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Review

The little we know on the structure and machinery of V-ATPase

Shai Saroussi and Nathan Nelson*
Biochemistry Department, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel
*Author for correspondence (e-mail: nelson@post.tau.ac.il)

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Summary

The life of every eukaryotic cell depends on the function of vacuolar H^+-ATPase (V-ATPase). Today we know that V-ATPase is vital for many more physiological and biochemical processes than it was expected three decades ago when the enzyme was discovered. These range from a crucial role in the function of internal organelles such as vacuoles, lysosomes, synaptic vesicles, endosomes, secretory granules and the Golgi apparatus to the plasma membrane of several organisms and specific tissues, and specialized cells. The overall structure and mechanism of action of the V-ATPase is supposed to be similar to that of the well-characterized F-type ATP synthase (F-ATPase). Both consist of a soluble catalytic domain (V_1 or F_1) that is coupled to a membrane-spanning domain (V_o or F_o) by one or more 'stalk' components. Owing to the complexity and challenging properties of V-ATPase its study is lagging behind that of its relative F-ATPase. Time will tell whether V-ATPase shares an identical mechanism of action with F-ATPase or its mode of operation is unique.

Key words: V-ATPase, secretory pathway, membrane proteins, mechanism of action, structure.

Introduction

The vacuolar H^+-ATPase (V-ATPase) functions in almost every eukaryotic cell. V-ATPase has a similar structure and mechanism of action to the F-ATPase, and several of their subunits probably evolved from common ancestors (Nelson, 1992). In contrast to F-ATPase, the primary function of which, in eukaryotic cells, is to form ATP at the expense of the proton motive force (pmf), V-ATPase functions exclusively as the ATP-dependent proton pump. The pmf generated by V-ATPase is utilized as a driving force for numerous secondary transport processes (Beyenbach and Wieczorek, 2006; Hirata et al., 1997; Nelson and Harvey, 1999). V-ATPase functions in various organelles such as endosomes, lysosomes, Golgi membranes, several types of secretory granules, clathrin-coated vesicles and the central vacuoles of plants and yeast (Anderson and Orci, 1988; Nelson and Harvey, 1999; Nishi and Forgac, 2002). Each of these intracellular compartments has a specific requirement for the internal pH that is generated by the V-ATPase (Nelson et al., 2000), moreover, V-ATPase may act as a sensor of their internal pH (Hurtado-Lorenzo et al., 2006). In a single cell, V-ATPases can be involved in a variety of essential cellular functions such as receptor-mediated endocytosis, posttranslational secondary transport (Nelson, 2003; Nelson and Harvey, 1999; Sun-Wada et al., 2004) and in the fusion of certain organelles with the plasma membrane (Morel, 2003). V-ATPases also play a major role in the energizing of the plasma membranes, especially apical plasma membranes of epithelial cells (Beyenbach and Wieczorek, 2006; Nelson and Harvey, 1999). Because of its essential role in many cellular processes, it is expected that V-ATPase will be present at high quantities in those membranes. However, most of the isolated organelles and membranes contain small amounts of V-ATPase (Nelson and Harvey, 1999). This together with the low specific activity that was measured *in vitro* represent a major challenge for the understanding of the function of V-ATPase in the various organelles.

Eukaryotic V-ATPase can be divided into two structural domains (Fig. 1): (1) a membrane-bound 260 kDa domain, V_o, composed of subunits a, d, e and the proteolipids subunits c, c′ and c″ with the stoichiometry of a1, d, e, $c_{4–5}$, c′ and c″, and (2) a soluble domain, V_1, composed of subunits A, B, C, D, E, F, G and H with suggested stoichiometry of A_3, B_3, C, D, E, F, G_2, $H_{1–2}$ and total mass of 600–650 kDa (Wang et al., 2007). Most of the structural information about eukaryotic V-ATPase comes from electron microscopy (Wilkens et al., 2005) and structures of prokaryotic homologs. To date, only two subunits of eukaryotic V-ATPase, C and H, have been determined by high-resolution X-ray crystallography (Drory et al., 2004a; Sagermann et al., 2001). It is the challenge for future research to come up with more high-resolution structures to get a better view of the architecture of the enzyme so that its mechanism of activity can be elucidated.

