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Mitochondrial Respiratory Complex I: Structure, Function and Implication in Human Diseases

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Abstract

Mitochondria are ubiquitous organelles in eukaryotic cells whose primary function is to generate energy supplies in the form of ATP through oxidative phosphorylation. As the entry point for most electrons into the respiratory chain, NADH:ubiquinone oxidoreductase, or complex I, is the largest and least understood component of the mitochondrial oxidative phosphorylation system. Substantial progress has been made in recent years in understanding its subunit composition, its assembly, the interaction among complex I and other respiratory components, and its role in oxidative stress and apoptosis. This review provides an updated overview of the structure of complex I, as well as its cellular functions, and discusses the implication of complex I dysfunction in various human diseases.

Keywords

Mitochondria; complex I; oxidative stress; reactive oxygen species; apoptosis; complex I functions

1. INTRODUCTION

Mitochondria are known as the "powerhouse" of the cell, generating ATP *via* oxidative phosphorylation (OXPHOS) complexes, which are present in the inner membrane of mitochondria. These complexes are known as NADH: ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II), ubiquinol–cytochrome c oxidoreductase (complex III, or cytochrome bc_1 complex), cytochrome c oxidase (complex IV), and ATP synthase (complex V).

Complex I is the first enzyme of the respiratory chain. It oxidizes NADH, which is generated through the Krebs cycle in the mitochondrial matrix, and uses the two electrons to reduce ubiquinone to ubiquinol. Ubiquinol is reoxidized by the cytochrome bc_1 complex and transfers electrons to reduce molecular oxygen to water at complex IV. The redox energy

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released during this process is used to transfer protons from the mitochondrial matrix to the periplasmic space that generates proton-motive force across the inner mitochondrial membrane at complex I, III, and IV. Complex V uses this proton-motive force to produce ATP from ADP and inorganic phosphate. This entire process constitutes OXPHOS. Because complex I is the major entry point for electrons to the respiratory chain and is suggested as the rate-limiting step in overall respiration, it plays a central role in energy metabolism.

Complex I is the largest and most complicated component of the respiratory chain. Using the bovine heart as the model system, previous work has characterized all the complex I subunits and cloned the encoding genes. However, the functions of the individual subunits are largely unclear. Complex I is the only component of the mammalian respiratory chain whose three-dimensional structure is available only at a low resolution. Tremendous progress has been made in recent years in exploring the role of mitochondria in different cellular functions. Besides being at the center of energy metabolism, mitochondria also play a critical role in apoptosis [1,2] and cell proliferation [3]. Mitochondria are also major factors in modulating calcium signaling [4,5], which is a universal secondary messenger. As we will discuss further, complex I has been implicated in the regulation of reactive oxygen species (ROS), which are important molecules in various signaling pathways, including apoptosis.

Over the last 20 years, mitochondrial dysfunction has been associated with various human diseases, including seizure, ataxia, cortical blandness, dystonia, exercise intolerance, ophthalmoplegia, optic atrophy, cataracts, diabetic mellitus, short stature, cardiomyopathy, sensorineural hearing loss, and kidney failure [6,7]. Isolated deficiency of complex I is the most frequent among mitochondrial disorders, which has been linked to the mutations in genes encoding complex I subunits and assembly factors.

Therefore, a comprehensive summary of current understanding of complex I is important. This review gives an overview of the structure and cellular functions of mammalian complex I and will also discuss the implication of complex I deficiency in human diseases.

2. COMPLEX I SUBUNITS GENES AND PROTEINS

In 1962, Hatefi *et al*. isolated complex I from bovine heart mitochondria [8]. They then divided the bovine enzyme into three functional parts containing the flavin mononucleotide (FMN), the iron–sulfur cluster, and a membrane, or hydrophobic, part by using chaotropic agents [9]. Others further resolved purified preparations of complex I by two-dimensional gel electrophoresis by combining isoelectric focusing and chromatographic methods [10,11]. The success in purifying this enzyme has enabled the potential identification and characterization of individual subunits for other biochemical and functional studies.

Soon after the sequence of human mitochondrial genome became available [12], seven hydrophobic complex I subunits (ND1–ND6 and ND4L) were identified, which are encoded by the mitochondrial genome (Fig. 1) [13,14]. The remaining nuclear-encoded subunits of complex I were divided into different parts (Iα, Iβ, Iλ, and Iγ) and identified by mass spectrometry [15,16]. A series of studies spanning two decades revealed that bovine complex I is a membrane-bound assembly of 45 different polypeptides with a combined

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molecular mass of about 1 MDa, together with noncovalently bound FMN and eight iron–sulfur clusters [17]. The human homologues of all 45 bovine subunits were also identified, which are summarized in Fig. (1) [18].

