

Review

Mitochondrial complex I and cell death: a semi-automatic shotgun model

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Mitochondrial dysfunction often leads to cell death and disease. We can now draw correlations between the dysfunction of one of the most important mitochondrial enzymes, NADH:ubiquinone reductase or complex I, and its structural organization thanks to the recent advances in the X-ray structure of its bacterial homologs. The new structural information on bacterial complex I provide essential clues to finally understand how complex I may work. However, the same information remains difficult to interpret for many scientists working on mitochondrial complex I from different angles, especially in the field of cell death. Here, we present a novel way of interpreting the bacterial structural information in accessible terms. On the basis of the analogy to semi-automatic shotguns, we propose a novel functional model that incorporates recent structural information with previous evidence derived from studies on mitochondrial diseases, as well as functional bioenergetics.

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Mitochondrial complex I is a large enzyme complex of over 40 subunits embedded in the inner mitochondrial membrane that forms the first site of oxidative phosphorylation.¹ It feeds the ubiquinone (Q) pool with reducing power collected from the mitochondrial matrix. Complex I dysfunction is the most common mitochondrial defect leading to cell death and disease.^{1–3} For a long time, the lack of structural information has prevented a molecular understanding of the pathological implications of complex I dysfunction and its overall mechanism of action. Recently, however, detailed structural information has been reported for the membrane-embedded region of complex I of two bacteria, *Escherichia coli* and *Thermus thermophilus* and of the yeast *Yarrowia lipolytica*.^{4,5}

This new structural information has revealed an unexpected architecture of the bioenergetic machinery of complex I, with a large physical separation between the Q reduction site and the membrane-embedded proton-pumping units. Such physical separation rules out the majority of models previously proposed for the mechanism of complex I, based on the assumption of direct coupling between the chemistry of Q reduction and the vectorial movement of protons across the membrane. However, recent proposals for complex I function still incorporate principles of direct coupling of Q redox chemistry with indirect proton-pumping devices^{6,7} that were introduced a long time ago.⁸ Moreover, the mechanisms proposed so far do not take into account the biochemical effects of mitochondrial DNA (mtDNA) pathogenic mutations and the different complex I specificity for quinone analogs.

Noticeably, the historical success of the elegant Q cycle scheme, which has proved so valuable in explaining the mechanism of cytochrome *bc*₁ complex,⁹ continues to influence the interpretation of complex I function, even in the face of the recent structural information. This structural information sets aside complex I from any previously known system reacting with Q and pumping protons across the membrane.

The purpose of this article is to produce a creative framework to render the recent structural information on bacterial complex I broadly accessible to scientists interested in mitochondrial complex I and its implications for cell death and disease. In this endeavor, we have taken a completely fresh view of how complex I may work, based on the analogy with the mechanism of semi-automatic shotguns.

Fitting the Mitochondria-Encoded NADH Dehydrogenase (ND) Proteins in the Bacterial Structure

The vast majority of mitochondrial genomes contain seven genes that form the membrane domain of complex I, which are labeled ND1 to ND6 plus ND4L^{10,11} (Table 1). All these genes encode very hydrophobic subunits that are co-translationally inserted into the mitochondrial membrane with multiple transmembrane stretches (TMS, Table 1). In some green algae, however, ND3 and ND4L are not present in the mitochondrial genome but are instead encoded by nuclear DNA,¹² suggesting that these subunits may be imported into

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Abbreviations: I₅₀, concentrations yielding 50% inhibition; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like syndrome; mtDNA, mitochondrial DNA; ND, NADH dehydrogenase (gene); Q, ubiquinone; Q-10, ubiquinone-10; Q-0, 2,3-dimethoxy,5-methyl, *p*-benzoquinone; QH2, ubiquinol; TMS, Trans Membrane Stretches

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Table 1 Complex I subunits in the membrane domain and pathogenic mutations

