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Subunit Structure, Function, and Arrangement in the Yeast and Coated Vesicle V-ATPases

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The vacuolar (H^+)-ATPases (or V-ATPases) are ATP-dependent proton pumps that function both to acidify intracellular compartments and to transport protons across the plasma membrane. Acidification of intracellular compartments is important for such processes as receptor-mediated endocytosis, intracellular trafficking, protein processing, and coupled transport. Plasma membrane V-ATPases function in renal acidification, bone resorption, pH homeostasis, and, possibly, tumor metastasis. This review will focus on work from our laboratories on the V-ATPases from mammalian clathrin-coated vesicles and from yeast. The V-ATPases are composed of two domains. The peripheral V_1 domain has a molecular mass of 640 kDa and is composed of eight different subunits (subunits A–H) of molecular mass 70–13 kDa. The integral V_0 domain, which has a molecular mass of 260 kDa, is composed of five different subunits (subunits a, d, c, c′, and c″) of molecular mass 100–17 kDa. The V_1 domain is responsible for ATP hydrolysis whereas the V_0 domain is responsible for proton transport. Using a variety of techniques, including cysteine-mediated crosslinking and electron microscopy, we have defined both the overall shape of the V-ATPase and the V_0 domain as well as the location of various subunits within the complex. We have employed site-directed and random mutagenesis to identify subunits and residues involved in nucleotide binding and hydrolysis, proton translocation, and the coupling of these two processes. We have also investigated the mechanism of regulation of the V-ATPase by reversible dissociation and the role of different subunits in this process.

KEY WORDS: V-ATPase; subunit arrangement; yeast vacuole; coated vesicle; subunit function.

FUNCTION OF VACUOLAR-ATPases

The Vacuolar-ATPases (V-ATPases) are a family of proton pumps that couple the energy of ATP hydrolysis to active proton transport across both intracellular and plasma membranes of eukaryotic cells (Graham *et al*., 2000; Kane and Parra, 2000; Nishi and Forgac, 2002). Intracellular V-ATPases are present in such compartments as clathrin-coated vesicles, endosomes, lysosomes, Golgi-derived vesicles, secretory vesicles, and the central vacuoles of fungi and plants. V-ATPases within these compartments play a variety of important roles (Nishi and Forgac, 2002). 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 carry 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. V-ATPases in secretory vesicles, such as synaptic vesicles, provide the driving force for uptake of neurotransmitters that are packaged in these compartments. The V-ATPase is likely present in brain clathrin-coated vesicles because of its retrieval from the presynaptic membrane following neurotransmitter release. Within lysosomes and the central vacuoles of fungi and plants, the V-ATPase provides both the acidic environment required for degradation of macromolecules as

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well as the driving force for various coupled processes. For example, the proton gradient across the yeast vacuolar membrane is required for coupled Ca^{2+} uptake into the vacuole. V-ATPases in the plasma membrane of various cell types function in such processes as renal acidification, bone resorption, pH homeostasis, coupled transport, and tumor metastasis (Nishi and Forgac, 2002; Wieczorek *et al*., 1999).

OVERALL STRUCTURE OF V-ATPases AND RELATIONSHIP TO F-ATPases

The V-ATPases are composed of two domains. The peripheral V_1 domain is a complex of 640 kDa responsible for ATP hydrolysis (Nishi and Forgac, 2002). It contains eight different subunits (A–H) of molecular mass 70–13 kDa that are present in a stoichiometry of $A_3B_3C_1D_1E_1F_1G_2H_{1-2}$ (Arai *et al*., 1988; Xu *et al*., 1999). Both the 70 kDa A subunit and the 60 kDa B subunit participate in nucleotide binding (Vasilyeva and Forgac, 1996; Zhang *et al*., 1995), with the catalytic nucleotide-binding sites located on the A subunit (Feng and Forgac, 1992a). The integral V_0 domain is a 260 kDa complex responsible for proton translocation (Zhang *et al*., 1994). V_0 contains five different subunits of molecular mass 100–17 kDa in a stoichiometry $a_1d_1c_{4-5}\,c'_1c''_1$ (Arai *et al*., 1988; Powell *et al*., 2000). Both the proteolipid subunits (c, c′, and c″) and the 100 kDa a subunit contain residues essential for proton transport (Hirata *et al*., 1997; Kawasaki-Nishi *et al*., 2001c). Our current structural model for the V-ATPases is shown in Fig. 1 and the subunit composition and function, including the genes encoding the various V-ATPase subunits in yeast, is shown in Table I.

The V-ATPases resemble in overall structure the F-ATPases of mitochondria, chloroplasts, and bacteria, that normally function in ATP synthesis (Cross, 2000; Fillingame *et al*., 2000; Weber and Senior, 2000). Like the V-ATPases, the F-ATPases contain a peripheral F_1 domain responsible for ATP hydrolysis and an integral F_0 domain responsible for proton transport. Amino acid sequence homology between the nucleotide-binding subunits (A and B for the V-ATPases, β and α for the F-ATPases) as well as between the proteolipid subunits indicates that the two complexes share a common evolutionary ancestor (Bowman *et al*., 1988; Mandel *et al*., 1988; Zimniak *et al*., 1988). The X-ray crystal structure of F_1 reveals a hexameric arrangement of alternating α and β subunits, with the highly alpha-helical gamma subunit extending from the center of the hexamer toward the F_0 domain (Abrahams *et al*., 1994; Bianchet *et al*., 1998). A partial structure of F_1F_0 reveals the $\alpha_3\beta_3$ hexamer attached to a ring of 10 proteolipid c subunits by a central stalk composed of the γ and ε subunits (Stock *et al*., 1999). F_1 and F_0 are also connected by a peripheral stalk composed of the δ subunit and the soluble domains of subunit b (McLachlin *et al*., 1998; Ogilvie *et al*., 1997).

