 (2001) 205–212.
[32] A. Videira, Complex I from the fungus *Neurospora crassa*, Biochim. Biophys. Acta 1364 (1998) 89–100.
[33] U. Weidner, U. Nehls, R. Schneider, W. Fecke, H. Leif, A. Schmiede, T. Friedrich, R. Zensen, U. Schulte, T. Ohnishi, et al., Molecular genetic studies of complex I in *Neurospora crassa*, *Aspergillus niger* and *Escherichia coli*, Biochim. Biophys. Acta 1101 (1992) 177–180.
[34] U. Schulte, W. Fecke, C. Krull, U. Nehls, A. Schmiede, R. Schneider, T. Ohnishi, H. Weiss, In vivo dissection of the mitochondrial respiratory NADH:ubiquinone oxidoreductase (complex I), Biochim. Biophys. Acta 1187 (1994) 121–124.
[35] A. Videira, M. Duarte, On complex I and other NADH:ubiquinone reductases of *Neurospora crassa* mitochondria, J. Bioenerg. Biomembr. 33 (2001) 197–203.
[36] A. Videira, M. Duarte, From NADH to ubiquinone in Neurospora mitochondria, Biochim. Biophys. Acta 1555 (2002) 187–191.
[37] L.I. Grad, B.D. Lemire, Riboflavin enhances the assembly of mitochondrial cytochrome c oxidase in *C. elegans* NADH-ubiquinone oxidoreductase mutants, Biochim. Biophys. Acta 1757 (2006) 115–122.
[38] H. Antonicka, I. Ogilvie, T. Taivassalo, R.P. Anitori, R.G. Haller, J. Vissing, N.G. Kennaway, E.A. Shoubridge, Identification and characterization of a common set of complex I assembly intermediates in mitochondria from patients with complex I deficiency, J. Biol. Chem. 278 (2003) 43081–43088.
[39] C. Ugalde, R. Vogel, R. Huijbens, B. Van Den Heuvel, J. Smeitink, L. Nijtmans, Human mitochondrial complex I assembles through the combination of evolutionary conserved modules: a framework to interpret complex I deficiencies, Hum. Mol. Genet. 13 (2004) 2461–2472.
[40] I.E. Scheffler, N. Yadava, P. Potluri, Molecular genetics of complex I-deficient Chinese hamster cell lines, Biochim. Biophys. Acta 1659 (2004) 160–171.
[41] M. Lazarou, M. McKenzie, A. Ohtake, D.R. Thorburn, M.T. Ryan, Analysis of the assembly profiles for mitochondrial- and nuclear-DNA-encoded subunits into complex I, Mol. Cell. Biol. 27 (2007) 4228–4237.
[42] R.O. Vogel, C.E. Dieteren, L.P. van den Heuvel, P.H. Willems, J.A. Smeitink, W.J. Koopman, L.G. Nijtmans, Identification of mitochondrial complex I assembly intermediates by tracing tagged NDUFS3 demonstrates the entry point of mitochondrial subunits, J. Biol. Chem. 282 (2007) 7582–7590.
[43] U. Nehls, T. Friedrich, A. Schmiede, T. Ohnishi, H. Weiss, Characterization of assembly intermediates of NADH:ubiquinone oxidoreductase (complex I) accumulated in Neurospora mitochondria by gene disruption, J. Mol. Biol. 227 (1992) 1032–1042.
[44] I. Bourges, C. Ramus, B. Mousson de Camaret, R. Beugnot, C. Remacle, P. Cardol, G. Hofhaus, J.P. Issartel, Structural organization of mitochondrial human complex I: role of the ND4 and ND5 mitochondria-encoded subunits and interaction with prohibitin, Biochem. J. 383 (2004) 491–499.
[45] P. Potluri, N. Yadava, I.E. Scheffler, The role of the ESSS protein in the assembly of a functional and stable mammalian mitochondrial complex I (NADH-ubiquinone oxidoreductase), Eur. J. Biochem. 271 (2004) 3265–3273.
[46] E. Perales-Clemente, E. Fernandez-Vizarra, R. Acin-Perez, N. Movilla, M.P. Bayona-Bafaluy, R. Moreno-Loshuertos, A. Perez-Martos, P. Fernandez-Silva, J.A. Enriquez, Five entry points of the mitochondrially encoded subunits in mammalian complex I assembly, Mol. Cell. Biol. 30 (2010) 3038–3047.
[47] N. Yadava, P. Potluri, E.N. Smith, A. Bisevac, I.E. Scheffler, Species-specific and mutant MWFE proteins. Their effect on the assembly of a functional mammalian mitochondrial complex I, J. Biol. Chem. 277 (2002) 21221–21230.
[48] N. Yadava, T. Houchens, P. Potluri, I.E. Scheffler, Development and characterization of a conditional mitochondrial complex I assembly system, J. Biol. Chem. 279 (2004) 12406–12413.