<i>Thermus thermophilus</i>	<i>Escherichia coli</i>	<i>Homo sapiens</i>	TMS ^a	Mutations: reported ^b	Mutations: confirmed ^b	Reported mutations probably damaging ^c
NQO8 (Q60019)	NuoH (P0AFD4)	ND1 (P03886)	8	26	3	13
NQO14 (Q56229)	NuoN (P0AFF0)	ND2 (P03891)	14	9	0	7
NQO7 (Q56217)	NuoA (P0AFC3)	ND3 (P03897)	3	1	3	1
NQO13 (Q56228)	NuoM (P0AFE8)	ND4 (P03905)	14	8	3	4
NQO11 (Q56226)	NuoK (P0AFE4)	ND4 L (P03901)	3	3	0	3
NQO12 (Q56227)	NuoL (P33607)	ND5 (P03915)	16	22	3	14
NQO10 (Q56225)	NuoJ (P0AFE0)	ND6 (P03923)	5	12	5	8

The table lists the bacterial subunits of the membrane domain of complex I aside to their ND counterparts in the mitochondria (identified by UniProt accession numbers for each species, in brackets). The mutations in ND genes related to human diseases that have been reported so far are shown. Mutations resulting in amino acid changes that are probably damaging (last column) have been selected within the reported mutations by using the prediction tool PolyPhen-2. ^aTMS, trans membrane stretches, estimated from crystal structures⁴ and also with Phobius: <http://phobius.sbc.su.se/index.html>. ^bAccording to the definitions and data reported in: <http://mitomap.org/bin/view.pl/MITOMAP/MutationsCodingControl>, accessed 5 July 2011. ^cNumber of probably or possibly damaging mutations at the protein level, which have been predicted using PolyPhen-2: <http://genetics.bwh.harvard.edu/pph2/index.shtml>

the mitochondria due to the reduced number of their TMS compared with the other ND subunits (Table 1). Plants also have an additional mitochondria-encoded subunit, ND7.¹³ Except ND7, the ND proteins have homologous counterparts in bacterial complex I, but their nomenclature differs considerably in the bacteria for which structural information is now available. In Table 1 we present the correspondence between the bacterial proteins and the mitochondrial ND subunits, with the human ones as reference for all organisms.

Structure–function insights in mitochondrial complex I have been previously obtained from the analysis of mutations in the ND genes that are associated with human diseases. Disruptive mutations in ND subunits, mostly affecting the assembly of complex I, are commonly found as somatic mutations in oncogenic tumors, but not as germline maternally inherited mutations associated with human diseases, because of their lethality.^{14,15} Instead, many missense mutations affecting conserved residues in ND subunits are causative of human diseases, ranging from mono-symptomatic pathologies, such as Leber's hereditary optic neuropathy (LHON), to more severe and multi-systemic clinical phenotypes, such as mitochondrial encephalomyopathy, lactic acidosis and stroke-like syndrome (MELAS) and Leigh syndrome.¹¹ Thus, different clinical entities are most probably determined by variable degrees of complex I dysfunction.^{11,16} The best-studied pathogenic mutations are those associated with LHON, which map in the ND1, ND4 and ND6 subunits (Table 1, see also Bridges *et al.*¹⁷). They have been modeled to lie in conserved hydrophilic loops protruding at the matrix side of the mitochondrial membrane, in the ND1 and ND4 subunits, and in a highly conserved TMS in the ND6 subunit.^{16–21}

Because the equivalent loops of the bacterial homologs of ND proteins are not yet resolved in the crystal structures,^{4,5} it is hard to precisely locate these and other pathological mutations in the reported structure of bacterial complex I. However, the combination of modeling studies with current structural information⁴ suggests the presence of hotspots in complex I structure that may contain most of these pathogenic mutations.^{16,18} A major hotspot in the ND1 subunit would lie at the interface between the hydrophilic domain and the membrane domain of complex I, encompassing part of the

large Q binding pocket and the structural elements that can transmit redox-driven conformational changes to the proton-pumping subunits.⁴ Moreover, the great majority of the pathogenic mutations that affect the ND6 subunit lie within the second TMS of the protein,^{17,19,21} suggesting a critical role of this region in complex I function.

