Molecular Characterization of the Yeast Vacuolar H\(^+\)-ATPase Proton Pore*

Ben Powell, Laurie A. Graham, and Tom H. Stevens†

From the Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

The \textit{Saccharomyces cerevisiae} vacuolar ATPase (V-ATPase) is composed of at least 13 polypeptides organized into two distinct domains, V\(_1\) and V\(_0\), that are structurally and mechanistically similar to the F\(_{1}\)-F\(_0\) domains of the F-type ATP synthases. The peripheral V\(_1\) domain is responsible for ATP hydrolysis and is coupled to the mechanism of proton translocation. The integral V\(_0\) domain is responsible for the translocation of protons across the membrane and is composed of five different polypeptides. Unlike the F\(_0\) domain of the F-type ATP synthase, which contains 12 copies of a single 8-kDa proteolipid, the V-ATPase V\(_0\) domain contains three proteolipid species, Vma3p, Vma11p, and Vma16p, with each proteolipid contributing to the mechanism of proton translocation (Hirata, R., Graham, L. A., Takatsuki, A., Stevens, T. H., and Anraku, Y. (1997) J. Biol. Chem. 272, 4795–4803). Experiments with hemagglutinin- and c-Myc epitope-tagged copies of the proteolipids revealed that each V\(_0\) complex contains all three proteolipid species, with only a single copy each of Vma11p and Vma16p but multiple copies of Vma3p. Since the proteolipids of the V\(_0\) complex are predicted to possess four membrane-spanning \(\alpha\)-helices, twice as many as a single F-ATPase proteolipid subunit, only six V-ATPase proteolipids would be required to form a hexameric ring-like structure similar to the F\(_0\) domain. Therefore, each V\(_0\) complex will likely be composed of four copies of the Vma3p proteolipid in addition to Vma11p and Vma16p. Structural differences within the membrane-spanning domains of both V\(_0\) and F\(_0\) may account for the unique properties of the ATP-hydrolyzing V-ATPase compared with the ATP-generating F-type ATP synthase.

The \textit{Saccharomyces cerevisiae} proton-translocating ATPase, located at the vacuolar membrane (V-ATPase), is to date the best characterized member of the V-type ATPase family (1–3). This family of ATP-driven proton pumps is located within the endomembrane systems of eukaryotic cells, where their primary function is the acidification of intracellular compartments. The pH gradient generated by the activity of the V-ATPase is utilized by secondary transporters to drive the transport of small molecules. The V-ATPase and the F-type ATP synthases share an overall similar structural organization (4–9). Both complexes contain a soluble catalytic domain (V\(_1\) and F\(_1\)) that is coupled to a membrane-spanning domain (V\(_0\) and F\(_0\)) by one or more “stalk” components (5, 6, 10). In the case of the F-ATP synthase the binding and hydrolysis of ATP by the catalytic domain induce rotation of a central stalk, which is fixed at one end to the membrane domain subunits (subunit c ring) (11–15). It still remains unclear how rotation of this central stalk complex brings about proton translocation through the membrane domain.

The membrane domain of the V-ATPase (V\(_0\)) is composed of five different subunits involved in the mechanism of proton translocation. The principal components involved in proton translocation in both V-ATPases and F-ATP synthases are a highly conserved family of hydrophobic proteins. These are termed proteolipids due to their solubility in organic solvents. The V\(_0\) domain is composed of three members of the proteolipid family, VMA3 (16.5-kDa; subunit c), VMA11 (17-kDa; subunit c\(^{\prime}\)), and VMA16 (29-kDa; subunit c\(^{\prime\prime}\)) (1). The proteolipids of the V-ATPase (subunits c, c\(^{\prime}\), and c\(^{\prime\prime}\)) share a high degree of sequence similarity and are predicted to contain at least four \(\alpha\)-helical transmembrane domains (14–17). Each proteolipid has been shown to be essential for the assembly and function of the yeast V-ATPase (18).

The F\(_0\) domain of the \textit{Escherichia coli} F-ATP synthase is composed of 12 copies of an 8-kDa proteolipid, forming two \(\alpha\)-helical transmembrane domains, arranged in a ring-like structure (19–21). Each F\(_0\) domain proteolipid possesses an acidic amino residue in the second helix that is critical for its role in proton translocation (22). It is thought that the V-ATPase proteolipids arose due to a gene duplication event from an ancestral 8-kDa progenitor gene, because the amino and carboxyl halves of the V-ATPase proteolipids are homologous to each other and to the 8-kDa proteolipid of the \textit{E. coli} F-ATP synthase (14, 23, 24). A fundamental difference between these proteolipids is that the larger V-ATPase proteolipids have lost one of the acidic residues from the amino-terminal half of the polypeptide (second helix). Within each of the V-ATPase proteolipids so far identified, this highly conserved acidic residue is located only within the extreme carboxyl-terminal transmembrane domain. Mutation of this conserved acidic residue results in a fully assembled complex that is unable to pump protons (18). This supports the critical role of this residue in the mechanism of proton translocation.

