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Ca<sup>2+</sup> as cofactor of the mitochondrial H<sup>+</sup>-translocating F<sub>1</sub>FO-ATP(hydrol)ase

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Nesci S., Pagliarani A. (2021). Ca<sup>2+</sup> as cofactor of the mitochondrial H<sup>+</sup>-translocating F<sub>1</sub>FO-ATP(hydrol)ase. PROTEINS, 869(5), 477-482 [10.1002/prot.26040].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/817907> since: 2021-04-03

*Published:*

DOI: <http://doi.org/10.1002/prot.26040>

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The final published version is available online at:  
<http://dx.doi.org/10.1002/prot.26040>

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Ca<sup>2+</sup> as cofactor of the mitochondrial H<sup>+</sup>-translocating F<sub>1</sub>F<sub>0</sub>-ATP(hydrol)ase

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Running title: H<sup>+</sup>-translocation driven by ATP hydrolysis by the Ca<sup>2+</sup>-activated F<sub>1</sub>F<sub>0</sub>-ATPase

Abstract

The mitochondrial F<sub>1</sub>F<sub>0</sub>-ATPase in the presence of the natural cofactor Mg<sup>2+</sup> acts as the enzyme of life by synthesizing ATP, but it can also hydrolyze ATP to pump H<sup>+</sup>. Interestingly, Mg<sup>2+</sup> can be replaced by Ca<sup>2+</sup>, but only to sustain ATP hydrolysis and not ATP synthesis. When Ca<sup>2+</sup> inserts in F<sub>1</sub>, the torque generation built by the chemomechanical coupling between F<sub>1</sub> and the rotating central stalk was reported as unable to drive the transmembrane H<sup>+</sup> flux within F<sub>0</sub>. However, the failed H<sup>+</sup> translocation is not consistent with the oligomycin-sensitivity of the Ca<sup>2+</sup>-dependent F<sub>1</sub>F<sub>0</sub>-ATP(hydrol)ase. New enzyme roles in mitochondrial energy transduction are suggested by recent advances. Accordingly, the structural F<sub>1</sub>F<sub>0</sub>-ATPase distortion driven by ATP hydrolysis sustained by Ca<sup>2+</sup> is consistent with the permeability transition pore signal propagation pathway. The Ca<sup>2+</sup>-activated F<sub>1</sub>F<sub>0</sub>-ATPase, by forming the pore, may contribute to dissipate the transmembrane H<sup>+</sup> gradient created by the same enzyme complex.

Keywords: Ca<sup>2+</sup> cofactor; F<sub>1</sub>F<sub>0</sub>-ATPase; mitochondria; H<sup>+</sup> pump; oligomycin; permeability transition pore; bioenergetics.