Comparison of V-ATPase and F-ATPase

The general architecture of the V-ATPase and the F-ATPase is similar. Both are composed of a soluble domain (V_1 and F_1, respectively) and a membrane embedded domain (V_o and F_o, respectively). They also share several homologous subunits, which probably evolved from a common ancestor (Nelson, 1992) (Fig. 1). Despite the similarity between the two holoenzymes, their localization, function and origin are different. In most cases F-ATPase is located in the plasma membrane of eubacteria, in the thylakoid membrane of chloroplasts and in the inner membrane of mitochondria. Its primary function is to synthesize ATP at the expense of the pmf, which is generated by the electron transport chains. By contrast, V-ATPase functions in eukaryotic plasma membranes as a membrane energizing system and in the membranes of vesicles and organelles mostly involved in the secretory pathway, as an interior acidifier (Nelson and Harvey, 1999). The origin of the eukaryotic F-ATPase is rooted in the eubacteria that evolved into chloroplasts and mitochondria.

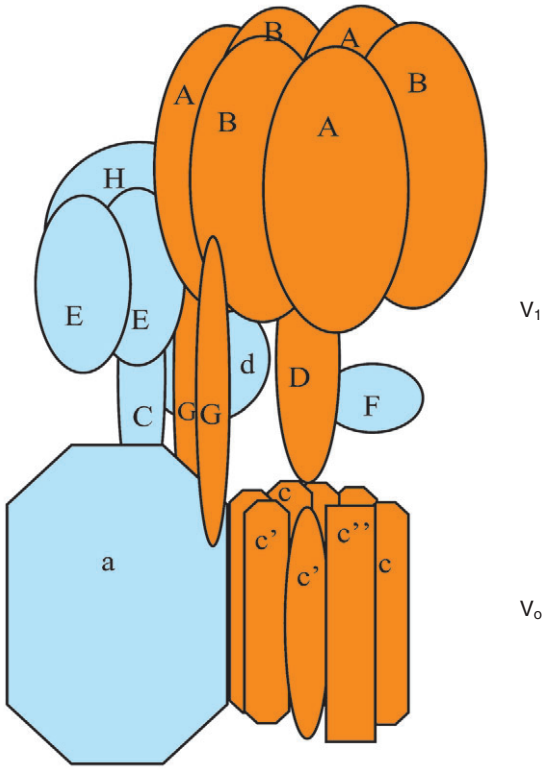

Fig. 1. Diagram of V-ATPase architecture. V-ATPases consist of two domains: a soluble domain, V_1, and a membrane-embedded domain, V_o. F-ATPase homologous subunits are colored orange. V-ATPase unique subunits are colored light blue.

