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The mitochondrial permeability transition pore in cell death: a promising drug binding bio-architecture

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Abstract

Bioenergetic failure often features programmed cell death involved in some severe pathologies. When the cell is fated to die, the inner mitochondrial membrane becomes permeable to ions and solutes, due to the formation and opening of a channel known as mitochondrial permeability transition pore (mPTP). Up to now, the still mPTP elusive structure and mechanism prevented any attempt to identify/design drugs to rule its formation and limit cell death. Latest advances, which strongly suggest that the F_1F_0 -ATPase can coincide with the mPTP, open new perspectives in therapy. Compounds targeting and inhibiting cyclophilin D, a known mPTP promoter, could be exploited to block mPTP formation. Moreover, if the mPTP- F_1F_0 -ATPase connection will be consolidated, selected F_1F_0 -ATPase inhibitors could represent novel therapeutic options to attenuate mPTP-related diseases by directly acting on the molecular mechanism of the mPTP. This intriguing perspective, which raises new hopes to counteract mPTP-related diseases, stimulates further studies to clarify the mPTP architecture and mechanism.

Keywords

Mitochondrial permeability transition pore; drugs; cell death; F_1F_0 -ATPase; cyclophilin D.

Main text

A number of human diseases associated with abnormal energy metabolism arise from mitochondrial dysfunctions, which eventually may lead to cell death. In regulated cell death, the pore formation in the inner mitochondrial membrane (IMM) during the mitochondrial permeability transition (mPT) is a common feature, but it remains an enigmatic phenomenon which would involve the formation of pores in the IMM. The “pore” responsible for the lethal cascade of events has been defined as mitochondrial permeability transition pore (mPTP), which would be directly responsible for the bioenergetic impairment. Among the different types of regulated cell death, which differ from each other morphologically, mechanistically and functionally, the mPT-driven necrosis is started by perturbations of the intracellular microenvironment, *i.e.* severe oxidative stress and cytosolic Ca^{2+} overload, which results from mPTP opening which in turn depend on cyclophilin D (CyPD)^{1,2}. Various insults and stresses can produce a Ca^{2+} cycle in which a selective Ca^{2+} efflux from Ca^{2+} -loaded mitochondria took place before mitochondrial swelling. Moreover, pro-apoptotic stimuli are required to promote intrinsic apoptosis by Cyt-C release and consequent caspase activation. Therefore, the mPTP could have a prominent role in regulated cell death and constitute the point of no return in the lethal pathway. The mPTP opening/closing dysregulation is a common element in a wide variety of human disorders in which the excessive mPTP opening induces tissue damage or, on the contrary, the mPTP closing limits apoptosis. The mPTP opening causes mitochondrial dysfunctions by abolishing the transmembrane H^+ gradient and preventing ATP synthesis. Accordingly, mitochondrial disorders involved in cellular demise mainly show these features¹. On these bases, the potential treatment of diseases featured by increased propensity to mPTP opening encourages to address drug design to modulate mPTP activity.

The molecular architecture of the mPTP is still unresolved, being the molecular components a matter of debate, but an overall consensus exists on the involvement of membrane-bound proteins. The initial belief that the mPTP forms at the IMM and outer mitochondrial membrane (OMM) adjoint sites. The mPTP identity was intensively searched for among the adenine nucleotide translocase (ANT), the mitochondrial phosphate carrier (PiC) and the spastic paraplegia 7 (SPG7). Then the ATP synthasome, an hetero-oligomeric membrane protein complex composed of ANT, PiC and F_1F_0 -ATPase, was also involved³. In addition, the OMM components, the voltage-dependent anion channel (VDAC), the translocator protein (TSPO) and BCL-2 family members have been suggested as candidates in mPTP formation¹. However, the mPTP is primarily an IMM event, while the OMM may play a role in its modulation. Several reports based on genetic manipulations, electrophysiological measurements and mutagenesis of specific aminoacid residues strongly suggest the mitochondrial F_1F_0 -ATPase as the main channel-forming component of the mPTP^{4,5}.