## 1. Introduction

The mitochondrial  $F_1F_0$ -ATPase is a multisubunit complex arranged in dimers or oligomers and placed at the edge of the *cristae* of the inner mitochondrial membrane (IMM) <sup>1</sup>. The monomer is formed by two domains, named  $F_1$  and  $F_0$  functionally and structurally linked to a stator (lateral stalk) and a rotor (central stalk). The  $F_1$  portion, namely the hydrophilic domain that protrudes in the mitochondrial matrix, has a conspicuous lollipop shape formed by  $\alpha_3$ ,  $\beta_3$ ,  $\gamma$ ,  $\delta$ , and  $\epsilon$  subunits. An alternated arrangement of  $\alpha$  and  $\beta$  subunits forms a globular hexamer around the  $\gamma$  subunit (Fig. 1A). The structure functions as a reversible rotary molecular motor which can build or hydrolyze ATP depending on the rotation direction, which in turn is driven by the transmembrane proton-motive force ( $\Delta p$ ). *In vitro* the  $\gamma$  subunit of  $F_1$ -ATPase was shown to rotate within the surrounding  $\alpha_3\beta_3$  subunits, synthesizing or hydrolysing ATP in three separate catalytic sites on the  $\alpha/\beta$  subunit interface. The catalytic sites are alternated with the non-catalytic sites, which can only bind adenine nucleotides <sup>2</sup>. During the kinetic reactions, the three non-equivalent conformation  $\beta_E$  (empty),  $\beta_{DP}$  (which hosts ADP) and  $\beta_{TP}$  (contains ATP or ADP) of the catalytic sites, with increasing affinity for ATP, change their conformation and binding properties every  $120^\circ$  with the rotation of the rotor <sup>3</sup>. In addition, the  $F_1F_0$ -ATPase catalytic and non-catalytic sites in their different conformations can also bind metal divalent cations <sup>1</sup>. In mammals, the membrane-embedded domain is composed by the  $a$  subunit, the transmembrane  $\alpha$ -helices of  $b$  subunit, the  $c_n$  subunits ( $n$ = eight in mammals) which arranged as a cylindric palisade form the  $c$ -ring, A6L subunit, and the supernumerary subunits  $e$ ,  $f$ ,  $g$ , DAPIT (Diabetes-Associated Protein in Insulin-sensitive Tissue), 6.8 KDa proteolipid (PL) (Fig. 1A) <sup>4</sup>. The  $H^+$  translocation sector arises from  $a/c$ -ring interactions by forming two asymmetric half-channels with unexpected horizontal membrane-intrinsic  $\alpha$ -helices in the  $a$  subunit. These two half-channels are mutually offset, while the  $H^+$  binding sites are located on the C-terminal  $\alpha$ -helix of each  $c$  subunit <sup>5</sup>. In the mammalian  $F_1F_0$ -ATPase the  $a$  and A6L membrane subunits are encoded by the mitochondrial DNA. The central stalk within the  $F_1$  domain contains the  $\gamma$  subunit, which joined to the  $\delta$  and  $\epsilon$  subunits, forms a sort of foot which interacts with the loop region of  $c$ -ring. The lateral or peripheral stalk joins the two  $F_0$  and  $F_1$  enzyme domains (Fig. 1A). The  $b$  subunit spans the complete length of the lateral stalk and interacts with OSCP, F6 and  $d$  subunits which belong to the soluble

enzyme section. All these subunits connect the soluble stator subunits with  $\alpha_{TP}$  subunit of  $F_1$  domain. In addition, the top of  $\alpha_{TP}$ ,  $\alpha_{DP}$ ,  $\alpha_E$  and the  $\beta_{DP}$  and  $\beta_E$  are only linked with OSCP. Some subunits of the lateral stalk, namely the membrane embedded portion of  $b$ ,  $f$  and A6L subunits<sup>6</sup> and the supernumerary subunits, are transmembrane subunits<sup>4</sup>. The lateral stalk shows a spectacular flexibility that plays the role of resisting the torque generation of the rotor by coupling  $F_1$  catalysis to  $H^+$  translocation<sup>7,8</sup>.

The  $H^+$ -translocating  $F_1F_0$ -ATPase sustains either ATP synthesis or hydrolysis<sup>9</sup>. In the “forward” mode the Mitchell’s proton motive force  $\Delta p$  created by mitochondrial respiration drives ATP formation from ADP and  $P_i$ . In the so-called “reverse” mode, the phosphorylation potential generated by ATP breakdown is exploited by the enzyme complex to pump  $H^+$  and energize the IMM when the  $\Delta p$  drops<sup>10</sup>. Both ATP synthase and hydrolase activities are opposite  $F_1F_0$ -ATPase functions that depend on the bioenergetic state of mitochondria. The bi-functional ATP synthesis/hydrolysis mode coupled to  $H^+$  translocation of  $F_1F_0$ -ATPase is a mechanism unique in biology sustained by the natural cofactor  $Mg^{2+}$ . The  $F_1F_0$ -ATPase can replace  $Mg^{2+}$  by  $Ca^{2+}$  losing the ATP synthesis function, but preserving the  $F_1F_0$ -ATP(hydrol)ase activity<sup>11</sup>. High  $Ca^{2+}$  concentrations in mitochondria activate  $F_1F_0$ -ATPase by direct  $Ca^{2+}$  binding to the  $\alpha_3\beta_3$  globular hexamer that dissociates ATP hydrolysis from  $H^+$  pumping. In this case  $F_1$  was reported to become uncoupled from  $F_0$  domain<sup>12</sup>. However, this assumption becomes questionable on considering the recent cryo-EM enzyme structure and structure activity relationship data on the effect of small molecules<sup>13</sup> and specific  $F_1$  and  $F_0$  domain inhibitors<sup>11,14</sup>. So, in search for a different interpretation of the findings up to now obtained, experimental and literature data<sup>15</sup> were combined to draw a pattern of the mechanism involved.

