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Minireview

Mitochondrial complex I deficiency of nuclear origin II. Non-structural genes

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ABSTRACT

Complex I deficiency is the most frequent cause of respiratory chain diseases. This large multiprotein complex is composed in human of 45 structural subunits, of which 7 are mitochondrial-encoded and 38 are nuclear-encoded. Most of the pathological mutations responsible for complex I deficiencies have been identified to date in complex I structural subunits. Numerous studies from last decade gave some insight into the biogenesis of this huge multi subunit complex of double genetic origin. A sequential incorporation of the structural subunits as well as ten complex I assembly factors has been described. Here, we present a short overview of the human complex I biogenesis and we review the pathological mutations identified to date in eight of the ten known complex I assembly factors.

Contents

Abbreviations: CI, complex I; OXPHOS, oxidative phosphorylation.

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1. Introduction

Complex I (CI), or NADH:ubiquinone oxidoreductase (EC 1.6.5.3), the largest of the five OXPHOS complexes, catalyzes the transfer of electrons from NADH to ubiquinone coupled to the generation of a proton gradient across the mitochondrial membrane which is consumed by ATP synthase to produce ATP. The mitochondrial complex I is composed in mammals of 45 structural subunits [1], while the prokaryotic enzyme consists of only 14 subunits [2,3]. The conserved core subunits of complex I are encoded by nuclear or mitochondrial DNA and they are organized in 3 functional modules (**N**, for NADH, H+ oxidation; **Q**, for ubiquinone reduction; **P**, for proton translocation) [4]. In addition to the core subunits, eukaryotic CI comprises up to 32 additional subunits known as supernumerary or "accessory" subunits which presumably do not participate in the enzymatic activity of CI [5]. The CI subunits are organized in "L" shaped structure, with two arms of similar length, a membrane arm being embedded in the mitochondrial membrane and a peripheral arm protruding in the mitochondrial matrix.

The large size and the double genetic origin of eukaryotic complex I make our knowledge of its biogenesis rather poor. However, numerous studies from last decade gave some insight into this process. The CI assembly was first described in the fungus *Neurospora crassa* in which a separate formation of the membrane and peripheral arms was observed [5]. On the other hand, studies performed on patients' cells with identified mutations in structural subunits or assembly chaperones helped to better understand the biogenesis of human CI [6].

Given the number of genes implicated in the biogenesis and function of this multi-subunit complex, it is not surprising that CI defects represent a large portion of OXPHOS deficiencies (up to 40%) [7,8]. Indeed, beside numerous pathogenic mutations found in structural subunits of mitochondrial complex I, isolated/predominant CI deficiencies are also related to mutations in a growing number of nuclear genes possibly involved in its biogenesis or stability.

In this review, we intend to summarize our knowledge on complex I biogenesis and report on the pathogenic mutations affecting its assembly related to isolated CI deficiencies.

2. Human complex I assembly: the state of the art

2.1. CI biogenesis

Direct evidence of the stepwise assembly of CI was provided by the use of inducible NDUFS3-green fluorescent protein (GFP) expression system in HEK293 cells [9]. The study showed that the labeled NDUFS3 was gradually incorporated into 6 subcomplexes assembled into holoenzyme. In addition to *de novo* CI synthesis, regeneration of existing complexes by subunit exchange was proposed as a complementary and efficient mechanism ensuring complex I biogenesis [12]. Analysis of mitochondrial-encoded ND4, ND5 and ND6 subunit mutants arrested in CI assembly, confirmed the presence of assembly intermediates containing mtDNA-encoded subunits highlighted by Lazarou et al. [12] and allowed the authors to propose the five different entry points by which the mitochondrial-encoded subunits are sequentially incorporated into the complex [10]. Moreover, by measuring the distribution of matrix-soluble and inner membrane-bound CI subunits expressing AcGFP1-tag, mobile (matrix-soluble) and/or immobile (membrane-bound) subassemblies could be detected in living cells [11].