[49] M. Gershoni, A. Fuchs, N. Shani, Y. Fridman, M. Corral-Debrinski, A. Aharoni, D. Frishman, D. Mishmar, Coevolution predicts direct interactions between mtDNA-encoded and nDNA-encoded subunits of oxidative phosphorylation complex i, J. Mol. Biol. 404 (2010) 158–171.
[50] R. Szklarczyk, B.F. Wanschers, S.B. Nabuurs, J. Nouws, L.G. Nijtmans, M.A. Huynen, NDUFB7 and NDUFA8 are located at the intermembrane surface of complex I, FEBS Lett. 585 (2011) 737–743.
[51] D. Milenkovic, T. Ramming, J.M. Muller, L.S. Wenz, N. Gebert, A. Schulze-Specking, D. Stojanovski, S. Rospert, A. Chacinska, Identification of the signal directing Tim9 and Tim10 into the intermembrane space of mitochondria, Mol. Biol. Cell 20 (2009) 2530–2539.
[52] T. Clason, T. Ruiz, H. Schagger, G. Peng, V. Zickermann, U. Brandt, H. Michel, M. Radermacher, The structure of eukaryotic and prokaryotic complex I, J. Struct. Biol. 169 (2010) 81–88.
[53] H. Angerer, K. Zwicker, Z. Wumaier, L. Sokolova, H. Heide, M. Steger, S. Kaiser, E. Nubel, B. Brutschy, M. Radermacher, U. Brandt, V. Zickermann, A scaffold of accessory subunits links the peripheral arm and the distal proton pumping module of mitochondrial complex I, Biochem. J. 437 (2011) 279–288.
[54] D.M. Kirby, M. Crawford, M.A. Cleary, H.H. Dahl, X. Dennett, D.R. Thorburn, Respiratory chain complex I deficiency: an underdiagnosed energy generation disorder, Neurology 52 (1999) 1255–1264.
[55] R.H. Triepels, L.P. Van Den Heuvel, J.M. Trijbels, J.A. Smeitink, Respiratory chain complex I deficiency, Am. J. Med. Genet. 106 (2001) 37–45.
[56] D. Skladal, J. Halliday, D.R. Thorburn, Minimum birth prevalence of mitochondrial respiratory chain disorders in children, Brain 126 (2003) 1905–1912.
[57] B.H. Robinson, L. De Meirleir, M. Glerum, G. Sherwood, L. Becker, Clinical presentation of mitochondrial respiratory chain defects in NADH-coenzyme Q reductase and cytochrome oxidase: clues to pathogenesis of Leigh disease, J. Pediatr. 110 (1987) 216–222.
[58] J.L. Loeffen, J.A. Smeitink, J.M. Trijbels, A.J. Janssen, R.H. Triepels, R.C. Sengers, L.P. van den Heuvel, Isolated complex I deficiency in children: clinical, biochemical and genetic aspects, Hum. Mutat. 15 (2000) 123–134.
[59] F. Distelmaier, W.J. Koopman, L.P. van den Heuvel, R.J. Rodenburg, E. Mayatepek, P.H. Willems, J.A. Smeitink, Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease, Brain 132 (2009) 833–842.
[60] M. Schuelke, J. Smeitink, E. Mariman, J. Loeffen, B. Plecko, F. Trijbels, S. Stockler-Ipsiroglu, L. van den Heuvel, Mutant NDUFV1 subunit of mitochondrial complex I causes leukodystrophy and myoclonic epilepsy, Nat. Genet. 21 (1999) 260–261.
[61] P. Benit, R. Beugnot, D. Chretien, I. Giurgea, P. De Lonlay-Debeney, J.P. Issartel, M. Corral-Debrinski, S. Kerscher, P. Rustin, A. Rotig, A. Munnich, Mutant NDUFV2 subunit of mitochondrial complex I causes early onset hypertrophic cardiomyopathy and encephalopathy, Hum. Mutat. 21 (2003) 582–586.
[62] P. Benit, D. Chretien, N. Kadhom, P. de Lonlay-Debeney, V. Cormier-Daire, A. Cabral, S. Peudenier, P. Rustin, A. Munnich, A. Rotig, Large-scale deletion and point mutations of the nuclear NDUFV1 and NDUFS1 genes in mitochondrial complex I deficiency, Am. J. Hum. Genet. 68 (2001) 1344–1352.
[63] S.J. Hoefs, O.H. Skjeldal, R.J. Rodenburg, B. Nedregaard, E.P. van Kaauwen, U. Spiekerkotter, J.C. von Kleist-Retzow, J.A. Smeitink, L.G. Nijtmans, L.P. van den Heuvel, Novel mutations in the NDUFS1 gene cause low residual activities in human complex I deficiencies, Mol. Genet. Metab. 100 (2010) 251–256.