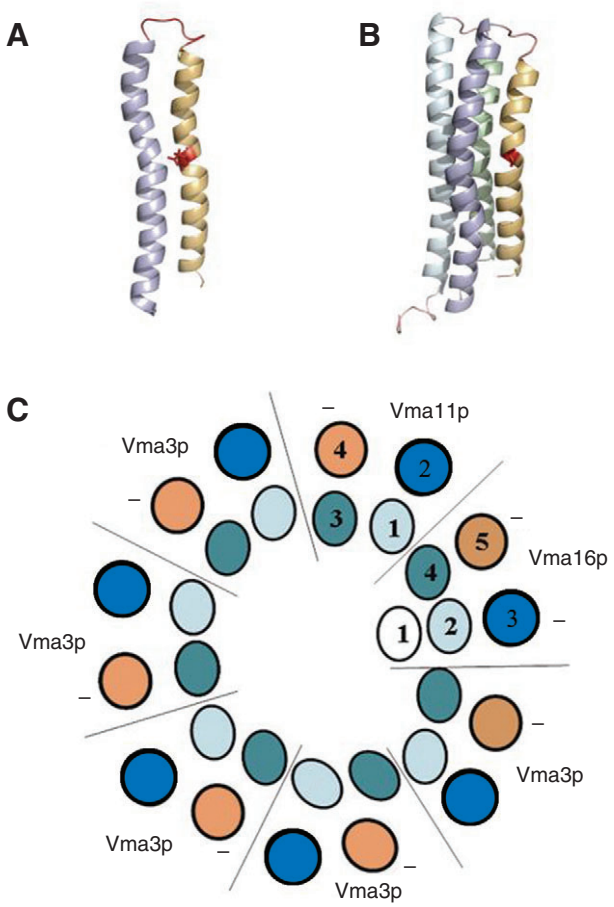

Fig. 2. Diagram of subunit c from F-ATPase (A) and V-ATPase (B). Subunit c of the V-ATPase consists of four transmembrane helices, two of which are homologs of the two transmembrane helices of F-ATPase (homologous helices are colored in brown and blue). The negatively charged amino acid Asp61 of *E. coli* F-ATPase and Glu137 of *S. cerevisiae* V-ATPase (colored in red) are essential for proton translocation. The structure of subunit c from *E. coli* F-ATPase is according to Protein Data Bank (PDB) ID 1c0v (Grivin et al., 1998). The model of subunit c from *S. cerevisiae* V-ATPase was generated with 3D-JIGSAW using PDB ID 2bl2 (Murata et al., 2005). All structural figures were rendered with PyMOL (www.pymol.org).
(C) Diagram of *S. cerevisiae* V-ATPase c-ring. The c-ring consists of three different subunits Vma3p, Vma11p and Vma16p (also termed c, c′ and c″, respectively), which assemble a six to seven subunit ring with a possible stoichiometry of $c_{4–5}$, c′, c″. Subunit c′ locates counterclockwise to subunit c″ (as viewed from the lumen) and the other four or five copies of subunit c complete the rest of the ring.

Because its assembly requires gene products of the organelles, it is confined to these organelles and shows no contact with the cell cytoplasm (Nelson, 1992). The origin of V-ATPase is related to the F-ATPase of Archaea (also called A-ATPase), and evolved in eukaryotes concomitantly with the secretory pathway. In contrast to the F-ATPase, V-ATPase is necessary for almost every eukaryotic cell, and except for yeast, the consequence of its destruction is lethality (Nelson and Harvey, 1999).

Looking closely into the structure of the two enzymes reveals a fundamental difference in the composition of V_o and F_o. The multimeric proteolipid subunit c (c-ring) of F-ATPase consists of 10–14 identical subunits (depending on the organism and organelle). Each subunit, with a molecular mass of 8 kDa, consists of two transmembrane α-helices. Helix 2 contains a negatively charged amino acid in position 61 (in *Escherichia coli*) that is essential for proton translocation (Fillingame et al., 2003) (Fig. 2A). The V-ATPase proteolipid is homologous to the F-ATPase proteolipid but the former is twice the size, 16 kDa, with four transmembrane α-helices (Mandel et al., 1988; Nelson and Nelson, 1989). The essential negatively charged glutamic acid in position 137 (in *Saccharomyces cerevisiae*) which also binds *NN′*-dicyclohexylcarbodi-imide (DCCD) is located in helix 4 (Fig. 2B).

Unlike F-ATPase, the c-ring of the yeast V-ATPase consist of three different subunits, so called c, c′ and c″, which are encoded by three different genes (in *S. cerevisiae*: *vma3*, *vma11* and *vma16*, respectively). Together they assemble a six- to seven-subunit ring in the following possible stoichiometry of $c_{4–5}$, c′, c″ (Powell et al., 2000). Subunits c′ and c″ are homologous to subunit c and consist of four and five transmembrane α-helices, respectively (Hirata et al., 1997). Null mutation of subunit c″ (*vma16Δ*), like most of the null mutations in yeast, results in a conditionally lethal phenotype (Nelson and Nelson, 1990). It was found that Vma16p from lemon fruit and *Arabidopsis thaliana* was able to complement the yeast *vma16Δ* null mutant. Moreover, sequence homology of subunit c″ between lemon fruit and yeast, reveals high identity among α-helices 2–5 (56%) but not with helix 1, which is lacking in plants. These findings led us to suggest that in yeast, helices 2–5 of subunit c″ are transmembrane and that the first α-helix is a cytoplasmic segment (Aviezer-Hagai et al., 2003). Taking into account the orientation of each proteolipid (Flannery et al., 2004), a series of gene fusion constructs between pairs of proteolipids was tested for assembly and activity and the results suggested a particular