In this paper we describe a biochemical approach designed to address the stoichiometry of Vma3p, Vma11p, and Vma16p proteolipids within the yeast V-ATPase enzyme complex. We have attached epitope tags (HA and c-Myc) to each of the proteolipid species. Immunoprecipitation and Western blot analysis has revealed that each V-ATPase V\(_0\) domain is composed of all three proteolipid species, with only a single copy each of Vma11p and Vma16p and therefore multiple copies of the Vma3p proteolipid.

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† To whom correspondence should be addressed: Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229. Tel.: 541-346-5884; Fax: 541-346-4854; E-mail: stevens@morel.uoregon.edu.

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Proteolipid Composition of the V-ATPase $V_0$ Domain

### TABLE I

| Yeast strains used in this study |
|----------------------------------|
| SF838-1Da MATa, ade6, leu2-3, 112, ura3-52, pep4-3, his4-519, gal2 | Source |
| BPY01 MATa, ade6, leu2-3, 112, ura3-52, pep4-3, his4-519, gal2, VMA3::HA::KanR, carrying pRH316 (VMA3::c-myc) | 44 |
| BPY02 MATa, ade6, leu2-3, 112, ura3-52, pep4-3, his4-519, gal2 | This study |
| BPY03 MATa, ade6, leu2-3, 112, ura3-52, pep4-3, his4-519, gal2, VMA11::HA, VMA11::c-myc | This study |
| BPY04 MATa, ade6, leu2-3, 112, ura3-52, pep4-3, his4-519, gal2, VMA3::HA::KanR, VMA16::c-myc | This study |
| BPY05 MATa, ade6, leu2-3, 112, ura3-52, pep4-3, his4-519, gal2, VMA16::HA, VMA16::c-myc | This study |

### EXPERIMENTAL PROCEDURES

**Strains and Culture Conditions**—Yeast strains and plasmids used in this study are listed in Table I and Table II. Yeast cells were grown in YEPD or YNBD medium plus appropriate amino acids (25). YEPD and YNBD media were buffered to pH 5.0 using 50 mM succinate/phosphate or to pH 7.5 with 50 mM HEPES. For biochemical analysis yeast cells were harvested during exponential growth.

**Protein Preparation, SDS-PAGE, and Western Blot Analysis**—Whole cell extracts and vacuolar membranes were prepared as described previously (26). Before storage, vacuolar membrane vesicles were washed with 10 mM Tris-HCl, pH 7.5, 1 mM EDTA (TE7.5), and 2 mM dithiothreitol. Following washing, vacuolar membrane vesicles were stored at −80 °C in TE7.5, 15% glycerol. SDS-PAGE analysis was performed using 12% polyacrylamide gels, and proteins were transferred to nitrocellulose. Immunoblots were probed with affinity-purified monoclonal anti-HA antibodies (Babco) at a dilution of 1:10,000 or rabbit secondary antibodies conjugated to horseradish peroxidase (1:10,000 dilution) (Bio-Rad) and visualized using chemiluminescent substrate (Amersham Pharmacia Biotech).

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| Strain and solubilized in PBS containing 1% C12E9 detergent were solubilized in PBS containing 1% C12E9 detergent and incubated on ice for 30 min (29). Solubilized membranes were subsequently centrifuged at 100,000 $\times g$ to remove any insoluble material. Supernatants were transferred to fresh tubes and normalized to a total protein concentration of 5 mg/ml. Following the addition of 20 μl of a 50% slurry of protein A-Sepharose CL-4B beads (Amersham Pharmacia Biotech) prepared in PBS + 1% C12E9 detergent samples were incubated at 4 °C with constant mixing for 1 h. After incubation the samples were centrifuged at 10,000 $\times g$ for 15 min at 4 °C. The pre-cleared supernatant was transferred to a fresh tube, and 5 μl of the affinity-purified monoclonal anti-HA antibody (Babco) was added and incubated at 4 °C for 2 h with constant mixing. 20 μl of a 50% slurry of protein A-Sepharose CL-4B was then added and incubated overnight at 4 °C with constant agitation. The protein A beads were collected by brief centrifugation and washed 6 times with 1 ml of cold PBS, followed by six washes with 1 ml of cold PBS + 1% C12E9. Pellets were resuspended in 150 μl of reducing SDS-PAGE sample buffer (5% β-mercaptoethanol), and 20 μl of the supernatant was loaded onto 12% SDS-polyacrylamide gels.