Among the 45 subunits, 14, including seven mtDNA-encoded subunits and others with motifs for binding redox centers, are the “core” subunits for a functional enzyme that are highly conserved across species, as well as homologous to the 14 subunits of the bacterial enzyme [19,20]. These core subunits are assumed to be sufficient for energy transduction. The remaining subunits were defined as “supernumerary/accessory” subunits, most likely with some new functions or regulatory sites.

3. COMPLEX I ARCHITECTURE

The structural information of complex I was first acquired using electron microscopy for the *Neurospora crassa* enzyme from a detergent-solubilized preparation on two-dimensional crystals. Complex I exhibited a characteristic L-shaped structure with two distinguishable arms [21]. The hydrophobic membrane arm, which contains all the mtDNA-encoded subunits, is embedded in the inner mitochondrial membrane, and the hydrophilic peripheral arm protrudes into the matrix. Comparative electron microscopic and biochemical analyses have indicated that these two arms are assembled together and probably emerged evolutionarily independently of each other [22]. For function, complex I can also be divided into three modules: the electron input module, or dehydrogenase module (N module), which accepts electrons from NADH; the electron output module, or hydrogenase module (Q module), which delivers electrons to ubiquinone; and the proton translocation module (P module), which pumps protons across the inner membrane [23]. Whereas the N and Q modules are parts of the matrix arm, the P module lies within the membrane arm.

The first three-dimensional structure of mammalian complex I was determined on single particles isolated from bovine cardiac muscle mitochondria by using electron cryomicroscopy [24]. Besides the overall L-shaped appearance closely related to complex I from *N. crassa*, the globular domain of the matrix arm is substantially larger, suggesting that additional intricacy was evolved. Complex I particles also display substantial flexibility [25] and exhibit notable redox-dependent conformational changes [26].

The first high-resolution crystal structure on complex I was recently determined in a simpler model system, with the peripheral arm in a bacterium, *Thermus thermophilus* [27]. This subcomplex consists of eight subunits and contains all redox centers of the enzyme, including nine iron–sulfur clusters. Taken together with the biochemical studies, these findings yield a clear picture on coupling of electron transfer and proton translocation in complex I.

4. COMPLEX I ASSEMBLY

Considering the intricacy of complex I, one perceives a delicate assembly system regulating the process. However, partly because of the lack of a sophisticated genetic system such as that of *Saccharomyces cerevisiae*, which does not contain complex I, the assembly of complex I is poorly understood compared with that of other respiratory complexes. Most of

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our current knowledge has been accumulated from the studies in *N. crassa* system and by investigating mammalian cells with defective complex I function.

4.1. Requirement of Subunits in Assembly of Complex I

Complex I assembles through an evolutionarily conserved module. Complex I assembly was studied primarily in the fungus *N. crassa*; two independent pathways exist that led to the preassembly of the membrane and matrix arms, which subsequently joined to form the whole complex [28–34]. The matrix arm is related to the small form of NADH: ubiquinone oxidoreductase and consists mostly of the nuclear-encoded subunits, FMN, three iron–sulfur clusters, and the site for the internal ubiquinone. The membrane arm consists of all the mtDNA-encoded subunits, one iron–sulfur cluster, and the catalytic site for the substrate ubiquinone [28,35].

The membrane and matrix arm assemble independently from each other, which was confirmed by using mammalian cells carrying specific mtDNA mutations. An efficient way to isolate such human and mouse cell lines, which carry mutations in any of the mtDNA-encoded subunits of complex I, is to expose them to high concentrations of the complex I inhibitor rotenone [36,37]. Analyses of these mutants have shown that subunits ND4 and ND6 are essential for the assembly of the other mtDNA-encoded subunits into complex I. Such studies were established by immunoprecipitation experiments using antibody against the 49-kDa subunit (NDUFS2) of the complex I iron–protein fragment [38,39]. These results indicate the decisive role of mtDNA-encoded subunits in holding the membrane arm collectively. In a separate line of investigation, a missense mutation, T14487→C, in the ND6 gene of a Leigh syndrome patient changed a highly conserved 63rd amino acid in its corresponding protein from M to V, thereby disrupting complex I assembly [40]. However, while diminishing NADH:Q_1 oxidoreductase activity and the stability of subunits of the membrane arm, the absence of ND4 or ND6 did not reduce NADH:$K_3Fe(CN)_6$ activity or the stability of subunits of the matrix arm, suggesting independent assembly of the two arms [39,41].