asymmetric arrangement of the proteolipid in the c-ring (Wang et al., 2007). Subunit c′ locates counterclockwise to subunit c″ (as viewed from the lumen) and the other copies of subunit c form the rest of the c-ring (Fig. 2C). The question we may ask is why V-ATPase needs such a large, multi-subunit, c-ring? We suggest that the constraint that led to gene duplication resulted in a so called proton slip mechanism (Moriyama and Nelson, 1988; Nelson et al., 2002; Perzov et al., 2001). This mechanism enables proton slippage in order to retain a membrane potential of about 120 mV and avoid thermodynamic equilibrium (about 240 mV). If proton slippage does not occur, the membrane potential will reach its maxima and ATP hydrolysis will cease.

Proton translocation from one side of the membrane to the other is assigned to subunit a. In *E. coli*, F-ATPase subunit a is 30 kDa. It is a highly hydrophobic protein consisting of five transmembrane α-helices (Vik and Ishmukhametov, 2005). Eukaryotic V-ATPase subunit a is much larger, about 100 kDa, and consists of two domains. The hydrophilic N-terminal domain and a large membrane-embedded, highly hydrophobic domain that spans the membrane with eight α-helices (Wang et al., 2008). *S. cerevisiae* contains two isoforms of subunit a, encoded by two genes: *VPH1* and *STV1*. Their gene products are usually assigned to the vacuole (Vph1p), and to the Golgi and endosomes (Stv1p) (Manolson et al., 1994). This suggests that the isoforms are responsible for enzyme localization. In contrast to other V-ATPase subunits, *VPH1* or *STV1* null mutants can grow on a pH 7.5 medium (the double mutant is lethal). We have shown that the *vph1Δ* mutant has an high levels of STV1 and that purified vacuoles contain assembled V-ATPase complexes (not contaminated with endosomal V-ATPase). In addition, the amount of proton pumping was measurable (Perzov et al., 2002).

Cold inactivation

The purification process of the V_oV_1 complex must be delicate and fast because of the high sensitivity of the enzyme, in particular to cold. Incubation of reconstituted V_oV_1 complexes purified from chromaffin granules at 0°C in the presence of Mg^{2+}, Cl^- and ATP resulted in a reduction of the ATPase activity and proton translocation. It was found that 0.1 mmol l^{-1} MgATP and 0.2 mol l^{-1} NaCl cause maximal inhibition. Similar results were found with V-ATPase of clathrin-coated vesicles, synaptic vesicles, kidney microsomes, red beet vacuoles and tomato vacuoles. The reason for the decline of the enzyme activity was the release of five polypeptides from the complex, which were found to be part of V_1 (Moriyama and Nelson, 1989). Later on, *in vivo* studies discovered that depletion of glucose and elevated amount of NaCl in the growth medium (in yeast) and molting (in insects), results in a reversible dissociation of V_1 (Kane, 1995; Perzov et al., 2001; Sumner et al., 1995).

It was suggested that the dissociation of V_1 is a result of the release of the unique subunit C which serves as a link between V_o and V_1 and that this phenomena is part of enzyme regulation (Beyenbach and Wieczorek, 2006). The dissociated V_1 was found to be not active, furthermore the membrane bound V_o was not able to translocate protons (Beltran and Nelson, 1992). We suggest that if the V_o were to keep its proton conductivity intact after the V_1 dissociation, the organism would be doomed under cold and other stress conditions.

Mechanism of action

It is accepted that F-ATPase and V-ATPase share similar mechanisms of action. Is this correct? Is the conventional mechanism for ATP hydrolysis correct? These two naive questions arise from the following facts.