Electron microscopy has revealed that for both the V and F-ATPases, the peripheral and integral domains are connected by a central and peripheral stalk (Boekema *et al*., 1997; Wilkens *et al*., 1999; Wilkens and Capaldi, 1998), although the V-ATPase structure is more complex, especially in the stalk region and at the top of the molecule. Moreover, the integral domain contains a ring of proteolipid subunits adjacent to the membrane-embedded subunit a (Birkenhäger *et al*., 1995; Wilkens and Forgac, 2001). Figure 2 summarizes the electron microscopy studies of the bovine enzyme. This review will focus first on what is known about the structure and function of individual subunits before discussing their arrangement in the V-ATPase complex and the proposed mechanism of ATP-driven proton transport. The final sections will discuss the role of certain subunits in targeting and regulation of V-ATPase activity.

STRUCTURE AND FUNCTION OF THE NUCLEOTIDE-BINDING SUBUNITS

Two V-ATPase subunits participate in nucleotide binding. Several lines of evidence indicate that the 70 kDa A subunit contains the catalytic nucleotide-binding sites. First, modification of a specific cysteine residue in subunit A (Cys254 in the bovine protein) by sulfhydryl reagents such as NEM and NBD-Cl leads to nucleotide protectable inhibition of activity (Feng and Forgac, 1992a). This cysteine residue is located in theWalker A consensus sequence (GXGKTV), which, from the crystal structure of the homologous F_1 β subunit, is wrapped around the terminal phosphates of ATP bound at the catalytic sites (Abrahams *et al*., 1994; Bianchet *et al*., 1998). Moreover, disulfide bond formation between Cys254 and Cys532 in the C-terminal domain of the A subunit leads to reversible inhibition of V-ATPase activity (Feng and Forgac, 1994). Because a significant fraction of the V-ATPase appears to exist in this disulfide-bonded state in vivo (Feng and Forgac, 1992b), we have proposed that reversible disulfide bond formation between cysteine residues at the catalytic site represents an important mechanism of regulating V-ATPase activity in cells.

Additional evidence that the A subunit contains the catalytic nucleotide-binding sites comes from modification by the nucleotide analog 2-azido-ATP, which

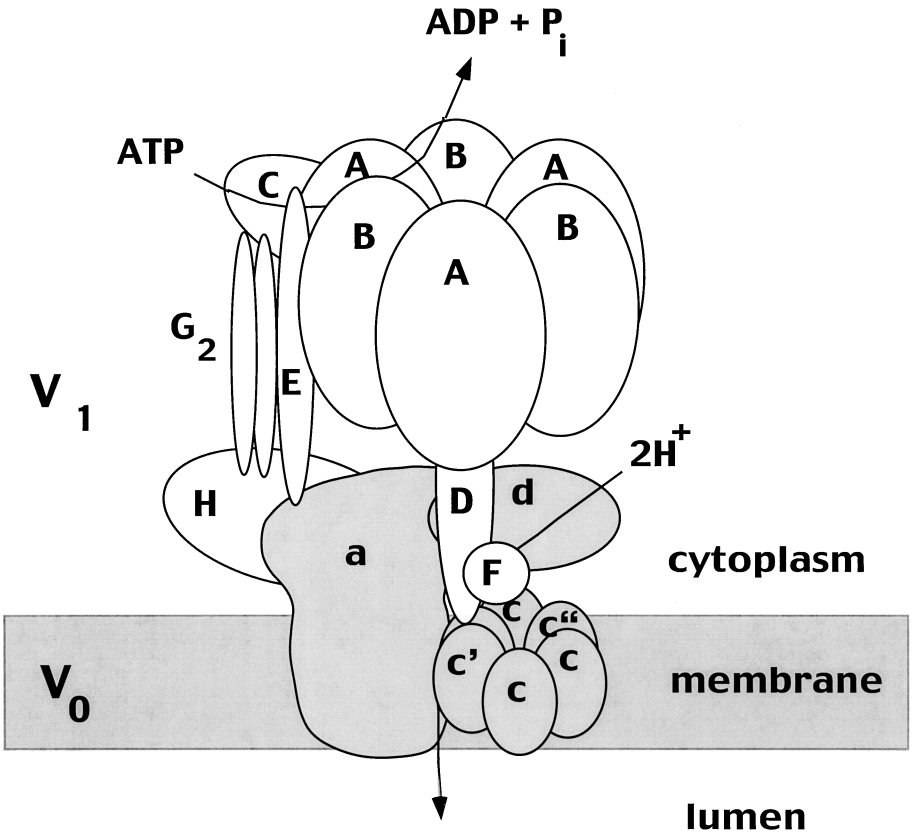

Fig. 1. Structural model of the V-ATPase complex. The V_1 domain (shown in white) is responsible for ATP hydrolysis whereas the V_0 domain (shaded) is responsible for proton translocation. ATP hydrolysis at the catalytic nucleotide-binding sites (located on the A subunits) is proposed to drive rotation of a central stalk (composed of the D and F subunits), which in turn drives rotation of the ring of proteolipid subunits (c, c′, c″) relative to subunit a.Subunit a is held fixed relative to the A_3B_3 head by a peripheral stalk composed of subunits C, E, G, and H and the soluble domain of subunit a.Movement of the ring of proteolipid subunits past subunit a is thought to drive unidirectional proton transport across the membrane.A stoichiometry of 2 H+/ATP was reported by Johnson *et al*. (1982). Model reprinted with permission from Arata *et al*. (2002a), copyright 2002, the American Society for Biochemistry and Molecular Biology.

