Biochimie 152 (2018) 85–93

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Mini-review

From the Ca^{2+}-activated F_1F_O-ATPase to the mitochondrial permeability transition pore: an overview

Salvatore Nesci, Fabiana Trombetti, Vittoria Ventrella, Alessandra Pagliarani*

Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, 40064, Ozzano Emilia, BO, Italy

ARTICLE INFO

Article history:
Received 5 April 2018
Accepted 26 June 2018
Available online 28 June 2018

Keywords:
F_1F_O-ATPase
Calcium ion
Mitochondrial permeability transition pore
Conformational mechanism

ABSTRACT

Based on recent advances on the Ca^{2+}-activated F_1F_O-ATPase features, a novel multistep mechanism involving the mitochondrial F_1F_O complex in the formation and opening of the still enigmatic mitochondrial permeability transition pore (MPTP), is proposed. MPTP opening makes the inner mitochondrial membrane (IMM) permeable to ions and solutes and, through cascade events, addresses cell fate to death. Since MPTP forms when matrix Ca^{2+} concentration rises and ATP is hydrolyzed by the F_1F_O-ATPase, conformational changes, triggered by Ca^{2+} insertion in F_1, may be transmitted to F_O and locally modify the IMM curvature. These events would cause F_1F_O-ATPase dimer dissociation and MPTP opening.

1. Introduction

Oxidative phosphorylation, which features mitochondria in eukaryotes, is based on the cooperation and interplay between multiple enzyme complexes. Briefly, these complexes are dehydrogenases which transfer electrons according to the electrochemical gradient from reduced respiratory substrates, namely NADH and $FADH_2$, to the final acceptor molecular oxygen, and, by pumping protons in the intermembrane space, generate a H^+ current through the inner mitochondrial membrane (IMM). Finally, the transmembrane electrochemical gradient of H^+ ($\Delta\mu_{H+}$) created by respiratory chain substrate oxidation drives ATP synthesis by the ATP synthase [1]. The formation of a large channel in the IMM, namely the so-called mitochondrial permeability transition pore (MPTP), dissipates the $\Delta\mu_{H+}$ and, differently from the accepted bases of chemiosmotic hypothesis [2], eludes ATP production and causes loss of substrates and nucleotides from the mitochondrial matrix [3–5]. MPTP opening, by dramatically changing the IMM electrophysiological features, leads to mitochondrial dysfunction. The MPTP regulation and role in different forms of cell death, including autophagy, and in various pathologies have been the subject of intense and fruitful research, sustained by the hope to exploit this mitochondrial event to fight cancer, ischemic damage and neurodegeneration [6]. On the other hand, recent studies suggest that the MPTP may also play a relevant role in mitochondrial function, cell differentiation and development [7]. The MPTP structure has long remained a mystery, even if its identity was intensively searched for among known membrane components, above all membrane-bound proteins. At first, the voltage-dependent anion channel (VDAC) and the peripheral benzodiazepine receptor on the outer mitochondrial membrane (OMM) together with the IMM adenine nucleotide translocase (ANT) seemed the most likely candidates to take part in the enigmatic mechanism of MPTP formation [8]. In this putative mechanism, ANT was thought to constitute the MPTP fulcrum since the ANT inhibitors atractyloside (ATR) and bongkrekic acid (BGK) modulated the MPTP. In detail, BGK inhibited the MPTP by locking ANT in the M conformation (closed MPTP), while ATR maintained it in the C conformation (open MPTP) [9]. However, the ANT channel showed a similar conductance to that of the MPTP [10]. Subsequent findings pointed out that MPTP formation involved a supramolecular complex, namely the assembly of different proteins [11]. Accordingly, differently localized proteins, namely hexokinase bound to the cytosolic surface of OMM, creatine kinase and nucleoside diphosphate kinase in the intermembrane space, and cyclophilin D (CypD) in the matrix apparently contributed to form the MPTP. An alternative model, in which the P_i carrier by interacting with ANT and CypD induced MPTP opening, was depicted [12]. However, all the models proposed over 40 years of studies did not fully match the electrophysiological MPTP features [6] or were undermined by genetic deletion tests, which, one by one, excluded that any of these proteins are essential for MPTP formation [13–16].

