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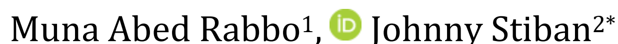
NUBPL: a mitochondrial Complex I deficiency disorder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Summary

Mitochondrial ailments are diverse and devastating. Defects in mitochondrial DNA or its products lead to a wide range of deficiencies in the mitochondrial electron transfer system and its ensuing energy transformation. Accessory proteins required for the assembly and function of the respiratory complexes are also required for healthy, coupled, and energy-transforming mitochondria. Recently, the protein nucleotide-binding protein-like (NUBPL or IND1) was identified as an iron-sulfur cluster transfer protein specifically for Complex I. Since the presence of multiple iron-sulfur clusters in Complex I is necessary for its activity, deficiency in NUBPL leads to severely dysfunctional mitochondria, with upregulated compensatory Complex II activity. Here we present a short review of the debilitating disease related to NUBPL deficiency.

Keywords - Complex I; NUBPL; IND1; iron-sulfur clusters; mtDNA helicase; bioenergetics

1. Definition

In humans, as in most other organisms, the oxidative phosphorylation (OXPHOS) process involves multimeric complexes embedded in the mitochondrial inner membrane (mtIM). Complexes CI, CIII, and CIV are proton pumps which create an electrochemical potential difference across the mtIM, namely the proton motive force, driving the synthesis of ATP via ATP synthase (Ohnishi et al 2018; Tang et al 2020).

NADH:ubiquinone oxidoreductase (CI) is the largest component of the OXPHOS system. It comprises 45 protein subunits encoded by nuclear and mitochondrial genes. Along with the apoprotein subunits, CI contains stably bound prosthetic groups, including flavin mononucleotide and eight to ten iron-sulfur (Fe-S) clusters (Ohnishi et al 2018).

Thus, even in the minimalistic CI from *E. coli*, the proper assembly of this composite protein is an essential factor determining its function (Schimpf et al 2022). The assembly of CI is governed by a set of assembly factors responsible for the insertion of the prosthetic groups into either the monomeric forms of the complex subunits or the fully assembled apoenzyme (Sheftel et al 2009).