The mtDNA-encoded ND subunits do not have the same effect on complex I assembly. For example, in cytoplasmic hybrids (cybrids) with mutations in ND5, even if at a lower efficacy, all the mtDNA-encoded subunits other than ND5 were coprecipitated, indicating flexibility in complex I assembly [37,42]. More interesting: another study screened for spontaneous revertants of a mouse cell line carrying a homoplasmic ND6 mutation; a galactose-resistant clone was isolated [42]. Although there is no ND6 protein synthesis, the assembly of other subunits into complex I was recovered. Furthermore, the respiration activity and membrane potential were fully restored. This restoration in mitochondrial function was associated with a change(s) in the nuclear background that could also suppress the defects caused by an ND5 mutation [42].

The necessity of subunits in complex I assembly was also investigated with complex I–deficient patient cell lines. In particular, absence of the ND3 subunit does not adversely affect assembly, whereas ND1 or ND2 disruption results in disturbed assembly with accumulated intermediate subcomplexes [43–46]. Determination of various subcomplexes in patient cells with mutations in genes encoding complex I subunits has clarified the assembly

process. Further, with a conditional assembly system in which reversible blockage of mitochondrial translation was applied, complex I assembled *via* combination and expansion of two parallel lines of assembly for these two arms [47]. On the basis of the appearance of those subcomplexes, previous work has proposed several models with more detailed assembly procedures [23,48].

4.2. Assembly Factors

Although the assembly process is poorly understood, several proteins involved in complex I biogenesis have been reported (Fig. 1). The first complex I assembly factor was identified by screening for complex I intermediate–associated (CIA) proteins in *N. crassa* deficient in complex I assembly [49]. The human homologue (NDUFAF1) of one of such protein, CIA30, was later identified and shown to be involved in the early stage of assembly [50]. Another protein, B17.2L, a paralogue of a small structural subunit in the matrix arm, was predicted as complex I chaperone in a whole-genome subtraction study between two yeast species with and without complex I [51,52]. Further, a mutation that abolished the synthesis of a functional B17.2L was identified in a patient with early-onset, progressive encephalopathy and low assembly of complex I [52]. Restoration of complex I assembly was achieved by introducing its cDNA into the patient's fibroblasts [52]. This approach helps subunits such as NDUFS4 or NDUFS6 to assemble with preexisting complexes [53]. Also, B17.2L was associated with a supercomplex containing both complex I and III.

Another assembly factor, *C60RF66*, was identified from the muscles of a patient with complex I deficiency by using the homozygosity mapping method [54]. A mutation in this gene diminishes complex I assembly with the accumulation of two smaller intermediates. This deficiency was fully restored when the *C60RF66* transgene was introduced exogenously [54]. Also interesting: apoptosis-inducing factor (AIF)–deficient cells exhibit a reduced content of complex I, pointing to a role of AIF in assembly and/or upholding of this complex [55].

The apoptotic function of AIF is independent of its role in complex I activity [56,57], whereas both B17.2L and C6ORF66 were previously identified as proteins involved in tumorigenesis [58,59]. A cytosolic protein termed Ecsit, also known as CIA30-binding protein, is also involved in complex I assembly [60].

Although knowledge of complex I assembly is increasing, the different complexes from the OXPHOS chain can organize together to form supramolecular structures termed supercomplexes or "respirasomes" [61–63]. The importance of interaction of different complexes has been indicated in the implication of both complex III [64] and IV [65,66] in the assembly and stability of complex I. Perhaps the preassembled complex III and/or IV serves as an anchor at the membrane to recruit complex I into the respiratory chain. Such studies clarify the functional dependence of different complexes on each other during electron transfer in OXPHOS.

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5. INHIBITORS AGAINST COMPLEX I

More than 60 different families of inhibitors of complex I have been isolated or synthesized [67] to elucidate the functional mechanism of complex I and to develop potential reagents for medical or pest-control purposes. The structure of natural inhibitors has modular features similar to those of ubiquinone, with a cyclic head analogous to the ubiquinone ring and a hydrophobic tail [68]. These inhibitors can be classified into two or three functional groups according to their action sites on complex I: a quinone antagonist acting at the entry of the hydrophobic site of the complex, such as rolliniastatin-2; a semiquinone antagonist acting at the intermediate step, such as rotenone; and a quinol antagonist acting at the formation or release of the product, such as myxothiazol [67–69].

Rotenone is the most commonly used potent specific complex I inhibitor. It has been used successfully in screening for mtDNA mutations in both human and mouse cells [36, 37]. In combination with another complex I inhibitor, flavone, rotenone was also used to distinguish the electron transfer activity of complex I from that of other NADH dehydrogenases (i.e., yeast NDI1) because of its specificity [70]. Rotenone induces ROS formation during forward electron transfer [71,72]. Another notable complex I inhibitor is 1-methyl-4-phenylpyridinium (MPP), which could induce neurodegeneration and parkinsonism in humans and experimental animals [73,74]. Annonaceous acetogenins, one of the most powerful lipophilic complex I inhibitors [67,68,75], caused neuronal cell death and induced the redistribution of tau protein (a feature of Parkinsonism) in primary cultures of striated neurons [76].