**RESULTS**

Detection of Epitope-tagged Copies of Vma3p, Vma11p, and Vma16p in Yeast Strains Expressing Two Forms of a Proteolipid—To determine whether every V-ATPase complex contains copies of each of the three proteolipids, epitope tags (HA or c-Myc) were attached to the carboxyl terminus of each of the three proteolipid species, Vma3p, Vma11p, and Vma16p. Strains expressing integrated copies of HA and/or c-Myc forms of the various proteolipids grew as well as wild-type cells on YEPD media buffered to neutral pH. The stable expression of two different epitope-tagged proteolipids within each strain was determined by immunoblot analysis (Fig. 1, A and B). Cell extracts were prepared from each of the strains expressing both HA- and c-Myc-tagged proteolipids and probed with anti-HA antibodies (Fig. 1A), anti-c-Myc antibodies (Fig. 1B). Comparing equivalent lanes in Fig. 1A and B, it is evident that both HA- and c-Myc-tagged proteolipids were expressed in each strain.

The next step was to demonstrate that each of the tagged proteolipids was present on the vacuolar membrane and incorporated into an active fully assembled V-ATPase complex. Vacuolar membrane vesicles were prepared from each of the yeast strains and solubilized in PBS containing 1% C12E9 detergent. Solubilized extracts were analyzed by immunoblotting for the presence of each of the tagged proteolipids using anti-HA (Fig. 1C) and anti-c-Myc (Fig. 1D) antibodies. Samples of equal total protein concentration were loaded onto the gels in order to determine the relative expression levels of each of the tagged proteolipids. All proteolipids were present on the vacuolar membrane and were found to exhibit a differential expression pattern, i.e. Vma3p, whether HA- or c-Myc-tagged proteolipid was present at a higher level than either Vma11p or Vma16p. From these data the exact molar ratio cannot be determined, but it is clear that there is more of Vma3p than Vma11p or Vma16p.
The V-ATPase Contains Copies of Vma3p, Vma11p, and Vma16p within Every Complex—We next examined whether all three proteolipids are part of the same complex and if each complex contains multiple copies of the proteolipids. Vacuolar membranes from each of the strains (BPY01-05) were solubilized with 1% C12E9 in PBS and used for a co-immunoprecipitation study. Following immunoprecipitation using monoclonal anti-HA antibodies, V1 subunits were detected in the immunoprecipitate indicating an assembled complex (V1 + V0) was effectively immunoprecipitated from each of the constructed strains (data not shown).

Plasmids used in this study

| Plasmids     | Description                                                                 | Ref.   |
|--------------|-----------------------------------------------------------------------------|--------|
| pRS314       | Centromere based, low copy plasmid (pCEN-TRP1)                               | 45     |
| pRS306       | Integrating plasmid (URA3)                                                  | 45     |
| pLG40        | 1.8-kb KpnI-Sac1 VMA11::HA fragment subcloned into pRS306                   | 18     |
| pLG64        | 1.8-kb Kpn1-Sac1 VMA11::c-myc fragment subcloned into pRS306                | This study |
| pLG34        | 1.9-kb EcoRI-BalI VMA16::HA fragment subcloned into pRS306                  | 18     |
| pLG35        | 1.9-kb EcoRI-BalI VMA16::c-myc fragment subcloned into pRS306               | This study |
| pRHA316      | 4.6-kb Sac1-XhoI fragment containing VMA3::c-myc fusion and URA3 (inserted as a 1.1-kb HindIII (blunted) fragment into EagI site (blunted) upstream of the VMA3 open reading frame) cloned into pRS314 | 18     |
| pLG68        | Same as pRHA316 except VMA3::HA::KanR gene fragment (URA3 was removed using PstI/SmaI and replaced with the KanR gene) | This study |
| pLG69        | Same as pLG68 except VMA3::c-myc::KanR                                     | This study |

* kb, kilobase pair.

