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Mini Review

Mitochondrial complex I in the network of known and unknown facts

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Abstract. The mitochondrial respiratory chain consists of five multi-subunit complexes embedded in the inner mitochondrial membrane. Complex I is the largest and most complicated proton pump of the respiratory chain encoded by both the mitochondrial and nuclear genomes. In this minireview, attention is given to recent knowledge on the structure, catalytic properties, supramolecular organisation of complex I and its possible role in the triggering of apoptosis.

Key words: Mitochondria — Complex I — Respiratory chain — Apoptosis

Abbreviations: CI, complex I; CIII, complex III; $\Delta\psi_m$, mitochondrial transmembrane potential; NO, nitric oxide; ROS/RNS, reactive oxygen/nitrogen species; SNO, nitrosothiols.

Introduction

Complex I (CI), NADH:ubiquinone oxidoreductase (EC 1.6.5.3), as the first multiprotein complex involved in the formation of the proton-motive force in mitochondria, carries out three well-defined functions of critical importance for cell physiology. The enzyme reoxidizes NADH, thus providing certain steady-state NAD^+/NADH ratios required for continuous operation of the oxidative metabolic pathway. It also serves as the major electron entry point to the respiratory chain for further energy transduction and the enzyme itself can work reversibly and thus significantly contributes to formation of the electrochemical gradient ($\Delta\mu_{H+}$). Mechanism of electron transfer coupling to proton translocation is a matter of debate (Vinogradov 2001; Brant 2006). Besides its well-known redox role in the electron transport chain, CI is considered one of the main sites of reactive oxygen species (ROS) production (Kussmaul and Hirst 2006). Moreover, it is believed that CI dysfunction and the subsequent impairment of mitochondrial respiration provoke the activation of the mitochondrial-dependent apoptotic machinery by directly triggering the release of apoptogenic molecule cytochrome *c* from defective mitochondria (Green and Kroemer 2004; Clayton et al. 2005; Perier et al. 2007). Finally, loss of mitochondrial CI catalytic activity in the electron transport chain is associated with a wide spectrum of neurodegenerative disorders (Table 1) or acute pathophysiological processes such as ischemia-reperfusion injury (Almeida et al. 1995; Tompkins et al. 2006; Racay et al. 2007) and heart failure as well as with normal aging process (Petrosillo et al. 2008).

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Complex I structure

CI is L-shaped protein, consisting of two perpendicular arms: a hydrophobic membrane arm which resides in the mitochondrial inner membrane and a hydrophilic matrix arm (Hofhaus et al. 1991; Janssen et al. 2006). Currently, 45 subunits have been described for bovine CI, seven of which are encoded by mitochondrial DNA (Carrol et al. 2006) and correspond to hydrophobic components named ND1, ND2, ND3, ND4, ND4L, ND5 and ND6 (Fig. 1). The minimal active form of the enzyme was found in bacteria, composed of 14 subunits, all of which are homologous to their mitochondrial counterparts. Three functional modules can be distinguished for mammalian CI (Brant et al. 2003). The first is the dehydrogenase module, which is responsible for oxidation of NADH and consists of the NDUFV1, the NDUFV2 and the NDUFS1 subunits. The second is the hydrogenase module, which guides the released electrons to electron acceptor ubiquinone and consists of the NDUFS2, NDUFS3, NDUFS7 and NDUFS8 subunits. The third is the proton translocation module, which consists of all ND

Table 1. Mutation in nuclear and mitochondrial DNA associated with CI deficiencies

Disease	Gene	Reference
Leigh syndrome	NDUFS4	Coene et al. (2001)
	NDUFS7	Triepels et al. (1999)
	NDUFS8	Loeffen et al. (1998)
	NDUFS1	Martin et al. (2005)
Leigh-like syndrome	NDUFS4	Budde et al. (2000)
	NDUFV1	Benit et al. (2001)
Hypertrophic cardiomyopathy and encephalomyopathy	NDUFS2	Loeffen et al. (2001)
Macrocephaly, leukodystrophy and myoclonic epilepsy	NDUFV1	Schuelke et al. (1999)
Leber's hereditary optic neuropathy (LHON)	ND1, ND4, ND6	Yen et al. (2006)
Parkinson disease	ND5 ?	Parker and Parks (2005) Ross and Smith (2007)