The first crystal structure of bovine heart mitochondrial F_1-ATPase was presented by the Walker group (Abrahams et al., 1994). The structure revealed a hexagonal, asymmetric catalytic unit, in which each β-catalytic site had different conformations. One catalytic site was occupied by AMP-PNP (adenylyl-imidodiphosphate), a non-hydrolysable analogue of ATP, the second was occupied by ADP and the last was empty. It was suggested that the conformational change is a consequence of the rotation of γ-subunit. This structure fits with Boyer's 'binding change mechanism' which suggests that the three catalytic sites have different nucleotide affinities (Boyer, 1993). A series of F_1-ATPase structures was published subsequently supporting these findings, but some of them raise doubt. There are few F_1-ATPase structures that do not have classical binding change mechanism properties. F_1-ATPase from rat liver mitochondria was found to have a symmetrical hexagonal structure with all three catalytic sites occupied by nucleotides (Bianchet et al., 1998). F_1-ATPase from the thermophilic *Bacillus* PS3 had a symmetrical hexagonal structure with no nucleotides bound to the catalytic sites (Shirakihara et al., 1997). Another example is the F_1-ATPase from spinach chloroplasts. Here, the catalytic sites adopt closed conformation, with no nucleotide binding, to form a symmetrical hexagonal structure (Groth and Pohl, 2001). Collectively, these results may suggest that some of the reported structures are not well

Table 1. Summary of eukaryotic and prokaryotic V-ATPases determined from their high resolution three-dimensional crystal structures

	Subunit	Function	Reference (PDB ID)
S. cerevisiae	V-ATPase subunit C	Stator, actin binding	Drory et al., 2004a (1u7l)
	V-ATPase subunit H	Regulation	Sagermann et al., 2001 (1ho8)
T. thermophilus	Bacterial V-ATPase subunit F (homolog to yeast subunit F)	Regulation	Makyio et al., 2005 (2d00)
	Bacterial V-ATPase subunit C (homolog to yeast subunit d)	Link between the central stalk and the membrane sector	Numoto et al., 2004 (1v9m)
	Bacterial V-ATPase subunit C (homolog to yeast subunit d)	Link between the central stalk and the membrane sector	Iwata et al., 2004 (1r5z)
H. hire	Na^+-dependent V-ATPase subunit K (homolog to yeast subunit c)	Rotor	Murata et al., 2005 (2bl2)
M. mazei	A-ATPase subunit B (homolog to yeast subunit B)	Regulation	Schaefer et al., 2006 (2c61)

PDB ID, Protein Data Bank, identification number.