The F_1F_0 -ATPase is a bi-functional enzyme complex composed by two sector (Fig. 1). A membrane-embedded F_0 sector formed from the subunits a , the c_n -ring, two membrane-inserted α -helices of b subunits and supernumeraries subunits e , f , g , A6L, DAPIT and 6.8 kDa proteolipid, which allows H^+ flow across the inner mitochondrial membrane (IMM). A soluble catalytic F_1 sector consisting of $(\alpha\beta)_3$, γ , δ and ϵ subunits which protrudes in the matrix and synthesizes/hydrolyzes ATP. The two sectors are functionally and structurally connected by a central stalk and a peripheral stalk (formed by the OSCP, d , F6 and the extrinsic α -helices of b subunits), so as to allow the protonmotive force to drive ATP synthesis; *vice versa* ATP hydrolysis is coupled to H^+ pumping from the matrix to the intermembrane space, which polarizes the IMM. The F_1F_0 -ATPases form supra-molecular dimeric complexes in the IMM by making closer their transmembrane F_0 domains. The dimeric arrangement contributes to the mitochondrial morphology and to the energy-transduction bioenergetics mechanism. Interestingly, according to an intriguing model, the F_1F_0 -ATPase known role of “enzyme of life” that produces ATP turns into the opposite task of “enzyme of death” as energy-dissipating mechanism when the mitochondrial Ca^{2+} concentration abruptly increases under pathological conditions by forming and opening of mPTP⁴. Even if the catalytic properties of substrate binding to enzyme are independent of the cation that acts as cofactor, the catalytic sites may adopt different conformations if the natural cofactor Mg^{2+} is replaced with Ca^{2+} . Recent findings strongly hint that in the latter case the F_1F_0 -

ATPase is directly involved in the mPTP formation⁶. Molecular dynamics simulations of the $(\alpha\beta)_3$ -OSCP complex in the F_1 domain strongly suggest that Ca^{2+} insertion in the enzyme catalytic sites when Ca^{2+} replaces Mg^{2+} triggers conformational changes⁷, which are mechanically transmitted from the hydrophilic sector F_1 to the membrane portion F_0 in the IMM. So, the mPTP arises from the dimer dissociation, namely from the detachment of the two membrane F_0 domains and forms at their interface, probably changing the curvature of the edge of the *cristae* in the IMM^{4,8}. Accordingly, the dimer disassembly during mPTP opening reduces the IMM convexity, thus causing the retraction of the *cristae* and modifying the mitochondrial morphology. However, the “c-ring hypothesis” sustains that the mechanism for channel formation lies within the c-ring of the F_0 sector⁹. Recently, c subunit-knockout cells, the larger cyclosporine A (CsA)-dependent current was not detected, but a detected lower conductance channel was shown to be inhibited by ADP and bongkreikic acid, probably due to the activation of the ANT channel¹⁰. The hypothesis that the mPTP could be linked to the F_1F_0 -ATPase c-ring was substantiated by the detection of serum levels of c subunits with surrogate markers of myocardial ischemia-reperfusion injury¹¹ when mPTP opening induces heart damage. In addition, the α -synuclein aggregate form interacts with the F_1F_0 -ATPase and induce mPTP opening¹² and may contribute as molecular event to Parkinson's disease.