Table I. Subunit Composition of the Yeast V-ATPase

	Subunit	Gene (Yeast)	Mr (kDa)	Function/location
V_1	A	*VMA1*	69	Catalytic site, homolog to β of F_1F_0-ATPase
	B	*VMA2*	58	Noncatalytic site, homolog to α of F_1F_0-ATPase
	C	*VMA5*	44	Peripheral stalk, released from V_1 complex during dissociation
	D	*VMA8*	29	Central stalk, homolog to γ of F_1F_0-ATPase
	E	*VMA4*	26	Peripheral stalk
	F	*VMA7*	14	Central stalk
	G	*VMA10*	13	Peripheral stalk
	H	*VMA13*	54	Peripheral stalk
V_0	a	*VPH1*	100	Proton translocation, targeting to vacuole, homolog to a of F_1F_0-ATPase
		STV1	100	Proton translocation, targeting to late Golgi, homolog to a of F_1F_0-ATPase
	d	*VMA6*	40	Cytoplasmic side, nontransmembrane protein
	c	*VMA3*	17	Proton translocation, DCCD- and concanamycin-binding sites, homolog to c of F_1F_0-ATPase
	c′	*VMA11*	17	Proton translocation, homolog to c of F_1F_0-ATPase
	c″	*VMA16*	23	Proton translocation, homolog to c of F_1F_0-ATPase

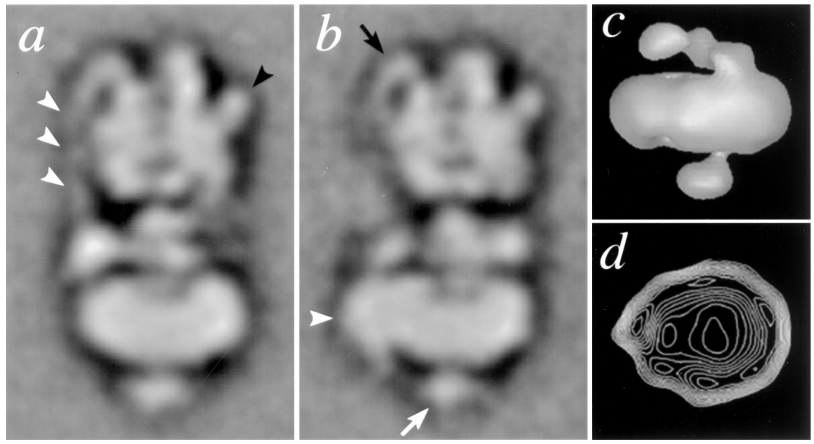

Fig. 2. Electron microscopy of the bovine V-ATPase. (*a*, *b*) Two-dimensional projections of the bovine V-ATPase showing the peripheral stalk (*a*, white arrowheads) and the "knob" like densities on the side of the V_1 (black arrowhead) next to a number of protein densities probably representing Ac45 (*b*, white arrow), the C-terminus of subunit a (white arrowhead) and (part of) subunits E and G (black arrow). Candidates for the densities seen in the interface between V_1 and V_0 are subunits D, F, H, and d and the N-terminus of subunit a. (*c*, *d*) Three-dimensional reconstruction of the coated vesicle V_0 domain shown as surface representation (*c*) and cross section (*d*). One protein density (Ac45) is bound on the lumenal side of the proteolipid ring while the two densities visible on the cytoplasmic side are formed by subunit d and the N-terminus of subunit a.The central mass (containing the proteolipid ring and the buried portion of subunit a) has dimensions of $11 \times 14 \times 6$ nm.The cross section shown in c is taken approximately 1.6 nm from the lumenal side of the central mass. Images modified from Wilkens *et al.* (1999) and Wilkens and Forgac (2001). Reprinted with permission from these articles, copyrights 1999 and 2001, the American Society for Biochemistry and Molecular Biology.

completely inhibits activity after reaction at a single A subunit site per complex (Zhang *et al*., 1995). Finally, mutagenesis studies on the yeast A subunit have identified a number of residues that play an important role in ATP binding or hydrolysis. Thus, both K263 in the Walker A sequence and E286 are essential for ATP hydrolysis (Liu *et al*., 1997). The X-ray structure of F_1 suggests that the corresponding lysine residue stabilizes the phosphate groups of bound ATP whereas the corresponding glutamic acid residue serves to abstract a proton from water during ATP hydrolysis (Abrahams *et al*., 1994). Modeling studies of the A subunit based upon sequence homology with the F-ATPases, the X-ray structure of F_1, and energy minimization have suggested the presence of aromatic residues forming an adenine-binding pocket at the catalytic site (MacLeod *et al*., 1998). The presence of these aromatic residues at the catalytic sites has been confirmed both by the effects of their mutation on nucleotide binding and by their modification by 2-azido-ATP (MacLeod *et al*., 1998, 1999).

Recent studies have suggested that a unique domain of the A subunit, termed the "non-homologous" domain, serves an important role in controlling coupling of ATP hydrolysis and proton transport as well as reversible dissociation of the V-ATPase complex (Shao *et al*., 2003). The nonhomologous domain is a 90 amino acid region not present in the F-ATPase β subunit, which is nevertheless conserved among all V-ATPase A subunit sequences (Puopolo *et al*., 1991; Zimniak *et al*., 1988). It is located approximately one third of the way from the amino terminus. There is evidence that the nonhomologous region forms a separate domain on the outer surface of the V_1 domain where it may contribute to the peripheral connection between V_1 and V_0. First, modeling studies suggest that the A_3B_3 hexamer is tightly packed, with little room inside to accommodate more than the γ subunit homolog (Arata *et al*., 2002a), and second, the insertion of the extra 90 amino acids occurs between conserved residues Leu132 and Leu 133 in the F-ATPase β subunit. Based on the X-ray structure of the mitochondrial F_1-ATPase (Abrahams *et al*., 1994), these residues are in a loop on the outer surface of the β subunit connecting the N-terminal and the nucleotide-binding domains. We have shown that several mutations in this region of the A subunit cause partial uncoupling of proton transport and ATP hydrolysis while one mutation causes a dramatic increase in coupling efficiency, suggesting that this domain may play a role in controlling the tightness of coupling (Shao *et al*.,

2003). In addition, several mutations in the nonhomologous region lead to significant inhibition of dissociation of the V-ATPase in response to glucose depletion (see below).