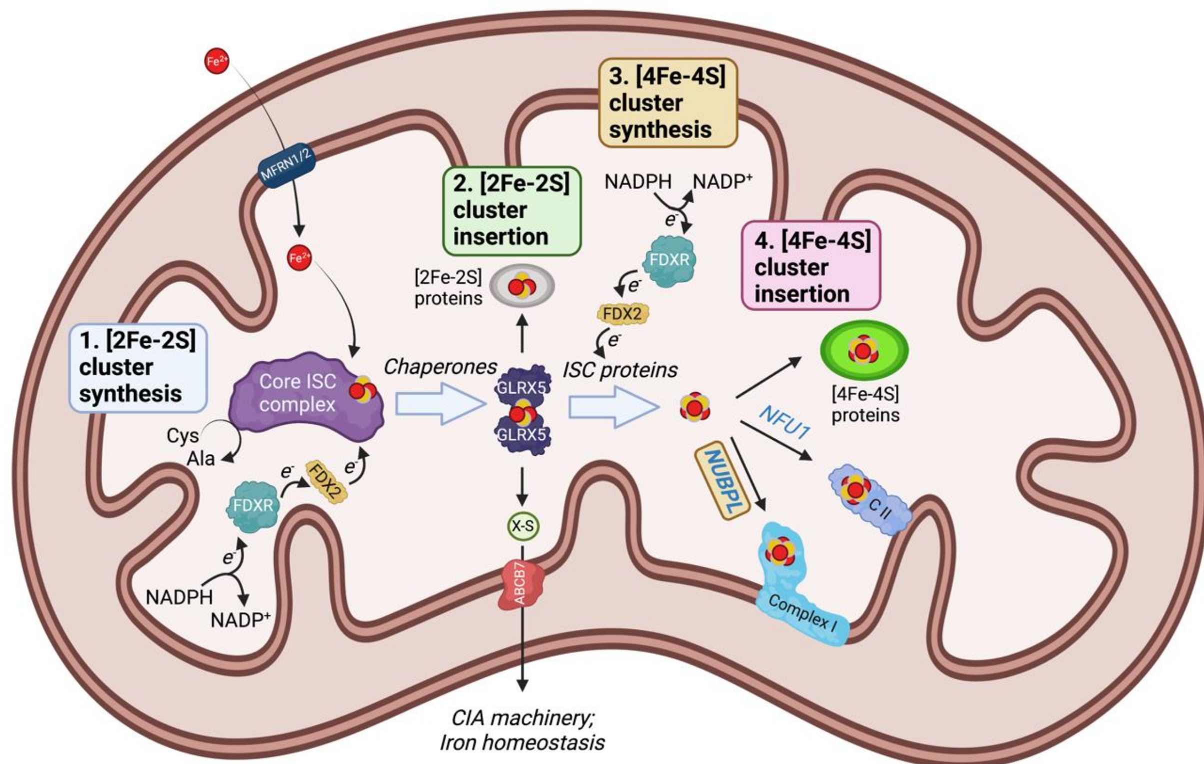

Figure 1. Fe-S cluster synthesis in the mammalian mitochondrion. Iron-sulfur cluster biosynthesis pathway (ISC) occurs in the matrix and can be subdivided into 4 steps. **(*1*)** *De novo* biogenesis of [2Fe-2S] cluster: a core complex composed of 6 proteins (the cysteine desulfurase complex comprising NFS1-ISD11-ACP1, frataxin (FXN), ferredoxin (FDX2) and ISCU2) catalyzes the extraction of sulfur from cysteine and its incorporation into a [2Fe-2S] cluster after a series of reactions. Ferrous iron enters into the matrix through mitoferrin (MFRN1/2) carrier proteins in the mtIM. The required electrons are supplied from NADPH through an electron transfer system requiring ferredoxin reductase (FDXR) and FDX2. **(*2*)** Insertion of [2Fe-2S] cluster into proteins: Using multiple chaperones the [2Fe-2S] cluster is transferred from ISCU2 to monothiol glutaredoxin (GLRX5). GLRX5 transfers the cluster to mitochondrial [2Fe-2S] proteins. Alternatively, GLRX5 has a role in the synthesis of sulfur-containing factor (X-S) which is exported from the mitochondrion via ABCB1 transporter to be used in the cytosolic iron-sulfur protein assembly (CIA). **(*3*)** Biosynthesis of [4Fe-4S] cluster: GLRX5 transfers the [2Fe-2S] cluster to downstream ISC proteins which builds the [4Fe-4S] cluster using the NADPH electron transferring system. **(*4*)** Insertion of [4Fe-4S] clusters into proteins: Dedicated chaperons transfer the nascent [4Fe-4S] clusters to their target proteins. NUBPL transfers [4Fe-4S] clusters to CI whereas NFU1 transfers them to CII. Other chaperons are needed to transfer these clusters to other mitochondrial [4Fe-4S] proteins. Figure adapted from Lill and Freibert (2020) and created with BioRender.com.

The Fe-S cofactors are ancient accessory parts of proteins that serve in a wide range of functions, from OXPHOS to cellular and nucleic acid metabolism (Stiban et al 2016; Khodour et al 2019). Despite the chemical spontaneity of Fe-S cluster assembly, the biogenesis and maturation of Fe-S-cluster-containing proteins in mammals is an elaborate enzymatic process (Lill, Freibert 2020) that can be divided into the mitochondrial Fe-S cluster assembly (ISC) (Figure 1) and the cytosolic Fe-S cluster assembly (CIA). Briefly, the ISC process begins in the mitochondrial matrix with the formation of sulfide from desulfurized cysteine by a cysteine desulfurase complex NFS1-ISD11-ACP1 (Sheftel et al 2009; Lill, Freibert 2020). It is then combined with ferrous ions and electrons from NADPH — through a system involving ferredoxin reductase (FDXR) and ferredoxin (FDX2) — at the scaffold protein ISCU1, which is a part of the core ISC complex. Multiple chaperons transfer the nascent cluster to monothiol glutaredoxin (GLRX5), which eventually transfers the cluster to an apoprotein (Sheftel et al 2009). The mitochondrial scaffold can then be exported by an Fe-S cluster export machinery to construct cytosolic and nuclear Fe-S proteins via the CIA machinery. Two cytosolic NTPase proteins, Nbp35 and Cfd1, which function as scaffolds for Fe-S cluster assembly, resume the biogenesis of Fe-S-containing proteins in the cytosol (Protasoni et al 2020). In the mitochondria, further downstream ISC proteins can generate [4Fe-4S] clusters using the synthesized [2Fe-2S] cluster and electrons from the NADPH electron transfer system. The nascent [4Fe-4S] cluster then is transferred by specific chaperones to its target proteins within the mitochondria.