Primary and provisionally pathogenic mutations in the overlapping spectrum of LHON/MELAS/Leigh syndrome have been found also in the ND5 and ND3 subunits. Conversely, the ND2 and ND4L subunits harbor only reported, not confirmed mutations (for the complete list of mtDNA mutations, see the MITOMAP repository²²). Table 1 shows the number of confirmed and reported mutations for each ND subunit, as well as the pathogenic potential of the amino acid substitutions associated with reported mutations as assessed by the on-line tool PolyPhen-2, which predicts the possible impact of amino acid substitutions on the structure and function of human proteins.²³ ND1, ND4, ND5 and ND6 are most affected by pathogenic mutations (Table 1), suggesting that these subunits form the functional core in the bioenergetic machinery mitochondrial complex I (Figure 1). ND3 and ND2 may also have a role in this machinery, because ND3 harbors confirmed pathogenic mutations and ND2 some potentially pathogenic mutations (Table 1). However, we note that ND2 has the most divergent structure from its bacterial counterparts among all mitochondrial ND proteins.^{17,25,28} Consequently, ND2 is less conserved than the ND4 and ND5 proteins,^{25,28} despite sharing a similar helical core related to sodium/proton antiporters.⁴ Moreover, the ND2 subunit has been associated with extra-mitochondrial proteins as well.²⁹ Considering this evidence, we propose an ancillary role for the ND2 subunit, even if current information cannot exclude its possible involvement in proton pumping.²⁴

Pathogenic LHON mutations have been shown to alter the reaction with either the Q substrate or the quinol product of complex I and decrease its binding to Q-antagonist inhibitors, for example, rotenone.^{20,21} These functional effects have been reproduced by site-directed mutagenesis in the equivalent subunits of the bacterial complex.^{17,19,30} Consistent with these findings, the ND1, ND4 and ND5 subunits have been shown to bind analogs of various potent inhibitors, from rotenone to plant acetogenins and synthetic pesticides.^{31–34}