In addition to the A subunit, the 60 kDa B subunit also participates in nucleotide binding, as evidenced by modification of the B subunit by the nucleotide analog BzATP (Vasilyeva and Forgac, 1996). Although the B subunit lacks the consensus nucleotide-binding motifs (like the Walker A sequence) present in the A, α, and β subunits (Bowman *et al*., 1988; Puopolo *et al*., 1992a), modeling studies of the B subunit based upon sequence homology with the α subunit, the X-ray coordinates of F_1, and energy minimization have predicted a structure which has been supported by cysteine scanning mutagenesis and chemical modification studies (Vasilyeva *et al*., 2000). Unique cysteine residues were introduced into a cys-less form of the B subunit and their modification by the sulfhydryl reagent biotin maleimide tested in the intact V-ATPase in the presence and absence of BzATP. While a number of the introduced cysteine residues were not accessible to modification by biotin maleimide, all those that were showed protection from labeling by BzATP, supporting their presence at the nucleotide-binding site (Vasilyeva *et al*., 2000). The role of the nucleotide-binding sites on the B subunit (termed "noncatalytic" sites) is not certain, but one of the mutations at this site leads to time-dependent increases in ATPase activity, suggesting that they may play a regulatory role (MacLeod *et al*., 1998). Other mutations have less dramatic effects on activity (Liu *et al*., 1996). As with the F-ATPases (Abrahams *et al*., 1994), evidence indicates that both the catalytic and noncatalytic sites are located at the interface of the A and B subunits (Liu *et al*., 1996; MacLeod *et al*., 1998), although, as indicated above, the catalytic sites are located primarily on the A subunits while the noncatalytic sites are located primarily on the B subunits.

STRUCTURE AND FUNCTION OF OTHER V_1 SUBUNITS AND ARRANGEMENT OF SUBUNITS IN THE V-ATPase COMPLEX

Mutational studies suggest that subunit D (product of the *VMA8* gene) is involved in coupling of ATP hydrolysis and proton transport (Xu and Forgac, 2000). Mutations clustered near the N- and C-terminus of subunit D have synergistic effects on coupling, suggesting that these regions may interact. Mutations causing uncoupling in the F-ATPase have also been identified in subunit γ (Shin *et al*., 1992), which adopts a coiled-coil structure that forms the main part of the central stalk connecting F_1 and F_0 (Abrahams *et al*., 1994). This has led us to suggest that subunit D corresponds to the γ subunit homolog in the V-ATPase (Xu and Forgac, 2000).

Evidence supporting this hypothesis has come from cysteine-mediated crosslinking studies. Using the molecular model of subunit B described above, unique cysteine residues were introduced into the yeast B subunit by site-directed mutagenesis at structurally defined sites. These unique cysteine residues were then modified using the photoactivated crosslinking reagent maleimido benzophenone followed by photactivated crosslinking. Crosslinking of subunits B and D was observed only at B subunit sites predicted to be oriented toward the center of the A_3B_3 hexamer (Arata *et al*., 2002b). By contrast, subunits E and G were shown to be crosslinked to subunit B at sites predicted to be oriented toward the outer surface of V_1 (Arata *et al*., 2002a,b). For subunit E, crosslinking could be observed at sites near the top of the B subunit, near the middle, and near the bottom, closest to the membrane, whereas for subunit G, crosslinking was observed only at sites near the top of V_1. These results suggest that subunit D is part of the central stalk connecting V_1 and V_0 whereas subunits E and G form part of the peripheral stalk connecting these domains.

Additional crosslinking studies performed on the coated vesicle V-ATPase indicate that subunits D and F are in contact whereas subunit E makes contact with subunits C, G, and H and the soluble domain of subunit a (Xu *et al*., 1999). Further evidence supporting this arrangement of subunits comes from the isolation of DF and EG heterodimers from yeast strains lacking certain V_1 subunits (Tomashek *et al*., 1997) and the ability to coimmunoprecipitate subunits C and E in vitro (Puopolo *et al*., 1992b).

Considerable information has been obtained from mutagenesis and overexpression studies on the function of subunits C, G, and H. Thus, subunit C appears to activate ATP hydrolysis by V_1 whereas subunit H inhibits this activity (Curtis and Kane, 2002; Parra *et al*., 2000). Subunit G, which has been proposed to correspond to the b subunit homolog in the V-ATPase complex (Hunt and Bowman, 1997), has been shown to tolerate short deletions in its sequence (Charsky *et al*., 2000), similar to the b subunit of the F-ATPase (Sorgen *et al*., 1998). These studies are described in detail in the paper by Kane and Smardon in this volume. A high resolution structure of subunit H has recently been obtained (Sagermann *et al*., 2001), representing the first such data on any V-ATPase subunit. Subunit H has been shown to contact both the A subunit and the soluble domain of subunit a by yeast 2-hybrid analysis and coimmunoprecipitation (Landolt-Matricorena *et al*., 2000).

STRUCTURE AND FUNCTION OF V_0 SUBUNITS

Unlike the F-ATPases, which contain a single type of proteolipid subunit, the V-ATPases contain three distinct proteolipids: subunit c (Vma3p), subunit c′ (Vma11p), and subunit c″ (Vma16p), all three of which are required for V-ATPase function (Hirata *et al*., 1997). Subunit c and c′ each contain four transmembrane helices with an essential glutamate residue in TM4. Subunit c″ was originally predicted to contain five transmembrane helices, but recent deletional analysis indicates that it instead contains four transmembrane helices with the critical glutamate residue in TM2 (Nishi *et al*., 2003). Moreover, the region originally predicted to correspond to TM1 is not required for function. Topological studies indicate that the C-terminus of subunit c is lumenal whereas the C-terminus of subunit c" is cytoplasmic (Nishi *et al*., 2001, 2003). The buried acidic residues in the proteolipid subunits, which have been shown to be essential by both chemical modification (Arai *et al*., 1987) and mutagenesis (Hirata *et al*., 1997), are believed to directly participate in proton translocation. Moreover, subunit c has recently been shown to form at least part of the binding site for the specific V-ATPase inhibitor bafilomycin (Bowman and Bowman, 2002).