subunits. All other subunits are called accessory subunits and their functions have to be clarified. It is hypothesized, they stabilize or protect the complex from damage by ROS and several of these subunits may have an additional function, too. For example, the NDUFA13 (GRIM-19, or gene associated with retinoid-IFN induced mortality in bovine CI) subunit is also a cell death regulatory protein induced by interferon-β and retinoic acid and was demonstrated to be released from the mitochondrion upon apoptosis (Fearnley et al. 2001; Huang et al. 2004). An apoptotic function is also described for subunit NDUFS1 (Ricci et al. 2004). The NDUFA9 subunit is known to harbour a NADH/NADPH binding site (Yamaguchi et al. 2000; Schulte 2001) while the NDUFS4 subunit was described to be phosphorylated by a cAMP-dependent protein kinase (PKA), possibly indicating a function in regulation of CI activity (Papa 2002; Papa et al. 2008). It proved later to be another 18 kDa subunit, the ESSS (NDUFB11 in humans) (Chen et al. 2004). For several other subunits some features are known, but the exact additional function is yet unclear.

Engagement of complex I in supramolecular organisation of the respiratory chain

Despite increasing knowledge on the structure of the individual complexes of the respiratory chain, their supramolecular organisation is still largely unknown. Two extreme alternative models for the arrangement of the respiratory chain complexes have been proposed. According to the currently favoured random collision model (Hackenbrock et al. 1986), all components of the respiratory chain diffuse freely in the membrane, and electron transfer depends on the random collision of the involved components. In the solid state model (Chance and Williams 1955) the substrate is channelled directly from one enzyme to the next. However, recent evidence, mainly based on native electrophoresis, has suggested that the mitochondrial respiratory chain is organized in the form of supercomplexes (Schägger and Pfeiffer 2000; Schäfer et al. 2006). Based on those results of mildly solubilised bovine heart mitochondria, the respirasome model was introduced. This model postulates the quantitative assembly of I, III and IV respiratory complexes into supercomplexes. They all contain a CI monomer, a complex III (CIII) dimer (I_1III_2), and a variable copy number of monomeric complex IV ($I_1III_2IV_{1-4}$) (Schägger and Pfeiffer 2001). What potential advantages could be provided by associations of complexes into respirasomes? It could include e.g. substrate channelling of quinones and/or cytochrome *c*, sequestration of reactive intermediates like ubisemiquinone, and stabilization of individual complexes by supramolecular assembly (Schägger and Pfeiffer 2000). Toward a molecular understanding of the structural and functional roles of respirasome formation, Schägger et al. (2004) focused on analysis of the structural integrity of human respirasomes in patients with defined defects of individual respiratory complexes. They showed that the formation of respirasomes is essential for the assembly/stability of CI. Genetic alterations leading to a loss of CIII prevented respirasome formation and led to the secondary loss of CI (Schägger et al. 2004). In a parallel and independent study Acín-Perez et al. (2004) came to the same conclusion. They confirmed that this structural dependency is not reciprocal and the absence of CI does not impair CIII biogenesis or function. Combined complex I/III defects associated with alterations in the NDUFS4 protein of CI have been observed and clarified by Budde et al. (2000). It has been suggested that the truncated NDUFS4 protein may interfere with the formation of supercomplexes thereby to lead to a combined deficiency of CI and CIII. Also enhanced radical production caused by CI deficiency may have a secondary influence on CIII activity.

The interdependence of CI and CIII could be expected on account of direct electron transfer between these two complexes which may be facilitated by physical interactions.

However, complex IV has not been shown to directly interact with CI but recent observations made by Rocher et al. (2005), Diaz et al. (2006), and Li et al. (2007) showed that CI assembly requires the presence of complex IV, even in very small amounts. It was reported that 17-kDa subunit of CI, which has been implied in the membrane arm assembly pathway (Ugalde et al. 2004), may need to be associated with complexes III and IV in order to associate with the ND6 subunit. Because CI is the major entry point for electron transfer, it is likely that this supercomplex is the central functional unit for mitochondrial respiration.