It is clear that the treatment of mPTP-related diseases may be greatly improved by the identification of drugs that rule the mPTP. Working in this direction, known molecules able to modulate the mPTP immediately raised a great interest. The mPTP activity is known to depend on CyPD, a protein modulator of mPTP opening, and to be inhibited by CsA. Even if CsA effects are routinely related to the mPTP activity, all mammalian cyclophilins are CsA susceptible. Unfortunately, CsA has limited solubility in water, poor bioavailability and a low blood-brain barrier permeability, all features which strongly limit its exploitation in therapy. Recent advances suggest that CyPD binds to the OSCP subunit and interacts with the N-terminal part of b subunit of the F_1F_0 -ATPase^{4,13}. Both OSCP and b subunits are part of the peripheral stalk of enzyme. CsA, probably by targeting CyPD can inhibit the Ca^{2+} -activated F_1F_0 -ATPase at increasing Ca^{2+} concentrations without affecting the Mg^{2+} -activated F_1F_0 -ATPase¹⁴. Noteworthy, high Ca^{2+} concentrations and oxidative stress in mitochondria are known to open the mPTP. On these bases, the elucidation of the mPTP structure, whose channel formation mechanism most likely is based on the multi-subunit architecture F_1F_0 -ATPase(s), will help to explore pharmacological strategies in drug design and discovery to identify novel, clinically useful mPTP inhibitors. Drugs acting as pore shutters, by targeting the mPTP and preventing the cell death pathway, may have a prominent role in many neurodegenerative diseases. Noteworthy, it is universally accepted that CyPD, without being an essential component of mPTP, is a protein which modulates mPTP formation and opening¹⁵. However, the drugs for brain diseases are a great challenge due to the blood-brain barrier which is only permeable to lipid-soluble small molecules. The mPTP inhibitors fall in two categories: (i) molecules that target CyPD; (ii) compounds that act as mPTP inhibitors via CyPD-independent mechanism. Up to now, CsA is the best characterized immunosuppressive peptide that binds CypD and inhibits the mPTP. However, other promising compounds are emerging. The macrocyclic natural product, as well as macrocyclic CsA-derivatives and other small molecules can bind the CypD and inhibit the mPTP. The non-immunosuppressive CsA derivatives such as NIM811 and Debio-025 are small molecules which can displace CyPD from its site and desensitize the mPTP¹⁶. Sanglifehrin A (SfA), like CsA, is a macrocyclic natural inhibitor of mPTP. SfA inhibits the peptidyl-prolyl *cis-trans* isomerase activity of CyPD and blocks the pore formation at different site from CsA. The sigmoidal dose-response curve suggests a cooperative effect on mPTP structure¹⁷. The CyPD is a positive mPTP regulator: the occurrence of CsA or genetic CyPD ablation desensitize the mPTP by increasing the Ca^{2+} threshold required to activate the mPTP, whose opening is still allowed. The CyPD binding site on the peripheral stalk could have a prominent role in the mechanical signal transduction from the F_1 to the F_0 sector where the pore forms^{4,7}. The selective deletion of b subunit or of OSCP subunit in cell lines maintains the mPTP features, even if the loss of these subunits of the peripheral stalk changes the solute permeation by affecting the mPTP size, which becomes smaller. Apart from CsA and CsA derivatives, other compounds such as cinnamic anilides, isoxazoles and benzamides delay mPTP opening and are not cytotoxic at effective

concentrations¹⁶. Indeed, “CyPD-independent drugs”, directly targeting the mPTP, could act on the Ca^{2+} -dependent mechanism of mPTP formation involving the F_1F_0 -ATPase. Interestingly, the F_1F_0 -ATPase is a bi-functional enzyme whose ATP synthesis/hydrolysis activities are only supported in the presence of the natural cofactor Mg^{2+} bound in the catalytic sites. Conversely, in presence of Ca^{2+} the Ca^{2+} -activated F_1F_0 -ATPase supports ATP hydrolysis but not ATP synthesis and this feature could be related to triggering mPTP opening (Fig. 2). The development of “CyPD-independent drugs” to block the mPTP formation is extremely attractive, but the main question that remains to be solved is to design or find inhibitors that specifically target the Ca^{2+} -activated F_1F_0 -ATPase without affecting the physiological bi-functional activity of the Mg^{2+} -activated F_1F_0 -ATPase, *i.e.* ATP synthesis and hydrolysis and likewise have no off-target effects outside the mitochondrial enzyme. The known inhibitors of F_0 domain of enzyme (oligomycin and DCCD) by blocking the H^+ translocation inhibit the Ca^{2+} and Mg^{2+} -activated F_1F_0 -ATPase, respectively⁶. The mPTP opening detected by calcein quenching rate was reduced by oligomycin and DCCD as efficiently as by CsA¹⁸. In addition, small molecules mimicking the southern moiety of oligomycin target the c subunit(s) of F_0 domain and inhibit the mPTP without affecting ATP synthesis¹⁹. Ca^{2+} binding to the F_1 domain involves a catalytic mechanism that may be cation-dependent⁶. The higher Ca^{2+} steric hindrance with respect to Mg^{2+} (Fig. 3) implies the onset a more flexible coordination geometry characterized by irregular bond distances and angles in the enzyme catalytic sites of β subunit. The geometry of the cofactor-binding site of the globular $(\alpha\beta)_3$ structure of F_1 domain can change and would be transmitted to the nearby OSCP subunit and through the peripheral stalk reach the membrane where the mPTP opens^{7,20}. To this end, the aim is to discover new drugs that selectively prevent the Ca^{2+} binding in the catalytic sites of F_1 domain thus preventing the mPTP formation.

