

# Mitochondrial Complex I Deficiency in Humans

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**Abstract:** The mechanism of NADH oxidation varies between living organisms, and is by far the most complex oxidizing system found in mitochondria. In human mitochondria, a unique, but huge structure, with more than 45 subunits, known as complex I, copes with NADH oxidation. This review compiles our present knowledge on the organization of this complex and the putative role of a small subset of its subunits. This review also describes the major progress that has been made in understanding the molecular bases of respiratory chain complex I deficiency in humans, with mutations identified in both the mitochondrial and the nuclear genes encoding complex I subunits. Finally, the puzzling questions raised by the varying clinical presentations of patients with complex I deficiency are discussed in light of our limited knowledge on complex I function in mammalian cells.

**Key Words:** Mitochondria, Respiratory Chain, Complex I deficiency, NADH.

## MITOCHONDRIAL OXIDATION OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDES: FROM THE MANY DEHYDROGENASES OF PLANTS TO THE UNIQUE MAMMALIAN COMPLEX I

The mechanism of NADH oxidation by mitochondria is highly variable across species [1, 2]. In a number of instances, NADH oxidation takes place on both sides of the inner membrane, and involves a varying number of dehydrogenases that cope with cytosolic and matrix NADH (Fig. 1). The highest complexity is observed in plant mitochondria (Fig. 1a), where several dehydrogenases are located on each side of the inner membrane [3]. Then, depending on their connection with either the proton-pumping cytochrome segment or the non-proton-pumping alternate oxidase present in most plant mitochondria, NADH oxidation generates from 0 to 3 ATP per oxygen consumed (Fig. 1A) [4]. In plants, this complex organization has been understood as an adaptation to the large changes in cell energy balance resulting from circadian rhythms of mitochondria-dependent and chloroplast-dependent ATP formation, and to varying environmental factors plants cannot escape (temperature, light, etc) [5]. A number of microorganisms also have quite complex systems allowing for NADH oxidation [6]. This enzymatic equipment varies among microorganisms (Fig. 1B, C), and a subset of these possesses a simplified version (fewer than 10 subunits) of the huge NADH-oxidizing complex (up to 46 subunits), spanning the inner membrane of mammalian mitochondria, known as the complex I of the respiratory chain (Fig. 1d) [7].

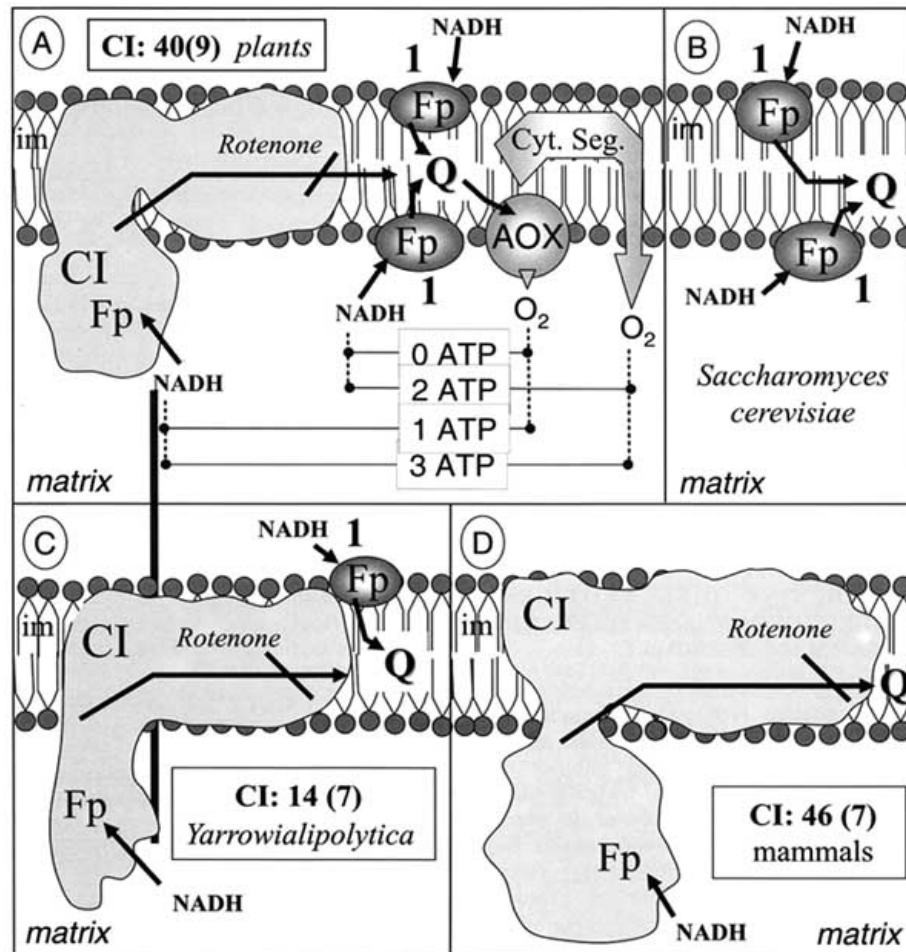
So far, this strikingly high number of subunits has not been associated with any extra function beside electron and proton transfers through the complex, although different suggestions have been made, that are summarized in figure 2. It follows that, by definition, complex I deficiency in human