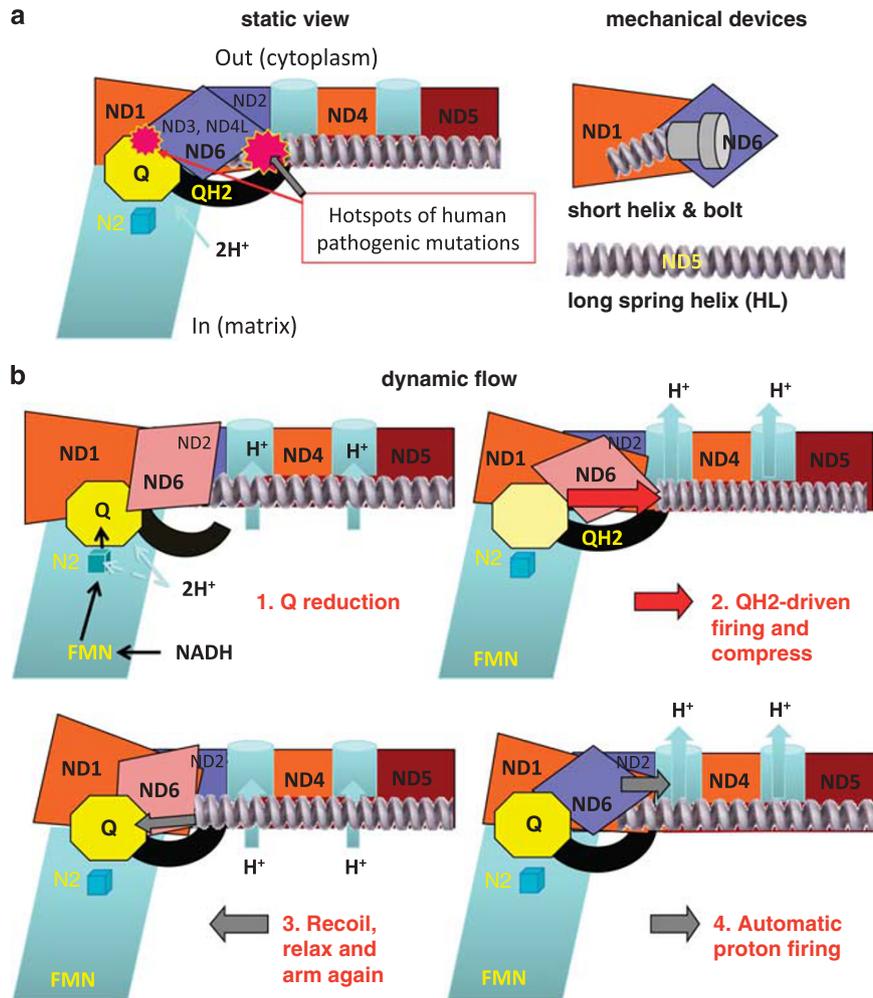


Figure 1 Complex I may work like a semi-automatic shotgun. The cartoon model previously published by Efremov *et al.*⁴ with the variation of not having the subunit equivalent to ND2 as a proton pump,²⁴ was first tilted vertically and then horizontally by 180° as in Figure 1 of Treberg and Brand.⁷ The silhouette, which respects the dimension of the X-ray structure of *Thermus* complex I,⁴ was then used to outline the modular assembly of the equivalent mitochondrial ND subunits (Table 1). The ND2 subunit is shown behind other subunits for it is postulated to have an ancillary role in proton pumping.²⁵ The major hotspots for the pathogenic mutations of different ND subunits are noted by the indicated symbol and arrows (Table 1, see also Bridges *et al.*¹⁷ for further details on pathogenic ND mutations). Helix HL of subunit ND5⁴ is represented by the long spring that may be analogous to the spring-loaded helices of viral agglutinins and similarly undergo proton-associated conformational changes.^{26,27} Here, these changes are considered to be analogous to the re-loading movements of semi-automatic shotguns. The ND1 subunit, perhaps together with adjacent subunits of the hydrophilic domain (e.g. NQO6⁴), provides the short spring that converts the inertia of the recoiling process into automatic forward movement of the 'bolt', which is proposed to be essentially formed by the ND6 subunit as depicted in the top right part of the figure (in subsequent parts, the image of the short spring and bolt device is not shown for sake of clarity). (a) Static view, in which The ND3 and ND4L subunits are labeled with lower font size to indicate that they may not form the core of the functional bioenergetic machinery of mitochondrial complex I, because they are not present in the mitochondrial genomes of some algae¹² and have fewer damaging mutations (Table 1). Cluster N2 is in light blue to denote its oxidized state. The images on the right illustrate the mechanical devices of complex I that are proposed to be analogous to those essential in semi-automatic shotguns. (b) Dynamic view in four snapshots (Supplementary Movie 1, for a cartoon showing these and other steps in motion). Step 1 – Reduction of Q via cluster N2, in turn reduced (dark blue) by NADH via other redox groups (only FMN is shown), is accompanied by the uptake of two protons from the matrix. Step 2 – The formation of the ubiquinol (QH2) product induces a large conformational change in the hydrophilic domain and the adjacent ND1 and ND6 subunits leading to the forward movement of the 'bolt', represented by the acute angle of the rhombic shape of ND6 along the membrane plane. This 'fires' the outward release of two protons by the ND4 and ND5 subunits, while compressing the long HL helix. Step 3 – The ubiquinol product leaves the complex allowing the decompression of helix HL and the re-loading of another molecule of Q substrate from a membrane reservoir (not pictured) into the Q-reacting cavity. In the recoil process, additional protons are taken up from the matrix side, while the spring and bolt mechanism is 'armed' again. This arming uses the mechanical energy provided by the relaxation of the long HL, which is connected to the short helix behind the 'bolt' by a lever device primarily formed by the ND1 and ND6 subunits, in analogy with the mechanics of semi-automatic shotguns. Step 4 – The automatic relaxation of the short helix pushes the ND6 'bolt' forward again, converting the mechanical energy driven by the HL helix recoil into an 'automatic' round of proton release by the ND4 and ND5 pumps, perhaps assisted by the ND2 subunit as well. Conformational type A inhibitors (Table 2) are predicted to block the recoiling of step 3 and 4, in particular

Potent Complex I Inhibitors May Physically Block its Conformational Changes

Some subunits belonging to the hydrophilic domain of complex I also contribute to the binding of Q and its antagonist

inhibitors, in particular the PSST and 49Kd proteins (beef complex I nomenclature¹).^{34,35} Indeed, the available X-ray structures indicate that complex I contains a large cavity formed by the bacterial equivalents of mitochondrial 49Kd and PSST subunits, together with ND1 and the ND6-containing

bundle in the hydrophobic domain of the complex.^{4,5} This structural evidence appears to be inconsistent with previous data, suggesting that the ND4 and ND5 subunits of mitochondrial complex I may bind Q²⁰ or its antagonist inhibitors such as fenpyroximate.³³ Clearly, the contribution of at least four distinct subunits to the large Q-binding pocket would be sufficient to account for the likely presence of reaction sites for the quinone substrate, its semiquinone intermediate and/or the quinol product.^{2,6,36,37}