## 2. Materials and Methods

### 2.1. Preparation of the mitochondrial fractions and $F_1F_0$ -ATPase activity assays

Swine hearts (*Sus scrofa domesticus*) were collected at a local abattoir and transported to the lab. From homogenized heart tissue and then subjected to differential centrifugation<sup>11</sup> the mitochondrial preparations, obtained in a divalent cation-free medium, were characterized as described in<sup>14</sup>. To

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2  
3 evaluate the mitochondrial  $F_1F_0$ -ATPase activities, the mitochondrial preparations obtained as  
4 described by Nesci et al.<sup>11</sup>, were added to the reaction system that contained 3 mM ATP and 2 mM  
5  $Ca^{2+}$  or  $Mg^{2+}$  in 75 mM ethanolamine-HCl buffer, pH 8.8. The enzyme activity was  
6 spectrophotometrically detected and evaluated after subtraction of the non-specific ATP hydrolysis  
7 in the blank<sup>16</sup>. The sensitivity of the  $F_1F_0$ -ATPase activity, either sustained by  $Mg^{2+}$  or by  $Ca^{2+}$ , to the  
8 specific  $F_1F_0$ -ATPase inhibitor oligomycin witnessed the functional and structural coupling between  
9 the two sectors  $F_1$  and  $F_0$ <sup>14</sup>.  
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## 21 2.2. Protein model

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23 The structural details of the protein arrangement in the  $F_1F_0$ -ATPase subunits were obtained by the  
24 Chem3D program of ChemOffice Professional 19.1.1 software<sup>17</sup> using the deposited structures in  
25 PDB.  
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## 33 2.3. Calculations and statistics