Fig. 1. Detection of epitope-tagged proteolipids from whole cell extracts and isolated solubilized vacuolar membranes. A and B, proteins in whole cell extracts isolated from strain BPY01-05 were subjected to SDS-PAGE using 12% polyacrylamide gels, and expression of each of the epitope-tagged proteolipids was determined by Western blot analysis after electrophoretic transfer to nitrocellulose. Each of the strains expresses HA- and c-Myc-tagged proteolipids of the correct molecular weight. C and D, vacuolar membranes were isolated from each of the yeast strains and solubilized to 5 mg/ml in PBS containing 1% C12E9 detergent. Purified vacuolar ATPase was subjected to SDS-PAGE using 12% polyacrylamide gels, and the relative expression of each of the epitope-tagged proteolipids was determined by Western blot analysis after electrophoretic transfer to nitrocellulose. Epitope-tagged proteolipids are indicated by arrows.

The V-ATPase Contains Copies of Vma3p, Vma11p, and Vma16p within Every Complex—We next examined whether all three proteolipids are part of the same complex and if each complex contains multiple copies of the proteolipids. Vacuolar membranes from each of the strains (BPY01-05) were solubilized with 1% C12E9 in PBS and used for a co-immunoprecipitation study. Following immunoprecipitation using monoclonal anti-HA antibodies, V1 subunits were detected in the immunoprecipitate indicating an assembled complex (V1 + V0) was effectively immunoprecipitated from each of the constructed strains (data not shown).

To determine the stoichiometry of proteolipids present in each complex, V-ATPase was immunoprecipitated using monoclonal anti-HA antibodies from solubilized vacuolar membranes prepared from yeast strain BPY02 expressing Vma3p-HA and Vma11p-c-myc. The immunoprecipitate and remaining supernatants were probed with both anti-HA and anti-c-Myc antibodies in order to determine whether a c-Myc-tagged copy of Vma11p was co-immunoprecipitated with Vma3p-HA (Fig. 2). Both Vma3p-HA-tagged proteolipid as well as >85% of the Vma11p-c-myc epitope-tagged proteolipid was detected in the immunoprecipitate (Fig. 2, A and C, lane 2, relative to Fig. 2, B and D, lane 2). The fact that both proteolipids can be immunoprecipitated with anti-HA antibody dem-
onstrates that each enzyme complex must contain copies of both Vma3p-HA and Vma11p-c-myc-tagged proteolipids. The V-ATPase complex was also immunoprecipitated out of a strain (BPY04) that expressed copies of both Vma3p-HA and Vma11p-c-myc proteolipids. Following immunoprecipitation using anti-HA antibodies, both Vma3p-HA and Vma11p-c-myc were present in the immunoprecipitate, as demonstrated by lane 4 in Fig. 2, A and C. Analysis of the immunoprecipitate and supernatants revealed that Vma3p-HA was efficiently precipitated (>80%; Fig. 2, A and B, lane 4) and that Vma11p-c-myc was efficiently co-immunoprecipitated (>80%; Fig. 2, C and D, lane 4).

Immunoprecipitation of HA-tagged Vma3p from strains expressing either c-Myc-tagged copies of Vma11p or Vma16p resulted in co-immunoprecipitation of both forms (Vma3p + Vma11p and Vma3p + Vma16p) of the tagged proteolipids. Therefore, each V-ATPase complex must contain copies of all three proteolipids, consistent with the genetic results that all three proteolipids are essential for function (18, 30).

One Copy Each of Vma11p and Vma16p Are Present within the V-ATPase Complex—In order to determine whether each V-ATPase complex contains more than one copy of either Vma3p, Vma11p or Vma16p, we immunoprecipitated the complex from cells expressing two epitope-tagged copies of the same proteolipid (e.g., Vma3p-HA and Vma3p-c-myc). Isolated vacuolar membranes were solubilized, and the V-ATPase was immunoprecipitated with monoclonal anti-HA antibodies. Polyclonal anti-c-Myc antibodies were then used to monitor by immunoblot analysis whether the complex also contains a second c-Myc-tagged copy of the same proteolipid.

The solubilized V-ATPase complex isolated from cells expressing copies of both Vma11p-HA and Vma16p-HA was immunoprecipitated with anti-HA antibodies, and −100% of Vma11p-HA was immunoprecipitated (Fig. 2, A and B, lane 3). Vma11p-c-myc was not detected in the immunoprecipitate when samples were probed using polyclonal anti-c-Myc antibodies (Fig. 2C, lane 3). However, Vma11p-c-myc was present in the cells and detected in the supernatant (Fig. 2D, lane 3). These data indicate that only a single copy of the proteolipid Vma11p is incorporated into the yeast V-ATPase complex.