[64] J. Loeffen, O. Elpeleg, J. Smeitink, R. Smeets, S. Stockler-Ipsiroglu, H. Mandel, R. Sengers, F. Trijbels, L. van den Heuvel, Mutations in the complex I NDUFS2 gene of patients with cardiomyopathy and encephalomyopathy, Ann. Neurol. 49 (2001) 195–201.
[65] H.A. Tuppen, V.E. Hogan, L. He, E.L. Blakely, L. Worgan, M. Al-Dosary, G. Saretzki, C.L. Alston, A.A. Morris, M. Clarke, S. Jones, A.M. Devlin, S. Mansour, Z.M. Chrzanowska-Lightowlers, D.R. Thorburn, R. McFarland, R.W. Taylor, The p.M292T NDUFS2 mutation causes complex I-deficient Leigh syndrome in multiple families, Brain 133 (2010) 2952–2963.
[66] P. Benit, A. Slama, F. Cartault, I. Giurgea, D. Chretien, S. Lebon, C. Marsac, A. Munnich, A. Rotig, P. Rustin, Mutant NDUFS3 subunit of mitochondrial complex I causes Leigh syndrome, J. Med. Genet. 41 (2004) 14–17.
[67] R.H. Triepels, L.P. van den Heuvel, J.L. Loeffen, C.A. Buskens, R.J. Smeets, M.E. Rubio Gozalbo, S.M. Budde, E.C. Mariman, F.A. Wijburg, P.G. Barth, J.M. Trijbels, J.A. Smeitink, Leigh syndrome associated with a mutation in the NDUFS7 (PSST) nuclear encoded subunit of complex I, Ann. Neurol. 45 (1999) 787–790.
[68] J. Loeffen, J. Smeitink, R. Triepels, R. Smeets, M. Schuelke, R. Sengers, F. Trijbels, B. Hamel, R. Mullaart, L. van den Heuvel, The first nuclear-encoded complex I mutation in a patient with Leigh syndrome, Am. J. Hum. Genet. 63 (1998) 1598–1608.
[69] V. Procaccio, D.C. Wallace, Late-onset Leigh syndrome in a patient with mitochondrial complex I NDUFS8 mutations, Neurology 62 (2004) 1899–1901.
[70] S. Scacco, V. Petruzzella, S. Budde, R. Vergari, R. Tamborra, D. Panelli, L.P. van den Heuvel, J.A. Smeitink, S. Papa, Pathological mutations of the human NDUFS4 gene of the 18-kDa (AQDQ) subunit of complex I affect the expression of the protein and the assembly and function of the complex, J. Biol. Chem. 278 (2003) 44161–44167.
[71] L. van den Heuvel, W. Ruitenbeek, R. Smeets, Z. Gelman-Kohan, O. Elpeleg, J. Loeffen, F. Trijbels, E. Mariman, D. de Bruijn, J. Smeitink, Demonstration of a new pathogenic mutation in human complex I deficiency: a 5-bp duplication in the nuclear gene encoding the 18-kD (AQDQ) subunit, Am. J. Hum. Genet. 62 (1998) 262–268.
[72] D.M. Kirby, R. Salemi, C. Sugiana, A. Ohtake, L. Parry, K.M. Bell, E.P. Kirk, A. Boneh, R.W. Taylor, H.H. Dahl, M.T. Ryan, D.R. Thorburn, NDUFS6 mutations are a novel cause of lethal neonatal mitochondrial complex I deficiency, J. Clin. Invest. 114 (2004) 837–845.
[73] D. Fernandez-Moreira, C. Ugalde, R. Smeets, R.J. Rodenburg, E. Lopez-Laso, M.L. Ruiz-Falco, P. Briones, M.A. Martin, J.A. Smeitink, J. Arenas, X-linked NDUFA1 gene mutations associated with mitochondrial encephalomyopathy, Ann. Neurol. 61 (2007) 73–83.
[74] S.J. Hoefs, C.E. Dieteren, F. Distelmaier, R.J. Janssen, A. Epplen, H.G. Swarts, M. Forkink, R.J. Rodenburg, L.G. Nijtmans, P.H. Willems, J.A. Smeitink, L.P. van den Heuvel, NDUFA2 complex I mutation leads to Leigh disease, Am. J. Hum. Genet. 82 (2008) 1306–1315.
[75] S.J. Hoefs, F.J. van Spronsen, E.W. Lenssen, L.G. Nijtmans, R.J. Rodenburg, J.A. Smeitink, L.P. van den Heuvel, NDUFA10 mutations cause complex I deficiency in a patient with Leigh disease, Eur. J. Hum. Genet. 19 (2011) 270–274.

[76] I. Berger, E. Hershkovitz, A. Shaag, S. Edvardson, A. Saada, O. Elpeleg, Mitochondrial complex I deficiency caused by a deleterious NDUFA11 mutation, Ann. Neurol. 63 (2008) 405–408.
