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Introduction: A Close Look at the Vacuolar ATPase

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The vacuolar ATPases (V-type ATPases) are a family of ATP-dependent ion pumps and found in two principal locations, in endomembranes and in plasma membranes. This family of ATPases is responsible for acidification of intracellulare compartments and, in certain cases, ion transport across the plasma membrane of eucaryotic cells. V-ATPases are composed of two distinct domains: a catalytic V_1 sector, in which ATP hydrolysis takes place, and the membrane-embedded sector, V_0, which functions in ion conduction. In the past decade impressive progress has been made in elucidating the properties structure, function and moleculare biology. These knowledge sheds light also on the evolution of V-ATPases and their related families of A-(A_1A_0-ATPase) and F-type (F_1F_0-ATPases)ATPases.

KEY WORDS: A_1A_0 ATPase; bioenergetics; F_1F_0 ATPase; vacuolar-type ATPase; V_1V_0 ATPase; V_1 ATPase.

Hardly a day goes by without the importance of the vacuolar type ATPase (V-ATPase; V_1V_0 ATPases) being brought to our attention. This enzyme complex belongs to a family of ion pumps that couples the energy of ATP hydrolysis to ion transport across intracellular and plasma membranes of eukaryotic cells (Nelson and Harvey, 1999; Nishi and Forgac, 2002). The V-ATPase resides in membranes of intracellular compartments, including lysosomes, endosomes, clathrin-coated vesicles, Golgi-derived vesicles, secretory vesicles, and the central vacuoles of fungi and plants. V-ATPases play a variety of roles crucial for the function of these subcellular organelles. For example, acidification of endosomes is required for ligand–receptor dissociation and receptor recycling following receptor-mediated endocytosis, as well as for the formation of endosomal carrier vesicles that transfer ligands from early to late endosomes. Certain viruses and toxins enter cells via acidic endosomal compartments. Acidification of intracellular compartments is also involved in targeting of newly synthesized lysosomal enzymes from the Golgi to lysosomes. In secretory vesicles, such as synaptic vesicles, V-ATPases provide the driving force for uptake of neurotransmitters that are packaged in these compartments. Within lysosomes and the central vacuoles of fungi and plants (Kluge *et al.*, this issue), the V-ATPase generates both the acidic environment required for degradation of macromolecules and the driving force for various coupled processes. V-ATPases identified in the plasma membrane of certain cells are involved in processes like renal acidification, bone resorption, pH homeostasis, coupled transport, and tumor metastasis (Kane and Parra, 2000; Nishi and Forgac, 2002; Wieczorek *et al.*, 1999). The relationship of V-ATPases to other ion-motive ATPases like the F-type (F_1F_0 ATPases) and A-type ATPases (A_1A_0 ATPases) has become clearer through the increasing number of genetic, biochemical, and structural studies (Grüber *et al.*, 2002; Hilario and Gogarten, 1998; Lolkema *et al.*, this issue; Müller and Grüber, 2003; Nelson, 1992).

A fundamental feature of V-ATPases is the reversible dissociation of V_1 from the V_0 complex as a way of in vivo regulation, as shown in insect cells (Sumner *et al.*, 1995) and yeast (Kane, 1995). In both insects and yeast, disassembly of V_1 results in the decrease of Mg-ATPase-activity and proton pumping at the membrane and reassembly restores these activities. Notably, neither disassembly nor reassembly requires new protein synthesis but an intact microtubular network (Holliday *et al.*, 2000; Vitavska *et al.*, 2003; Wieczorek *et al.*, this issue; Xu and Forgac, 2001). In addition, such reassembly is proposed to be promoted by a complex termed RAVE (regulator of H^+-ATPase of vacuolar and endosomal membranes; (Kane and Smardon, this issue)). The recovery of disassembled V_1 particles from the cytoplasm made structural