To sum up, the discovery of molecular identity and mechanism of mPTP phenomenon will significantly improve the search for new mitochondrial drugs that will have an effective pharmaceutical solution in the treatment of many diseases by preventing the formation of the channel with selective mPTP inhibitors. Conversely, the extreme refractoriness to death of cancer cells suggests that these cells have evolved mechanisms to escape mPTP opening by desensitizing the mPTP to mPTP regulators. Since the decreased propensity to mPTP opening favours tumour progression, the mPTP re-activation arises as promising strategy to circumvent oncogenic defects.

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Figure Legends

Figure 1. F₁F₀-ATPase monomer in the inner mitochondrial membrane. Protein subunits are drawn as ribbon representations (modified PDB ID codes: 5ARA and 6B2Z). Olive, α subunits; red, β subunits; blue, γ subunit; fuchsia, δ subunit; turquoise, ϵ subunit; orange, ring of c subunits; violet, a subunit; purple, A6L subunit; gold, f subunit; green, b subunit; salmon, d subunit; sky-blue, F6 subunit; grey, OSCP subunit. e and g subunits drawn in ball and stick mode, are blue and light blue, respectively; pink, 6.8 kDa proteolipid (i/j subunit in yeast); brown, DAPIT (k subunit in yeast) is truncated.

Figure 2. Putative involvement of the F₁F₀-ATPase complexes in mPTP formation by switching between Mg²⁺-activated and Ca²⁺-activated F₁F₀-ATPase. Protein subunits are drawn as ribbon representations (modified PDB ID codes: 5ARA and 6B2Z). The colours of the subunits are the same as in Fig. 1. ATP synthesis and hydrolysis are physiological features of the Mg²⁺-activated F₁F₀-ATPase (on the left), while only ATP hydrolysis can be supported by the Ca²⁺-activated F₁F₀-ATPase (on the right) which triggers the mPTP opening. The bidirectional red arrow depicts the presumed region of mPTP opening.

Figure 3. Putative arrangement of Mg²⁺ and Ca²⁺ within key aminoacid residues in the catalytic β subunit of the F₁F₀-ATPase. A) MgATP (Mg²⁺ as turquoise sphere and ATP in stick mode) and B) CaATP (Ca²⁺ as grey sphere and ATP as stick mode). The electrondensity map of Mg²⁺ and Ca²⁺ is drawn as a sphere. The divalent cations are directly coordinated by Thr 163 and the oxygen atoms of β and γ -phosphates of ATP, while Arg 189, Asp 256 and Glu 192 are indirectly coordinated by three ordered water molecules.

Bio-sketch

His research is focused on the mitochondrial bioenergetics. In particular, his interest is addressed to the study of enzymatic catalysis of mitochondrial ATP synthase due to post-translational chemical modifications and the physiological role of the enzyme activated by calcium.