is only depicted as a perturbation of electron/proton transfers, although other yet unknown consequences resulting from complex I deficiency will certainly be revealed in the future.

## THE DIFFICULTY IN ASSAYING COMPLEX I IN HUMAN SAMPLES

Measuring complex I activity in human samples relies on the use of a set of methods devised in the 60's using mainly either polarographic and/or spectrophotometric studies. However, these methods had to be scaled down tremendously as to be workable in this particular context, especially when working with children. In addition, highly reproducible experimental conditions have to be used in order to safely identify an eventual deficiency [14, 15]. Although a consensus method is not available yet, most assay methods successfully applied to skeletal muscle tissue, and biopsy material obtained from a variety of human tissues, liver, heart, kidney, yield a high rate of rotenone-sensitive NADH quinone reductase activity (Fig. 3B, trace a). However, until recently, this was not true for most circulating cells (platelets, lymphocytes) or cultured cells (skin fibroblasts, lymphoblastoid cell lines) for which only the isolation of mitochondria allowed a confident estimation of complex I activity, except when dealing with very severe deficiency [16]. In the case of these cells, both the presence of a highly active non-mitochondrial NADH dehydrogenase and the limited access of the substrates (NADH, donor; quinone analog, acceptor) to complex I concurred to hamper the use of the above mentioned standard methods. As a result, complex I activity, specifically inhibited by rotenone, could not be measured in these cells by standard method (Fig. 3B, trace b). The introduction of a new procedure based on cell membrane permeabilization by making use of digitonin and on soluble enzyme trapping by polyvinylpyrrolidone-enrobed silica (Percoll) (Fig. 3A) has nevertheless reduced this technical difficulty, allowing the measurement of reasonable rates of rotenone-sensitive complex I activity in

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**Fig. (1). The various mitochondrial NADH-oxidizing systems in living organisms.**

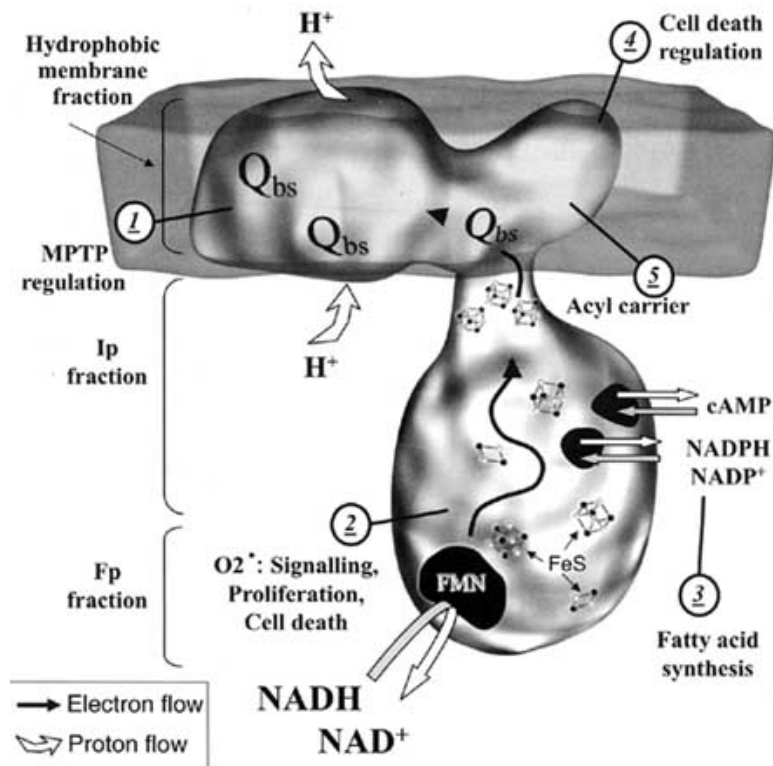
**A:** The plant system has three NADH dehydrogenases (Fp), located on each side of the mitochondrial inner membrane, leading to different ATP yield depending on their connection with either the cyanide-insensitive non-proton-motive alternate oxidase (AOX) or the proton-motive cytochrome segment (Cyt. Seg.) of the respiratory chain. Notice that additional NADPH dehydrogenase, active on the outer surface of the inner membrane, is not represented on this scheme. **B:** The reduced system, without the proton-motive complex I, found in the yeast *Saccharomyces cerevisiae*. **C:** The NADH-oxidizing system present in *Yarrowia lipolytica* comprises an alternative NADH dehydrogenase located on the outer surface of the inner membrane plus a 14-subunit Complex I. **D:** The mammals' NADH-oxidizing system reduced to a huge 46-subunit Complex I. CI: complex I of the respiratory chain; im: mitochondrial inner membrane; Q: coenzyme Q<sub>10</sub>. Numbers refer to the subunit composition of the different dehydrogenases. Numbers between brackets represent the number of subunits encoded by the mtDNA.