[77] E. Ostergaard, R.J. Rodenburg, M. van den Brand, L.L. Thomsen, M. Duno, M. Batbayli, F. Wibrand, L. Nijtmans, Respiratory chain complex I deficiency due to NDUFA12 mutations as a new cause of Leigh syndrome, J. Med. Genet. (2011), doi:10.1136/jmg.2011.088856.
[78] C. Ugalde, R.H. Triepels, M.J. Coenen, L.P. van den Heuvel, R. Smeets, J. Uusimaa, P. Briones, J. Campistol, K. Majamaa, J.A. Smeitink, L.G. Nijtmans, Impaired complex I assembly in a Leigh syndrome patient with a novel missense mutation in the ND6 gene, Ann. Neurol. 54 (2003) 665–669.
[79] D.M. Kirby, R. McFarland, A. Ohtake, C. Dunning, M.T. Ryan, C. Wilson, D. Ketteridge, D.M. Turnbull, D.R. Thorburn, R.W. Taylor, Mutations of the mitochondrial ND1 gene as a cause of MELAS, J. Med. Genet. 41 (2004) 784–789.
[80] G. Hofhaus, G. Attardi, Lack of assembly of mitochondrial DNA-encoded subunits of respiratory NADH dehydrogenase and loss of enzyme activity in a human cell mutant lacking the mitochondrial ND4 gene product, EMBO J. 12 (1993) 3043–3048.
[81] C. Ugalde, R. Hinttala, S. Timal, R. Smeets, R.J. Rodenburg, J. Uusimaa, L.P. van Heuvel, L.G. Nijtmans, K. Majamaa, J.A. Smeitink, Mutated ND2 impairs mitochondrial complex I assembly and leads to Leigh syndrome, Mol. Genet. Metab. 90 (2007) 10–14.
[82] R. McFarland, D.M. Kirby, K.J. Fowler, A. Ohtake, M.T. Ryan, D.J. Amor, J.M. Fletcher, J.W. Dixon, F.A. Collins, D.M. Turnbull, R.W. Taylor, D.R. Thorburn, De novo mutations in the mitochondrial ND3 gene as a cause of infantile mitochondrial encephalopathy and complex I deficiency, Ann. Neurol. 55 (2004) 58–64.
[83] D.M. Kirby, A. Boneh, C.W. Chow, A. Ohtake, M.T. Ryan, D. Thyagarajan, D.R. Thorburn, Low mutant load of mitochondrial DNA G13513A mutation can cause Leigh's disease, Ann. Neurol. 54 (2003) 473–478.
[84] C. Ugalde, R.J. Janssen, L.P. van den Heuvel, J.A. Smeitink, L.G. Nijtmans, Differences in assembly or stability of complex I and other mitochondrial OXPHOS complexes in inherited complex I deficiency, Hum. Mol. Genet. 13 (2004) 659–667.
[85] C. Sugiana, D.J. Pagliarini, M. McKenzie, D.M. Kirby, R. Salemi, K.K. Abu-Amero, H.H. Dahl, W.M. Hutchison, K.A. Vascotto, S.M. Smith, R.F. Newbold, J. Christodoulou, S. Calvo, V.K. Mootha, M.T. Ryan, D.R. Thorburn, Mutation of C20orf7 disrupts complex I assembly and causes lethal neonatal mitochondrial disease, Am. J. Hum. Genet. 83 (2008) 468–478.
[86] M. Helm, H. Brule, F. Degoul, C. Cepanec, J.P. Leroux, R. Giege, C. Florentz, The presence of modified nucleotides is required for cloverleaf folding of a human mitochondrial tRNA, Nucleic Acids Res. 26 (1998) 1636–1643.
[87] L. Pintard, J.M. Bujnicki, B. Lapeyre, C. Bonnerot, MRM2 encodes a novel yeast mitochondrial 21S rRNA methyltransferase, EMBO J. 21 (2002) 1139–1147.
[88] S. Carilla-Latorre, M.E. Gallardo, S.J. Annesley, J. Calvo-Garrido, O. Grana, S.L. Accari, P.K. Smith, A. Valencia, R. Garesse, P.R. Fisher, R. Escalante, MidA is a putative methyltransferase that is required for mitochondrial complex I function, J. Cell Sci. 123 (2010) 1674–1683.
[89] J. Carroll, I.M. Fearnley, J.M. Skehel, M.J. Runswick, R.J. Shannon, J. Hirst, J.E. Walker, The post-translational modifications of the nuclear encoded subunits of complex I from bovine heart mitochondria, Mol. Cell. Proteomics 4 (2005) 693–699.
[90] M. Gerards, W. Sluiter, B.J. van den Bosch, L.E. de Wit, C.M. Calis, M. Frentzen, H. Akbari, K. Schoonderwoerd, H.R. Scholte, R.J. Jongbloed, A.T. Hendrickx, I.F. de Coo, H.J. Smeets, Defective complex I assembly due to C20orf7 mutations as a new cause of Leigh syndrome, J. Med. Genet. 47 (2010) 507–512.