Interestingly, in 2008, a mitochondrial protein with sequence similarity to the cytosolic NTPases was identified and initially termed IND1 for an Fe-S protein required for NADH dehydrogenase. This protein harbors a conserved C-terminal CXXC motif that is thought to be the site where Fe-S clusters bind during the biogenesis of Fe-S-cluster-containing proteins of respiratory CI (Bych et al 2008). Therefore, a defect in IND1, now known as nucleotide-binding protein-like (NUBPL), is considered a subclass of primary mitochondrial diseases that cause mitochondrial CI deficiency, nuclear type 21; MC1DN21(Kimonis et al 2021). As an Fe-S transfer protein, NUBPL has only one CXXC motif and, therefore, is likely to be a dimer with the 2Fe-2S cluster bridging the two monomers (Figure 2). It is worth noting that the *Drosophila* homolog of the mammalian NUBPL was recently found to alleviate mitochondrial DNA (mtDNA) replication stalling in a similar fashion to overexpression of mtDNA helicase, indicating a possibility of interaction between the helicase and NUBPL (So et al 2021). This finding in fruit flies may have implications as NUBPL may transfer an Fe-S cluster to the N-terminal domain of mtDNA helicase (Stiban et al 2014) initiating its activity. How this relates to the human mtDNA helicase which lacks an Fe-S cluster is currently under investigation.

2. Etiology

Sheftel et al (2009) reported that RNA-interference-mediated-NUBPL/IND1 knockdown in HeLa cells resulted in mitochondrial ultrastructural changes. These changes included massive remodeling of the respiratory supercomplexes, loss of cristae membranes, and increased lactate production, likely due to a higher dependence on fermentation in the absence of proper function of the electron transfer system (ETS). They also revealed that NUBPL/IND1 depletion resulted in significant remodeling of the membrane arm of respiratory CI, as an abnormal subcomplex – comprising parts of the original membrane complex – appears upon NUBPL/IND1 absence (Sheftel et al 2009).

Calvo et al (2010) provided the first clinical genetic evidence by reporting a compound heterozygous patient with two different mutations in the *NUBPL* gene. The first allele, inherited from the patient's father, consisted of c.166G>A/p.G56R and another splice site mutation (c.815-27T>C). The former resulted in a missense mutation in a glycine conserved across 36 vertebrate species, whereas the latter caused skipping of exon 10, and thus aberrant splice products. On the other hand, the maternal allele had a complex rearrangement comprising of exon 7 duplication along with a deletion in exons 1-4. As a result, this patient suffered developmental delay, leukodystrophy, and elevated cerebrospinal fluid lactate due to CI deficiency (Calvo et al 2010).