Catalytic properties of complex I

The central position of CI in the metabolic network implies that the enzymes activity must be finely regulated both in the long-term scale at the level of its biogenesis and in shorter-term scale *via* covalent modification or alternation of its catalytic activity by certain ligands. However, due to an intricate structure of the enzyme, very little is known about regulatory mechanism of CI. The first regulatory mechanism can involve specific serine phosphorylation in numerous subunits of CI by a PKA (Papa et al. 2008). *In vivo* activation of cAMP cascade promotes the NADH: ubiquinone oxidoreductase activity of CI and decreases the cellular level of ROS. These effects of cAMP were found to be associated with PKA-mediated serine phosphorylation in the conserved C-terminus of the subunit encoded by the nuclear gene NDUFS4. Chen et al. (2004) found that PKA activity associated with the matrix side of the inner membrane of the organelle appears to be responsible for the phosphorylation of inner membrane proteins of 18 (ESSS) and 6.5 kDa (MWFE). Subunit ESSS has no known role, but subunit MWFE is required for assembly into CI of seven hydrophobic subunits encoded by the mitochondrial genome. Yadava et al. (2008) investigated the significance of potential phosphorylation of these two subunits in CI assembly and function by mutational analysis of the phosphorylation sites. It was found that these mutations of the phosphorylation sites significantly reduced CI activity.

Another possible regulatory mechanism that may operate under physiological conditions is the slow reversible interconversion between the catalytically active (A) and inactive (D) forms of CI (Vinogradov 1998; Vinogradov and Grivennikova 2001). The A form catalyzes the physiologically relevant rotenone-sensitive NADH:ubiquinone reductase reaction while the D form can be fully reduced by NADH and oxidized by artificial electron acceptors like ferricyanide or hexammineruthenium (Zickermann et al. 2000), but it is unable to transfer electrons to ubiquinone. Incubation of CI at elevated temperature under conditions where enzyme turnover is not permitted either because ubiquinone is fully reduced or because NADH is absent, results in a slow decline of enzyme activity measured as the initial rate of the NADH:quinone reductase or oxidase reactions. This process is called deactivation. The A to D transition for mammalian enzyme is extremely sensitive to temperature (Kotlyar and Vinogradov 1990) and it is detectable only over 30°C. The presence of D form is seen by as more or less pronounced lag phase in the onset of the catalytic activities assayed in forward – NADH oxidation or reverse – NAD^+ reduction by ubiquinol (Kotlyar and Vinogradov 1990). The lag phase is completely eliminated after preincubation of enzyme preparation with NADH under conditions that permit enzyme turnover (Kotlyar and Vinogradov 1990). The duration of this lag is strongly pH-dependent and activation is inhibited by bivalent cations (Ca^{2+}, Mg^{2+}) (Kotlyar et al. 1992) and free fatty acids (Loskovitch et al. 2005). Activation of D form of the enzyme is a result of slow activating turnover, which includes fast reduction by NADH and slow oxidation by quinone (Maklashina and Vinogradov 1994).

The phenomenon of the reversible active/deactive transition described above has been shown for preparations of different degrees of resolution i.e. purified CI (Maklashina et al. 1994), submitochondrial particles (Kotlyar and Vinogradov 1990) or intact rat heart mitochondria (Grivennikova et al. 2001), but was not observed in membranes of prokaryote (Kotlyar et al. 1998; Grivennikova et al. 2003). However, could CI undergo the A↔D transition *in vivo*? Under normoxic conditions, where CI catalyzes steady-state NADH oxidation, it seems likely that the majority of the enzyme would be in the active form (Grivennikova et al. 2001). Under hypoxic conditions such as global ischemia, where lack of oxygen leads to high levels of ubiquinol, it is expected that CI would be deactivated. This question has been examinated by Maklashina et al. (2002) in *ex vivo* experiments on perfused rat hearts during anoxia-oxygen reperfusion cycles. They presented that in freshly isolated rat heart, as well as after 60 min of normoxic perfusion, CI is present in an almost fully active state. Either global ischemia or anoxic perfusion converts the majority of CI to the deactive state. Thus, alternation of normoxic/anoxic perfusion causes reversible transition of CI from highly active to predominantly deactive state. The existence of the transition between two functional states of CI arouses interest in which of subunits could play a critical role in the transition. Galkin and coworkers (2008) propose that this subunit is the ND3 subunit. They reported the identification of the cysteine residue selectively accessible only in the D-form of CI in the loop connecting two transmembrane helices of the mitochondrial encoded ND3 subunit. This loop connects the ND3 subunit of the membrane arm with the PSST subunit of peripheral arm of CI, placing it in a region that is known to be critical for the catalytic mechanism of CI. Mutations in three positions of the loop were previously reported to cause Leigh syndrome