these cells (Fig. 3B, trace c) [17]. Notably, the activity of complex V can be subsequently measured in the same spectrophotometer cuvette (Fig. 3B, trace c).

#### THE MOLECULAR BASES OF RESPIRATORY CHAIN COMPLEX I DEFECT: MITOCHONDRIAL AND NUCLEAR DNA

If it was not for these difficulties, isolated complex I deficiency would represent about 30% of respiratory chain deficiencies encountered in humans, making this deficiency one of the most frequently observed as compared to deficiencies affecting other respiratory chain complexes (Fig. 4) [18]. The identification of the molecular bases of complex

I deficiency therefore represents an important task, and in view of the high number of potential genes involved, a true challenge. Both the mitochondrial and the nuclear genomes encode subunits of this complex (Fig. 4), the former encoding 7 subunits that presumably play an important role, if only considering their conservation across species. The structural study of bovine complex I suggests that it is constituted of three major fractions (Fig. 2) [19]. One, highly hydrophobic, is embedded in the core of the inner membrane and is mostly involved in proton pumping. It contains, among a few dozen nuclear-encoded subunits, the seven mtDNA-encoded subunits. The other part of the complex protrudes into the matrix space and consists of the



**Fig. (2). Mammalian complex I.**

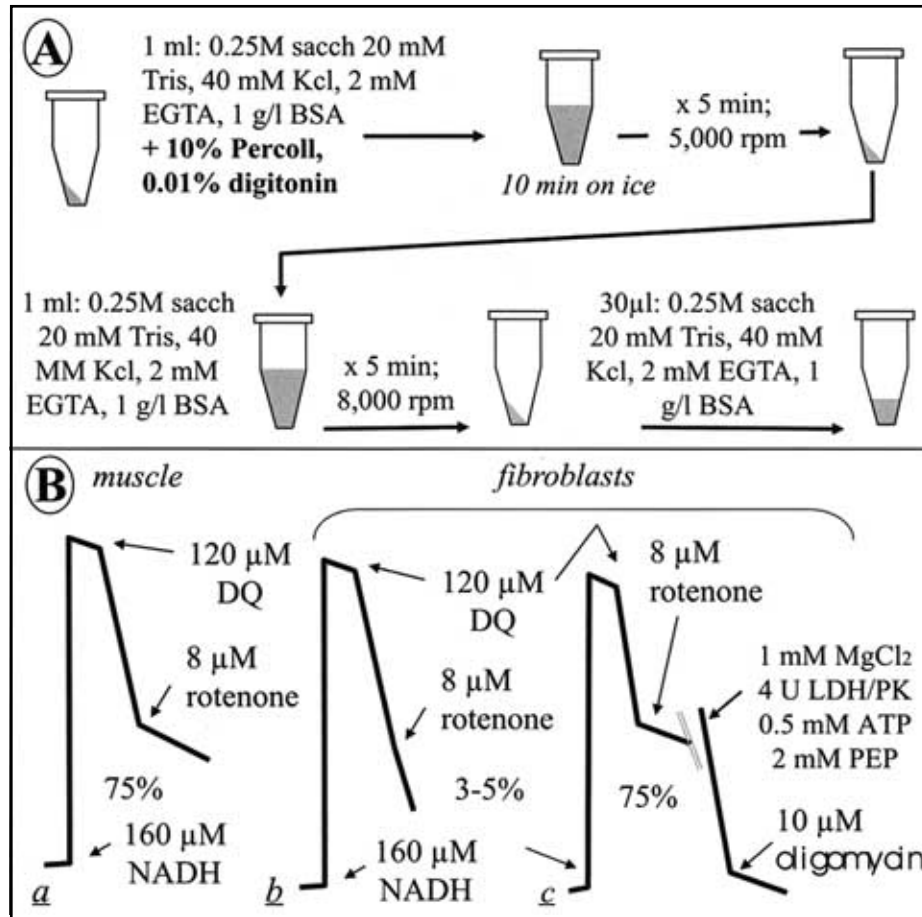
Adapted from the work of Grigorieff [8], this drawing features the three fractions of mammalian complex I, with the NADH active site in the Fp fraction, as well as the phosphorylation and NADPH fixation site in the Ip fraction. Although one major quinone-binding site (Q<sub>bs</sub>) appears to function in electron flow, three putative Q<sub>bs</sub> are represented on the last hydrophobic membrane fraction. Beside standard electron and proton transfer, five additional properties/functions (numbered 1 to 5 on the scheme) have been proposed for complex I: 1) Regulation of the mitochondrial permeability transition pore (MPTP) [9]; 2) Signalling for proliferation/cell death through superoxides [10]; 3) Putative intramitochondrial fatty acid synthesis [6]; binding of NADPH might also be linked to a reductase/isomerase activity of complex I and be involved in the biogenesis of the complex [11]; 4) GRIM-19, recently claimed to be one of the complex I subunits, is one of the cell death-regulatory proteins [12]; 5) The NDUFA1 subunit may act as an acyl carrier in complex I [13]. Fp, flavoprotein fraction; Fe-S, iron-sulphur cluster; FMN, Flavin mononucleotide; Ip, Iron-sulphur protein fraction; MPTP, Mitochondrial Permeability Transition Pore.