Although some complex I inhibitors (e.g., rotenone, piericidin A, myxothiazol) can induce a strong increase in ROS production, others (e.g., stigmatellin, capsaicin, mucidin) prevent ROS generation from this enzyme [77]. These results have shown the diversity of complex I inhibitors.

6. COMPLEX I AND ROS GENERATION

Complex I and III are generally considered the main sites of ROS production where electron leaks and single electrons react with oxygen, producing superoxide anion [78]. These products are thought to play a role as signaling molecules to activate various pathways including protein kinase C, mitogen-activated protein kinase (MAPK), PI3K, Akt, and p38 MAPK, as well as Ca^{2+} signaling.

Recently, new evidence has emerged that complex I contributes most of the ROS generated in intact mitochondria, which are involved directly or indirectly in the signaling pathways, as shown in Fig. (2) [79]. There are two possible sites on complex I where oxygen could access the electrons at the end of cofactor chain: the flavin moiety and the quinine-binding site [80]. Some ROS stimulators also generate superoxide though complex I. For example, paraquat is taken up by the mitochondrial inner membrane and then reduced by complex I to form its cationic radical, which then reacts with oxygen to form superoxide and cause oxidative stress [81].

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Mutations in the genes encoding complex I subunits and complex I dysfunction have often been linked with alteration in ROS levels [78]. In *Caenorhabditis elegans*, defective complex I (*gas-1* mutant) increases oxidative damage, which in turn shortens the lifespan. [82]. Similarly, overproduction of ROS was also recorded [83,84] in human cybrids carrying Leber's hereditary optic neuropathy (LHON) mutations.

Complex I also appears to be the most vulnerable site to oxidative stress. In *C. elegans*, mutation of *NDUFV1* results in hypersensitivity to oxidative stress [85], and the alternative NADH dehydrogenase NDI1 protects the cells effectively from oxidative stress in both human cell and animal models [70,86].

Because ROS are also involved in a variety of cellular functions ranging from apoptosis and necrosis to cell proliferation and carcinogenesis, the regulation of complex I activity is also implicated in these processes.

7. COMPLEX I AND APOPTOSIS

Mitochondria play a critical role in regulating apoptosis [87,88]. A detailed analysis on the modulation of the respiratory chain (RC) on apoptosis was carried out with mutants that either lacked specific respiratory complexes or from which the RC had been eliminated [89]. Cells lacking the RC are protected against both mitochondrial and endoplasmic reticulum stress–induced apoptosis. Cells that have the RC but cannot generate electron flux are protected against mitochondrial apoptosis, although they have increased sensitivity to endoplasmic reticulum stress. Interestingly, cells with a partial reduction in electron flux have increased apoptosis under both conditions [89]. In another study, the resistance to apoptosis of certain cancer cells was attributed to the increased complex I substrate NADH, probably due to the dysfunction of complex I [90]. In such conditions, cell survival was mediated by Akt activation through a redox modification mechanism due to inactivation of PTEN [90].

On the other hand, several studies implicated inhibition of complex I activity in neuronal cell death and degeneration [91–93]. In particular, pharmacological usage of complex I inhibitors, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), and rotenone in rodents have helped to generate animal models for Parkinson's disease that exhibited degeneration of dopaminergic neurons of the substantia nigra [91] through activation of apoptotic pathways [94,95]. Further investigations in these animals revealed a downregulation of several mitochondrial genes, including five subunits of complex I and several ATP synthase subunits [96].

In another study, the role of complex I in activation-induced T-cell death has been demonstrated, where complex I was not only the source of mitochondrial ROS but also a prerequisite for subsequent ROS production *via* the NADPH oxidase. Inhibition of complex I assembly chaperone (NDU-FAF1) by small interfering RNA blocked ROS production, thereby inhibiting activation-induced T-cell death. This finding was further supported by the use of metformin, an antidiabetic drug and mild complex I inhibitor, which also inhibited activation-induced ROS production [97].