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35 In each set of experiments, the data represent the mean  $\pm$  SD of the number of analyses carried out  
36 on at least three distinct mitochondrial preparations. The coupling index was calculated as the ratio  
37 between the total  $F_1F_0$ -ATPase activity and the oligomycin-sensitive  $F_1F_0$ -ATPase activity, being the  
38 latter obtained from the difference between the total  $F_1F_0$ -ATPase activity and the enzyme activity  
39 detected in the presence of 3mg/mL oligomycin, a dose which ensured maximal inhibition of the  
40  $F_1F_0$ -ATPase<sup>18</sup>. The differences between the enzyme activity data in differently treated mitochondria  
41 were evaluated by one way ANOVA followed by Student-Newman-Keuls' test when  $F$  values  
42 indicated significance ( $P \leq 0.05$ ). The significance of the difference between the mean coupling index  
43 of the  $Mg^{2+}$ -dependent  $F_1F_0$ -ATPase and the  $Ca^{2+}$ -dependent  $F_1F_0$ -ATPase was evaluated by  
44 Student's  $t$  test ( $P \leq 0.05$ ). Percentage data were *arcsin*-transformed before statistical analyses to  
45 ensure normality.  
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3 3. Results and Discussion  
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6 The catalytic and non-catalytic subunits of the  $F_1F_0$ -ATPase show specific amino acid residues and  
7 secondary structure motifs required for the molecular interaction with adenine nucleotides and  
8 divalent cations. An eight amino acids sequence, *GXXXXGKT*, conserved in all ATPases <sup>19</sup> is the  
9 basic structural feature of the P-loop, known as a phosphate binding loop, on  $\alpha$  and  $\beta$  subunits (Fig.  
10 1B). The motif interacts with  $Mg^{2+}$  and phosphate (Pi) groups of ATP by coordinating  $\beta$ -Pi and  $\gamma$ -Pi  
11 to exchange the terminal  $\gamma$ -Pi when the ATP is synthesized or hydrolysed. The positions and specific  
12 amino acid composition in the pig sequence are <sub>158</sub>*GGAGVGKT*<sub>165</sub> and <sub>169</sub>*GDRGTGKT*<sub>176</sub> in the  $\beta$   
13 and  $\alpha$  subunits, respectively. *T*<sub>165</sub> is the only residue that plays a key role in coordinating  $Mg^{2+}$  in the  
14  $\beta$  subunits of enzyme during ATP hydrolysis, while *T*<sub>176</sub> of  $\alpha$  subunits could bind the cofactor. Similarly  
15 to  $Mg^{2+}$ ,  $Ca^{2+}$  can also bind to all the catalytic sites and probably also to the non-catalytic sites <sup>4</sup>. The  
16 relative affinities for divalent cations and ATP in the reverse reaction of ATP hydrolysis are  
17 modulated by mutagenesis of these specific residues of  $\beta$  subunits <sup>20</sup>. However,  $Ca^{2+}$ , which has  
18 higher steric hindrance than  $Mg^{2+}$ , can change the coordination geometry of the  $Mg^{2+}$ -binding site  
19 from the octahedral bipyramide which binds six ligands up to allow eight ligands when  $Ca^{2+}$  is  
20 inserted in replacement of  $Mg^{2+}$  <sup>21</sup>. Therefore, the rigid octahedral complex changes to a less rigid  
21 geometry with irregular bond distances and angles and variable coordination number. This flexible  
22 arrangement may explain the non-competitive  $Ca^{2+}$  inhibition of the  $Mg^{2+}$ -activated  $F_1F_0$ -ATPase <sup>14</sup>.  
23 The  $Ca^{2+}$ -dependent  $F_1F_0$ -ATP(hydrol)ase is capable of sustaining torque generation of the rotor.  
24 The rotational motion was found to be similar to that induced by  $Mg^{2+}$  in the  $F_1$ -ATPase <sup>22</sup>.  $Ca^{2+}$   
25 binding could have the functional consequence to prevent the building of the transmembrane  $H^+$   
26 gradient, as shown by ACMA fluorescence quenching <sup>23</sup>. However, these results cannot exclude that  
27 the rotation driven by ATP hydrolysis stimulated by  $Ca^{2+}$  <sup>22</sup> is coupled to  $H^+$  translocation. The  $Mg^{2+}$ -  
28 activated  $F_1F_0$ -ATPase can display  $H^+$  flow across  $F_0$  in the absence of adenine nucleotides bound  
29 to  $F_1$ . This uncoupled proton leakage, known as “proton slip”, is associated with a free-wheeling of  
30 the central stalk under non-physiological conditions <sup>10</sup>. In addition, the proton slip is abolished by  $F_0$   
31 inhibitors (e.g. oligomycin), but it is insensitive to  $F_1$  inhibitors. Conversely, the  $Ca^{2+}$ -dependent  $F_1F_0$ -  
32 ATP(hydrol)ase activity was shown to inhibited by various  $F_1$  inhibitors <sup>14</sup> and insensitive to other Ca-