All three V-ATPase proteolipids are homologous to the F-ATPase subunit c, from which they appear to have arisen from gene duplication and fusion (Mandel *et al*., 1988). The F-ATPase c subunit adopts a helical hairpin structure (Girvin *et al*., 1998), with the orientation of the buried acidic group changing depending upon its state of protonation (Rastogi and Girvin, 1999). The F_0 domains from *E. coli* and yeast mitochondria contain 10 copies of subunit c which form a ring (Jiang *et al*., 2001; Stock *et al*., 1999), although other stoichiometries for the c subunits in the F_0 domain have also been reported (Seelert *et al*., 2000; Vonck *et al*., 2002). Stoichiometry measurements indicate that the V_0 domain contains 5–6 copies of subunits c plus c′ and a single copy of subunit c″ (Arai *et al*., 1988), whereas epitope tagging experiments indicate that both subunits c′ and c″ are present in single copies (Powell *et al*., 2000). Together, these results suggest a subunit stoichiometry of $c_{4-5}\ c'_1c''_1$ for V_0.

The largest subunit in the V_0 domain is the 100 kDa subunit a. In yeast, this is the only subunit encoded by more than one gene (*VPH1* and *STV1*) (Manolson *et al*., 1992, 1994). Vph1p has been shown to target the V-ATPase to the vacuole whereas Stv1p targets the V-ATPase to the late Golgi (Kawasaki-Nishi *et al*., 2001a; Manolson *et al*., 1994). In mammalian cells, the a subunit is encoded by four genes (a1–a4), which are expressed in a tissue-specific manner (Nishi and Forgac, 2000; Oka *et al*., 2001). The a3 isoform targets the V-ATPase to the plasma membrane in osteoclasts (Toyomura *et al*., 2000), and defects in this gene lead to a genetic defect known as osteopetrosis (Li *et al*., 1999). The a4 isoform targets the V-ATPase to the plasma membrane of renal intercalated cells (Oka *et al*., 2001), and disruption of this gene causes the disease renal tubule acidosis (Smith *et al*., 2000).

Topological studies of subunit a using cysteine mutagenesis and modification by membrane permeant and impermeant maleimides indicate that subunit a contains an amino terminal hydrophilic domain of about 50 kDa oriented toward the cytoplasmic side of the membrane followed by a carboxyl terminal hydrophobic domain containing nine transmembrane segments, with the C-terminus located on the lumenal side of the membrane (Leng *et al*., 1999). Mutational studies indicate that Arg735, located in TM7 of Vph1p, is essential for proton transport (Kawasaki-Nishi *et al*., 2001c), and that other residues located in TM7 and TM9, including E789, H743, and R799, likely play some role in proton translocation (Leng *et al*., 1996, 1998). Although no sequence homology exists between the a subunits of V_0 and F_0, we have suggested that the V_0 subunit a plays a similar role in proton transport. For the F_0 a subunit, Arg210 in TM4 is critical for proton pumping (Cain, 2000) and is postulated to directly interact with the buried carboxyl groups of the ring of proteolipid subunits (Jiang and Fillingame, 1998; Vik *et al*., 2000). Other F-ATPase a subunit residues are postulated to contribute to access channels that allow protons to reach and leave these buried sites (Cain, 2000; Vik *et al*., 2000).

In addition to localization to different intracellular membranes, the two isoforms of the yeast a subunit have also been shown to differ in their degree of assembly with V_1, the efficiency of coupling of proton transport and ATP hydrolysis, and the in vivo dissociation of complexes containing these two isoforms in response to glucose withdrawal (Kawasaki-Nishi *et al*., 2001b). Thus Vph1p-containing V_0 complexes show approximately 10-fold greater assembly with V_1 than do Stv1p-containing complexes, and the resultant assembled V-ATPase complexes containing Vph1p display four- to Fivefold tighter coupling of proton transport to ATP hydrolysis. This may reflect the need to maintain a lower lumenal pH in the vacuole (where Vph1p-containing complexes reside) than in the Golgi (where Stv1p-containing complexes are found). Using chimeras constructed from the amino and carboxyl-terminal domains of Vph1p and Stv1p, it has been shown that the information controlling both the degree of assembly of V_1 and V_0 and the tightness of coupling of proton transport and ATP hydrolysis is located in the carboxyl-terminal domain, whereas information

controlling intracellular targeting is located in the amino-terminal hydrophilic domain (Kawasaki-Nishi *et al*., 2001a).

MECHANISM OF ATP-DEPENDENT PROTON TRANSPORT

Because of the structural similarity between the V- and F-ATPases, they are thought to operate by a similar rotary mechanism (Junge *et al*., 1996; Vik and Antonio, 1994). For the F-ATPases, ATP hydrolysis in the F_1 domain drives rotation of the central rotor, composed of the γ and ε subunits, which in turn drives rotation of the ring of c subunits relative to subunit a. Rotation of both the γ subunit in F_1 (Duncan *et al*., 1995; Noji *et al*., 1997; Sabbert *et al*., 1996) and the ring of c subunits in F_1F_0 (Sambongi *et al*., 1999) have been observed. The a subunit is thought to both provide access channels for the protons to reach and leave the buried carboxyl groups on the c subunit ring and to stabilize these groups in the deprotonated state (Cain, 2000; Fillingame *et al*., 2000; Vik *et al*., 2000). Rotation of the c ring relative to the a subunit thereby drives unidirectional proton transport across the membrane.

