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ATP synthase oligomerization: From the enzyme models to the mitochondrial morphology☆

Johan Habersetzer[a,b], Widade Ziani[a,b], Isabelle Larrieu[a,b], Claire Stines-Chaumeil[a,b], Marie-France Giraud[a,b], Daniel Brèthes[a,b,*], Alain Dautant[a,b,**], Patrick Paumard[a,b,***]

[a] University of Bordeaux, IBGC, UMR 5095, F-33000 Bordeaux, France
[b] CNRS, IBGC, UMR 5095, F-33000 Bordeaux, France

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ABSTRACT

Mitochondrial F_1F_o ATP synthase is an enzymatic complex involved in the aerobic synthesis of ATP. It is well known that several enzymes are organized in supramolecular complexes in the inner mitochondrial membrane. The ATP synthase supramolecular assembly is mediated through two interfaces. One leads to dimer formation and the other to oligomer formation. In yeast, the presence of ATP synthase oligomers has been described as essential to the maintenance of the mitochondrial *cristae* ultrastructure. Indeed, the destabilization of the interactions between monomers was shown to alter the organization of the inner mitochondrial membrane, leading to the formation of onion-like structures similar to those observed in some mitochondrial pathologies. By using information obtained this decade (structure modeling, electron microscopy and cross-linking), this paper (i) reviews the actual state of the art and (ii) proposes a topological model of the transmembrane domains and interfaces of the ATP synthase's tetramer. This review also discusses the physiological role of this oligomerization process and its potential implications in mammal pathology.

This article is part of a Directed Issue entitled: Bioenergetic Dysfunction, adaptation and therapy.

1. Introduction

Mitochondria are cytoplasmic organelles organized in cells as a network that can adopt a tubular shape (*Saccharomyces cerevisiae*) or interconnected filaments (mammalian cells). This network is highly dynamic, and reflects the perpetual movements of mitochondria which fuse, move or divide in the cytoplasm. Mitochondria are composed of two distinct membranes, a limiting outer membrane (OM) and an inner membrane (IM) that encloses a protein-rich matrix. These two membranes delineate the inter membrane space (IMS). The IM forms invaginations called *cristae*. The rise of 3D electron tomographic studies revealed a new description of *cristae*, which are connected to the IMS by *cristae* junctions (Frey and Mannella, 2000). *Cristae* are now considered as a subcompartment formed by the invagination of the IM (Mannella, 2006). Mitochondria host different functions essential for cell life, among which one of the most important is the conversion of energy into a bio-available form known as ATP. The last step of the aerobic synthesis of ATP is catalyzed by a large complex embedded in the IM, the F_1F_o ATP synthase, also called complex V of the oxidative phosphorylation (OxPhos) pathway. Conserved from yeast to human, this enzyme is composed of two sectors named F_1 and F_o (Table 1). The F_1 sector contains the catalytic head piece. When separated from its membranous counterpart, it retains the ability to hydrolyze ATP. The F_o sector is embedded in the membrane and is mainly composed of hydrophobic subunits forming a specific proton-conducting pathway. The stoichiometry is 3 for the subunits α and β, 8 (mammals) or 10 (yeasts) for the subunit c, and 1 for the other subunits. When the two sectors are coupled, the complex acts as a $\Delta\mu_{H^+}$-driven ATP synthase. ATP is synthesized from ADP and inorganic phosphate using the electrochemical proton gradient generated by the respiratory chain (Fillingame, 1999; for reviews: Ackerman and Tzagoloff, 2005; Devenish et al., 2008; von Ballmoos et al., 2009).

Abbreviations: IM, inner membrane; OM, outer membrane; IMS, intermembrane space; TM, transmembrane spanning segment; EM, electron microscopy; OxPhos, oxidative phosphorylation; BN-PAGE, Blue-Native PAGE; CN-PAGE, Clear-Native PAGE.