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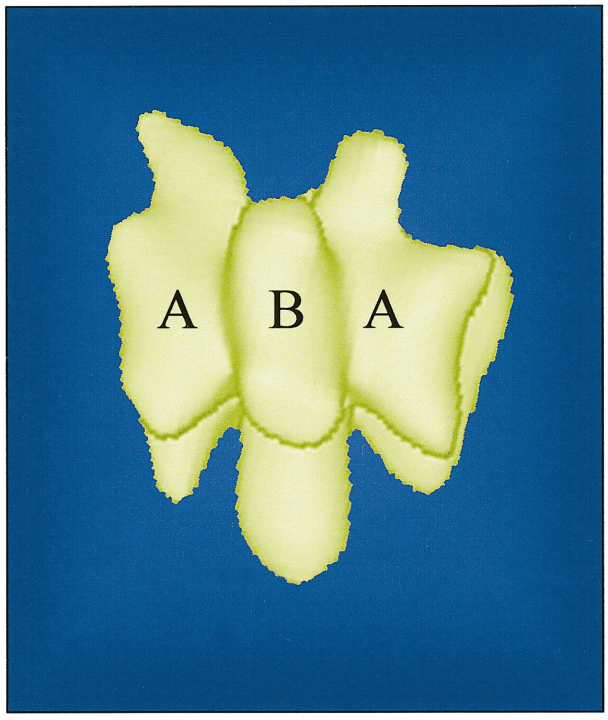

Fig. 1. The 18 Å structure of the V_1 ATPase without subunit C from *M. sexta* (modified from Radermacher *et al.*, 2001).

determinations of this complex possible (Müller and Grüber, 2003; Radermacher *et al.*, 2001).

The V_1 domain comprises the catalytic subunit A and the nucleotide-binding subunit B in a stoichiometry of A_3:B_3 and the so-called stalk subunits C-H in a proposed stoichiometry of C_1:D_1:E_1:F_1:G_2:H_1 (Xu *et al.*, 1999). A comparison of independently identified V_1 structures (Boekema *et al.*, 1998; Radermacher *et al.*, 1999; Svergun *et al.*, 1998; Wilkens, 2001) reveals that the A and B subunits are arranged hexagonally and that they alternate around a central cavity in which a seventh mass is located (Radermacher *et al.*, 1999, 2001). A recent three-dimensional structure of the V_1 ATPase from *Manduca sexta* at 18 Å resolution (Fig. 1) shows that the seventh mass is not located in the center of the cavity of the A_3B_3 hexamer, but slightly offset to one side, thereby strengthening the interaction between two nonneighboring A subunits (Radermacher *et al.*, 2001). This feature is comparable to the asymmetric location of the rotating γ subunit in the $\alpha_3\beta_3$ subcomplex of the related F-ATPase (Abrahams *et al.*, 1994), indicating significant movements of the central mass in V-ATPases during catalysis. In the side view, the structure shows three protuberances at the top of the A_3B_3 headpiece, which might belong to the N-termini of subunit A (Domgall *et al.*, 2002; Grüber *et al.*, 2000; Radermacher *et al.*, 2001; Wilkens *et al.*, 1999). At the bottom side of the A_3B_3 domain the stalk protrudes with an angle of approximately 7° with the vertical axis of the cavity. The only component of the V_1-ATPase solved at atomic resolution (3.0 Å) is subunit H (Graham *et al.*, this issue; Sagermann *et al.*, 2001). This elongated subunit is characterized by a large, primarily α-helical N-terminal domain, forming a shallow groove, and a C-terminal domain, both connected by a four-residue loop.

Structures of the V_1 ATPase from *Calorama-tor fervidus* (Boekema *et al.*, 1998) and *M. sexta* (Radermacher *et al.*, 2001; Svergun *et al.*, 1998) identified recently revealed a molecule with a single compact stalk, but no peripheral stalk. Side view projections of negatively stained V_1V_0 ATPases from *C. fervidus* show a peripheral stalk, proposed to be formed by the V_0 portion and to function as a stator (Boekema *et al.*, 1998). More recently, a second peripheral stalk in the V_1V_0 ATPase has been described from negatively stained images (Domgall *et al.*, 2002; Wilkens *et al.*, 1999). Based on these structural data, the question arose whether the subunits building this putative second stator and the V_1 complex might become more compact following dissociation, with the peripheral stalk collapsing into a single stalk in the free V_1 ATPase.