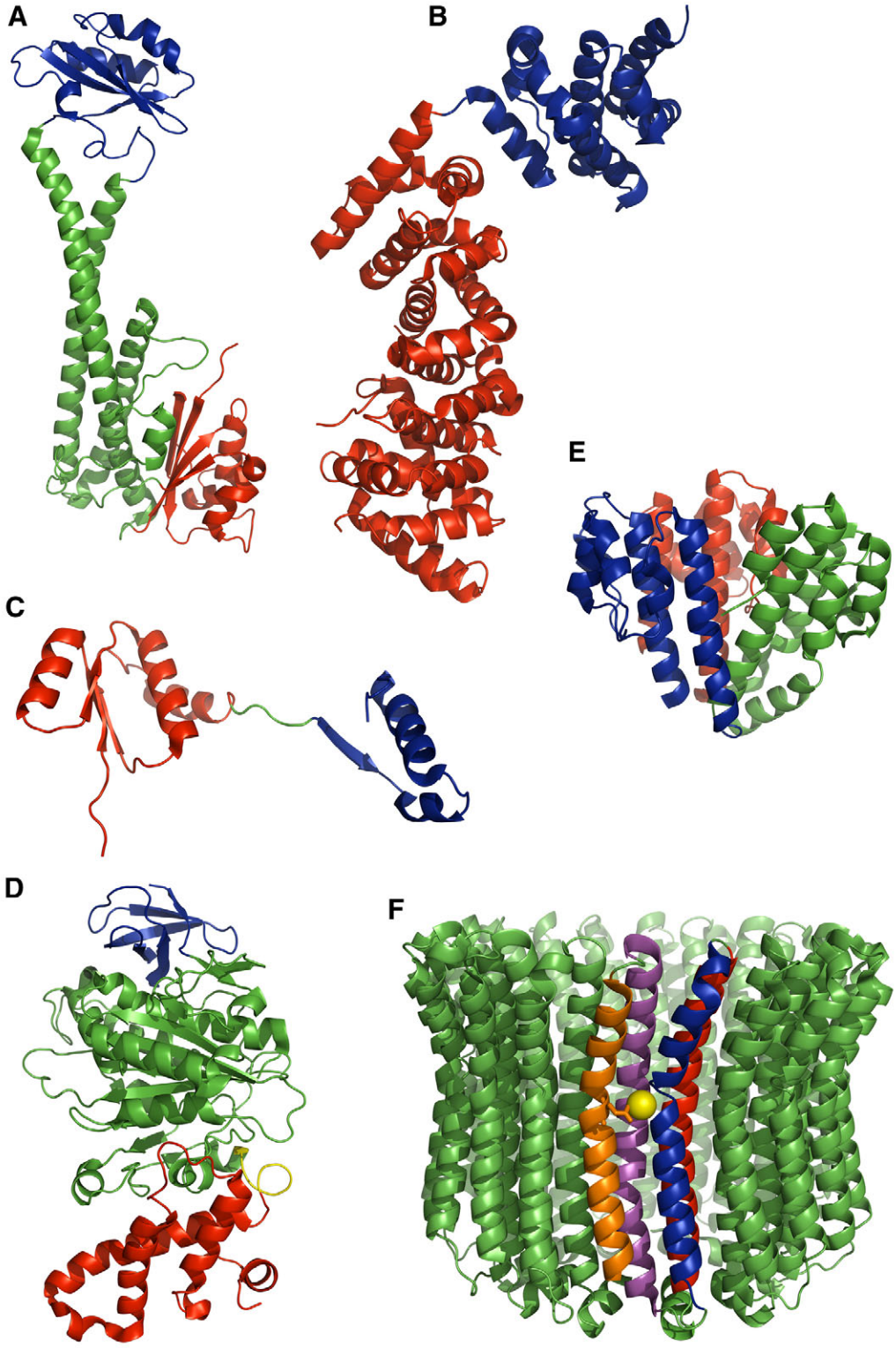

Fig. 3. Crystal structures of V-ATPase subunits and their prokaryotic homologs. (A) Crystal structure of subunit C from *S. cerevisiae* (PDB ID 1U7L). The structure consists of three distinct domains. An upper globular domain, the 'head' (blue). An elongated domain, the 'neck' (green) and lower globular domain, the 'foot' (red). (B) Crystal structure of subunit H from *S. cerevisiae* (PDB ID 1Ho8). There are two distinct domains. A large N-terminal domain (red) and a smaller C-terminal domain (blue). (C) Crystal structure of subunit F from *T. thermophilus* (PDB ID 2d00) in its 'extended' form. It is elongated with two distinct domains. An N-terminal domain (red) and a C-terminal domain (blue) connected by a flexible loop (green). (D) Crystal structure of subunit B from *M. mazei* (PDB ID 2c61). It consists of three domains: an N-terminal domain (blue), an intermediate domain (green) and a C-terminal domain (red). A P-loop is colored yellow. (E) Crystal structure of subunit C from *T. thermophilus* (PDB ID 1r5z). It is funnel shaped with three similar structural domains (colored in red, green and blue). (F) Crystal structure of the K-ring from *E. hire* (PDB ID 2bl2). The K-ring consists of 10 identical subunits. Each subunit contains of four transmembrane α-helices (H1–4) and one soluble α-helix. Negatively charged Glu137, situated in H4 (orange stick), blocks the Na^+ atom (yellow sphere). H0 is colored green, H1 is red, H2 is blue, H3 is magenta and H4 is orange.

resolved. However, there are other possibilities such as crystallographic constrains that have to be kept in mind.

The strongest support for the rotary mechanism came from the observation of a spectacular experiment in which the globular part of bacterial F-ATPase was fixed onto a solid support and an ATP-dependent rotation of a fluorescent rod attached to the γ-subunit or the c-ring (Noji et al., 1997; Omote et al., 1999; Panke et al., 2000). Amazingly, the fact that less than 1% of the total fixed F-ATPase molecules exhibited rotation was totally neglected. Is it possible that the rotating F-ATPase molecules were chemically modified by processes such as proteolytic digestion of one of the subunits? Even if we take the rotation experiments at face value, some discrepancies between the structural information and the rotation mechanism were reported (Sielaff et al., 2008). Can we adopt the wealth of information obtained for F-ATPase to explain the mechanism of action of eukaryotic V-ATPase? Only time will tell.