ATPase inhibitors<sup>24</sup>. Since the  $F_1F_0$ -ATPase in the presence of  $Ca^{2+}$  shows a four orders of magnitude lower enzyme activity than the  $Mg^{2+}$ -activated  $F_1F_0$ -ATPase (Fig. 2A), ATP hydrolysis sustained by  $Ca^{2+}$  may be unable to support a significant  $H^+$  pumping to energize the membrane. Moreover, the  $Ca^{2+}$ -activated  $F_1F_0$ -ATPase is now generally recognized to play an important role in the permeability transition pore (PTP) formation and opening<sup>25–29</sup>, which can dissipate the  $\Delta p$ <sup>11</sup>. The loss of  $F_1F_0$ -ATPase structural-functional integrity emerges as the most likely event involved in the decreased oligomycin sensitivity when the  $F_1$  catalysis is not coupled to  $H^+$  transport by  $F_0$ <sup>30</sup>. However, the  $Ca^{2+}$ -dependent  $F_1F_0$ -ATP(hydrol)ase is inhibited by oligomycin<sup>24</sup>. A similar behaviour was described in pea stem mitochondria, where, since the Ca-ATPase activity was fully sensitive to oligomycin, ATP hydrolysis could be coupled to  $H^+$  translocation<sup>31</sup>. In swine heart mitochondria oligomycin displays a higher inhibition efficiency on the  $Ca^{2+}$ -activated  $F_1F_0$ -ATPase than on the  $Mg^{2+}$ -activated  $F_1F_0$ -ATPase, as shown by the lower  $IC_{50}$  value (Fig. 2B). The coupling index (the ratio between the total  $F_1F_0$ -ATPase activity and the oligomycin-sensitive  $F_1F_0$ -ATPase activity) is statistically similar, namely  $94.7 \pm 1.8\%$  and  $91.6 \pm 3.7\%$  in presence of  $Mg^{2+}$  and  $Ca^{2+}$ , respectively. Therefore oligomycin blocks  $H^+$  translocation coupled to ATP hydrolysis irrespective of the divalent cation (Fig. 2C). Consistently, these data suggest that mechanochemical coupling of  $Ca^{2+}$ -dependent  $F_1$ -ATP(hydrol)ase works as a rotary chemical motor to drive  $H^+$  translocation in the  $F_0$  domain<sup>11,16</sup>. The fact that the  $H^+$ -pumping activity driven by  $Ca^{2+}$  may not energize IMM is not surprising, being supported by the new “bent-pull” model of the c-ring gated channel<sup>32</sup> and by the cryo-EM maps of the enzyme exposed to  $Ca^{2+}$ <sup>4</sup>. The lack of apparent  $H^+$  translocation with  $Ca^{2+}$ -dependent  $F_1F_0$ -ATP(hydrol)ase may be rather due to  $H^+$  backflow through the open PTP<sup>33</sup>. Indeed, different  $Ca^{2+}$   $F_1F_0$ -ATPase states during ATP hydrolysis were not identified in the  $Mg^{2+}$ -activated  $F_1F_0$ -ATPase. Moreover recent data show that the PTP opens when the  $Ca^{2+}$ -enzyme in disassembled conformation has the peripheral stalk twisted and the  $F_1$  detached from  $F_0$ <sup>4</sup> (Fig. 3). In all likelihood, oligomycin inhibits ATP hydrolysis sustained by  $Ca^{2+}$  in the first conformational stages of the  $Ca^{2+}$ -dependent  $F_1F_0$ -ATP(hydrol)ase when  $F_1$  is still coupled to  $F_0$ . Indeed, oligomycin, venturicidin, and DCCD, which block  $H^+$  translocation by binding to the c-ring, can reduce the calcein quenching rate<sup>34</sup>, while small-molecules obtained from the oligomycin structure target



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the *c* subunits and inhibit the PTP<sup>35</sup>. Previous experiments in our lab showed that DCCD, which specifically blocks H<sup>+</sup> translocation by covalently binding to the *c* subunit carboxylic groups which constitute H<sup>+</sup> binding sites and inhibits ATP hydrolysis, more promptly reacts and binds to the Ca<sup>2+</sup>-activated F<sub>1</sub>F<sub>0</sub>-ATPase than the Mg<sup>2+</sup>-activated F<sub>1</sub>F<sub>0</sub>-ATPase<sup>11</sup>. Moreover, in inside-out submitochondrial particles the oligomycin sensitive ATP hydrolysis was shown to be similarly affected by Δ*p* when the enzyme activity is sustained by Ca<sup>2+</sup> or by Mg<sup>2+</sup><sup>11</sup>. Therefore, most likely, when the activating cation is Ca<sup>2+</sup>, H<sup>+</sup> translocation may be masked by the high ionic conductance of the open PTP.