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A clearer image of complex I implicated in apoptosis came into play when one of its subunits, NDUFA13, was recognized as a cell death regulatory protein (GRIM-19 [gene associated with retinoid-interferon–induced mortality]) [98]. GRIM-19 is essential for complex I assembly and electron transfer activity [99,100]. Upon apoptotic induction, it is released from the mitochondria to associate with the proapoptotic serine protease HtrA2 to promote cell death [101]. The disruption of complex I activity in cell death was indicated when another complex I subunit, NDUFS1, was characterized as a substrate of caspases during apoptosis [102]. More recently, an iron–sulfur subunit, NDUFS3, was identified as the substrate of the killer lymphocyte protease granzyme A. The cleavage of NDUFS3 at the mitochondrial matrix triggers caspase-independent target cell death through disrupting membrane potential and interfering with ATP generation [103].

Complex I also plays an important role in human immunodeficiency virus (HIV)–induced apoptosis. Specifically, *NDUFA6* was downregulated, as analyzed by differential expression of genes in the apoptotic and nonapoptotic population of cells infected by HIV. Inhibiting complex I activity reduced ATP levels and membrane potential. Inhibiting NDUFA6 expression also induced apoptosis, and overexpression could rescue HIV-induced apoptosis [104]. Similarly, during human cytomegalovirus infection, a protection mediated by a noncoding 2.7-kb viral RNA transcript (β2.7) against the rotenone-induced apoptosis was reported: β2.7 interacts with complex I and prevents the relocalization of its subunit GRIM-19 in response to apoptotic stimuli. This targeting of complex I by viral RNA is important for maintaining the mitochondrial membrane potential and ATP production essential for the viral propagation cycle [105].

8. ADDITIONAL FUNCTIONS OF COMPLEX I OR SUBUNITS OF COMPLEX I

One easy explanation for the large number of subunits and intricacy in mammalian complex I is that it might have acquired regulatory sites and new functions during evolution.

Sequence comparisons suggest that the bovine 39-kDa (human NDUFA9) subunit is related to the short-chain dehydrogenase/reductases such as steroid/cholesterol dehydrogenases, dihydroflavonol reductases, and nucleotide sugar epimerases [106,107]. These enzymes require NADH or NADPH as cofactor, and NDUFA9 also has a nucleotide binding motif that may bind either NADH or NADPH. Because association between short-chain dehydrogenase/reductases and acyl carrier proteins (ACPs) are known, this subunit might have a role as a biosynthetic module with the ACPs.

Similarly, bovine SDAP subunit (human homologue NDUFAB1) is closely related to the ACPs from bacteria and chloroplasts. These ACPs are involved in fatty acid biosynthesis; therefore, this subunit might have a role in mitochondrial de novo fatty acid synthesis, elongation, or desaturation [18]. In *N. crassa*, disrupting the gene for the ACP subunit of complex I resulted in incorrect assembly of complex I and a fourfold increase of the lysophospholipid content of the mitochondrial membranes [108,109]. Thus, a possible role for the mitochondrial ACPs is to recycle lysophospholipids, synthesized from lipid hydroperoxides by a phospholipase and by using an ACP-bound fatty acid for reacylation.

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Mitochondrial ACPs might also be involved in the biosynthesis of lipoic acid or to transfer lipoic acid to proteins such as the pyruvate dehydrogenase complex [110–112].

Also, bovine MWFE (human NDUFA1) protein is required in the synthesis of the mtDNA-encoded ND subunits and their incorporation into the complex [113,114]. The nuclear-encoded subunit B14.7 (human NDUFA11) shows homology with the Tim proteins (Tim17, Tim22, and Tim23) of the translocase of the inner membrane 17/22 (TIM17/22) family, which are involved in protein import from cytosol to the mitochondrial matrix and inner membrane [16,51]. Similarly, two other subunits, NDUFA2 and NDUFA10, have shown homology to mitochondrial ribosomal proteins (L43 and S25) and to mitochondrial thymidine kinase 2, respectively, suggesting a possible connection between complex I and mitochondrial protein synthesis [51].

In recent years, a correlation between mitochondrial complex I OXPHOS capacity and volatile anesthetic sensitivity in *C. elegans* has been demonstrated. More specifically, defective complex I and OXPHOS capacity is directly proportional to the degree of volatile anesthetic sensitivity [115]. Also, patients with mitochondrial diseases impairing complex I activity were specifically sensitive to the volatile anesthetic sevoflurane [116].

9. COMPLEX I DEFICIENCY AND HUMAN DISEASES

As mitochondria are maternally inherited, primary mutations and their respective phenotypes are transmitted only from mothers to their progeny. Because the number of mtDNA copies is rather large, the heteroplasmic condition for a given mutation is possible in a cell or tissue. Therefore, a threshold level for a pathogenic mtDNA mutation is required to alter the phenotype of cell/tissue in a way that leads to clinical manifestation of disease. Over the years, mutations in the subunits/assembly factors of complex I have been observed in various clinical phenotypes, as summarized in Table 1.