2.2. Import and assembly of CI subunits

The NDUFS3 subunit is incorporated at an early stage of the assembly process whereas the NDUFV1, NDUFV2, NDUFA2, and NDUFA12 subunits are incorporated at a late stage during the assembly of CI. Consistent with the presence of a transmembrane domain in NDUFB6, this latter subunit was found either immobile or in one of the more slowly diffusing membrane-bound subassembly complexes [11]. Vogel and co-authors showed that CI subunits are gradually incorporated into 6 different subcomplexes. Authors demonstrated that mitochondrial DNA-encoded subunits are required for the formation of the subcomplexes 4 to 6 and of the fully assembled CI (Fig. 1) [9]. The sequential incorporation of the mtDNA-encoded subunits into CI, previously demonstrated [12], was further elucidated with identification of 5 different entry points. Perales and co-workers showed that ND1 and ND2 belong to 2 different assembly intermediates that evolve differently. They defined the first entry point for ND1 in a ~400 kDa intermediate anchored to the inner mitochondrial membrane and formed by peripheral arm subunits NDUFS3, NDUFS2, NDUFS7 and NDUFS8. The second entry point was defined for ND2, ND3 and ND4L composing a ~460 kDa membrane subcomplex that joins the ND1-containing subassembly with addition of ND4 in a third entry point. The fourth and the fifth entry points were defined for ND6 and ND5, respectively, with their successive incorporation into the subsequent CI subassemblies [10].

Despite some unresolved questions concerning CI biogenesis a generally accepted model of its assembly pathway could be proposed. According to this model, an early subassembly of the Q module, composed of the peripheral nuclear-encoded NDUFS2, NDUFS3, NDUFS7 and NDUFS8 subunits, is first anchored to the membrane by the mitochondrial-encoded subunits constituting the P module. The Q and P modules expand then by gradual addition of other nuclear-encoded subunits of both peripheral and membrane arms. Finally, insertion of the N module, composed of the nuclear-encoded NDUFV1, NDUFV2 and NDUFS1 subunits, completes the assembly process (Fig. 1) [6].

2.3. Protein chaperones in the CI assembly

As CI is composed of 45 structural subunits of dual genetic origin, its assembly is likely to entail many factors involved in subunit maturation, insertion, co-factor attachment or chaperoning of assembly intermediates [6]. However, while 16 assembly factors have already been identified for human complex IV (cytochrome *c* oxidase; 13 structural subunits) [13], only 10 CI assembly factors have been found so far: NDUFAF1 (human CIA30), Ecsit, ACAD9, NDUFAF2 (B17.2L), NDUFAF4 (C6ORF66), NDUFAF3 (C3ORF60), C8ORF38, C20ORF7, AIF and human Ind1 (huInd1).

2.3.1. Chaperones with identified roles in CI assembly

NDUFAF1 was first identified by Vogel and co-workers [14] as being the human homologue of a CI assembly factor found in the fungus *N. crassa*, CIA30 [15]. NDUFAF1 in human mitochondria is involved in the initial assembly of the ND2-containing ~460 kDa membrane subcomplex [10] and is present in two high molecular weight CI assembly intermediates [14]. However 500–850 kDA subcomplexes containing NDUFAF1 could be also detected in cells deficient in mitochondrial translation and thus devoid of CI assembly intermediates which suggests that NDUFAF1 may bind to other protein complexes [16]. It is therefore possible that NDUFAF1 participates in CI assembly indirectly by affecting mitochondrial functions such as import, processing or translation [16]. NDUFAF1 was subsequently found in the complex with an assembly chaperone Ecsit [17], which in turn interacts with Acyl-CoA dehydrogenase 9 (ACAD9), a member of the acyl-CoA dehydrogenase family similar to very long-chain acyl-CoA dehydrogenase (VLCAD) specifically required for the assembly of CI [18]. Using whole genome subtraction of yeasts with and without a complex I, Ogilvie and colleagues identified a new assembly factor *NDUFAF2*, a paralogue of B17.2, one of the mammalian CI structural subunit (also known as DAP13 or NDUFA12) [19]. *NDUFAF2*, also named *B17.2L* (*B17.2* like) or *NDUFA12L* (*NDUFA12* like), arose as an ancient duplication of the gene coding