To sum up, the H<sup>+</sup>-translocating Ca<sup>2+</sup>-dependent F<sub>1</sub>F<sub>0</sub>-ATP(hydrol)ase is a (mono)functional mode of the mitochondrial F-type ATPase complex. The F<sub>1</sub> domain which hydrolyzes ATP in the presence of Ca<sup>2+</sup> drives the mechanical-power transmission which results in F<sub>0</sub> conductance to H<sup>+</sup>. Consistently, the poor H<sup>+</sup>-pumping activity of the Ca<sup>2+</sup>-dependent F<sub>1</sub>F<sub>0</sub>-ATP(hydrol)ase fails to energize the IMM, mainly because the same enzyme activity is a key PTP constituent, and most likely the PTP opening prevents and masks Δ*p* formation<sup>33,36</sup>.

4. Conclusion

Since long-time Ca<sup>2+</sup> has been involved in the F<sub>1</sub>F<sub>0</sub>-ATPase modulation in heart mitochondria<sup>37</sup>. Most knowledge comes from *in vitro* experiments, mainly due to difficulties in the detection of individual action mechanisms *in vivo*<sup>38</sup>. The structural data which cast light on the Ca<sup>2+</sup>-driven conformational changes of the F<sub>1</sub>F<sub>0</sub>-ATPase shoulder the idea that, among the multiple Ca<sup>2+</sup> actions in mitochondria, the Ca<sup>2+</sup> intervention in the PTP is one of the most relevant mitochondrial roles of this multitasking cation in physiology and pathology.

Conflicts of interest

None.

## Acknowledgments

This work was supported by the CARISBO Foundation Grants n° 2019.0534, Bologna, Italy to SN.

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Figure 1. Representative structure of F<sub>1</sub>F<sub>0</sub>-ATPase monomers in mammalian mitochondria (A). The enzyme subunits are drawn as ribbon representations obtained from modified PDB ID codes: 6TT7.  $\Delta p$ , Mitchell's proton motive force, IMM, inner mitochondrial membrane. The letter colors are the same as those of the subunit to which belong. B) Catalytic binding site of F<sub>1</sub>F<sub>0</sub>-ATPase. The ATP substrate and Mg<sup>2+</sup> cofactor (in ball and stick representation) are located in the  $\beta$  and  $\alpha$  subunits, drawn as ribbon model (modified PDB ID code: 6J5J) in  $\beta_{TP}$  and  $\alpha_{TP}$  conformation, respectively, which show the position of key amino acid residues that bind Mg<sup>2+</sup>. The P-loop is in light blue in both subunits. The binding sites are viewed from the  $\gamma$  subunit (upper panel) and between the observer and the  $\gamma$  subunit (lower panel).

Figure 2. Effect of divalent cations on ATP hydrolysis by the mitochondrial F<sub>1</sub>F<sub>0</sub>-ATPase. A) F-ATPase activities in the presence of Ca<sup>2+</sup> or Mg<sup>2+</sup> are shown as bar chart. B) Dose-response curve of oligomycin on the F<sub>1</sub>F<sub>0</sub>-ATPase activated by Ca<sup>2+</sup> or Mg<sup>2+</sup> expressed as percentage of the enzyme activity in the absence of oligomycin. C) The oligomycin-sensitive ATPase activity (■) and the oligomycin-insensitive ATPase activity in presence of 3  $\mu$ g/ml of oligomycin (■) are expressed as percentages of the total mitochondrial ATPase activity sustained by Ca<sup>2+</sup> or Mg<sup>2+</sup>, respectively. Data expressed as column chart represent the mean  $\pm$  SD (vertical bars) from three experiments carried out on different mitochondrial preparations. \* indicates significantly different values ( $P \leq 0.05$ ).

Figure 3. F<sub>1</sub>F<sub>0</sub>-ATPase activity raised by Mg<sup>2+</sup> or Ca<sup>2+</sup> as cofactors. ATP hydrolysis sustained by Mg<sup>2+</sup> (i) or Ca<sup>2+</sup> (ii) is coupled to H<sup>+</sup> translocation. The different size of the two cofactors changes the F<sub>1</sub>F<sub>0</sub>-ATPase conformation. Indeed, the transition of the Ca<sup>2+</sup>-dependent F<sub>1</sub>F<sub>0</sub>-ATP(hydrol)ase from the assembled (ii) to the disassembled state (iii) could induce the loss of H<sup>+</sup>-translocation. Consequently, the PTP opens when a retracted e subunit pulls the lyso-phosphatidylserine plug out of the c-ring at the inner mitochondrial membrane side, while the F<sub>1</sub>F<sub>0</sub> destabilization pulls out phosphatidylserine at the matrix side.