LHON is one of first mitochondrial diseases to be identified genetically [117]. In particular, primary mutations in the ND1, ND4, and ND6 genes, with resulting complex I deficiency, play a causative role in the development of the disease [118]. The mtDNA mutations in complex I subunit genes have also been associated with other classical mitochondrial diseases such as MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes), MERRF (myoclonus epilepsy associated with ragged-red fibers), Leigh syndrome, and progressive epilepsy [119].

With the identification of nuclear genes encoding complex I subunits, the corresponding mutations have also been associated with Leigh syndrome, hypertropic cardiomyopathy and encephalomyopathy, hypoglycemia and lactic acidemia, and leukodystrophy [119,120]. Mutations in highly conserved regions of *NDUFS1, NDUFS2, NDUFS3, NDUSF4*, *NDUFS7, NDUFS8, NDUFV1*, and *NDUFV2* were identified [47] and listed in Mitomap database (a human mitochondrial genome database; http://www.mitomap.org). As discussed earlier, defects in the assembly proteins (CIA30, C6ORF66, and B17.2L) were also found in patients with cardioencephalomyopathy and encephalomyopathy [50,52,54].

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Recently, defects in complex I activity were implicated in more common age-related disorders such as sporadic neurodegenerative disorders such as Parkinson's disease [121], type II diabetes, and cardiac diseases [122,123]. In particular, deficiency in the activity of complex I in Parkinson's disease substantia nigra was identified [124], and later an excess of heteroplasmic modifications in *ND5* in patients with Parkinson's disease was also reported [125].

Although not without controversies [126], mtDNA mutations exist in many cancers [127–131]. Mutations have been reported in subunits of all respiratory complexes. We recently did a survey on mtDNA mutations reported in various tumors and cancers; more mutations had been found in complex I genes than in other complexes (Lu, Sharma and Bai, unpublished data). Some of them are also listed in Table 1.

Cancer cells accumulate defects in the mitochondrial genome such as deficient mitochondrial respiration and increased ROS production, which make it more vulnerable to oxidative injury and may account for some of the increased mtDNA mutations often observed in cancer. Usually such mutations are acquired during or after oncogenesis, depending on the type of mutation. Still, how mtDNA mutations contribute to the phenotype of cancer cells remains a question of debate. We recently investigated the contribution of mtDNA mutation and mitochondrial dysfunction in tumorigenesis, using human cell lines carrying a frameshift mutation at the ND5 gene, similar to the mutation identified in colorectal cancer [146]. The heteroplasmic mutation led to enhanced tumorigenesis, possibly through alteration of ROS generation and apoptosis [148].

Again, a common concern is whether mtDNA mutations are primary or secondary events. On the basis of our own data, as mentioned earlier, we propose that the mtDNA mutations in cancer development could function as follows: in the initial stage, cancer cells are mutagenic either because of a carcinogenic insult or the compromised repair mechanism, and mtDNA is more likely to be mutated at this stage. Because of the replicative advantage of mutant mtDNA molecules, such as previously described for mtDNA carrying the mutation associated with mitochondrial encephalomyopathy, mtDNA mutations are enriched to a certain level of heteroplasmy. This process would enhance tumor progression because of elevated ROS generation, which in turn activates the oncogenic pathways; increased genome instability; or both.

Thus, the importance of mitochondrial complex I in energy production, ROS generation, and apoptosis regulation, combined with the genetics of mtDNA, provide a rational explanation for many of the metabolic and stochastic features of human diseases.

10. ANIMAL MODELS FOR COMPLEX I DEFICIENCY

One of the substantial advances in mitochondrial medicine in recent years is the production of various animal models with mitochondrial deficiency, including pharmacologically and genetically generated complex I–defective mice. The mouse Parkinson's disease model involving the neurotoxin MPTP has been widely used [149]. It is hypothesized that MPTP is oxidized to MPP^+, which is then transported into dopaminergic neurons by the dopamine transporter. MPP^+ is accumulated in the mitochondrial matrix and inhibits complex I. The

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damaged complex I appears to generate ROS, which may induce death of neurons. Complex I inhibitors such as rotenone and acetogenins also produced similar effects in rats that resembled those of human Parkinson's disease, such as death of dopamine neurons and formation of Lewy body–like cytoplasmic inclusions [92].

Loss of complex I activity was achieved in transgenic mice with a ribozyme targeting the complex I subunit NDUFA1. In such animals, increased ROS levels in retinal ganglion cells and optic nerve degeneration were recorded [93]. More recently, in an effort to produce a mouse model of LHON, allotopic expression of mutant human ND4 in the mouse visual system resulted in the characteristic swelling of the optic nerve and acute visual loss that have been found for LHON [150]. Unexpectedly, the ROS level in such mice was 10-fold higher than that observed in cultured cells carrying the same mutation.