flavoprotein fraction, which comprises three subunits, and the iron-sulphur cluster-binding fraction, composed of at least seven subunits. This protruding part is likely to be involved in electron transfer (Fig. 2).

### MITOCHONDRIAL DNA MUTATIONS

The first deleterious mutations reported to cause complex I deficiency were identified in the mitochondrial genome in the 1990's, mostly associated with the Leber Hereditary Optic Neuropathy (LHON) [20, 21]. To date, up to 40 mutations in mtDNA ND genes have been reported with proven (or suggested) associations with strikingly variable phenotypes (MITOMAP; <http://www.mitomap.org/>). These phenotypes range from early-onset severe encephalopathy, Leigh or Leigh-like syndrome (progressive neurological degradation with developmental delay associated with brainstem and/or basal ganglia anomalies, neuropathological examination showing spongiform lesions, mostly bilateral and symmetrical, demyelination, gliosis and capillary proliferation), to hypertrophic cardiomyopathy (evolving to dilation), or to late-onset LHON with very selective targeting

of the optic nerve (Table 1). There has been a great effort made to understand this phenotypic variability over the last decade. As for any mtDNA mutations, the undisputed importance of the cellular load in wild-type *versus* mutant mtDNA - a feature termed heteroplasmy and resulting from the coexistence of both mtDNA species in each cell - has been recognized [22]. To a certain extent, the presence of the wild-type genome allows for a natural genetic complementation to take place within the mitochondrial network between the different genomes [23]. In keeping with this, a threshold effect would be required in order to actually observe a tissue-dependent biochemical phenotype, as reported for other mtDNA mutations affecting genes encoding tRNA or respiratory chain subunits [24]. However, such tissue specificity, as that observed in the cases of the LHON-causing mtDNA-mutation, remains a total mystery. Indeed, the specific involvement of the optic nerve in the LHON syndrome, observed in patients with a widespread homoplasmic mutation in the mtDNA complex I genes, can certainly not be accounted for by any simple mechanism. Thus, alternative explanations have been proposed. A particular high energy demand of the affected cells has been