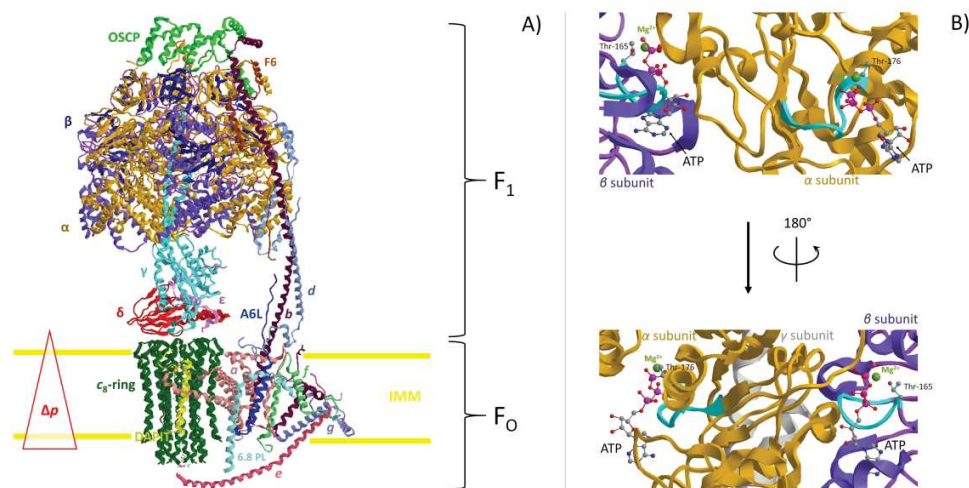


Figure 1. Representative structure of F1FO-ATPase monomers in mammalian mitochondria (A). The enzyme subunits are drawn as ribbon representations obtained from modified PDB ID codes: 6TT7.  $\Delta p$ , Mitchell's proton motive force, IMM, inner mitochondrial membrane. The letter colors are the same as those of the subunit to which belong. B) Catalytic binding site of F1FO-ATPase. The ATP substrate and  $Mg^{2+}$  cofactor (in ball and stick representation) are located in the  $\beta$  and  $\alpha$  subunits, drawn as ribbon model (modified PDB ID code: 6J5J) in  $\beta$ TP and  $\alpha$ TP conformation, respectively, which show the position of key amino acid residues that bind  $Mg^{2+}$ . The P-loop is in light blue in both subunits. The binding sites are viewed from the  $\gamma$  subunit (upper panel) and between the observer and the  $\gamma$  subunit (lower panel).

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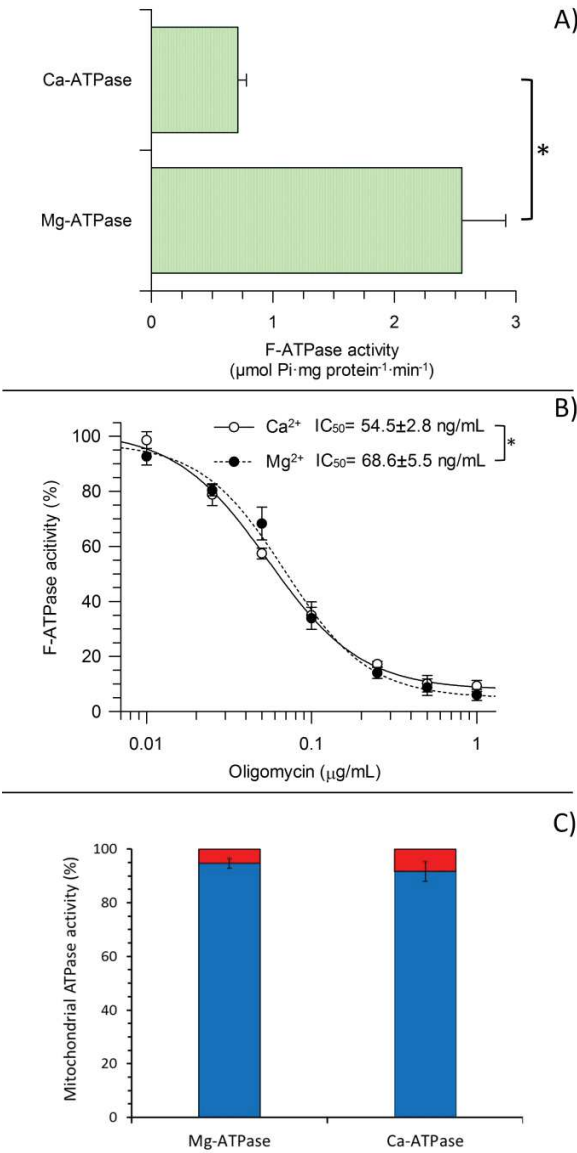