11. PERSPECTIVES

Remarkable progress has been achieved in illustrating the components, structure, and function of mammalian complex I in recent years; however, multiple gaps remain. Because the importance of complex I function has gained more and more attention, new and exciting findings in this field are expected soon.

Success in combining powerful biochemical and genetic approaches using different model systems for complex I (i.e., yeast *Yarrowia lipolytica*) might provide new information on the function of more or individual complex I subunits. As a complementary approach to the successful investigation of samples from human patients with complex I deficiency, the genetic system bears the hope for providing a platform to isolate more assembly factors.

We expect structural studies, including those with improved electron cryomicroscopy on the intact complex and high-resolution analysis on crystal structure of more sub-complexes and with different ligands, to provide new insights.

Information on the regulation of complex I activity will probably come from characterizing the posttranslational modification, phosphorylation/dephosphorylation, and acetylation/deacetylation of complex I subunits and inducible binding of *trans*-acting elements on complex I. One notice- able development in recent years is the emergence of system biology approaches. Combining mass spectrometry, green fluorescent protein tagging, and machine learning, a previous work identified several new proteins related to complex I activity [151].

Finally, with the availability of increasing numbers of cellular and animal models with complex I deficiencies, more sophisticate and comprehensive investigations are expected to define not only the molecular pathogenesis of mitochondrial diseases but also the regulatory network of complex I functions.

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ABBREVIATIONS

OXPHOS	oxidative phosphorylation
ROS	reactive oxygen species
mtDNA	mitochondrial DNA
NDUFAF1	NADH dehydrogenase (ubiquinone) 1α subcomplex assembly factor 1
NDUFS4	NADH dehydrogenase (ubiquinone) Fe–S protein 4
AIF	apoptosis-inducing factor
CIA30	complex I intermediate–associated protein 30
Ecsit	cytosolic adaptor protein essential for inflammatory response and embryonic development
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PKC	protein kinase C
MAPK	mitogen-activated protein kinase
PI3K	phosphoinositide kinase 3
LHON	Leber's hereditary optic neuropathy
MELAS	mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes
MERRF	myoclonic epilepsy associated with ragged-red fibers

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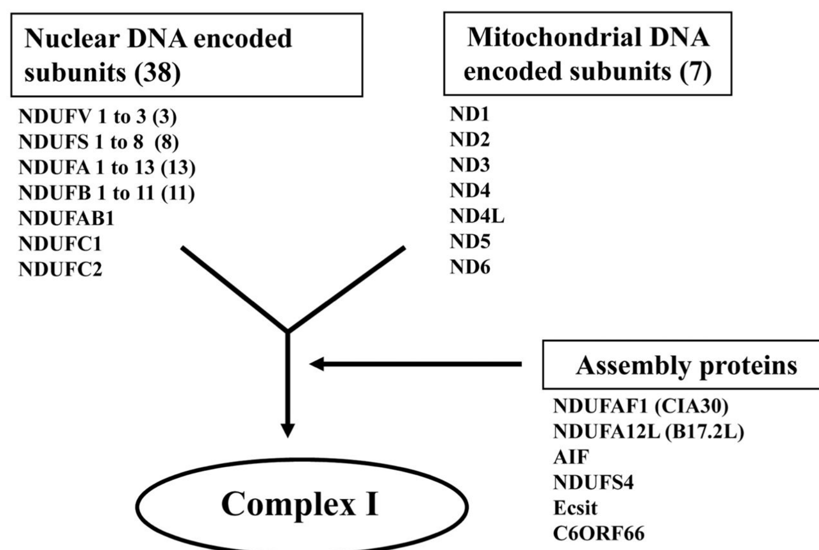

Fig. (1).

Structural constituents of mammalian complex I. The subunit composition of complex I encoded by nuclear and mtDNA along with known assembly proteins are shown. The nuclear-encoded 38 subunits and seven mtDNA-encoded subunits form an L-shaped structure of complex I. Numbers in parentheses signify number of subunits. Assembly proteins, proteins which have been reported to mediate complex I assembly or maintain the complex I stability.