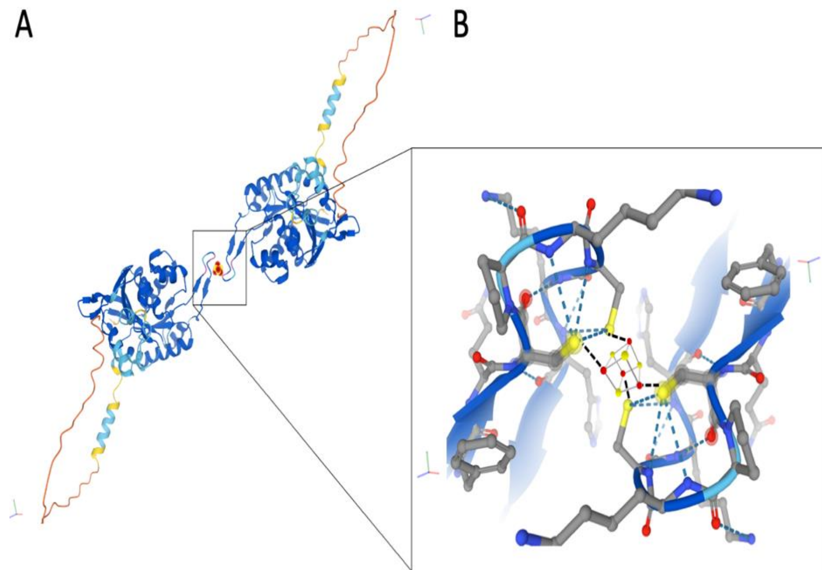

Figure 2. Possible structure of a dimeric human NUBPL (Q8TB37). The full protein structure **(A)** was modeled using AlphaFold protein structure database (Jumper et al 2021; Varadi et al 2022). The presence of a 4Fe-4S cluster is modeled in the midst of the two monomers. A zoomed in structure showing the interacting CXXC motifs from both monomers and the interacting Fe-S structure is shown in **(B)**.

Disease	Gene Defect	Other Names
NUBPL	NUBPL/IND1	Mitochondrial Complex I Deficiency, nuclear type 21; MC1DN21
OMIM: 618242	OMIM: 613621	Nucleotide-binding protein-like gene (NUBPL) Deficiency
		Iron-sulfur protein required for NADH dehydrogenase (IND1) Deficiency

Figure 3. NUBPL disease card. Online Mendelian Inheritance in Man (OMIM) entries of NUBPL deficiency along with deficient genes and other names of the disease (obtained from https://www.omim.org/entry/613621?search=nubpl&highlight=nubpl)

Kevelam et al (2013) identified six unrelated patients with Complex I deficiency. All patients shared at least one copy of the previously identified haplotype G56R/c.815-27T-C in the *NUBPL* gene. Consequently, all patients had a characteristic leukoencephalopathic MRI pattern, including white matter lesions, abnormally swelled corpus callosum, motor ataxia, and other cortical and brainstem abnormalities (Kevelam et al 2013). Wydro and Balk (2913) investigated the impact of the c.815-27T>C mutation on CI expression and function. They concluded that this mutation could introduce changes in CI, eventually

leading to protein misfolding and instability. Thus, a disease was associated with NUBPL deficiency (Figure 3).

Other cases of compound heterozygosity were identified by Friederich et al (2020) who reported a novel missense M117I mutation in the *NUBPL* gene. According to Kimonis et al (2021) cerebellar dysfunction is prevalent in patients carrying splice-site mutation c.815-27T>C alone or as part of the c.815-27T-C/G46R haplotype and is absent in patients carrying other NUBPL mutations.

To conclude, CI deficiency is inherited in an autosomal recessive pattern of mutations/pathogenic haplotypes in the *NUBPL* gene mapped to chromosome 14q12, leading to a range of neurodegenerative manifestations.

3. Clinical Manifestations

Like most diseases related to OXPHOS malfunction, NUBPL manifests in several systems with different severities (Table 1). In the brain, genetic deficiency of NUBPL is associated with neurological symptoms and some forms of Parkinson's disease (Kimonis et al 2021). Interestingly, the use of chemical agents, such as pesticides, has been implicated in CI deficiency and enhanced susceptibility to Parkinson's (Richardson et al 2019). Pesticides such as rotenone and 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine are strong CI inhibitors and have been used to generate chronic Parkinson's disease mouse models (Zhang et al 2022). It is unclear whether clinical manifestations of genetic CI deficiency differ from chemically induced inhibition of CI. Nevertheless, since the outcome of both is a severely reduced ETS activity and reactive oxygen species generation, it is not farfetched that the manifestations would not be very different.