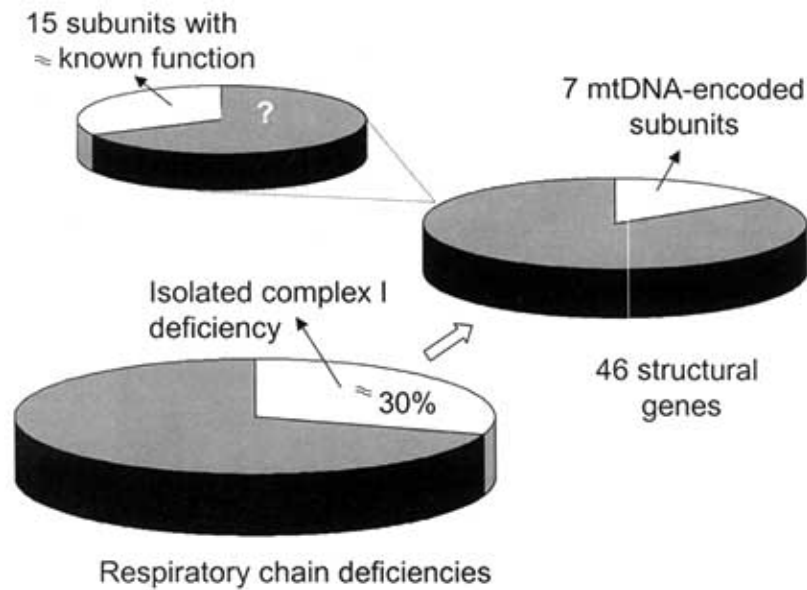


**Fig. (3). Biochemical assay of complex I activity in human cells**

**A:** Circulating lymphocytes, cultured skin fibroblasts, and lymphoblastoid cell lines all present high activity of non-mitochondrial contaminating NADH dehydrogenases and/or reduced access of NADH and acceptor quinone for complex I. However, cell permeabilization and treatment with digitonin and Percoll as described allows the specific assay of complex I [17]. **B:** Complex I assay measured as the NADH decylubiquone (DQ) reductase is readily measured using skeletal muscle homogenate with high sensitivity to rotenone (about 75%), a specific inhibitor of complex I (trace a). In contrast, freeze-thaw permeabilized cultured skin fibroblasts display a high rate of rotenone-insensitive NADH DQ reductase (trace b). However, treating cells as described in (A) permits the measurement of a high rate of rotenone-sensitive NADH DQ reductase as well as of the oligomycin-sensitive ATPase (complex V) in the presence of additional MgCl<sub>2</sub>, lactic dehydrogenase (LDH)/pyruvate kinase (PK), ATP and phosphoenolpyruvate (PEP) [17].

put forward to explain their hypersensitivity to respiratory chain impairment [25]. However, the fact that the same nerve cells can be left unaffected in severe and widespread respiratory deficiency, in particular those resulting from mutations in nuclear genes encoding the CI subunit, makes such a simple hypothesis rather unlikely. Incidentally, the extent of CI activity decrease resulting from some of these LHON-causing mutations is still debated. Later on, the deleterious effect of superoxides that might be generated by defective CI has also been proposed to explain selective involvement, either due to higher generation of superoxides in these cells or to decreased cell defenses [26]. This attractive hypothesis remains however to be firmly demonstrated and presupposes a specific sensitivity to oxidative stress in this particular cell type. Impairment of either a cell-specific, intricate metabolic pathway, or a yet unknown function associated with complex I, could also be responsible for cell-to-cell differential susceptibility to complex I deficiency.

In this context, as for all mtDNA mutations, the demonstration of a potentially deleterious effect of a base change in mtDNA represents a true challenge. Transforming mitochondria with exogenous genetic information and studying the resulting biochemical phenotype would obviously be the straightforward strategy. However, this approach is still in its infancy and no attempt to use any of the complex I genes to do so has ever been reported [27, 28]. Instead, a tedious method, making use of cybrid cell lines in which the whole cell's mitochondrial population is placed under the control of an exogenous nucleus allows the association of a given phenotype to a given mutant mtDNA population [29]. The detailed study of clones with various mutant mtDNA loads further supports the association of the defective phenotype with a mutant mtDNA [30]. Interestingly enough, similar information can be sometimes obtained simply by culturing cells simultaneously in either a medium containing glucose (allowing for respiratory chain-defective cells to grow) or devoid of any sugar but