[91] A. Saada, S. Edvardson, A. Shaag, W.K. Chung, R. Segel, C. Miller, C. Jalas, O. Elpeleg, Combined OXPHOS complex I and IV defect, due to mutated complex I assembly factor C20ORF7, J. Inherit. Metab. Dis. (2011), doi:10.1007/s10545-011-9348-y.
[92] A. Saada, S. Edvardson, M. Rapoport, A. Shaag, K. Amry, C. Miller, H. Lorberboum-Galski, O. Elpeleg, C6ORF66 is an assembly factor of mitochondrial complex I, Am. J. Hum. Genet. 82 (2008) 32–38.
[93] A. Saada, R.O. Vogel, S.J. Hoefs, M.A. van den Brand, H.J. Wessels, P.H. Willems, H. Venselaar, A. Shaag, F. Barghuti, O. Reish, M. Shohat, M.A. Huynen, J.A. Smeitink, L.P. van den Heuvel, L.G. Nijtmans, Mutations in NDUFAF3 (C3ORF60), encoding an NDUFAF4 (C6ORF66)-interacting complex I assembly protein, cause fatal neonatal mitochondrial disease, Am. J. Hum. Genet. 84 (2009) 718–727.
[94] O. Rackham, S.M. Davies, A.M. Shearwood, K.L. Hamilton, J. Whelan, A. Filipovska, Pentatricopeptide repeat domain protein 1 lowers the levels of mitochondrial leucine tRNAs in cells, Nucleic Acids Res. 37 (2009) 5859–5867.
[95] R.O. Vogel, R.J. Janssen, C. Ugalde, M. Grovenstein, R.J. Huijbens, H.J. Visch, L.P. van den Heuvel, P.H. Willems, M. Zeviani, J.A. Smeitink, L.G. Nijtmans, Human mitochondrial complex I assembly is mediated by NDUFAF1, FEBS J. 272 (2005) 5317–5326.
[96] C.J. Dunning, M. McKenzie, C. Sugiana, M. Lazarou, J. Silke, A. Connelly, J.M. Fletcher, D.M. Kirby, D.R. Thorburn, M.T. Ryan, Human CIA30 is involved in the early assembly of mitochondrial complex I and mutations in its gene cause disease, EMBO J. 26 (2007) 3227–3237.
[97] S. Liu, Y.F. Lee, S. Chou, H. Uno, G. Li, P. Brookes, M.P. Massett, Q. Wu, L.M. Chen, C. Chang, Mice lacking TR4 nuclear receptor develop mitochondrial myopathy with deficiency in complex I, Mol. Endocrinol. 25 (2011) 1301–1310.
[98] C. Xiao, J.H. Shim, M. Kluppel, S.S. Zhang, C. Dong, R.A. Flavell, X.Y. Fu, J.L. Wrana, B.L. Hogan, S. Ghosh, Ecsit is required for Bmp signaling and mesoderm formation during mouse embryogenesis, Genes Dev. 17 (2003) 2933–2949.
[99] E. Kopp, R. Medzhitov, J. Carothers, C. Xiao, I. Douglas, C.A. Janeway, S. Ghosh, ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway, Genes Dev. 13 (1999) 2059–2071.
[100] R.O. Vogel, R.J. Janssen, M.A. van den Brand, C.E. Dieteren, S. Verkaart, W.J. Koopman, P.H. Willems, W. Pluk, L.P. van den Heuvel, J.A. Smeitink, L.G. Nijtmans, Cytosolic signaling protein Ecsit also localizes to mitochondria where it interacts with chaperone NDUFAF1 and functions in complex I assembly, Genes Dev. 21 (2007) 615–624.
[101] J. Zhang, W. Zhang, D. Zou, G. Chen, T. Wan, M. Zhang, X. Cao, Cloning and functional characterization of ACAD-9, a novel member of human acyl-CoA dehydrogenase family, Biochem. Biophys. Res. Commun. 297 (2002) 1033–1042.
[102] J. Nouws, L. Nijtmans, S.M. Houten, M. van den Brand, M. Huynen, H. Venselaar, S. Hoefs, J. Gloerich, J. Kronick, T. Hutchin, P. Willems, R. Rodenburg, R. Wanders, L. van den Heuvel, J. Smeitink, R.O. Vogel, Acyl-CoA dehydrogenase 9 is required for the biogenesis of oxidative phosphorylation complex I, Cell Metab. 12 (2010) 283–294.
[103] T.B. Haack, K. Danhauser, B. Haberberger, J. Hoser, V. Strecker, D. Boehm, G. Uziel, E. Lamantea, F. Invernizzi, J. Poulton, B. Rolinski, A. Iuso, S. Biskup, T. Schmidt, H.W. Mewes, I. Wittig, T. Meitinger, M. Zeviani, H. Prokisch, Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency, Nat. Genet. 42 (2010) 1131–1134.
