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Mitochondrial permeability transition, F_1F_0 -ATPase and calcium: an enigmatic triangle

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The Permeability Transition Pore (PTP) is a mysterious structure that induces cell death by increasing the permeability of the inner mitochondrial membrane (IMM) to ions and soluble molecules. In 2015, the F_1F_0 -ATPase was identified as being involved in forming the PTP in eukaryotes [1]. Two hypotheses were put forward to explain the mechanism of PTP opening, both of which involve the mitochondrial F_1F_0 -ATPase: the channel forms within the *c*-ring [2] or results from the conformational changes of F_1F_0 -ATPase dimers [3]. F_1F_0 -ATPase is a multi-subunit enzyme at the cristae edges of the IMM that either synthesizes (forward reaction) or hydrolyses ATP (reverse reaction) in the catalytic hydrophilic F_1 domain while translocating H⁺ through the *c*-ring in the hydrophobic F_0 domain.

The first hypothesis by Alavian and colleagues proposes that the PTP channel is created within the c-ring of the F₀ subunit by Ca²⁺-dependent extrusion of the extrinsic F₁ domain from the membrane-embedded F₀ domain of the enzyme [2]. However, two points remain obscure: the occurrence of cyclophilin D in the basic structure of the "c-ring channel", and the F₁ domain detachment from the F₀ domain, which usually occurs only under extreme conditions, *e.g.* high urea concentration. However, downregulation with siRNA and dephosphorylation of c-ring subunits support a role in PTP opening [1]. In addition, a mitochondrial water-permeable channel has been proposed based on a *de novo* complex composed of c subunits, polyphosphate, polyhydroxybutyrate and Ca²⁺ [4].

Yet, the F₀ *c*-ring channel properties have recently been found incompatible with the characteristics of the PTP [5]. First, the lipid-filled *c*-ring cavity blocks the ions and osmolytes permeation. Moreover, calculations of ionic conductance over the *c*-ring lumen are inconsistent with the high conductance properties of the PTP, even if the interior of the *c*-ring becomes hydrated [5]. Similarly, results by He et al. [6], argue against the *c*-ring being involved in PTP formation. They showed that cells unable to build the F₁F₀-ATPase *c*-ring can still form the PTP. Furthermore, if this structurally defective or vestigial F₁F₀-ATPase lacks the *a* subunit, *c*-ring and A6L subunit, the PTP still forms even if the defective enzyme is unable to translocate H⁺. It remains to be seen if any (or all) of the other F₁F₀-ATPase membrane subunits, namely *b*, *e*, *f* and *g*, may be involved in PTP formation [6].

The alternative hypothesis – that PTP channel formation is induced by conformational changes of F_1F_0 -ATPase dimers [3] when Ca^{2+} replaces the Mg^{2+} in the catalytic site – is supported by some intriguing clues. This involves ATP hydrolysis by Ca^{2+} -activated F_1F_0 -ATPase, an alternative

function of the natural Mg^{2+} -dependent F_1F_0 complex in response to high Ca^{2+} concentrations in the mitochondrial permeability transition [7,8] that does not reenergize the IMM .

Interestingly, PTP opening is linked to dissipation of the proton motive force (Δp) and IMM depolarization. When mitochondrial Ca²⁺ concentration increases, Ca²⁺ replaces the cofactor Mg²⁺ in the catalytic site of the F₁F₀ complex and only supports ATP hydrolysis but not ATP synthesis. Moreover, ATP hydrolysis in the Ca²⁺-activated F₁F₀-ATPase does not build up the Δp by H⁺ translocation [7], or H⁺ translocation is counteracted by H⁺ backflow through the PTP [8]. When ATP hydrolysis by Ca²⁺-activated F₁F₀-ATPase depletes the ATP pool, the IMM cannot be polarized as long as the PTP is open.

