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Structure, subunit function and regulation of the coated vesicle and yeast vacuolar (H^+)-ATPases

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Abstract

The vacuolar (H^+)-ATPases (or V-ATPases) are ATP-dependent proton pumps that function to acidify intracellular compartments in eukaryotic cells. This acidification is essential for such processes as receptor-mediated endocytosis, intracellular targeting of lysosomal enzymes, protein processing and degradation and the coupled transport of small molecules. V-ATPases in the plasma membrane of specialized cells also function in such processes as renal acidification, bone resorption and pH homeostasis. Work from our laboratory has focused on the V-ATPases from clathrin-coated vesicles and yeast vacuoles.Structurally, the V-ATPases are composed of two domains: a peripheral complex (V_1) composed of eight different subunits (A–H) that is responsible for ATP hydrolysis and an integral complex (V_0) composed of five different subunits (a, d, c, c′ and c″) that is responsible for proton translocation. Electron microscopy has revealed the presence of multiple stalks connecting the V_1 and V_0 domains, and crosslinking has been used to address the arrangement of subunits in the complex. Site-directed mutagenesis has been employed to identify residues involved in ATP hydrolysis and proton translocation and to study the topology of the 100 kDa a subunit. This subunit has been shown to control intracellular targeting of the V-ATPase and to influence reversible dissociation and coupling of proton transport and ATP hydrolysis.

Keywords: V-ATPase; Proton transport; Membrane protein; Vacuolar acidification; Proton pump structure; Regulation of acidification

1. V-ATPase function

V-ATPases have been identified in a wide variety of intracellular compartments, including endosomes, lysosomes, clathrin-coated vesicles, secretory vesicles, Golgi-derived vesicles and the central vacuoles of fungi and plants [1,2]. Acidification of these compartments in turn plays an important role in many cellular processes. Acidification of early endosomes is required for dissociation of internalized ligand receptor complexes and recycling of receptors to the cell surface. It also plays a role in formation of endosomal carrier vesicles that move ligands from early to late endosomes [3] and in the entry of certain envelope viruses and toxins into cells. Acidification of late endosomes is required for the targeting of newly synthesized lysosomal enzymes from the Golgi to lysosomes, whereas acidification of a late Golgi compartment appears to play a role in vacuolar targeting in yeast [1]. The low pH of lysosomes (and the central vacuoles of yeast) is necessary to maintain the activity of acid hydrolases present in the lumen of these compartments and to provide a driving force for coupled transport of small molecules and ions. Both the proton gradient and the membrane potential generated by the V-ATPases are used to drive coupled transport in secretory vesicles, such as synaptic vesicles. The V-ATPase in brain clathrin-coated vesicles is likely en route from the plasma membrane (following membrane retrieval) to synaptic vesicles. The V_0 domain has also recently been suggested to play a role in membrane fusion [4]. V-ATPases present in the plasma membrane of specialized cells function in such processes as renal acidification, bone resorption, tumor metastasis and K^+ transport [1].

2. V-ATPase structure

Our current model for the structure of the V-ATPase is shown in Fig. 1. The V-ATPase is composed of two domains

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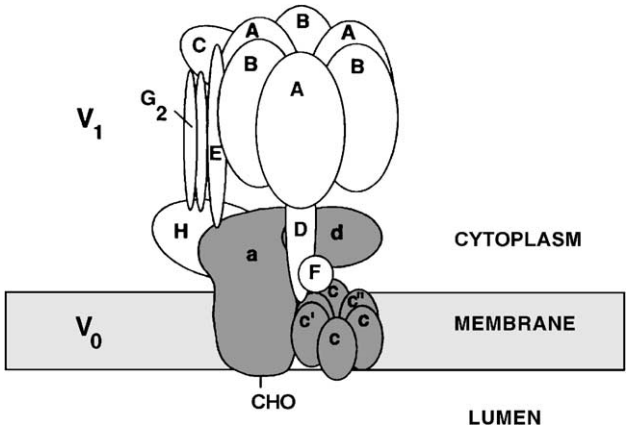