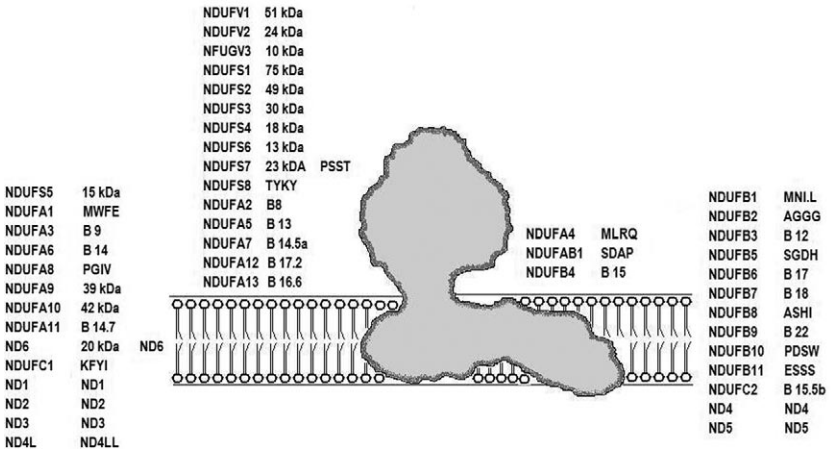

Figure 1. Complex I subunit topology (free according to Janssen et al. 2006).

or progressive mitochondrial disease (McFarland et al. 2004; Sarzi et al. 2007).

Could complex I be an early mitochondrial target for nitrosative stress?

It might be expected that triggering cell signalling pathways may affect the A↔D transition and this way to control mitochondrial respiration. Regulation of CI activity may have important consequences for production of ROS/RNS (reactive oxygen/nitrogen species) which might result in the initiation of caspase cascade culminating in the death of cell. Considering that transition from the A- to the D-form may take place during pathological conditions when the turnover of the enzyme is limited at physiological temperatures, such as during hypoxia or when the NO : oxygen ratio increases (Moncada and Erusalimsky 2002) it can be expected that the conformation change of CI hides more important meaning. Further, considering that mitochondria contain sizeable thiol pools, are abundant in transition metals and have an internal alkaline pH, all of which modulate S-nitrosothiols (SNO) biochemistry (Foster and Stamler 2004), it begs the question of possible posttranslation modifications of cysteine thiols to form SNO as a principle mechanism of NO-based signalling. Studies of the regulation and biological impact of these modifications are technically difficult, due to degradation of SNO in response to light, low-molecular-mass thiols and metal ions, as well as limited methodology to detect S-nitrosated targets. However, indirect evidence exists for S-nitrosation of CI (Brown and Borutaite 2004; Hsu et al. 2005). These studies showed CI inhibition by NO, peroxynitrite and SNO that was reversible by light or reduced thiols, and therefore may be due to S-nitrosation or Fe-nitrosylation of the complex. There is also an irreversible inhibition by peroxynitrite, other oxidants and high levels of NO, which may be due to tyrosine nitration, oxidation of residues or damage to iron sulphur centres. Burwell and co-workers (2006) provided direct evidence for S-nitrosation of CI (75 kDa subunit). These authors were the first to show increased S-nitrosation of mitochondrial proteins from hearts subjected to the cardioprotective process known as ischemic preconditioning. Reversibly inhibition of CI by S-nitrosation would decrease the electron flux through the chain, thereby decreasing ROS generation. Previously, it has been shown that inhibition of complex IV by NO can cause back-up of electrons in the respiratory chain and increases ROS generation at CIII (Brookes and Darley-Usmar 2002). Whereas CI inhibition may increase ROS at complex itself (Taylor et al. 2003), it is known that CI is quantitatively a much smaller source of ROS than CIII (Chen et al. 2003). Thus, a small SNO-induced increase in production of ROS by CI may be beneficial if it inhibits large-scale ROS generation at CIII. Still and all, little is known about whether such nitrosation occurs in physiological conditions and what the possible cellular mechanisms are. Galkin and Moncada (2007) found that the active/deactive conformational state of CI is a significant factor for the interaction of the enzyme with nitrosothiols and peroxynitrite. They showed only the D-form of CI was susceptible to inhibition while the A-form appeared insensitive to such treatment. The susceptibility of CI to inactivation and increased superoxide production on S-nitrosation indicates that it has a significant role during nitrosative stress in pathophysiological conditions. Because NO is capable of interacting with mitochondria produced superoxide anion before the latter can be converted by superoxide dismutase to hydrogen peroxide (Huie and Padmaja 1993), authors speculate that superoxide production in pathological condi-