[104] M. Gerards, B.J. van den Bosch, K. Danhauser, V. Serre, M. van Weeghel, R.J. Wanders, G.A. Nicolaes, W. Sluiter, K. Schoonderwoerd, H.R. Scholte, H. Prokisch, A. Rotig, I.F. de Coo, H.J. Smeets, Riboflavin-responsive oxidative phosphorylation complex I deficiency caused by defective ACAD9: new function for an old gene, Brain 134 (2011) 210–219.
[105] T. Saijo, K. Tanaka, Isoalloxazine ring of FAD is required for the formation of the core in the Hsp60-assisted folding of medium chain acyl-CoA dehydrogenase subunit into the assembly competent conformation in mitochondria, J. Biol. Chem. 270 (1995) 1899–1907.
[106] M. Tsuneoka, K. Teye, N. Arima, M. Soejima, H. Otera, K. Ohashi, Y. Koga, H. Fujita, K. Shirouzu, H. Kimura, Y. Koda, A novel Myc-target gene, mimitin, that is involved in cell proliferation of esophageal squamous cell carcinoma, J. Biol. Chem. 280 (2005) 19977–19985.
[107] I. Ogilvie, N.G. Kennaway, E.A. Shoubridge, A molecular chaperone for mitochondrial complex I assembly is mutated in a progressive encephalopathy, J. Clin. Invest. 115 (2005) 2784–2792.
[108] S.J. Hoefs, C.E. Dieteren, R.J. Rodenburg, K. Naess, H. Bruhn, R. Wibom, E. Wagena, P.H. Willems, J.A. Smeitink, L.G. Nijtmans, L.P. van den Heuvel, Baculovirus complementation restores a novel NDUFAF2 mutation causing complex I deficiency, Hum. Mutat. 30 (2009) E728–E736.
[109] K. Bych, S. Kerscher, D.J. Netz, A.J. Pierik, K. Zwicker, M.A. Huynen, R. Lill, U. Brandt, J. Balk, The iron–sulphur protein Ind1 is required for effective complex I assembly, EMBO J. 27 (2008) 1736–1746.
[110] A.D. Sheftel, O. Stehling, A.J. Pierik, D.J. Netz, S. Kerscher, H.P. Elsasser, I. Wittig, J. Balk, U. Brandt, R. Lill, Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I, Mol. Cell. Biol. 29 (2009) 6059–6073.
[111] S.E. Calvo, E.J. Tucker, A.G. Compton, D.M. Kirby, G. Crawford, N.P. Burtt, M. Rivas, C. Guiducci, D.L. Bruno, O.A. Goldberger, M.C. Redman, E. Wiltshire, C.J. Wilson, D. Altshuler, S.B. Gabriel, M.J. Daly, D.R. Thorburn, V.K. Mootha, High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency, Nat. Genet. 42 (2010) 851–858.
[112] S.A. Susin, H.K. Lorenzo, N. Zamzami, I. Marzo, B.E. Snow, G.M. Brothers, J. Mangion, E. Jacotot, P. Costantini, M. Loeffler, N. Larochette, D.R. Goodlett, R. Aebersold, D.P. Siderovski, J.M. Penninger, G. Kroemer, Molecular characterization of mitochondrial apoptosis-inducing factor, Nature 397 (1999) 441–446.
[113] N. Joza, J.A. Pospisilik, E. Hangen, T. Hanada, N. Modjtahedi, J.M. Penninger, G. Kroemer, AIF: not just an apoptosis-inducing factor, Ann. N. Y. Acad. Sci. 1171 (2009) 2–11.
[114] N. Modjtahedi, F. Giordanetto, F. Madeo, G. Kroemer, Apoptosis-inducing factor: vital and lethal, Trends Cell Biol. 16 (2006) 264–272.
[115] N. Apostolova, A.M. Cervera, V.M. Victor, S. Cadenas, A. Sanjuan-Pla, A. Alvarez-Barrientos, J.V. Esplugues, K.J. McCreath, Loss of apoptosis-inducing factor leads to an increase in reactive oxygen species, and an impairment of respiration that can be reversed by antioxidants, Cell Death Differ. 13 (2006) 354–357.
[116] E.C. Cheung, N. Joza, N.A. Steenaart, K.A. McClellan, M. Neuspiel, S. McNamara, J.G. MacLaurin, P. Rippstein, D.S. Park, G.C. Shore, H.M. McBride, J.M. Penninger, R.S. Slack, Dissociating the dual roles of apoptosis-inducing factor in maintaining mitochondrial structure and apoptosis, EMBO J. 25 (2006) 4061–4073.
[117] A. Urbano, U. Lakshmanan, P.H. Choo, J.C. Kwan, P.Y. Ng, K. Guo, S. Dhakshinamoorthy, A. Porter, AIF suppresses chemical stress-induced apoptosis and maintains the transformed state of tumor cells, EMBO J. 24 (2005) 2815–2826.