Extension of this mechanism to the V-ATPases would suggest that ATP hydrolysis in V_1 drives rotation of the D and F subunits together with the proteolipid ring relative to subunit a. Rotation of the D and F subunits of the V_1 domain of the V-ATPase from *Thermos thermophilus* was recently demonstrated (Imamura *et al*., 2003), consistent with the placement of these subunits in the central stalk (Arata *et al*., 2002b). Interaction of the proteolipid carboxyl groups with Arg735 on subunit a (Kawasaki-Nishi *et al*., 2001c) would then lead to release of protons into the access channel leading to the lumenal side of the membrane. Continued rotation of the proteolipid ring would bring these groups into contact with the cytoplasmic aqueous channel, allowing them to become reprotonated before entering the hydrophobic phase of the bilayer.

REGULATION OF V-ATPase ACTIVITY

In addition to reversible disulfide bond formation between conserved cysteine residues at the catalytic site of the V-ATPase (discussed above), several other mechanisms have been proposed to be involved in regulation of V-ATPase activity in vivo. Differences in coupling efficency have been shown to be triggered by a variety of conditions, including high concentrations of ATP (Arai *et al*., 1989), partial proteolysis (Adachi *et al*., 1990), and mutations in several V-ATPase subunits, including subunit D (Xu and Forgac, 2000), subunit a (Kawasaki-Nishi *et al*., 2001c), and the nonhomologous region of subunit A (Shao *et al*., 2003), suggesting that the enzyme may be poised to change the tightness of coupling in vivo. As described above, differences in coupling efficiency of V-ATPase complexes containing different isoforms of subunit a have also been observed (Kawasaki-Nishi *et al*., 2001b).

Perhaps the most well-established mechanism for regulating V-ATPase activity in vivo involves reversible dissociation of the V_1 and V_0 domains. A rapid and reversible dissociation of the V-ATPase was observed in yeast in response to removal of glucose from the media (Kane, 1995). Dissociation of the V-ATPase has also been demonstrated in insects, where it occurs during molting (Sumner *et al*., 1995). In yeast, many of the signal transduction pathways activated by glucose depletion are not involved in reversible dissociation (Parra and Kane, 1998). In vivo dissociation has been shown to require catalytic activity (MacLeod *et al*., 1999; Parra and Kane, 1998) and an intact microtubular network (Xu and Forgac, 2001), although reassembly of the complex occurs even in the absence of microtubules. Mutations in the nonhomologous region have been shown to block in vivo dissociation even without perturbing activity (Shao *et al*., 2003), suggesting that this domain may function in controlling the dissociation process. Mutations have also been identified in subunit G that cause reduced dissociation of the V-ATPase through stabilization of the complex (Charsky *et al*., 2000).

As described above, V-ATPase complexes containing different isoforms of the a subunit also differ in their in vivo dissociation behavior (Kawasaki-Nishi *et al*., 2001b). Thus, Vph1p-containing complexes located in the vacuole undergo dissociation in response to glucose withdrawal whereas Stv1p-containing complexes located in the Golgi do not. If Stv1p-containing complexes are retargeted to the vacuole by overexpression of Stv1p, in vivo dissociation is now observed. If Vph1p-containing complexes are prevented from reaching the vacuole by disruption of the normal targeting pathway, in vivo dissociation is still observed, but is less complete than for Vph1p-containing complexes located in the vacuole. These results suggest that in vivo dissociation is controlled both by the a subunit isoform present in the complex and by the cellular environment in which the complex resides (Kawasaki-Nishi *et al*., 2001b). Studies of chimeric constructs of the a subunit indicate that dissociation is controlled by the amino-terminal hydrophilic domain (Kawasaki-Nishi *et al*., 2001a). Recent studies have identified a novel complex (termed RAVE) involved in normal and regulated

assembly of the V-ATPase (Seol *et al*., 2001), and its interaction with the V_1 domain has begun to be elucidated (Smardon *et al*., 2002).

CONCLUSIONS

The V-ATPases are multisubunit complexes responsible for ATP-driven proton transport across both intracellular and plasma membranes. They are composed of a peripheral V_1 domain that hydrolyzes ATP and an integral V_0 domain that transports protons. They are thought to operate by a rotary mechanism, similar to that demonstrated for the F-ATPases. The complexity of their regulation likely reflects the diversity of roles they serve in cellular physiology.