However, also the ND2 subunit has been reported to bind a Q-antagonist, the acetogenin asimicin.³⁸ Q binding and reduction at the interface between ND1 and the hydrophilic domain of the complex may thus produce large conformational changes that are mechanically transmitted along the plane of the membrane, progressively involving ND2, ND4 and ND5.^{4,39} Fenperoxymate, asimicin, piericidin, rolliniastatins and related potent inhibitors may not only displace Q, but also prevent the mechanical transmission of these redox-linked conformational changes, presumably due to their large energy of binding to the complex. Indeed, they have binding constants in the nanomolar range.²

Partial reduction and binding of potent inhibitors change the conformation of complex I and the mutual relationships of its subunits.^{4,5,37,39–43} Analogous conformational changes occur in the cytochrome *bc*₁ complex.⁹ However, in complex I the structural alterations induced by the above inhibitors may produce a profound structural disruption of the connection between Q reduction and proton pumping.⁵ Hence, potent Q-antagonist inhibitors functionally overlapping piericidin – previously called type A-inhibitors,² now including also fenperoxymate – may physically act as spanners that block the mechanical transmission of redox-linked conformational changes to the proton-pumping subunits.

Table 2 reports experimental evidence that support this concept. The most potent inhibitors of mitochondrial complex I, such as the acetogenin rolliniastatin-1 and the antibiotic piericidin, inhibit more potently the proton-pumping activity than the redox activity of mitochondrial NADH-Q reductase (Table 2).⁸ These potent complex I inhibitors could then ‘jam’ its protonmotive machinery. Conversely, weak inhibitors like myxothiazol may not elicit equivalent structural changes, because they inhibit with comparable potency the

redox and bioenergetic function of NADH:Q reductase (Table 2).⁸

Other Evidence Suggesting a Different Way of Interpreting Complex I Mechanism

Among the experimental facts that recent models for complex I can hardly explain is the variability in the bioenergetic efficiency of NADH:Q reductase catalysis. Although the general consensus assigns a stoichiometry of four protons per NADH oxidized or ubiquinol produced (without counting the two scalar protons required for quinol formation^{6,35}), the stoichiometry, which can be experimentally measured varies considerably depending on the Q substrate used. Although hydrophobic quinones like decyl-Q elicit bioenergetic reactions equivalent to those of endogenous Q-10, hydrophilic Q analogs such as Q-0 induce little proton pumping in their rotenone-sensitive reaction with complex I.^{44,45} In the Q tail there appears to be a very narrow chemical threshold for inducing the shift from the half-efficient to the fully-efficient capacity of proton pumping.^{44,45} These differences cannot be due to mere partition effects in the membrane or interaction with redox groups in the hydrophilic domain of the complex,⁴⁶ because the measured reactions remained sensitive to Q-antagonist inhibitors.^{2,44,45,47} Direct coupling mechanisms (e.g., Ohnishi *et al.*⁶; Treberg and Brand⁷), as well as the ‘two-stroke’ mechanism proposed by Brandt while this work was in progress,⁴⁸ can hardly explain the narrow chemical threshold found in the proton-pumping efficiency of complex I.⁴⁵

The recent structure of *Thermus* complex I shows a cavity that can easily accommodate at least one isoprenoid unit in the hydrophobic tail of Q, even if a Q molecule was not visible in the X-ray images.⁴ This Q-reacting cavity is embedded in a region that is exposed beyond the limit of the lipid membrane, protruding at its matrix (negative) side.^{5,17} There, bound Q would be at a sufficient distance from the closest redox group of the hydrophilic arm, iron–sulfur cluster N2, to allow rapid electron transfer yielding the semiquinone and quinol products.^{4–7}

