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Unraveling the complexity of mitochondrial complex I assembly: A dynamic process☆

Laura Sánchez-Caballero [1], Sergio Guerrero-Castillo [1], Leo Nijtmans

Radboud Center for Mitochondrial Medicine, Department of Pediatrics, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands

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ABSTRACT

Mammalian complex I is composed of 44 different subunits and its assembly requires at least 13 specific assembly factors. Proper function of the mitochondrial respiratory chain enzyme is of crucial importance for cell survival due to its major participation in energy production and cell signaling. Complex I assembly depends on the coordination of several crucial processes that need to be tightly interconnected and orchestrated by a number of assembly factors. The understanding of complex I assembly evolved from simple sequential concept to the more sophisticated modular assembly model describing a convoluted process. According to this model, the different modules assemble independently and associate afterwards with each other to form the final enzyme. In this review, we aim to unravel the complexity of complex I assembly and provide the latest insights in this fundamental and fascinating process. This article is part of a Special Issue entitled Respiratory complex I, edited by Volker Zickermann and Ulrich Brandt.

1. Introduction

One of the largest macromolecular complexes is mitochondrial complex I [1,2] which plays an important role in the cellular energy production. Complex I is involved in respiratory electron transport and contributes to the generation of the proton motive force needed for ATP synthesis. In addition complex I produces reactive oxygen species (ROS), which can be detrimental, but is also of importance for cell signaling [3]. Defects in this enzyme cause a severe disturbance of energy metabolism and often lead to severe inherited metabolic disorders such as Leigh Syndrome [4] (MIM #256000).

Mammalian complex I is composed of 44 different subunits [5,6] and the assembly of the holocomplex requires the involvement of at least 13 established assembly factors. From the 44 subunits, 37 are encoded in the nuclear DNA and seven are encoded in the mitochondrial DNA. Out of these, the seven mitochondrially encoded and seven nuclear-encoded subunits are called central subunits, because they are also constituents of bacterial complex I and execute the core bioenergetic functions. The remaining 30 proteins are called accessory or supernumerary subunits. To comprehend the mechanisms involved, it is of pivotal importance to understand how these macromolecular complexes are assembled and how this assembly is regulated. Assembly factors play a role in the admittance of mitochondrial and nuclear encoded subunits or have been linked to the biogenesis of cofactors such as iron–sulfur clusters to form the functional enzyme.

Complex I is an L-shaped molecule with a peripheral arm, protruding into the matrix, and a membrane arm, residing in the inner membrane [7,8]. The peripheral arm contains two functional blocks, the N (NADH binding) and the Q (ubiquinone binding) modules, whereas the membrane arm consists of the P (proton pumping) module [1].

Mechanistically, complex I assembly covers several processes that need to be tightly regulated and interconnected to ensure a mature and functional complex. The process starts with the mitochondrial and nuclear transcription of complex I subunits and assembly factors, continues with mitochondrial and cytosolic translation, import of nuclear-encoded proteins, insertion of cofactors, and proceeds until the assembled complex is completely inserted into the inner membrane. Many of these processes, ranging from transcription to mitochondrial import are common with other complexes of the oxidative phosphorylation system [9]. Others, like addition of cofactors and insertion into the membrane are specific for every complex and involve dedicated assembly factors [10,11].

In this review, we will give some insights about how proteins of complex I, encoded in the nucleus and in the mitochondria, reach their position in this gigantic enzyme and describe the steps of the assembly pathway taking into account all the players described up to now. We will place particular emphasis on human complex I assembly.

2. Mitochondrial import of nucleus-encoded subunits

During evolution of eukaryotes, seven of the 'ancestral' complex I genes were transferred from the mitochondrial DNA to the nuclear DNA [12]. Additionally, the products of another 30 nuclear-encoded genes, which originated in eukaryotes, contribute to the fully assembled

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[1] Contributed equally.

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Table 1
Assembly factors involved in complex I assembly.
Description of 13 assembly factors with a pivotal role in complex I assembly.

Gene name[a]	*Other gene names*	*Protein names*	*M_r (kDa)*	*Putative role in complex I assembly*	*Module*	*Trans-membrane domains*	*Isoform*[b]	*MTS*[c]	*Proven as disease causing*	*Identification*
NUBPL	*C14orf127*	IND1, hInd1, Nucleotide-binding protein-like	24,4	Insertion of iron-sulfur clusters [86,105] in N and Q module subunits		0	2	Yes [86,105]	Yes [56–58,106]	Homology [86,105,106]
NDUFAF2	NDUFA12L	B17.2 L, Mimitin, mitochondrial, MMTN, NDUFA12-like protein	19,8	Binding of N module	N-Q interface	0		Yes	Yes [57,59,107]	Bioinformatics/exome sequencing [59]
NDUFAF3	C3orf60	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 3	20,3	Binding of Q with Ppa	Q	0	2	Yes [50]	Yes [50]	Homology/exome sequencing [50]
NDUFAF4	C6orf66, HRPAP20, HSPC125, My013	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 4	20,3	Binding of Q with Ppa	Q	0		Yes	Yes [50,60]	Exome sequencing [60]
TIMMDC1	C3orf1, UNQ247/PRO284	Protein M5–14, TIMM domain containing-protein 1	32,2	Insertion of ND1	P_P-a	4 [54]				Protein-protein interaction [54]
NDUFAF1	CIA30, CGI-65	Complex I intermediate-associated protein 30, mitochondrial, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex assembly factor 1	37,8	Insertion of ND2	P_P-b	0		Yes [53]	Yes [108–111]	Homology [53,81]
ECSIT		Evolutionarily conserved signaling intermediate in Toll pathway, mitochondrial, Protein SITPEC	49,1	Insertion of ND2	P_P-b	0	4	Yes [64]		Protein-protein interaction [64]
ACAD9		Acyl-CoA dehydrogenase family member 9, mitochondrial	68,8	Insertion of ND2	P_P-b	0		Yes	Yes [65,112–117]	Protein-protein interaction [65]
TMEM126B	HT007	Complex I assembly factor TMEM126B, mitochondrial, Transmembrane protein 126B	25,9		P_P-b	4	5 [54]			Co-migration [67]
FOXRED1	FP634	FAD-dependent oxidoreductase domain-containing protein 1	53,8		P_D	0 [77]	3		Yes [57,61]	Exome sequencing/bioinformatics [57,118]
NDUFAF5	C20orf7	NADH dehydrogenase (ubiquinone) complex I, assembly factor 5	36,1	Methyltransferase activity [62]		0	2 [63]	Yes	Yes [62,63,119]	Exome sequencing [62]
NDUFAF6	C8orf38	NADH dehydrogenase (ubiquinone) complex I, assembly factor 6	38,2	Squalene/phytoene synthetase activity [55]		0	3	Yes	Yes [55]	LC–MS/MS, exome sequencing [55]
NDUFAF7	C2orf56, PRO1853	NADH dehydrogenase [ubiquinone] complex I, assembly factor 7, Protein midA homolog, mitochondrial	49,2	Methyltransferase activity [66]		0		Yes		Co-precipitation [66]

[a] Primary gene name.
[b] Described in Uniprot.
[c] Predicted by MitoprotII, TargetP, and Predotar.