The V_0 domain is composed of at least five subunits *a*, *d*, *c*, *c*′, and *c*″. Subunit *a* is a two-domain protein. The large N-terminal hydrophilic domain interacts with both subunit H and A (Landolt-Marticorena *et al.*, 2000) and therefore most likely is part of the peripheral stalk. The C-terminal domain of subunit *a* is very hydrophobic and predicted to span the membrane nine times. Mutagenesis studies have identified several membrane-buried residues important for function, but only one Arg residue (Arg-735 of *Saccharomyces cerevisiae*) was shown to be essential for ion transport (Kawasaki-Nishi *et al.*, 2001). A feature very unique to the V-ATPase is the presence of three hydrophobic subunits (*c*, *c*′, and *c*″) that form a hexameric (Graham *et al.*, this issue; Harrison *et al.*, 2003) or heptameric (Murata *et al.*, 2003) proteolipid ring. Subunits *c*, *c*′, and *c*″ are similar to each other and to other proteolipids from A- and F-ATPases. Subunits *c* and *c*′, which are twice the size of the 8-kDa proteolipids from bacteria, probably arose by duplication and fusion of an ancestral proteolipid gene giving rise to a 16-kDa protein with four transmembrane (TM) helices (Hirata *et al.*, 1997; Mandel *et al.*, 1988). Subunit *c*″ is even bigger and has five predicted transmembrane helices, with the active carboxylate conserved only in TM3. Previous studies clearly demonstrated that each V_0 domain contains at least one copy of *c*, *c*′, and *c*″ (Hirata *et al.*, 1997), and it is assumed that the number of transmembrane helices in A-, F-, or

V-ATPases is similar (about 24 transmembrane helices, see above). Therefore, the number of proton translocating residues in V_0 is only half of that in F_0.

Subunit *d* is a completely hydrophilic, cytoplasmic protein but copurifies with the V_0 complex and therefore is regarded as a V_0 subunit. Its function is unknown, but it could be involved in regulation of ion conductance through V_0. Recently, a sixth subunit (*e*) of the V_0 domain has been described for the V-ATPase of the *M. sexta* midgut (Merzendorfer *et al.*, 1999) and bovine chromaffin granules (Ludwig *et al.*, 1998). Until now, nothing is known concerning its function. Interestingly, the homologue of these polypeptides in yeast, Vma21, resides in the endoplasmic reticulum and is there involved in the assembly processes of the V-ATPase (Graham *et al.*, 1998, this issue).

The coupling ion in most of the V-ATPases studied to date is the proton. However, two anaerobic bacteria, *C. fervidus* (Speelmans *et al.*, 1994) and *Enterococcus hirae* (Kakinuma and Igarashi, 1995), contain Na^+-translocating ATPases of the A-/V-type (Lolkema *et al.*, this issue). Both are fermenting anaerobes that have a rather low energy yield obtained only through substrate level phosphorylation. *C. fervidus* is a thermophile, and *E. hirae* produces the Na^+-translocating V-ATPase only at alkaline conditions. In both cases, Na^+-based membrane energization is energetically advantageous over the use of protons. The proteolipid of the V-ATPases from *E. hirae* contains the sodium ion binding motif, and the active carboxylate has been identified experimentally to be involved in Na^+-binding (Murata *et al.*, 1999).

A key remaining question is how catalytic-site events in the A_3B_3-headpiece are coupled to proton translocation through V_0. It seems clear from recent work in a number of different laboratories that both sectors communicate through conformational changes and rearrangements of multiple stalk subunits (Grüber *et al.*, 2001; Hirata *et al.*, 2003; Landolt-Marticorena *et al.*, 2000; Müller and Grüber, 2003). The previous demonstrations of the exquisite operation of the F_1F_0-ATPase at a mechanistical and structural level revealed a biological nanomachine (Yoshida *et al.*, 2001). From what is already known about the mechanism of V-ATPases (Hirata *et al.*, 2003; Sun-Wada *et al.*, this issue), such analogies will be obvious for these ion translocating enzymes.

This volume with 10 minireviews on the V-ATPase both collects historical and recent developments, current results, controversies, ideas from many of the leading investigators and provides an up-to-date snapshot of what is known about this enzyme from a variety of viewpoints. I hope that these publications will stimulate further experiments on V-ATPases as well as summarize the present position to those outside the immediate field.

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