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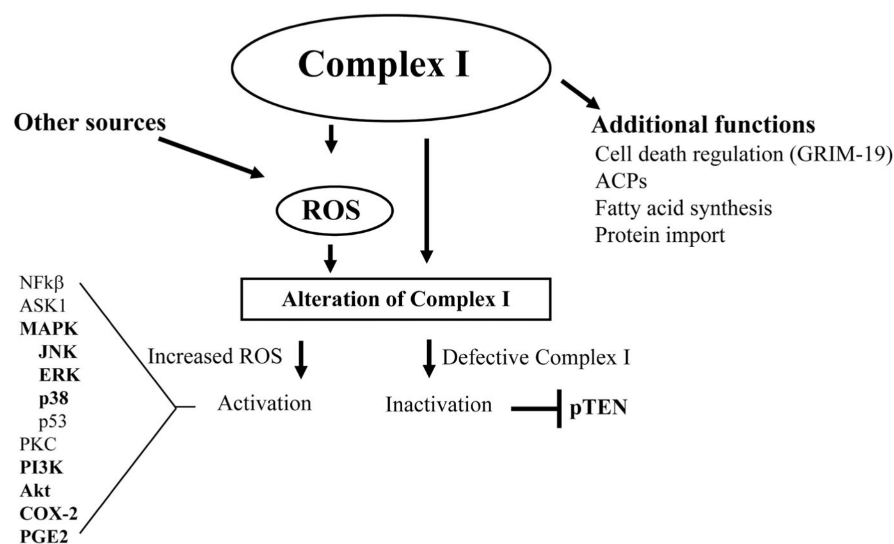

Fig. (2).

Functionality of complex I in different cellular pathways. Signaling pathways triggered by ROS production through complex I or other sources are shown. ROS can also affect complex I to generate more free radicals. Some additional functions are suggested based on the similarity of complex I subunits to other proteins. NFkβ, nuclear factor κB; ASK1, apoptosis signal–regulating kinase 1; MAPK, mitogen-activated protein kinase; JNK, c-jun N-terminal kinase; ERK, extracellular signal–regulated kinase; PKC, protein kinase C; PI3K, phosphoinositide kinase 3; Akt, serine/threonine protein kinase; COX-2, cyclooxygenase 2; PGE2, prostaglandin E2; pTEN, phosphatase and tensin homolog; NADH, reduced nicotinamide adenine dinucleotide; ACPs; acyl carrier proteins. Pathways involved in the direct association of complex I are in boldface type.

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Table 1

Complex I Deficiency and Human Disease*

Disorder	Genetic origin	Clinical phenotype	Functional defects	Ref.
Classical mitochondrial diseases (complex I deficiency)	mtDNA-encoded subunits: ND1–ND6, ND4L	LHON, MELAS, adult-onset dystonia, Leigh syndrome, CPEO, exercise intolerance, MERRF, bipolar disorder, MCI, ptosis, MW, NIDDM	Increased or altered mitochondrial ROS production, reduction in complex I activity, disrupted complex I assembly, decreased mitochondrial membrane potential and complex I activity	[40, 118, 132, 133]
	Nuclear-encoded subunits: NDUFS1, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFV1, NDUFS2, NDUFV2, NDUFS6	Leukoencephalopathy, Leigh-like syndrome, hypertrophic cardiomyopathy and encephalopathy, lethal infantile mitochondrial disease with hypotonia, lethargy and seizures	Reduced residual activity of complex I, defective complex I assembly	[134–137]
	Assembly proteins: CIA30, C6ORF66, B17.2L, C20orf7	Cardioencephalomyopathy, cardiomyopathy, progressive encephalopathy, lethal neonatal complex I deficiency	Reduced levels and activity of complex I, complex I assembly defect	[50, 52, 54, 138]
Neuro degenerative disorder	mtDNA-encoded subunits: ND1, ND5	Idiopathic Parkinson's disease	Loss of complex I activity and a tendency toward apoptotic cell death, reduction of protein level of complex I subunits, misassembled and oxidatively damaged complex	[125,139– 141]
	Nuclear-encoded subunits: NDUFV2, NDUFS1	Down syndrome and Alzheimer disease		
Cancer	mtDNA-encoded subunits: ND1–ND6, ND4L	Prostrate, pancreatic, colon, thyroid, bladder, head and neck cancer, breast, medulloblastoma	Metabolic alterations with mitochondria, i.e., increased gluconeogenesis, glycolysis, lactic acid production, decreased oxidative phosphorylation	[142–146]
	Nuclear-encoded subunits: GRIM-19	Hürthle cell tumors	Defective complex I assembly, apoptosis, and defective mitochondrial metabolism	[99, 147]

*Table describes the most common clinical phenotypes associated with mutations in different subunits of complex I. The common functional defects observed in the given phenotypes were inferred from the cited references. LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes; CPEO, chronic progressive external ophthalmoplegia; MERRF, myoclonic epilepsy associated with ragged-red fibers; MCI, multiple cerebral infarction; MW, muscle weakness; NIDDM, non–insulin-dependent diabetes mellitus.