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* Corresponding author at: University of Bordeaux, IBGC, UMR 5095, F-33000 Bordeaux, France. Tel.: +33 556999047.
** Corresponding author at: University of Bordeaux, IBGC, UMR 5095, F-33000 Bordeaux, France. Tel.: +33 556999044.
*** Corresponding author at: University of Bordeaux, IBGC, UMR 5095, F-33000 Bordeaux, France. Tel.: +33 556999034; fax: +33 556999051.
E-mail addresses: daniel.brethes@ibgc.cnrs.fr (D. Brèthes), A.Dautant@ibgc.cnrs.fr (A. Dautant), Patrick.Paumard@ibgc.u-bordeaux2.fr (P. Paumard).

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Table 1
Saccharomyces cerevisiae and *Bos taurus* ATP synthase subunits.

Sector	Function[a]	Name[b]	*S. cerevisiae*[c]	*B. taurus*[c]	% identity	TM[d]	Color[a]
F_1	F_1-stator	α	510(545)	510(553)	74		Red
		β	478(511)	480(528)	79		Yellow
	Rotor	γ	278(311)	273(298)	42		Blue
		δ	138(160)	146(168)	36		Magenta
		ε	61(62)	50(51)	33		Green
F_o		***9*/c**(c)[e]	**76(76)**	75(136)	59	↑↓	Purple
	Stator –peripheral stalk	OSCP	195(212)	190(213)	34		Teal
		4(*b*)	209(244)	214(256)	22	↓↑	Pink
		h(F_6)	92(124)	76(108)	15		Pea green
		d	173(174)	160(161)	21		Orange
		f	95(101)	87(88)	21	↓	Gray
	Membrane stator	***6*(*a*)**[e]	**249(259)**	**226(226)**	29	↑↓↑↓↑	Marine
		***8*(A6L)**	**48(48)**	**66(66)**	20	↑	Dark green
		i/*j*	59(59)	–	–	↓	Gold
	Dimerization subunits	*e*	95(96)	70(71)	17	↓	
		g	115(115)	102(103)	25	↓	
		k	68(68)	–	–		
		(*s*)	–	175(200)	–		
	Regulation	IF1	63(85)	84(109)	26		

[a] See Fig. 1.
[b] Protein name of the yeast (*S. cerevisiae*) and mammalian (*Bos taurus*) subunits of the F_1F_o ATP synthase. The mammalian name is written in parenthesis when it differs. mtDNA-encoded proteins are in bold.
[c] Sequence length (in amino acids) of the mature subunit. The sequence length of the precursor protein was given in parenthesis.
[d] Orientation of predicted TMs. Up arrow indicates a TM spanning the IM toward the matrix and down arrow in the opposite direction.
[e] Subunits forming the proton conducting channel.

Respiratory chain complexes are associated into supercomplexes in yeast, mammalian (Schägger and Pfeiffer, 2000) and plant mitochondria (Van Lis et al., 2003; Eubel et al., 2004; Dudkina et al., 2006a). In yeast, cardiolipins stabilize these supercomplexes (Pfeiffer et al., 2003). It has also been established that interactions between ATP synthase monomers leads to the formation of dimers and oligomers. Blue-Native (BN-PAGE) and Clear-Native (CN-PAGE) polyacrylamide gel electrophoresis of the yeast F_1F_o ATP synthase permitted the observation of dimeric and oligomeric forms of the enzyme when solubilized with digitonin (Arnold et al., 1998; Paumard et al., 2002b). By using the same technique, supramolecular forms of ATP synthase have been identified in mammals, fungi and plants (Krause et al., 2005; Cortes-Hernandez et al., 2007). It has been reported that in bovine ATP synthase, the subunit *s* called factor B (Lee et al., 2008) or the inhibitor peptide IF1 (Walker and Gledhill, 2005; Garcia et al., 2006) could promote the dimerization.