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REFERENCES

Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994). *Nature* **370**, 621–628.
Arata, Y., Baleja, J. D., and Forgac, M. (2002a). *J. Biol. Chem*. **277**, 3357–3363.
Arata, Y., Baleja, J. D., and Forgac, M. (2002b). *Biochemistry* **41**, 11301–11307.
Arai, H., Berne, M., and Forgac, M. (1987). *J. Biol. Chem*. **262**, 11006–11011.
Arai, H., Pink, S., and Forgac, M. (1989). *Biochemistry* **28**, 3075–3082.
Arai, H., Terres, G., Pink, S., and Forgac, M. (1988). *J. Biol. Chem*. **263**, 8796–8802.
Adachi, I., Arai, H., Pimental, R., and Forgac, M. (1990). *J. Biol. Chem*. **265**, 960–966.
Bianchet, M. A., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1998). *Proc. Natl. Acad. Sci. U.S.A.* **95**, 11065–11070.
Birkenhäger, R., Hoppert, M., Deckers-Hebestreit, G., Mayer, F., and Altendorf, K. (1995). *Eur. J. Biochem*. **230**, 58–67.
Boekema, E. J., Ubbink-Kok, T., Lolkema, J . S., Brisson, A., and Konings, W. N. (1997). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 14291–14293.
Bowman, B. J., Allen, R., Wechser, M. A., and Bowman, E. J. (1988). *J. Biol. Chem.* **263**, 14002–14007.
Bowman, B. J., and Bowman, E. J. (2002). *J. Biol. Chem*. **277**, 3965–3972.
Cain, B. D. (2000). *J. Bioenerg. Biomembr*. **32**, 365–371.
Charsky, C. M., Schumann, N. J., and Kane, P. M. (2000). *J. Biol. Chem*. **275**, 37232–37239.
Cross, R. L. (2000). *Biochim. Biophys. Acta* **1458**, 270–275.
Curtis, K. K., and Kane, P. M. (2002). *J. Biol. Chem.* **277**, 2716–2724.
Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L., and Cross, R. L. (1995). *Proc. Natl. Acad. Sci. U.S.A.* **92**, 10964–10968.
Feng, Y., and Forgac, M. (1992a). *J. Biol. Chem*. **267**, 5817–5822.
Feng, Y., and Forgac, M. (1992b). *J. Biol. Chem*. **267**, 19769–19772.
Feng, Y., and Forgac, M. (1994). *J. Biol. Chem*. **269**, 13224–13230.
Fillingame, R. H., Jiang, W., and Dmitriev, O. Y. (2000). *J. Exp. Biol*. **203**, 9–17.
Girvin, M. E., Rastogi, V. K., Abildgaard, F., Markley, J . L., and Fillingam, R. H. (1998). *Biochemistry* **37**, 8817–8824.
Graham, L. A., Powell, B., and Stevens, T. H. (2000). *J. Exp. Biol.* **203**, 61–70.
Hirata, R., Graham, L. A., Takatsuki, A., Stevens, T. H., and Anraku, Y. (1997). *J. Biol. Chem*. **272**, 4795–4803.
Hunt, I. E., and Bowman, B. J. (1997). *J. Bioenerg. Biomembr*. **29**, 533–540.
Imamura, H., Nakano, M., Noji, H., Muneyuki, E., Ohkuma, S., Yoshida, M., and Yokoyama, K. (2003). *Proc. Natl. Acad. Sci. U.S.A.* **100**, 2312–2315.
Jiang, W., and Fillingame, R. H. (1998). *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6607–6612.
Jiang W., Hermolin J., Fillingame, R. H. (2001). *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4966–4971.
Johnson, R. G., Beers, M. F., and Scarpa, A. (1982). *J. Biol. Chem*. **257**, 10701–10707.
Junge, W., Sabbert, D., and Engelbrecht, S. (1996). *Ber. Bunsenges. Phys. Chem*. **100**, 2014–2019.
Kane, P. M. (1995). *J. Biol. Chem*. **270**, 17025–17032.
Kane, P. M., and Parra, K. J. (2000). *J. Exp. Biol.* **203**, 81–87.
Kawasaki-Nishi, S., Bowers, K., Nishi, T., Forgac, M., and Stevens, T. H. (2001a). *J. Biol. Chem*. **276**, 47411–47420.
Kawasaki-Nishi, S., Nishi, T., and Forgac, M. (2001b). *J. Biol. Chem*. **276**, 17941–17948.
Kawasaki-Nishi, S., Nishi, T., and Forgac, M. (2001c). *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12397–12402.
Landolt-Marticorena, C., Williams, K. M., Correa, J., Chen, W., and Manolson, M. F. (2000). *J. Biol. Chem.* **275**, 15449–15457.
Leng, X. H., Manolson, M., and Forgac, M. (1998). J. *Biol. Chem*. **273**, 6717–6723.
Leng, X. H., Manolson, M. F., Liu, Q., and Forgac, M. (1996). *J. Biol. Chem*. **271**, 22487–22493.
Leng, X. H., Nishi, T., and Forgac, M. (1999). *J. Biol. Chem*. **274**, 14655–14661.
Li, Y. P., Chen, W., Liang, Y., Li, E., and Stashenko, P. (1999). *Nat. Genet.* **23**, 447–451.
Liu, Q., Kane, P. M., Newman, P. R., and Forgac, M. (1996). *J. Biol. Chem*. **271**, 2018–2022.
Liu, Q., Leng, X. H., Newman, P. R., Vasilyeva, E., Kane, P. M., and Forgac, M. (1997). *J. Biol. Chem*. **272**, 11750–11756.
MacLeod, K. J., Vasilyeva, E., Baleja, J. D., and Forgac, M. (1998). *J. Biol. Chem.* **273**, 150–156.
MacLeod, K. J., Vasilyeva, E., Merdek, K., Vogel, P. D., and Forgac, M. (1999). *J. Biol. Chem*. **274**, 32869–32874.
Mandel, M., Moriyama, Y., Hulmes, J. D., Pan, Y. C., Nelson, H., and Nelson, N. (1988). *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5521–5524.
Manolson, M. F., Proteau, D., Preston, R. A., Stenbit, A., Roberts, B. T., Hoyt, M., Preuss, D., Mulholland, J., Botstein, D., and Jones, E. W. (1992). *J. Biol. Chem*. **267**, 14294–14303.
Manolson, M. F., Wu, B., Proteau, D., Taillon, B. E., Roberts, B. T., Hoyt, M. A., and Jones, E. W. (1994). *J. Biol. Chem.* **269**, 14064–14074.
McLachlin, D. T., Bestard, J. A., and Dunn, S. D. (1998). *J. Biol. Chem*. **273,** 15162–15168.
Nishi, T., and Forgac, M. (2000). *J. Biol. Chem*. **275**, 6824–6830.
Nishi, T., and Forgac, M. (2002). *Nat. Rev. Mol. Cell Biol.* **3**, 94–103.
Nishi, T., Kawasaki-Nishi, S., and Forgac, M. (2001). *J. Biol. Chem.* **276**, 34122–34130.
Nishi, T., Kawasaki-Nishi, S., and Forgac, M. (2003). *J. Biol. Chem*. **278**, 5821–5827.
Noji, H., Yasuda, R., Yoshida, M., and Kazuhiko, K. (1997). *Nature* **386**, 299–302.
Ogilvie, I., Aggeler, R., and Capaldi, R. A. (1997). *J. Biol. Chem*. **272,** 16652–16656.
Oka, T., Murata, Y., Namba, M., Yoshimizu, T., Toyomura, T., Yamamoto, A., Sun-Wada, G. H., Hamasaki, N., Wada, Y., and Futai, M. (2001). *J. Biol. Chem*. **276**, 40050–40054.