tions and in the presence of NO would result in formation of peroxynitrite ($ONOO^-$) in the mitochondrial matrix followed by irreversible inhibition of the D-form of CI that may accumulate during hypoxic conditions. On the other hand, Gostimskaya et al. (2006) assume that an exposure of highly reactive SH-groups in the D-form is unlikely to be the only difference between the active and deactivated CI. Due to the exceptionally high activation energy for the A↔D transition in mammalian mitochondria, they expect gross structural rearrangements of the complex and the sensitivity of the enzyme to other than SH-targeted regulatory signals, such as multiple protein phosphorylation or dephosphorylation, which could also be A↔D-transition sensitive. Overall, it is evident that S-nitrosation of CI may play important roles in mitochondrial physiology and pathology, and that further work is required to elucidate the upstream mechanisms that regulate this important event.

Role of complex I in triggering of apoptosis

It is generally known that reduced activity in mitochondrial CI is associated with wide spectrum of neurodegenerative diseases. Moreover, it is believed that CI dysfunction and the subsequent impairment of mitochondrial respiration provoke the activation of the mitochondrial-dependent apoptotic pathway by directly triggering the release of cytochrome *c* and activation caspase cascade executing cell death (Green and Kroemer 2004; Clayton et al. 2005). Contrary to this view, Perier et al. (2005) argue that CI inhibition produces a mitochondrial oxidative damage that includes cardiolipin peroxidation. That affects the binding of cytochrome *c* to the mitochondrial inner membrane, leading to an increased soluble pool of cytochrome *c* in the intermembrane space. Upon mitochondrial permeabilization by the cell death agonist Bax, more cytochrome *c* is released to the cytosol from brain mitochondria and this way inhibition of CI decreases the threshold for activation of apoptosis. How CI deficiency leads to Bax activation is currently unknown. Going on their research into CI dysfunction, Perier and co-workers (2007) propose a pathogenic scenario in which CI deficiency results in neuronal death. In this scenario, inhibition of CI by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) blocks the flow of electrons along the mitochondrial respiratory chain, which results in increased production of ROS. Mitochondrial ROS then increase the soluble pool of cytochrome *c* in the intermembrane space, whereas ROS outside the mitochondria cause damage to different cellular elements, including DNA. DNA damage activates both p53 with subsequent transcriptional upregulation of Bax and JNK which participates in Bax mitochondrial translocation. Once Bax is inserted into the mitochondrial outer membrane, induces the release of cytochrome *c* followed by caspase activation and cell death at the end (Fig. 2).

The process of apoptosis is orchestrated by caspases through cleavage of specific substrates in the cell (Green and