The supramolecular organization of ATP synthase along the edge of tubular *cristae* was evidenced for the first time in *Paramecium multimicronucleatum* mitochondria by using freeze-fracture electron microscopy (EM) (Allen et al., 1989). This study correlates the linear and regular arrays of dimer assembly on *cristae* to the formation of tubular *cristae* (Allen, 1995). Single-particle analysis of detergent-solubilized ATP synthase dimers by transmission EM from yeast (Dudkina et al., 2006b; Thomas et al., 2008; Couoh-Cardel et al., 2010), bovine (Minauro-Sanmiguel et al., 2005) or fungi (Dudkina et al., 2006b) mitochondria revealed (i) a main interface through the membrane domains, (ii) a variable distance between the catalytic domains and (iii) the shape of the peripheral stalk. Recently, rows of dimers have been observed by atomic force microscopy (Buzhynskyy et al., 2007) and EM (Thomas et al., 2008) in yeast and by cryo-EM tomography in rat and bovine mitochondria (Strauss et al., 2008) or potato and fungi (Davies et al., 2011). To date, the existence of rows of ATP synthase dimers in mitochondria is widely accepted. Cross-linking studies were performed to establish the molecular basis of the contact interfaces (Belogrudov et al., 1996; Spannagel et al., 1998; Velours et al., 2011). Two interfaces have been characterized: (i) the dimerization interface and (ii) the oligomerization interface. The oligomers observed in the membrane appear to be constructed by the association of dimers.

Our aim in this review is to propose a topological model of the ATP synthase oligomer and to discuss the potential role of the oligomerization in the cell. As such, we will combine the various available structural data to provide a homology model of the yeast monomeric ATP synthase. Further, we will propose a raw topological model of the dimeric and the oligomeric enzyme. Finally, we will describe the role of this process in the maintenance of the mitochondrial shape in yeast and discuss its physiological regulation and potential pathophysiological role in yeast and mammals.

2. The model of the yeast F_1F_o ATP synthase monomer

AG Leslie and JE Walker's numerous atomic models of various conformations of the F_1 sector, of the soluble part of the peripheral stalk (*bd*F_{6sol}) (Dickson et al., 2006), of the F_1 sector in complex with entire or truncated subunits of the stator (OSCP, F_6, b_T and d_T (F_1-stator$_T$) (Rees et al., 2009) and of the sub-complex F_1c_8 (Watt et al., 2010) are invaluable for knowledge of the bovine enzyme. Although the structures of the F_1 sector (Kabaleeswaran et al., 2006) and of the sub-complex F_1c_{10} (Stock et al., 1999; Dautant et al., 2010; Giraud et al., 2012) were solved for the yeast enzyme, there is no atomic model for the yeast peripheral stalk. Finally, few recent studies into membrane stator subunits concern bacterial enzymes (Priya et al., 2009; Lau and Rubinstein, 2011). Conversely, there is more cross-linking data for the yeast dimers and oligomers than for the bovine ones. The cryo-EM structures of both bovine and yeast monomeric ATP synthases evidenced the strong overall similarity of the two enzymes (EMDB ID: 1357; Rubinstein et al., 2003; EMDB ID: 2011; Lau et al., 2008) and provided helpful guidance for molecular modeling.

Since the OSCP and the subunits *4*(*b*) and *d* are highly similar to their bovine counterparts they can be accurately modeled for the soluble part of the yeast peripheral stalk (Table 1, Fig. 1). Indeed, the predicted secondary structures of the subunits that constitute the bovine stator were valid (Rees et al., 2009). Cross-linking studies have indicated that the C-terminal part of the subunit *4*(*b*) is in contact with the subunits *b*, *d*, *h* and OSCP, (Soubannier et al., 1999). These cross-links were confirmed by the bovine F_1-stator$_T$ structure where the peripheral stalk follows a noncatalytic subunit interface along the surface of the F_1 head (Rees et al., 2009). The