[118] N. Vahsen, C. Cande, J.J. Briere, P. Benit, N. Joza, N. Larochette, P.G. Mastroberardino, M.O. Pequignot, N. Casares, V. Lazar, O. Feraud, N. Debili, S. Wissing, S. Engelhardt, F. Madeo, M. Piacentini, J.M. Penninger, H. Schagger, P. Rustin, G. Kroemer, AIF deficiency compromises oxidative phosphorylation, EMBO J. 23 (2004) 4679–4689.
[119] S.J. Chinta, A. Rane, N. Yadava, J.K. Andersen, D.G. Nicholls, B.M. Polster, Reactive oxygen species regulation by AIF- and complex I-depleted brain mitochondria, Free Radic. Biol. Med. 46 (2009) 939–947.
[120] V. El Ghouzzi, Z. Csaba, P. Olivier, B. Lelouvier, L. Schwendimann, P. Dournaud, C. Verney, P. Rustin, P. Gressens, Apoptosis-inducing factor deficiency induces early mitochondrial degeneration in brain followed by progressive multifocal neuropathology, J. Neuropathol. Exp. Neurol. 66 (2007) 838–847.
[121] J.A. Klein, C.M. Longo-Guess, M.P. Rossmann, K.L. Seburn, R.E. Hurd, W.N. Frankel, R.T. Bronson, S.L. Ackerman, The harlequin mouse mutation downregulates apoptosis-inducing factor, Nature 419 (2002) 367–374.
[122] D. Ghezzi, I. Sevrioukova, F. Invernizzi, C. Lamperti, M. Mora, P. D'Adamo, F. Novara, O. Zuffardi, G. Uziel, M. Zeviani, Severe X-linked mitochondrial encephalomyopathy associated with a mutation in apoptosis-inducing factor, Am. J. Hum. Genet. 86 (2010) 639–649.
[123] P. Torija, J.J. Vicente, T.B. Rodrigues, A. Robles, S. Cerdan, L. Sastre, R.M. Calvo, R. Escalante, Functional genomics in Dictyostelium: MidA, a new conserved protein, is required for mitochondrial function and development, J. Cell Sci. 119 (2006) 1154–1164.

[124] D.J. Pagliarini, S.E. Calvo, B. Chang, S.A. Sheth, S.B. Vafai, S.E. Ong, G.A. Walford, C. Sugiana, A. Boneh, W.K. Chen, D.E. Hill, M. Vidal, J.G. Evans, D.R. Thorburn, S.A. Carr, V.K. Mootha, A mitochondrial protein compendium elucidates complex I disease biology, Cell 134 (2008) 112–123.
[125] E. Fassone, A.J. Duncan, J.W. Taanman, A.T. Pagnamenta, M.I. Sadowski, T. Holand, W. Qasim, P. Rutland, S.E. Calvo, V.K. Mootha, M. Bitner-Glindzicz, S. Rahman, FOXRED1, encoding an FAD-dependent oxidoreductase complex-I-specific molecular chaperone, is mutated in infantile-onset mitochondrial encephalopathy, Hum. Mol. Genet. 19 (2010) 4837–4847.
[126] M. McKenzie, M.T. Ryan, Assembly factors of human mitochondrial complex I and their defects in disease, IUBMB Life 62 (2010) 497–502.
[127] H. Swalwell, D.M. Kirby, E.L. Blakely, A. Mitchell, R. Salemi, C. Sugiana, A.G. Compton, E.J. Tucker, B.X. Ke, P.J. Lamont, D.M. Turnbull, R. McFarland, R.W. Taylor, D.R. Thorburn, Respiratory chain complex I deficiency caused by mitochondrial DNA mutations, Eur. J. Hum. Genet. (2011) 1–7.
[128] H. Pagniez-Mammeri, A. Lombes, M. Brivet, H. Ogier-de Baulny, P. Landrieu, A. Legrand, A. Slama, Rapid screening for nuclear genes mutations in isolated respiratory chain complex I defects, Mol. Genet. Metab. 96 (2009) 196–200.
[129] M.L. Valentino, P. Barboni, A. Ghelli, L. Bucchi, C. Rengo, A. Achilli, A. Torroni, A. Lugaresi, R. Lodi, B. Barbiroli, M. Dotti, A. Federico, A. Baruzzi, V. Carelli, The ND1 gene of complex I is a mutational hot spot for Leber's hereditary optic neuropathy, Ann. Neurol. 56 (2004) 631–641.
[130] M. Crimi, A. Papadimitriou, S. Galbiati, P. Palamidou, F. Fortunato, A. Bordoni, U. Papandreou, D. Papadimitriou, G.M. Hadjigeorgiou, E. Drogari, N. Bresolin, G.P. Comi, A new mitochondrial DNA mutation in ND3 gene causing severe Leigh syndrome with early lethality, Pediatr. Res. 55 (2004) 842–846.