Structural biology

Studying the structure of the entire V_oV_1 complex requires a concerted effort of various analytical methods such as electron microscopy (EM), small angle X-ray scattering (SAXS), X-ray crystallography, etc. The combination of these methods in addition to biochemical methods such as cross linking assays, localization assays and purification processes may advance our understanding of the structure of V-ATPase.

The increasing use of electron microscopy provides a picture of the holoenzyme. Low resolution structures of V-ATPase from mammals, yeast and plants reveal a general architecture resembling F-ATPase. The complex is cylindrical with overall dimension of 28 nm×14 nm×14 nm. The two major domains, the 6.5 nm V_o and the 9 nm V_1 are connected by a 6 nm stalk (Domgall et al., 2002; Harrison et al., 2003; Wilkens et al., 1999; Wilkens et al., 2005; Zhang et al., 2003). Electron density maps can be used to identify the docking of high resolution structures of a single subunit (or complexes) from eukaryotic and prokaryotic V-ATPase, and thus a structural model can be built. The next level of structure determination is achieved by high resolution three-dimensional structure. Here we can deduce and understand both the structure and functionality of the protein. Unlike F-ATPase, there is no structure evidence of a soluble domain or a membrane embedding domain in the eukaryotic V-ATPase. Notwithstanding, with the relatively minor structural information from single subunit structures of the eukaryotic V-ATPase or its bacterial and archaeal homologs (Table 1), we are trying to learn about the mode of action of the enzyme, the function of each subunit and the interaction between them (reviewed by Drory and Nelson, 2006b).

Structures related to V_1

The crystal structure of the unique V-ATPase subunit C (Vma5p) from *S. cerevisiae* was determined at 1.75 Å resolution in our laboratory a few years ago (Drory et al., 2004a; Drory et al., 2004b; Drory and Nelson, 2006b). It has three distinct domains: an upper globular domain, the 'head', an elongated domain, the 'neck', and a lower globular domain, the 'foot' (Fig. 3A). We suggest that the protein head domain is attached to the catalytic sector and the 'foot' and the neck' domains interact with the membrane domain and act as a stator. Disassembly of the C subunit during glucose depletion or cold treatment followed by the dissociation of the V_1 from V_o, support the notion of a static connection between the catalytic and the membrane sectors (Beyenbach and Wieczorek, 2006). A second crystal structure was determined at 2.9 Å resolution. This structure is highly similar to the first, with the foot and lower neck domain but a large movement of the head domain was found. This phenomenon leads us to attribute a flexible ratchet property to this stator. We also found a remarkable similarity between subunit C foot and head domains and the actin binding protein from the gelsolin family. Moreover, V-ATPase was found to bind F and G actin with high affinity and it also leads to cross linking of actin filaments (Vitavska et al., 2003). Taking all together, we modeled the interaction between actin filaments and subunit C and we found that actin can bind both head and foot domains without any interference between the two (Drory and Nelson, 2006a).

The structure of subunit H (*S. cerevisiae*, Vma13P) was determent at 2.95 Å resolution (Sagermann et al., 2001). It is an α-helical, elongated structure with two distinct domains connected by four residues, probably a flexible loop (Fig. 3B). It has five HEAT motifs (also called armadillo motifs) similar to the karyopherins family, a nuclear localization signal (NLS) binding protein. This similarity may suggest that subunit H binds other proteins using a similar mechanism to the karyoproteins, and may also provide an indication for the regulation properties of ATPase activity that are attributed to this subunit.

Prokaryotic V-ATPase subunit F (Vma7p) is known to function in ATPase activity and in the association of V_1 with V_o. The crystal structure of subunit F was determined at 2.2 Å resolution from the archaean *Thermus thermophilus* (Makyio et al., 2005). The N-terminal and the C-terminal domains are composed of an α/β-fold and they are connected with a flexible loop (Fig. 3C). In addition to the crystal structure, single pair fluorescence resonance energy transfer (FRET) analysis indicates that subunit F exist in two conformations, 'extended' and 'retracted', depending on the presence and absence of ATP, respectively. Moreover, it supports the idea that only one copy of subunit F exist in the V-ATPase complex.