A recently proposed mechanism considers the known pH-dependence of N2 oxido–reduction as a vehicle for proton uptake at the matrix side of the membrane, in a way that leads to full translocation of the same (and at least another) proton to the opposite side of the membrane.⁷ This uptake of a proton at the N2-Q junction might compensate for the partial counter-charge separation due to electron transfer between N2 and bound Q, leaving a net charge separation of approximately one-half negative inside, that is, equivalent to that observed experimentally.⁴⁷ However, at the moment it appears totally conjectural how a proton taken up by N2 in the matrix then ends up at the opposite side of the membrane, from which bound Q would be separated by no less than 4 nm.^{4,5} The same structural evidence does not support the presence of a semiquinone-gated proton pump associated with Q reduction by complex I.⁶ Hence, we find our similar proposal of semiquinone-gated pumps⁸ to be clearly untenable. Here, we provide a new way to envisage how complex I works, which leaves aside any similarity with other Q-reacting enzymes.

Table 2 Potent inhibitors of complex I may act as conformational blockers

Inhibitor	I ₅₀ proton pumping (nmol/mg of protein)	Ratio of I ₅₀ proton pumping With I ₅₀ redox ^a	Notes
Rolliniastatin-1	0.03	0.68	Type A
Rolliniastatin-2	0.05	0.59	Type A
Piericidin	0.03	0.49	Type A
Rotenone	0.04	1.08	Not A
Myxothiazol	22.00	1.45	Weak

Tightly coupled submitochondrial particles from beef or sheep heart were prepared and assayed as described earlier,^{44,45} with 0.1 mM NADH and saturating concentrations of undecyl-Q, in the presence of complex II and cytochrome *bc*₁ complex inhibitors. The table shows values of I₅₀ for inhibitors that are nearly stoichiometric with complex I⁸—except for myxothiazol, which inhibits complex I more weakly than the cytochrome *bc*₁ complex.² ^aAverage of at least four separate experiments

The Quinol-Triggered Semi-Automatic Shotgun Model for Mitochondrial Complex I

Our proposed model flips upside down the structure of complex I,⁴ as recently shown in Treberg and Brand,⁷ to make it resemble a gun (Figure 1). The shape similarity, although accidental, has suggested us a functional analogy with the mechanism of shotguns. The mechanism of semi-automatic shotguns relies on two essential devices (Figure 1, top right): a long spring that drives the recoil action of the barrel and a short spring that is located in the back of the firing chamber and transfers its inertial momentum to the bolt via a lever system connected to the long spring. Spring-loaded conformational changes have been previously proposed for viral agglutinins with membrane fusion capacity, which extend the length of amphipathic α helices in response to a lower pH.^{26,27} Similar changes have been reported also for mammalian membrane proteins.⁴⁹

The structure of bacterial complex I shows a very long α helix, helix HL, that runs parallel to the membrane plane spanning most of the hydrophobic domain, with a central interruption.⁴ A similar long helix is present in the mitochondrial complex I⁵ and apparently also in its plastidial homolog.²⁸ The long helix HL has been proposed to act as a piston or rod driving the proton-pumping action of the antiporter-like subunits of complex I.^{4,6,48} We consider instead that this HL helix may store energy in a spring-loaded fashion as viral agglutinins^{26,27} and is therefore analogous to the long spring exploiting the recoil of semi-automatic shotguns. Expanding the analogy further, one or more of the tilted helices observed at the rear of the Q cavity in the bacterial complex⁴ may function as the short spring transferring inertia to a shotgun bolt. These helices belong to the bacterial subunit corresponding to ND1, the protein most consistently associated with the binding of Q and its inhibitors in the mitochondrial complex I.^{16,20,31,32,34,37,50} Of note, the elastic capacity of tilted helices has been already described.^{49,51} In mammalian mitochondria, it is entirely possible that additional subunits of both the hydrophobic and the hydrophilic domain¹ may contribute to this proposed spring-loaded mechanism and its fine regulation.