**Fig. (4). Figures for complex I and its deficiency in humans**

Complex I deficiency represents one of the most frequent isolated respiratory chain deficiencies. Up to 46 structural genes and an unknown number of other genes (involved in assembly, maintenance, regulation, etc.) may be at the origin of this deficiency. So far, a function has been attributed to only a subset of the structural genes.

containing glutamine (a medium highly selective for respiratory chain-competent cells) (Fig. 5) [31]. Thorough study of both genotype and phenotype may then indicate the association of a given mutant mtDNA with a defective phenotype, growth in the selective medium resulting in the specific loss of cells with high load of mutant mtDNA and return to normal complex I activity (Fig. 5). It should however be stressed again that a whole mtDNA genome rather than one given mutation will be associated with defective phenotypes in such studies. Indeed, it is often the sum of several arguments/suggestions that establishes the deleterious nature of a given mutation: its absence from the control population (including information now available through databases), *plus* an eventual transmission in the family (maternal inheritance, phenotype-to-genotype association), *plus* the variable level of mutant mtDNA load among tissues (predictably higher in affected tissues; the heteroplasmy itself being in addition often taken as an additional element, since polymorphisms are supposedly barely heteroplasmic).

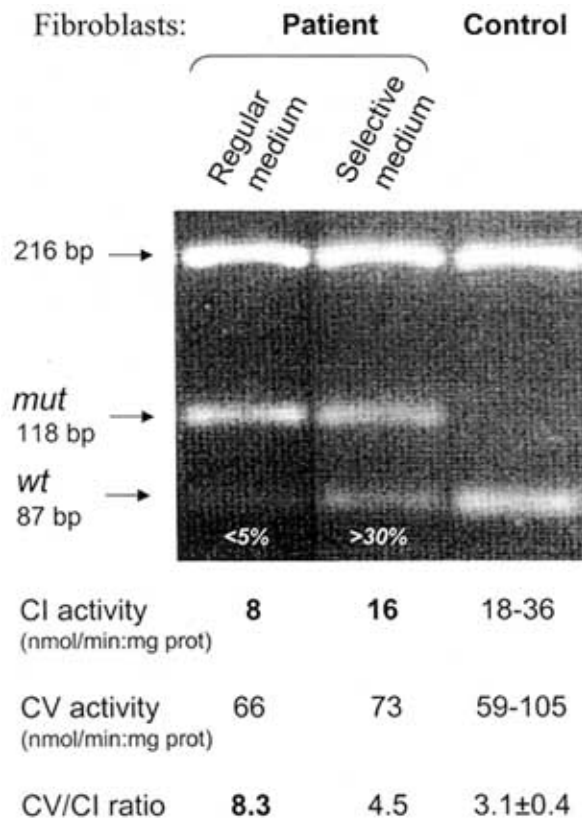
The mechanism resulting in mutation of mtDNA ND genes is also a debatable issue. The location of mtDNA in the pro-oxidant environment created by the respiratory chain activity might be advanced as a hypothesis to account for the unexpected high frequency of thymine and guanine residue mutations (T>C; G>A) in the ND genes, and more generally in mtDNA (T>C and G>A transitions representing about 70% of mtDNA mutations reported so far). Indeed, according to this hypothesis, oxidative mutagenic lesions mostly affect thymine and guanine residues [32]. Selective changes in a class of residues can also be favored by the over-representation of the corresponding precursor

nucleotide pool utilized for mtDNA replication. Such mechanism has been recently illustrated in the case of mutant thymidine phosphorylase that, due to increased cellular nucleotide pools of thymidine and deoxyuridine, favors T to C transitions, particularly when preceded by 5'-AA sequences [33].

Additional explanations are needed to account for the recurrence of *de novo* mutations in mtDNA. Indeed, even if so far histones have not been shown to be associated with mtDNA, specific features of the mitochondrial nucleoids (discrete structures associated to the inner mitochondrial membranes and comprising a subset of proteins forming a scaffold around the mtDNA [34, 35]) may favor the exposure of specific mtDNA regions to deleterious modifications. Whatever the mechanism at work, the limited repair capacity of the mitochondrial replication machinery may allow these *de novo* mutations to be spared from repairing, and subsequently amplified in specific tissue through random segregation.