Another observation that supports the second hypothesis is that a Thr-163>Ser mutation in the Walker motif of the β subunit causes an increase in activity with Mg²⁺ and a decrease with Ca²⁺. and confers resistance to PTP opening [7]. Ca^{2+} can bind to the same amino acid residues as Mg^{2+} but with greater affinity, and only the Thr-163 is directly implied in binding divalent cations. Since Ca^{2+} bound to the catalytic subunits implies a higher steric hindrance than Mg^{2+} , the geometry of the cofactor-binding site of the enzyme changes. The larger atomic radius of Ca²⁺ allows a less rigid coordination geometry compared to Mg²⁺, from six-fold octahedral to up to eight ligands, involving irregular bond distances and angles. The conformational change of the catalytic sites would be transmitted to the nearby OSCP subunit. As OSCP is a component of the peripheral stalk, these conformation changes may pull the stalks together and decrease the stalk-to-stalk distance between dimers to reduce the convex membrane curvature in the IMM [9]. The stalk tilt would be transmitted to the F₁F₀-ATPase membrane subunits, probably causing the PTP to open at the interface between monomers (Fig. 1). Daum et al [9] assert that curvature loss of mitochondrial cristae induces dissociation of F_1F_0 -ATPase dimers, which, through mitochondrial swelling and burst owing to PTP opening, leads to cell death. In line with this hypothesis, PTP opening can be inhibited by overexpression of Inhibitory Factor 1 (IF1) or the e subunit [10] to promote dimerization of the F₁F₀-ATPase.

Some other observations would support this hypothesis. For instance, modifying the *c*-ring conformation reduces PTP opening [10]. Moreover, the dissociation of F_1F_0 -ATPase dimers is associated with decreased mitochondrial bioenergetics, ageing and cell death [9]. Inhibiting ATPase activity by IF1 and incorrect *c*-ring conformation can preclude the Ca²⁺-activated F_1F_0 -ATPase

hydrolysis and central stalk rotation respectively, which occur during the PTP formation [8]. Moreover, the *e* subunit has already been considered a potential component of PTP [6].

The recent insights in the disputed molecular identity of PTP support the theory that the dissociation of Ca²⁺-activated F₁F₀-ATPase dimers starts a multistep process of pore formation. Conversely, the hypothesis that *c* subunits are structural elements of the pore does not hold [6] even if an appropriate *c*-ring conformation is required to induce PTP formation after the dissociation of dimers [10]. Furthermore, the Ca²⁺-activated ATP hydrolysis without building Δp are fully consistent with ATP depletion and Δp dissipation events, which lead to cell death through PTP opening [1].

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Figure 1. "From F₁F₀-ATPase dimer to pore formation": a model of PTP opening mechanism. A) Mg-activated F₁F₀-ATPase dimer. B) The Ca-activated F₁F₀-ATPases involved in PTP opening. On the left of the A and B panels the ground-state structure of catalytic site in the β_{DP} -subunit (PDB ID code: 2JDI) binding Mg²⁺ or Ca²⁺ cofactor is shown. The $_{\beta}$ Arg-189, $_{\beta}$ Glu-192, $_{\beta}$ Asp-256 residues are drawn in stick mode; each aminoacid through a water molecules (do not show) establish a bridge interaction with divalent cation. The $_{\beta}$ Thr-163 (drawn as ball and stick model) of Walker motif directly binds the cofactor. The dashed black arrows indicate the likely modification of stalk-to-stalk position in closed/open PTP. The F₁F₀-ATPase subunits (modified PDB ID code: 5ARA) are in ribbon representation. Green, α subunits; violet, β subunits; dark blue, γ subunit; yellow, δ subunit; brown, ε subunit; red, *c* subunits; olive, *a* subunit; fuchsia, *b* subunit; orange, *d* subunit; cyan, F₆ subunit; blue, OSCP subunit. The bidirectional arrow indicates the putative region of PTP channel formation. The membrane subunits *e*, *f*, *g*, A6L, DAPIT (Diabetes-Associated Protein in Insulin-sensitive Tissues), 6.8 kDa proteolipid, of still undefined structure, are drawn as space-filling models.