In a final step of the analogy with shotguns, we propose that the formation of the ubiquinol product acts as a sort of trigger for 'firing' protons outside mitochondria, first directly and then automatically. The free energy associated with the second electron transferred from the iron-sulfur clusters of the hydrophilic domain to bound Q⁷ would explosively drive the forward movement of the 'bolt', thus 'firing' the proton-pumping activity of the distal membrane subunits. It will also be partially converted into mechanical energy, via conformational changes that compress the long HL helix along the membrane plane. In the subsequent recoil of the HL helix, protons could be taken up from the matrix side by the deprotonated pumps, while an additional Q substrate could be inserted in the reaction pocket, aided by the sliding movement of membrane elements associated with HL helix recoil. Notably, the energy released by the mechanical relaxation of the same HL helix would then 'arm' again the spring and bolt device via conformational changes transmitted by inter-connected parts of the ND6 and ND1 subunits (Figure 1b,

step 3). The process would link the sliding movement of the long helical spring (C-terminal part of helix HL) to an elastic compression of the short spring, whose relaxation will then push forward the 'bolt' again, leading to an automatic firing of the protons previously inserted in the pumps. Hence, two functional proton pumps^{8,24,48} would be sufficient to produce the maximal stoichiometry of proton translocation by complex I, because they would automatically fire *twice* with a single release of the product ubiquinol. Although ND4 and ND5 are postulated to form the proton pumps in complex I (Figure 1 and Supplementary Movie 1), it is possible that the ND2 subunit provides backup reinforcement for the automatic firing of protons.

Conclusions and Perspectives for the New Shotgun Model of Complex I

The basic feature of the new shotgun model for complex I (Figure 1 and Supplementary Movie 1) is that it eliminates the requirement of semiquinone-linked pumps^{6,8} or other devices of direct coupling, which do not seem to be compatible with the structural information available for bacterial complex I.⁴ Our model implies that most of the bioenergetic function of complex I resides in the proton-pumping subunits ND4 and ND5. These proteins can fire protons outside in two automatic rounds per every quinol produced, via a mechanism provided by spring-loaded helical structures intimately inter-locked within the hydrophobic domain of the complex, dynamically connecting proximal Q reduction to distal proton pumping (Figure 1). Additionally, the present model provides a new explanation for the following pieces of evidence:

- (1) The involvement of the distal subunits ND5, ND4 – and also the ND2 homolog of bacteria – in the binding of potent Q antagonist inhibitors such as plant acetogenins,^{6,20,33} even if these subunits are far away from the Q-reacting pocket in the complex.⁴ The potent inhibitors may physically disrupt the long distance connections between distal proton pumping and proximal Q reduction at different points of the hydrophobic domain of the complex.
- (2) The increased potency of potent type A inhibitors for the proton-pumping activity of NADH:Q reductase (Table 2),⁸ similarly to the action of amiloride-like inhibitors of the antiporter activity of the complex.^{6,32} By disrupting key conformational changes, these inhibitors also block the re-loading of the Q substrate into its reaction pocket, thereby jamming the spring-loaded action of proton pumping.
- (3) The charge separation that occur upstream of the rotenone site.^{8,36} Besides the redox-linked protonation of the N2 cluster,⁷ it may derive from the spring-loading of ND4 and ND5 with matrix-derived protons during recoiling.⁴⁷
- (4) The Q-dependent variability in the bioenergetic efficiency of complex I.^{44,45} Hydrophilic Q analogs would be unable to enter the membrane sliding process driven by the HL helix that re-loads the empty Q reacting chamber – therefore, they can elicit only a single firing of protons by the complex.
- (5) That most of the energy of Q reduction seems to be released with the second electron forming the quinol

product.^{7,52} Indeed, quinol formation would act as the trigger for firing protons outside the membrane.

- (6) The concentration of pathological mutations in hotspots is contributed by multiple ND subunits. A central hotspot is proposed to lie in the intricate part of the complex that connects Q reduction with distal proton pumping (Figure 1). Multiple points of attrition are likely to be present in such a delicate part of the functional mechanism⁵³ and pathogenic mutations in the ND proteins may stress these points, then affecting the whole bioenergetic capacity of the complex.

Other models proposed to date do not seem to account for all the above pieces of evidence. However, this hypothesis is just a new view of assembling together recent structural information for bacterial complex I with previous evidence accumulated for mitochondrial complex I and render it attractive to the cell death field. Clearly, the model and its postulates (Figure 1) are open to intellectual and experimental challenges, in the perspective of improving even further the understanding of one of the most complex enzymes in nature and its frequent involvement in disease.

Conflict of Interest

The authors declare no conflict of interest.

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