#### NUCLEAR GENE MUTATIONS

The situation should be simpler when dealing with a mutation in a nuclear gene encoding one of the complex I subunits. In particular, the expression of the mutant cDNA in a selected model system should establish the deleterious effect of a given mutation [36]. But even before facing this question, a very difficult challenge is to find the guiding thread that will lead to the identification of mutation in one of the numerous possible candidate nuclear genes. In fact, mutations could reside in any of the more than 39 obvious candidate genes (those encoding complex I subunits) [7], without mentioning of the assembly genes, although only



**Fig. (5). Associating a biochemical defect with a defective mitochondrial genome.**

Fibroblasts from a control (right) and a patient harboring the deleterious G13513A ND5 gene mutation (left) have been grown for 3 weeks in normal RPMI medium supplemented with 200  $\mu$ M uridine and 2.5 mM pyruvate (regular medium). PCR amplification of the ND5 gene was performed using a mismatch forward primer (nt13491-nt13512) and a reverse primer (nt13825-nt13806) to generate a 334 bp fragment as described by Chol *et al.* [31]. Digestion of this fragment with the restriction enzyme MboII in control fibroblasts generates three fragments, 216 bp, 87 bp, 31 bp, the smallest not shown on the gel. Digestion of the mutant fragment in patient fibroblasts generates only two fragments, 216 bp, 118 bp. The normal sequence represented less than 5% of the total mtDNA in the patient as shown by the faint band on the gel for the 87 bp species. Enzyme assays established that these fibroblasts had a severe complex I defect, both in absolute activity and relative to complex V activity. After three weeks growth in a medium devoid of any sugars, patients' cells growing in glutamine only were re-analysed for both mitochondrial genotype and phenotype. It was found that growth in this highly selective medium for respiratory-competent cells resulted in a significant loss of mutant DNA species, the wild-type species representing more than 30% of total mtDNA. At the same time, enzyme assays show that a threshold value for the normal mutant mtDNA load has been reached, above which normal activity of complex I is observed both in absolute value and relative to complex V activity.

one (CIA30) is known in humans. Once a mutation in mtDNA has been ruled out, we are left with very few tools in order to incriminate one or another of these 39 genes. Respective degrees of conservation of the different subunits through evolution has been the initial clue and has permitted the report of the first mutations in complex I subunit-encoding genes. To date, 8 nuclear genes have been found to bear mutations in patients with complex I deficiency (Table 1). Systematic screening of these genes through modern but costly molecular methods (DHPLC followed by automatic sequencing, etc.) is now currently carried out in a few places around the world. This should result in the future in more information made available dealing with the potential relationship between clinical presentations and these mutant genes. However, it is already clear that to draw genotype-to-phenotype correlations promises to be as difficult for CI

subunit-encoding nuclear genes as for mtDNA genes. Indeed, the examination of the clinical phenotypes associated with the handful of mutations already identified in nuclear genes (Table 1) reveals that the central nervous system is affected most (as it is for mtDNA mutations), despite the fact that all these genes are widely expressed across human tissues, with no identified isoforms. Diseases due to CI deficiency of nuclear origin, however, range from pure neurological involvement to more complex presentations associating cardiac with neurological symptoms. It may well be that, independent on the gene being mutated, the actual dysfunction of complex I (residual activity, overproduction of superoxide, etc) indeed governs the clinical presentation, making any attempt to correlate genotypes to phenotypes meaningless.

**Table 1. From Clinical Presentation to Gene Mutation in Complex I Deficiency**

Clinical presentation	Gene	Mutations	References
Leigh syndrome	NDUFV1	E214K/IVS8nt+4	[37]
	NDUFV1	A432P/del nt 989-990	[37]
	NDUFV1	Y204C/C206G	[37]
	NDUFS1	R241W/R557X	[37]
	NDUFS1	M707V/large scale deletion	[37]
	NDUFS3	T145I/R199W	[38]
	NDUFS4	Homologous 5bp duplication	[39]
	NDUFS4	IVS1nt-1/IVS1nt-1	[40]
	NDUFS7	V122M/V122M	[41]
	NDUFS8	P79L/R102H	[42]
	ND3	T10158C	[32]
	ND3	T10191C	[32]
	ND5	T12706C	[43]
ND6	T14487C	[32]	
Leigh-like syndrome	NDUFS4	R316X/R316X	[44]
	NDUFS4	W96X/W96X	[45]
	ND5	G13513A	[31]
Encephalomyopathy	NDUFV1	R59X/T423M	[46]
Leucodystrophy	NDUFS1	D252G/delcodon 222	[37]
Leucodystrophy and myoclonic epilepsy	NDUFV1	A341V/A341V	[46]
Hypertrophic cardiomyopathy end encephalomyopathy	NDUFS2	R228Q/R228Q	[47]
	NDUFS2	P229Q/P229Q	[47]
	NDUFS2	S413P/S413P	[47]
	NDUFV2	IVS2+5_+8delGTAA	[48]
Multisystemic disorder (at least 3 organs involved)	ND5	T12706C	[32]
	ND6	A13514G	[32]
LHON	ND1	G3460A	[20]
	ND4	G11778A	[49]
	ND6	G14459A	[50]
	ND6	T14484C	[51]
MELAS	ND5	G13513A	[52]
		A13514G	[53]
ESOC	ND3	T10191C	[54]