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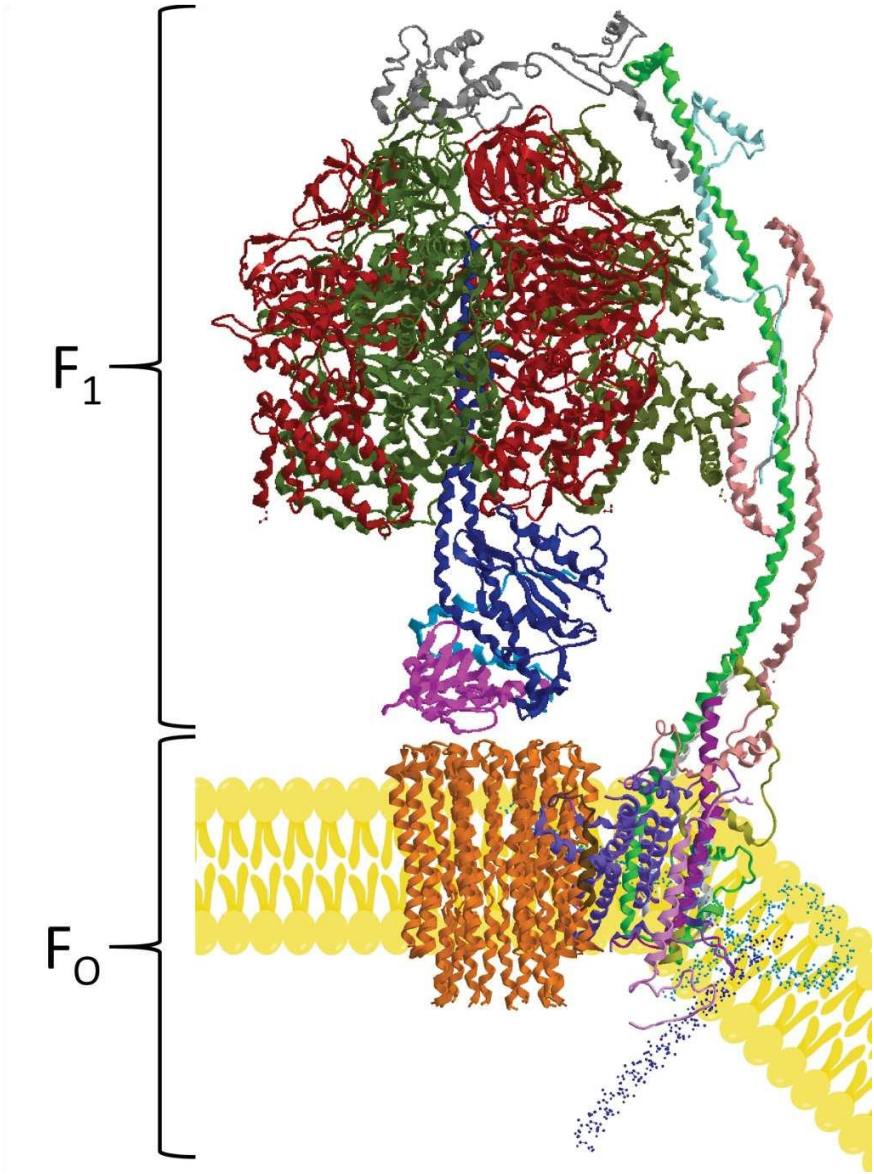


Figure 1. F1FO-ATPase monomer in the inner mitochondrial membrane. Protein subunits are drawn as ribbon representations (modified PDB ID codes: 5ARA and 6B2Z). Olive, α subunits; red, β subunits; blue, γ subunit; fuchsia, δ subunit; turquoise, ϵ subunit; orange, ring of c subunits; violet, a subunit; purple, A6L subunit; gold, f subunit; green, b subunit; salmon, d subunit; sky-blue, F6 subunit; grey, OSCP subunit. e and g subunits drawn in ball and stick mode, are blue and light blue, respectively; pink, 6.8 kDa proteolipid (i/j subunit in yeast); brown, DAPIT (k subunit in yeast) is truncated.