Fig. 1. Structural model of the V-ATPase. The V-ATPase is composed of two domains. The peripheral V_1 domain (shown in white) is responsible for ATP hydrolysis and the integral V_0 domain (shown shaded) is responsible for proton translocation. The V_1 and V_0 domains appear from electron microscopy [8] to be connected by at least two stalks. Based upon crosslinking studies [7,18], the central stalk appears to contain subunits D and F while the peripheral stalk is composed of subunits C, E, G, H and the soluble domain of subunit a. Reprinted with permission from reference 17 (2002) The American Society for Biochemistry and Molecular Biology.

[1,2]. The peripheral V_1 domain is a 640 kDa complex containing eight different subunits (A–H) of molecular mass 70 to 13 kDa that is responsible for ATP hydrolysis. The integral V_0 domain is a 260 kDa complex containing five different subunits (a, c, c′ , c″ and d) of molecular mass 100 to 17 kDa that is responsible for proton translocation. The V-ATPases thus resemble the F-ATPases, which are normally involved in ATP synthesis [5–7]. Electron microscopy has revealed that, like the F-ATPases, the peripheral and integral domains are connected by two stalks [8]. For the F-ATPases, ATP hydrolysis in F_1 drives rotation of a central γ subunit, which in turn drives rotation of a ring of c subunits relative to the a subunit in F_0 [5–7]. The a subunit is held fixed relative to the $\alpha_3\beta_3$ head of F_1 by a peripheral stalk composed of the b and δ subunits. It is rotation of the ring of c subunits relative to a that causes unidirectional proton transport. The V-ATPases are also postulated to operate via a rotary mechanism.

3. Structure and function of V_1 subunits

The nucleotide binding sites in the V-ATPase are located on the 70 kDa A and 60 kDa B subunits, which are each present in three copies per complex [1,2]. The catalytic sites are located on the A subunits and appear, from site-directed mutagenesis and covalent modification using nucleotide analogs [9–11], to contain residues similar to those shown by X-ray crystallography of F_1 to be present at the catalytic sites on the β subunits [12]. These include E286, which is believed to be involved in proton abstraction from water, K263, which helps stabilize the negatively charged phosphates, and three aromatic residues that appear to interact with the adenine ring of the bound nucleotide [9–11]. The B subunits also bind nucleotides, forming so-called "non-catalytic" nucleotide binding sites [13,14]. Despite the absence of Walker A and B motifs, the structure of these sites is well predicted from molecular modeling studies, as indicated by the ability of nucleotide analogs to protect cysteine residues introduced into the B subunit from modification by biotin maleimide [15]. While the function of these sites is uncertain, mutations at these sites suggest they may play a role in regulation of activity [10].

The arrangement and function of other subunits in the V_1 domain has been addressed using a combination of mutagenesis and crosslinking. Mutations in subunit D that lead to uncoupling of proton transport and ATP hydrolysis appear to cluster in two discrete locations of the molecule, suggesting that this subunit may function, like the γ subunit of the F-ATPase, as the central rotor in the V-ATPases [16]. By contrast, subunit E, which like subunit D is predicted to be highly alpha helical, has been shown to reside on the outer surface of the complex, where it likely forms part of the peripheral stator [17]. This was demonstrated by crosslinking of subunit E to subunit B at unique cysteine residues introduced into the exposed surface of subunit B using a photoactivatable maleimide. Other crosslinking studies suggest that, in addition to subunit E, the peripheral stator also contains subunits C, G, H and the amino-terminal soluble domain of subunit a, whereas the central rotor is composed of subunits D and F [18].