Archeal V-ATPase subunit B, the non-catalytic subunit in the catalytic domain, shares a 58% identity with its *S. cerevisiae* homolog, subunit B. Its structure was determined at 1.5 Å resolution from the archaean *Methanosarcina mazei* (Schafer et al., 2006). Subunit B is 80 Å long and 50 Å wide. It consists of three domains: a six-strand β-barrel N-terminal domain, an intermediate domain composed of α/β-fold and an α-helical C-terminal domain (Fig. 3D). It has been shown that this regulatory subunit can bind nucleotides, but as in eukaryotic subunit B the nucleotide binding site, the P-loop, does not exist in the conserved position as in subunit α of F-ATPase, and both counterparts share identical sequence it the apparent P-loop position. The question whether this shared identical sequence binds nucleotides in both prokaryotic and eukaryotic V-ATPase is still open. Co-crystallization of prokaryotic subunit B with nucleotides may answer this question.

Structures related to V_o

Prokaryotic subunit C shares low but significant sequence similarity (18%) with its yeast homologous subunit d (Vma6p). It is solely ascribed to V-ATPase, and it is located at the central stalk. The structure of subunit C was determined first from the eubacterium *T. thermophilus* at 1.95 Å resolution (Iwata et al., 2004) and subsequently, at 1.85 Å resolution (Numoto et al., 2004). Subunit C is funnel shaped with dimensions of 45 Å×50 Å×50 Å. It is mainly α-helical and consists of three similarly structured domains (Fig. 3E). The lower central region of the protein is about 30 Å in diameter, which matches the 30 Å diameter cavity of the c-ring of *E. coli*. Moreover, the structure reveals a positively charged amino acid region, mainly lysine, at the lower central region, which may interact with the negatively charged glutamic acid of the L

subunit (part of the membrane embedded L-ring, the homolog of F-ATPase and V-ATPase subunit c). Recently a low resolution structure of subunit d from *S. cerevisiae* V-ATPase was determined from solution X-ray scattering data. According to these findings, subunit d is a boxing glove shape with two distinct domains (Thaker et al., 2007). These data contradict the suggested structural similarity between subunit C of *T. thermophilus* and subunit d from *S. cerevisiae*. Therefore, the question of whether prokaryotic subunit C and eukaryotic subunit d are homologous and share similar function remains unanswered.

Na^+-dependent vacuolar ATPase is a close relative of the eukaryotic V-ATPase. The structure of its ring (K-ring) was determined from the prokaryotic *Enterococcus hire* at 2.1 Å resolution (Murata et al., 2005). The ring consists of 10 similar subunits, encoded by the same gene, *NtpK*. Each subunit contains five α-helices, H0–H4. The first α-helix (H0) is cytoplasmic and the other four α-helices (H1–H4) are transmembrane. Similar to its eukaryotic homolog, the essential negatively charged glutamic acid is placed in H4 in position 137, which blocks the Na^+ atom within the binding pocket (Fig. 3F). The ring dimensions are 68 Å in length and 83 Å maximal diameter. As expected from sequence similarity, the ring length is similar to the *S. cerevisiae* and *Ilyobacter tartaricus* F-ATPase c-ring, 58 Å and 70 Å, respectively (Meier et al., 2005; Stock et al., 1999). Interestingly, the ring maximal diameter is much larger the F-ATPase homologs, 55 Å and 50 Å, respectively). These differences may reflect differences in the association of the ring with the central stalk.

Despite the primary structure similarity and the apparent resemblance in architecture, the understanding of the exact structure and the mode of action of each subunit within the eukaryotic V-ATPase c-ring is yet to be determined.

At this point we would like to emphasize that at the time of submission of this review, the high resolution structure of the membrane-embedded proton channel (subunit a in V-ATPase and F-ATPase) has not been determined. Such structural information will facilitate the understanding of the proton translocation mechanism and as a consequence the clarification of the mechanism of energy conversion by the mechano-chemical machines of F- and V-ATPases.

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