Table 1. Clinical manifestations of NUBPL deficiency (El-Hattab, Scaglia 2016; Kimonis et al 2021)

Systems	Major (signs/symptoms)	Minor (signs/symptoms)
brain	onset of neurological symptoms at 3-18 months global developmental delay cerebellar dysfunction (including ataxia, dysarthria, nystagmus, and tremor) some forms of Parkinson's disease seizures leukoencephalopathy macrocephaly sensorineural deafness	-
heart	xardiomyopathy	-
muscle	spasticity muscle atrophy	hypotonia
liver	hepatic dysfunction	-
plasma	-	lactic acidosis hypoglycemia

4. Diagnosis

A plethora of rare childhood leukoencephalopathies renders the specific diagnosis extremely challenging. Elevated lactate levels in the plasma and cerebrospinal fluid are the first diagnostic markers for identifying mitochondrial leukoencephalopathies. This is usually followed by OXPHOS analysis of the patient's muscle biopsy. These diagnostic tests are shared among multiple mitochondrial leukoencephalopathies. Consequently, for a specific diagnosis of NUBPL, a DNA analysis (e.g. whole-exome sequencing) guided by the previous results usually follows. An MRI pattern analysis can also support this molecular diagnostic test since patients with NUBPL variants possess a unique combination of T2-hyperintense signal of the cerebellar cortex bilaterally and supratentorial white matter abnormalities (Roosendaal et al 2021). Moreover, there is a distinct MRI progressive pattern among NUBPL patients carrying the splice-site mutation. This pattern begins with cerebellar, deep white matter, and corpus callosum abnormalities at the early stages. At later stages, the white matter and corpus callosum abnormalities improve. In contrast, new brainstem abnormalities develop (Kevelam et al 2013). Recently, in five new patients, brain MRI showed cerebellar atrophy (Kimonis et al 2021).

5. Management

Despite the lack of management and treatment approaches, a pre-clinical investigation of candidate therapeutic strategies for NUBPL disease is currently being conducted at The Children's Hospital of Philadelphia. To achieve this goal, researchers are developing three evolutionarily distinct NUBPL knockout models to help in the optimization of the therapeutic regimen (Mathew 2022).

6. Conclusion and future directions

A debilitating rare disease that affects a miniscule percentage of the world’s population is challenging on several fronts. There is limited basic research on this topic and hence treatment options are lacking. Nevertheless, several questions pertaining to this disease can be articulated and merit scientific investigation. (*1*) What is the bona fide function of NUBPL/IND1? (*2*) What is the structure of NUBPL/IND1? (*3*) What are the molecular bioenergetics ramifications of NUBPL/IND1 deficiency — especially with enhanced CII function? (*4*) How is NUBPL/IND1 related to CI, and does it actually transfer some or all Fe-S clusters to it? (*5*) Does human NUBPL/IND1 interfere positively or negatively with mtDNA helicase? (*6*) What is the mechanism of Fe-S transfer from NUBPL/IND1 to C I? (*7*) Is the protein actually a dimer and if so, how stable is the Fe-S in the dimer? (*8*) Are there any drugs that alleviate the symptoms of NUBPL disease? Answering these general questions will certainly move along this field and provide some hope for the known patients who have this disease (Ohnishi et al 2018).

Abbreviations

CIA	cytosolic iron-sulfur assembly	GLXR5	monothiol glutaredoxin
CI	Complex I, NADH:ubiquinone oxidoreductase	ISC	mitochondrial iron-sulfur cluster assembly
C II	Complex II, succinate dehydrogenase	MFRN1/2	mitoferrin 1 or 2
ETS	electron transfer system	mtIM	mitochondrial inner membrane
FDX2	ferredoxin	mtDNA	mitochondrial DNA
FDXR	ferredoxin reductase	NUBPL	nucleotide-binding protein-like
Fe-S	iron-sulfur	OXPHOS	oxidative phosphorylation
FXN	Frataxin		

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