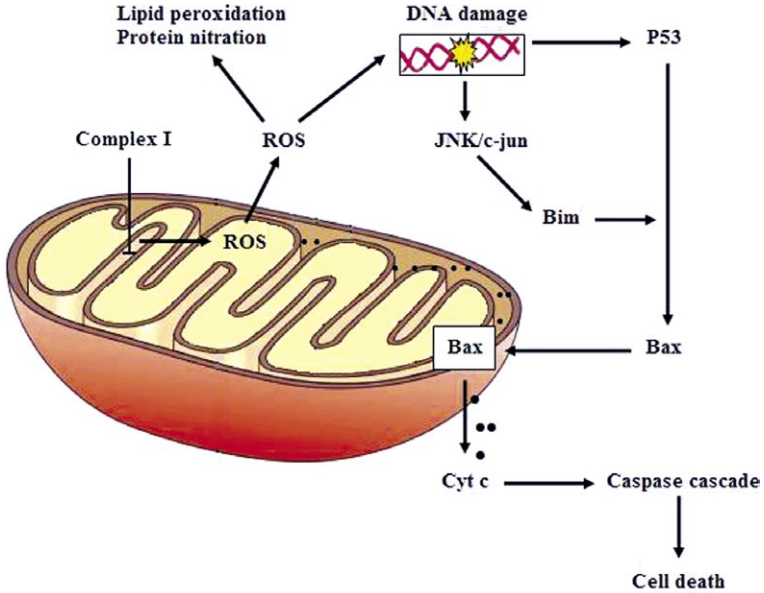

Figure 2. Assumed pathogenic scenario induced by CI impairment. CI inhibition results in increased production of ROS which cause damage to different cellular elements, including DNA. DNA damage activates both p53 with subsequent transcriptional upregulation of Bax and JNK which participates in Bax mitochondrial translocation. Once Bax is inserted into the mitochondrial outer membrane, induces the release of cytochrome *c* (cyt c) followed by caspase activation and cell death.

Evan 2002). While some caspase substrates are known to be responsible for DNA fragmentation and nuclear condensation, potential caspase substrates in mitochondria must be determined. Making an effort to meet this target, Ricci and co-workers (2004) identified one key caspase substrate, the 75 kDa subunit of CI and showed that the cleavage this subunit by caspases is responsible for disruption of electron transport and transmembrane potential, leading to production of ROS, loss of ATP production and mitochondrial damage.

Importance of CI position in both metabolism and apoptosis starts being exposed in the light of identification of novel cell death-regulatory molecule, GRIM-19 (Angell et al. 2000). GRIM-19 was originally identified as a nuclear protein with apoptotic nature in interferon (IFN)- and all-*trans*-retinoic acid (RA)-induced tumour cells. Subsequently, its homolog was found in mitochondrial CI purified from bovine heart (Fearnley et al. 2001) and human heart mitochondria (Murray et al. 2003). These results raise a question about the roles of GRIM-19 in cells. Huang et al. (2004) attempted to address these questions by investigating the biological roles of GRIM-19 with genetic approaches. Their results demonstrate that GRIM-19 is a component of CI that is essential for the assembly of CI and the integrity of the whole mitochondrial electron transfer chain. In the recent review by Kalvakolanu (2004), it was proposed that GRIM-19 may initiate apoptosis upon IFN-β/RA stimulation and to translocate from mitochondria to the nucleus. However, Huang's group (2007) testing this hypothesis, did not find nuclear translocation in both nonapoptotic and apoptotic cells. Although the genetic evidence showed the effect of GRIM-19 on mitochondrial CI assembly and activity, it is unknown whether this protein is a structural subunit or a functional one for CI activity. To further understand its function in CI, Lu and Cao (2008) dissected the functional domains of GRIM-19 to acquire a systematic series of internal and truncation mutants. By doing so, they defined that the N-terminal domain (1–60 aa) is essential for the mitochondrial localization and also for its incorporation to CI. The middle region (70–100 aa) is required for the electron transfer activity and the C-terminal promote GRIM-19 assembly to CI. Furthermore, they showed that the C-terminal region of GRIM-19 has a unique role in the maintenance of mitochondrial transmembrane potential ($\Delta\psi_m$), which overlapped with its electron transfer activity, while the last 10 residues affected ability of GRIM-19 to be assembled to CI. Because disruption of $\Delta\psi_m$ by GRIM-19 mutants was not sufficient to trigger the release of cytochrome *c,* the authors assume that it sensitizes cells to undergo cell death after the classical death signals. The exact mechanism is currently unclear.

In spite of the primary role in mitochondrial CI, other functions of GRIM-19 are not excluded. To reveal its biological function as well as the possible links among diverse biological processes, that it is involved in, will require further effort and investigation.

Conclusion

Complex I, the largest and the most complicated enzyme complex of the respiratory chain, has been subjected to considerable investigation in recent years. Significant progress has been made in the field of understanding of electron transfer, catalytic properties, structure, composition, assembly or its engagement in a wide spectrum of mitochondrial diseases and aging. Recent observations on CI involvement in apoptotic pathway *via* its structural subunits imply that the complex is not independent entity but rather a part of a physically and functionally interconnected cellular network. Unravelling the intricate mechanisms underlying the functioning of this membrane-bound enzyme complex in health and disease will pave the way for developing adequate diagnostic procedures and advanced therapeutic treatment strategies in the future.

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