* Corresponding author.
E-mail address: alessandra.pagliarani@unibo.it (A. Pagliarani).

https://doi.org/10.1016/j.biochi.2018.06.022
0300-9084/

ANT, the P_i carrier and the F_1F_O-ATPase may mutually interact through cardiolipin which would somehow connect these proteins to form the ATP synthasome. Consistently, conformational changes triggered by Ca^{2+} within the ATP synthasome may perturb the interface between these structures and produce the pore [17]. The ATP synthasome dynamics is ruled by the metabolic demand and is CypD-dependent [18]. Moreover, changes in the contact sites between the inner and outer mitochondrial membranes could intervene in MPTP opening [17]. At present, it seems likely that the MPTP may coincide with a conserved mitochondrial protein of key role in mitochondria. Recently, the F_1F_O-ATPase -a splendid molecular machine- [19] has been proposed to form the pore structure [20,21].

2. The F_1F_O-ATPase: from an old to a new story as pore former

From its discovery around the middle of the 20th century, the F_1F_O-ATPase has undergone a sort of on-going evolution, stimulated by the increasing development of techniques and of knowledge, which lead to a continuous re-evaluation of the roles of this intriguing enzyme complex [19,22]. At present, we can say that new and up to now unsuspected roles for this ubiquitous enzyme are emerging in mitochondria. As widely known, in eukaryotic mitochondria the F_1F_O-ATPase constitutes the amazing molecular machine that exploits the electrochemical energy produced by the respiratory chain in the form of Mitchell's proton motive force (Δp) to produce ATP via a chemo-mechanical coupling mechanism [23]. Even if ATP synthesis represents the classical enzyme task, the catalytic mechanism is long known to work also in *reverse* to energize the IMM by ATP hydrolysis [22,24,25]. In practice, the direction of catalysis depends on Δp, with ATP synthesis consuming Δp. and, conversely, ATP hydrolysis re-building Δp. The F_1F_O-ATPase structure is quite complex and can be roughly defined as an oligomer structurally composed by a hydrophilic F_1 catalytic domain and by a membrane-embedded F_O domain. These two domains are joined by a central and a peripheral stalk (Fig. 1). In turn the F_1 sector, which protrudes in the mitochondrial matrix, shows a $\alpha_3\beta_3\gamma\delta\varepsilon$ subunit composition and stoichiometry [26,27]. The three α subunits alternate with three β subunits to form the F_1 globular hexamer. The adenine nucleotide binding sites, namely three non-catalytic sites on α subunits and three catalytic sites on the β subunits, open at the interfaces between the α and β subunits of this spherical complex [28,29]. The membrane-embedded F_O sector is also formed by multiple proteins, namely the *a* subunit, the short amphipathic *b* subunit with the two transmembrane α-helices, *e*, *f*, *g*, A6L, DAPIT (diabetes-associated protein in insulin-sensitive tissue) subunits, a 6.8 kDa proteolipid and the c_n-ring, in which the subunit number is species-dependent [30,31]. The γ subunit extends from the center of the $(\alpha\beta)_3$ structure of F_1 to the F_O domain where it joins the δ and the ε subunits to form the foot of central stalk [32]. The core of F_O is formed by the *c*-ring, which is directly attached to the central stalk and constitutes the enzyme rotor, which transmits the rotational energy to F_1. Laterally, the *b*, *d*, F_6 and OSCP subunits form the peripheral stalk, which not only links the $(\alpha\beta)_3$-catalytic structure to the *a* subunit in the F_O domain, forming the integral enzyme stator, but also plays the role of resisting the torque generation of rotor [33]. Actually, the F_1F_O-ATPase/synthase is a rotary engine which matches rotation to catalysis. The clockwise rotation (seen from the intermembrane space) is driven by Δp which makes H^+ downhill translocate across the IMM through the *a* subunit/*c*-ring complex interface. This rotation transmitted from F_O to F_1 produces one ATP molecule per each β subunit, namely three ATP molecules are built in a 360° cycle. The opposite rotation, which pumps H^+, in the intermembrane space and re-constitutes Δp, is coupled to ATP hydrolysis. The