4. Structure and function of V_0 subunits

Of the five V_0 subunits, three (c, c′ and c″) are highly hydrophobic proteolipid subunits that are homologous both to each other and to the c subunit of F_0 [1,2]. Subunits c and c′ are both 16 kDa proteins containing four putative transmemebrane helices, with a critical buried acidic residue located in the middle of TM4. Subunit c″ is a 23 kDa polypeptide containing five putative transmembrane segments, with critical acidic residue in TM3 [19]. Because there are a total of five to six copies of the 16 kDa proteolipids (c plus c′) per complex [20], but only single copies of c′ and c″ [21], the likely stoichiometry is $c_{4-5}c'_1c''_1$. Expression of epitope tagged forms of c and c″ suggest that the C-terminus of subunit c is lumenal whereas the C-terminus of subunit c″ is cytoplasmic [22]. Subunit c has also been shown to contain the binding site for the highly specific inhibitor bafilomycin [23]. Electron microscopy of V_0 suggests a ring of proteolipid subunits with the a subunit to one side, although some interdigitation of a and the c ring is suggested within the membrane [24]. Both the d subunit and the amino-terminal domain of the a subunit appear as a "cuff" exposed on the cytoplasmic side of the membrane.

The 100 kDa a subunit possesses an amino-terminal hydrophilic domain of 50 kDa and a hydrophobic car-

boxy-terminal domain (also 50 kDa) containing multiple transmembrane helices [1,2]. Studies employing cysteine-mutagenesis of the yeast 100 kDa a subunit Vph1p and labeling by membrane permeant and impermeant sulfhydryl reagents has led us to propose a model for the a subunit in which the amino-terminal domain is cytoplasmic and the carboxy-terminal domain contains nine transmembrane segments (TM1-9), with the C-terminus located on the lumenal side of the membrane [25]. The carboxy-terminal domain also contains a number of buried charged residues that appear to play a role in proton transport [26–28]. In particular, Arg-735 in TM7 appears to be absolutely essential for proton translocation [28], and may play a role analogous to Arg-210 of the F-ATPase a subunit [29,30], in promoting proton release from the buried carboxyl groups of the proteolipid subunits.

5. Targeting and regulation of the V-ATPases

In yeast, the a subunit of V_0 is encoded by two genes (*VPH1* and *STV1*) [31], with Vph1p present in the vacuole and Stv1p present in another intracellular compartment recently identified as the late Golgi [32]. Vph1p-containing complexes have also been shown to be more assembled with V_1 and to show tighter coupling of proton transport and ATP-hydrolysis than Stv1p-containing complexes [33]. While targeting of the V-ATPase appears to be controlled by signals located in the amino-terminal domain of the a subunit, assembly and coupling efficiency of the V-ATPase appears to be controlled by the carboxy-terminal domain [32]. In mouse, the a subunit is also present in multiple isoforms that appear to be responsible for differential targeting of the V ATPase [34,35]. Interestingly, one splice variant of the a3 isoform is predicted to contain only the amino terminal soluble domain of the protein, and might play a role in controlling assembly of the V ATPase [34].

An important regulatory mechanism for controlling V ATPase activity, first identified in yeast in response to glucose depletion, is reversible dissociation of the V_1 and V_0 domains [36]. We have found that this process, like intracellular targeting, is controlled by signals located in the amino terminal domain of subunit a [32]. Dissociation, but not reassembly, is blocked by disruption of microtubules [37], suggesting that they are independently controlled processes. Interestingly, V ATPase complexes located in the vacuole undergo dissociation, whereas those located in the late Golgi do not [33]. V-ATPase complexes containing Vph1p that are prevented from reaching the vacuole still show glucose-dependent dissociation, but at a reduced level, suggesting that dissociation is controlled both by the a subunit isoform present and by the membrane environment in which the complex resides. A second mechanism proposed for controlling V-ATPase activity involves disulfide bond formation between conserved cysteine residues located at the catalytic site on the A subunit that leads to reversible inhibition of activity [1,2,38].

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