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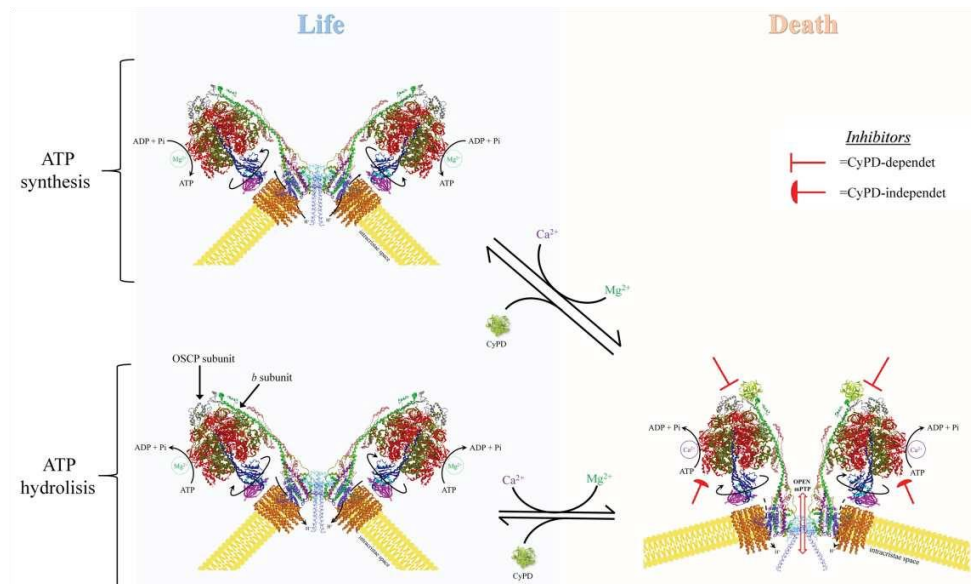


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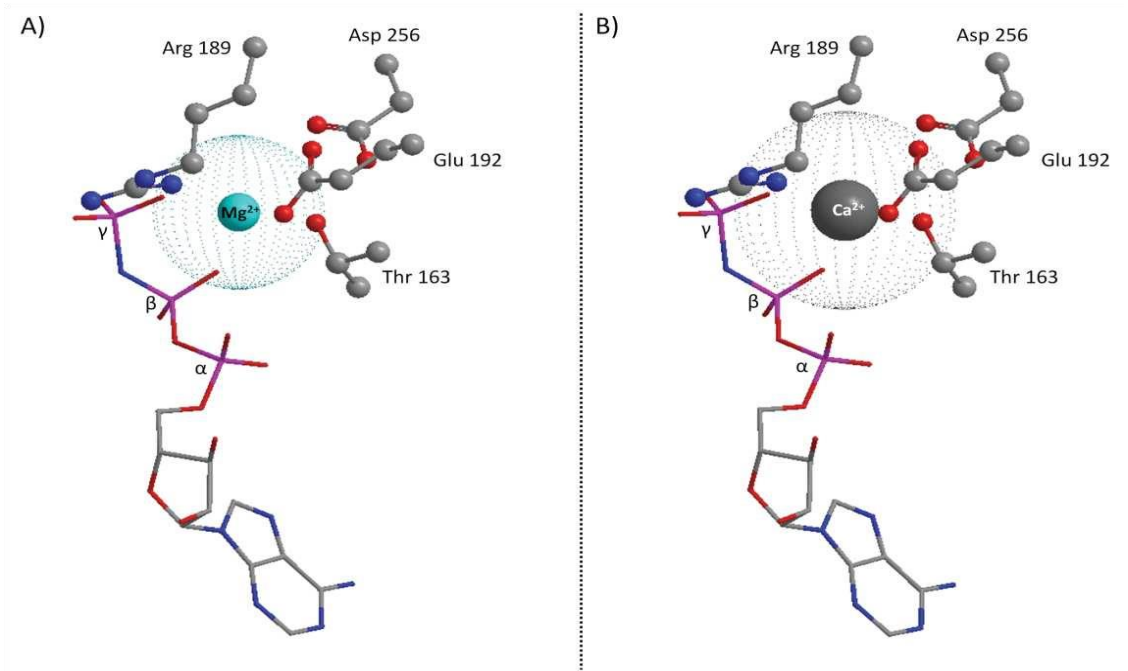


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108x72mm (600 x 600 DPI)