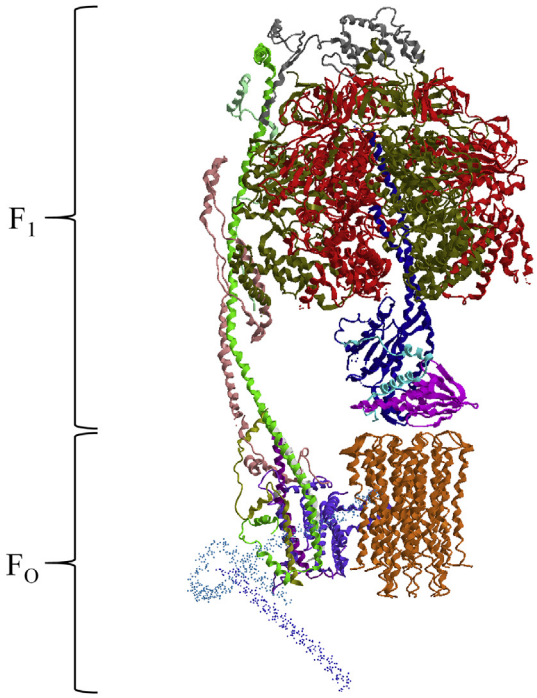

Fig. 1. Subunit composition and structural arrangement of the F_1F_O-ATPase monomer. Protein subunits are drawn as ribbon representations (modified PDB ID codes: 5ARA and 6B2Z). Olive, α subunits; red, β subunits; blue, γ subunit; fuchsia, δ subunit; turquoise, ε subunit; orange, ring of *c* subunits; violet, *a* subunit; purple, A6L subunit; gold, *f* subunit; green, *b* subunit; pink, *d* subunit; sky-blue, F_6 subunit; grey, OSCP subunit. *e* and *g* subunit drawn in ball and stick mode, are blue and light blue, respectively. DAPIT and the 6.8 kDa proteolipid, still undefined membrane subunits, are not represented.

nucleotide binding in the catalytic site requires the coordination of the essential cofactor Mg^{2+}, which contributes to ATP synthesis/hydrolysis and to the asymmetry of the three catalytic sites, which produces the differences in affinity for nucleotides [34]. Accordingly, each β subunit is asymmetric and during the rotation, by interacting with the γ subunit, undergoes three distinct conformational states β_E (always empty), β_{DP}, which contains bound MgADP and β_{TP} which binds MgATP [35].

Interestingly, in mitochondria, the F_1F_O-ATPases are assembled in supra-molecular dimeric complexes by the transmembrane F_O domain [36] which form extensive rows [37,38] distributed along the tightly curved ridges of the IMM *cristae* [39]. This localization exploits the higher H^+ density on the surface in the curved membrane regions [40] created by the respiratory complexes crowding at either side of the rows [41]. Structure, localization and function are tightly connected. Accordingly, the F_1F_O-ATPase structural arrangement and localization have relevant implications for the mechanism of mitochondrial energy transduction [39] and substantiate the F_1F_O-ATPase active role in membrane bending and *cristae* formation [40,42], thus contributing to mitochondrial morphology. Indeed, the enzyme complex assembly locally produces an extreme membrane curvature in either concave (negative curvature) where the membrane invaginates or convex (positive curvature) at the edge of the *cristae*, as seen from the matrix [40].

The F_1F_O-ATPase energy-transduction mechanism of bioenergetics [24] and its modeling ability on mitochondria [29,36] turns into an energy-dissipating machinery when the mitochondrial Ca^{2+} concentration abruptly increases under pathological conditions [43]. In this case the F_1F_O-ATPase activated by Ca^{2+} instead of Mg^{2+} would form a channel which matches the