ESOC: Epilepsy, Strokes, Optic atrophy and Cognitive decline; LHON: Leber Hereditary Optic Neuropathy; MELAS: Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke like episode. NDUFV (nuclearly-encoded NADH dehydrogenase ubiquinone flavoprotein subunits); NDUFS (nuclearly-encoded NADH dehydrogenase ubiquinone Fe-S subunits), ND (mtDNA-encoded NADH dehydrogenase subunits). Subunits of complex I are numbered according to MitoPick ([http://www-dsv.cea.fr/thema/MitoPick/Search/Homo\\_sapiens.html](http://www-dsv.cea.fr/thema/MitoPick/Search/Homo_sapiens.html)).

## CONCLUDING REMARKS

As a brief conclusion, it should be stressed that major progress has been made in both the biochemical and molecular characterization of complex I deficiency over the last few years, complex I deficiency being encountered in the largest group of patients with respiratory chain dysfunction. Mutational analysis has revealed deleterious mutations in 30 to 40% of the cases either in mtDNA or in 8 of the nuclear genes encoding CI subunits, and so far no simple phenotype-to-genotype relationship can be made. Aside from the determination of complementation groups, identification of major genes responsible for complex I efficiency in human

can probably be helped by using the elegant strategy proposed by the group of Shoubridge for COX deficiency [55]. This method makes use of a panel of viral constructs containing genes of interest that functionally complement respiratory-defective cell lines, and to possibly identify defective genes. It however assumes that cell lines do actually express the defect - only a subset of the cases - and that the deficiency is sufficiently severe as to allow unambiguous conclusion. An alternative approach consisting in analyzing sub-complex assembly in cells harboring various mutations may indicate genes involved [56, 57]. However, it remains to be established that sub-complex assembly is so dissimilar between different types of mutants

as to provide valuable information that speeds gene identification. The different “-omics” approaches may be considered, since by delineating the consequences of various mutations in genes encoding complex I subunits, they may help define profiles specific for known mutants, which may thereafter allow to direct research towards given genes in other patients. However, it is conceivable that cells respond to a deficiency of complex I in a rather similar way whatever the mutant gene, making this information not necessarily very helpful. The difficulty also lies in that, beside the genes encoding structural subunits, mutations in a large series of nuclear genes can possibly result in complex I deficiency as well. The number of these genes, involved in complex I assembly, maintenance, and regulation, intervening in the correct handling of iron-sulphur clusters and FMN components, in the phosphorylation of specific subunits, in controlling protein thiol homeostasis, is yet to be established.

Having stressed the limitations of each strategy, it has to be also recognized that each one may also result in unpredictable discoveries and probably merits careful exploration. In our introductory remarks to this short review, we recalled the diversity of NADH-oxidizing systems encountered in living organisms. The tremendous complexity of mammalian complex I could recall this striking diversity. So far, a function has been assigned to only a limited subset of complex I components. Unravelling the molecular bases and dissecting the consequences of complex I deficiency in humans might help to shed some light on yet unknown functions of some of these complex I components.

In a significant number of cases, the improvement of biochemical methods, but also and above all an understanding of the molecular bases of complex I deficiency, has allowed families access to prenatal diagnosis. In contrast, in term of therapy, we are stuck where we were 10 years ago. In a vast majority of cases, therapeutic supplementation with riboflavin, carnitine, coenzyme Q<sub>10</sub> and other supplements fails to improve patients' conditions. It will be, however, important to establish if, as suggested by some reports [58, 59], idebenone, a coenzyme Q<sub>10</sub> analog, should be used in the particular case of the LHON disease [60].

To find a clue to counteract these diseases, we absolutely need either to delineate which are the exact consequences of the various complex I deficiencies, hoping to identify steps which might be pharmacologically targeted, or to observe major progresses in the genotherapy or stem cell fields.

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