Figure 2. Effect of divalent cations on ATP hydrolysis by the mitochondrial F1FO-ATPase. A) F-ATPase activities in the presence of Ca<sup>2+</sup> or Mg<sup>2+</sup> are shown as bar chart. B) Dose-response curve of oligomycin on the F1FO-ATPase activated by Ca<sup>2+</sup> or Mg<sup>2+</sup> expressed as percentage of the enzyme activity in the absence of oligomycin. C) The oligomycin-sensitive ATPase activity (■ blue) and the oligomycin-insensitive ATPase activity in presence of 3  $\mu\text{g/ml}$  of oligomycin (■ red) are expressed as percentages of the total mitochondrial ATPase activity sustained by Ca<sup>2+</sup> or Mg<sup>2+</sup>, respectively. Data expressed as column chart represent the mean  $\pm$  SD (vertical bars) from three experiments carried out on different mitochondrial preparations. \* indicates significantly different values ( $P \leq 0.05$ ).

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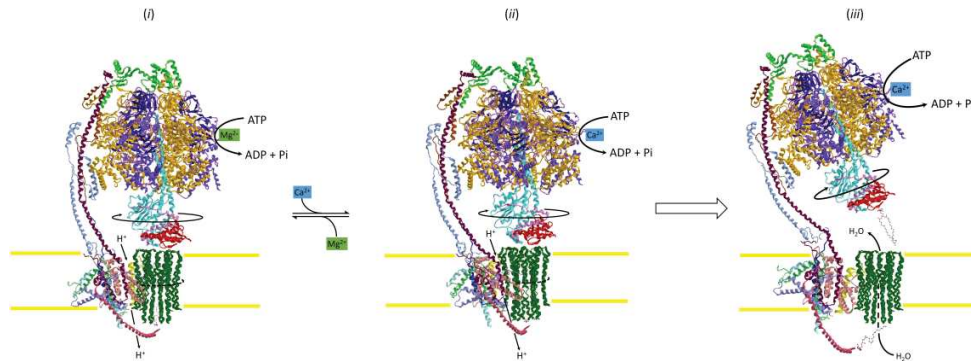


Figure 3. F1FO-ATPase activity raised by Mg<sup>2+</sup> or Ca<sup>2+</sup> as cofactors. ATP hydrolysis sustained by Mg<sup>2+</sup> (i) or Ca<sup>2+</sup> (ii) is coupled to H<sup>+</sup> translocation. The different size of the two cofactors changes the F1FO-ATPase conformation. Indeed, the transition of the Ca<sup>2+</sup>-dependent F1FO-ATP(hydrol)ase from the assembled (ii) to the disassembled state (iii) could induce the loss of H<sup>+</sup>-translocation. Consequently, the PTP opens when a retracted e subunit pulls the lyso-phosphatidylserine plug out of the c-ring at the inner mitochondrial membrane side, while the F1FO destabilization pulls out phosphatidylserine at the matrix side.

381x143mm (600 x 600 DPI)