Parra, K. J., and Kane, P. M. (1998). *Mol. Cell. Biol.* **18**, 7064–7074.
Parra, K. J., Keenan, K. L., and Kane, P. M. (2000). *J. Biol. Chem*. **275,** 21761–21767.
Powell. B., Graham, L. A., and Stevens, T. H. (2000). *J. Biol. Chem*. **275**, 23654–23660.
Puopolo, K., Kumamoto, C., Adachi, I., and Forgac, M. (1991). *J. Biol. Chem*. **266**, 24564–24572.
Puopolo, K., Kumamoto, C., Adachi, I., Magner, R., and Forgac, M. (1992a). *J. Biol. Chem*. **267**, 3696–3706.
Puopolo, K., Sczekan, M., Magner, R., and Forgac, M. (1992b). *J. Biol. Chem*. **267**, 5171–5176.
Rastogi, V. K., and Girvin, M. E. (1999). *Nature* **402**, 263–268.
Sabbert, D., Engelbrecht, S., and Junge, W. (1996). *Nature* **381**, 623–625.
Sagermann, M., Stevens, T. H., and Matthews, B. W. (2001). *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7134–7139.
Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999). *Science* **286**, 1722–1724.
Seelert, H., Poetsch, A., Dencher, N. A., Engel, A., Stahlberg, H., Müller, D. J. (2000). *Nature* **405**, 418–419.
Seol, J. H., Shevchenko, A., Shevchenko, A., and Deshaies, R. J. (2001). *Nat. Cell Biol*. **3**, 384–391.
Shao, E., Nishi, T., Kawasaki-Nishi, S., and Forgac, M. (2003). *J. Biol. Chem.* **278**, 12985–12991.
Shin, K., Nakamoto, R. K., Maeda, M., and Futai, M. (1992). *J. Biol. Chem*. **267**, 20835–20839.
Smardon, A. M., Tarsio, M., and Kane, P. M. (2002). *J. Biol. Chem*. **277**, 13831–13839.
Smith, A. N., Skaug, J., Choate, K. A., Nayir, A., Bakkaloglu, A., Ozen, S., Hulton, S. A., Sanjad, S. A., Al-Sabban, E. A., Lifton, R. P., Scherer, S. W., and Karet, F. E. (2000). *Nat. Genet.* **26**, 71–75.
Sorgen, P. L., Caviston, T. L., Perry, R. C., Cain, B. D. (1998). *J. Biol. Chem.* **273**, 27873–27878.
Stock, D., Leslie, A. G., and Walker, J. E. (1999). *Science* **286**, 1700–1705.
Sumner, J. P., Dow, J. A., Early, F. G., Klein, U., Jäger, D., and Wieczorek, H. (1995). *J. Biol. Chem*. **270**, 5649–5653.
Tomashek, J. J., Graham, L. A., Hutchins, M. U., Stevens, T. H., and Klionsky, D. J. (1997). *J. Biol. Chem*. **272**, 26787–26793.
Toyomura, T., Oka, T., Yamaguchi, C., Wada, Y., and Futai, M. (2000). *J. Biol. Chem*. **275**, 8760–8765.
Vasilyeva, E., and Forgac, M. (1996). *J. Biol. Chem*. **271**, 12775–12782.
Vasilyeva, E., Liu, Q., MacLeod, K. J., Baleja, J. D., and Forgac, M. (2000). *J. Biol. Chem*. **275**, 255–260.
Vik, S. B., and Antonio, B. J. (1994). *J. Biol. Chem*. **269**, 30364–30369.
Vik, S. B., Long, J. C., Wada, T., and Zhang, D. (2000). *Biochim. Biophys. Acta* **1458**, 457–466.
Vonck, J., von Nidda, T. K., Meier, T., Matthey, U., Mills, D. J., Kühlbrandt, W., and Dimroth, P. (2002). *J. Mol. Biol.* **321**, 307–316.
Weber, J., and Senior, A. E. (2000). *Biochim. Biophys. Acta* **145**8, 300–309
Wieczorek, H., Brown, D., Grinstein, S., Ehrenfeld, J., Harvey, W. R. (1999). *Bioessays* **21**, 637–648.
Wilkens, S., and Capaldi, R. A. (1998). *Nature* **393**, 29.
Wilkens, S., and Forgac, M. (2001). *J. Biol. Chem*. **276**, 44064–44068.
Wilkens, S., Vasilyeva, E., and Forgac, M. (1999). *J. Biol. Chem*. **274**, 31804–31810.
Xu, T., and Forgac, M. (2000). *J. Biol. Chem*. **275**, 22075–22081.
Xu, T., and Forgac, M. (2001). *J. Biol. Chem*. **276**, 24855–24861.
Xu, T., Vasilyeva, E., and Forgac, M. (1999). *J. Biol. Chem*. **274**, 28909–28915.
Zhang, J., Feng, Y., and Forgac, M. (1994). *J. Biol. Chem*. **269**, 23518–23523.
Zhang, J., Vasilyeva, E., Feng, Y., and Forgac, M. (1995). *J. Biol. Chem*. **270**, 15494–15500.
Zimniak, L., Dittrich, P., Gogarten, J. P., Kibak, H., and Taiz, L. (1988). *J. Biol. Chem.* **263**, 9102–9112.