[131] E. Sarzi, M.D. Brown, S. Lebon, D. Chretien, A. Munnich, A. Rotig, V. Procaccio, A novel recurrent mitochondrial DNA mutation in ND3 gene is associated with isolated complex I deficiency causing Leigh syndrome and dystonia, Am. J. Med. Genet. A 143 (2007) 33–41.
[132] E. Leshinsky-Silver, D. Lev, G. Malinger, D. Shapira, S. Cohen, T. Lerman-Sagie, A. Saada, Leigh disease presenting in utero due to a novel missense mutation in the mitochondrial DNA-ND3, Mol. Genet. Metab. 100 (2010) 65–70.
[133] M.D. Brown, I.A. Trounce, A.S. Jun, J.C. Allen, D.C. Wallace, Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mitochondrial DNA mutation, J. Biol. Chem. 275 (2000) 39831–39836.
[134] H. Komaki, J. Akanuma, H. Iwata, T. Takahashi, Y. Mashima, I. Nonaka, Y. Goto, A novel mtDNA C11777A mutation in Leigh syndrome, Mitochondrion 2 (2003) 293–304.
[135] M.D. Brown, E. Starikovskaya, O. Derbeneva, S. Hosseini, J.C. Allen, I.E. Mikhailovskaya, R.I. Sukernik, D.C. Wallace, The role of mtDNA background in disease expression: a new primary LHON mutation associated with Western Eurasian haplogroup J, Hum. Genet. 110 (2002) 130–138.
[136] F.M. Santorelli, K. Tanji, R. Kulikova, S. Shanske, L. Vilarinho, A.P. Hays, S. DiMauro, Identification of a novel mutation in the mtDNA ND5 gene associated with MELAS, Biochem. Biophys. Res. Commun. 238 (1997) 326–328.
[137] A.S. Jun, M.D. Brown, D.C. Wallace, A mitochondrial DNA mutation at nucleotide pair 14459 of the NADH dehydrogenase subunit 6 gene associated with maternally inherited Leber hereditary optic neuropathy and dystonia, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 6206–6210.
[138] D.M. Kirby, S.G. Kahler, M.L. Freckmann, D. Reddihough, D.R. Thorburn, Leigh disease caused by the mitochondrial DNA G14459A mutation in unrelated families, Ann. Neurol. 48 (2000) 102–104.
[139] P. Potluri, A. Davila, E. Ruiz-Pesini, D. Mishmar, S. O'Hearn, S. Hancock, M. Simon, I.E. Scheffler, D.C. Wallace, V. Procaccio, A novel NDUFA1 mutation leads to a progressive mitochondrial complex I-specific neurodegenerative disease, Mol. Genet. Metab. 96 (2009) 189–195.
[140] J.A. Mayr, O. Bodamer, T.B. Haack, F.A. Zimmermann, F. Madignier, H. Prokisch, C. Rauscher, J. Koch, W. Sperl, Heterozygous mutation in the X chromosomal NDUFA1 gene in a girl with complex I deficiency, Mol. Genet. Metab. 103 (2011) 358–361.
[141] M.A. Martin, A. Blazquez, L.G. Gutierrez-Solana, D. Fernandez-Moreira, P. Briones, A.L. Andreu, R. Garesse, Y. Campos, J. Arenas, Leigh syndrome associated with mitochondrial complex I deficiency due to a novel mutation in the NDUFS1 gene, Arch. Neurol. 62 (2005) 659–661.
[142] M. Ferreira, A. Torraco, T. Rizza, F. Fattori, M.C. Meschini, C. Castana, N.E. Go, F.E. Nargang, M. Duarte, F. Piemonte, C. Dionisi-Vici, A. Videira, L. Vilarinho, F.M. Santorelli, R. Carrozzo, E. Bertini, Progressive cavitating leukoencephalopathy associated with respiratory chain complex I deficiency and a novel mutation in NDUFS1, Neurogenetics 12 (2011) 9–17.
[143] R. Spiegel, A. Shaag, H. Mandel, D. Reich, M. Penyakov, Y. Hujeirat, A. Saada, O. Elpeleg, S.A. Shalev, Mutated NDUFS6 is the cause of fatal neonatal lactic acidemia in Caucasus Jews, Eur. J. Hum. Genet. 17 (2009) 1200–1203.
[144] S. Lebon, D. Rodriguez, D. Bridoux, A. Zerrad, A. Rotig, A. Munnich, A. Legrand, A. Slama, A novel mutation in the human complex I NDUFS7 subunit associated with Leigh syndrome, Mol. Genet. Metab. 90 (2007) 379–382.
[145] F. Barghuti, K. Elian, J.M. Gomori, A. Shaag, S. Edvardson, A. Saada, O. Elpeleg, The unique neuroradiology of complex I deficiency due to NDUFA12L defect, Mol. Genet. Metab. 94 (2008) 78–82.