Immunoprecipitation of the V-ATPase complex from solubli-
lized vacuum membranes isolated from the strain BPY01, expressing both Vma3p-HA and Vma3p-c-myc, demonstrates that every V-ATPase complex contains multiple copies of Vma3p. Following immunoprecipitation using monoclonal anti-HA antibodies, Vma3p-HA was detected in the immunoprecipitate (Fig. 2, lane 1). By using polyclonal anti-c-Myc antibodies, Vma3p-c-myc was also detected in the immunoprecipitate, indicating that both epitope-tagged copies of the proteolipid are present within each V-ATPase complex. The same results were also observed when anti-c-Myc antibodies were used to immunoprecipitate the complex (data not shown).

We observed that the expression of both epitope-tagged forms of the same proteolipid are similar at the protein level as demonstrated in Fig. 1, indicating that either copy could be inserted into the V-ATPase complex with equal probability. If multiple copies of Vma11p and Vma16p were present per complex, then both tagged versions of the same proteolipid would have been detected in the immunoprecipitate. The data obtained clearly demonstrate that the V-ATPase complex contains only a single copy of both Vma11p and Vma16p and multiple copies of Vma3p.

**DISCUSSION**

Both the V-ATPases and F-ATP synthases share an overall similar structural organization (4–7, 31) and are thought to operate through a similar mechanism, coupling ATP hydrolysis or synthesis to proton translocation using a rotary mechanism (12, 13, 32). A striking difference between these two complexes is the presence of three different species of proteolipid within the V0 domain compared with a single proteolipid species within F0. The yeast V-ATPase requires all three proteolipid species to assemble and function correctly, since the loss of any one proteolipid or mutation of any one of the single conserved acidic residues results in a complete loss of V-ATPase activity (18).

In this paper we have demonstrated that each V-ATPase complex contains only a single copy of both Vma11p and Vma16p but multiple copies of Vma3p. The data clearly demonstrate that immunoprecipitation of Vma11p-HA or Vma16p-HA from solubilized vacuolar membranes does not co-immunoprecipitate Vma11p-c-myc or Vma16p-c-myc from cells expressing both tagged copies of the same proteolipid. Therefore, each complex must contain only a single copy of both Vma11p and Vma16p. However, when any one of the proteolipids is expressed in the same cells as Vma3p-HA, either Vma3p-c-myc, Vma11p-c-myc, or Vma16p-c-myc can be co-immunoprecipitated from solubilized vacuolar membranes demonstrating the presence of all three proteolipids within each complex. Complexes composed of only Vma3p-Vma11p, Vma3p-Vma16p, or any single proteolipid can be ruled out, as the loss of any one of the proteolipid genes results in a complete loss of V-ATPase function in yeast (1).

Our data indicate that each V0 domain contains only a single copy of Vma11p (c) and Vma16p (c'). However, the exact number of Vma3p (c) subunits per V0 domain remains uncertain. The data of Araï et al. (33) indicate ~5.5 copies of a 16-kDa hydrophobic species (presumably c plus c') and ~0.85 copies of a 19-kDa hydrophobic polypeptide (possibly c') per V0 domain for the bovine V-ATPase. Unfortunately, these data cannot distinguish between a stoichiometry of c,c,c' (6 proteolipids/V0) and c,c',c' (7 proteolipids/V0) for the V0 domain. Whereas a V0 domain stoichiometry of c,c,c' seems most likely for a number of reasons (see below), further studies will be required to define unambiguously the overall proteolipid stoichiometry as well as the structure of the V0 domain.

Recently it has been demonstrated that the F0 domain of the *E. coli* F-ATP synthase is composed of 12 copies of a single 8-kDa subunit c arranged in a ring-like complex comprising 24 transmembrane a-helices (34). Since both the F-ATP synthase and V-ATPases share a similar architectural plan and are likely to operate through a similar mechanism, our model is that the V0 domain will be composed of a total of six proteolipid subunits (four copies of Vma3p and one each of Vma11p and Vma16p) also contributing 24 transmembrane a-helices. The fact that the V0 domain contains three proteolipid species unlike the F0 domain may help explain some of the functional differences observed between these two enzymes. An important difference between these two complexes concerns the number of buried acidic residues in the V0/F0 domain, which presumably explains the different in vivo functions of the enzymes, hydrolysis versus synthesis of ATP (35). The V0 domain contains half the number of buried acidic residues compared with the F0 domain and would account for the difference in H+/ATP stoichiometry between the two complexes. The *E. coli* F-ATP synthase has been shown to rotate in discrete 120° steps per ATP hydrolyzed, presenting four protonated proteolipids to subunit a per step (12). Therefore, the F-ATPase has a H+/ATP stoichiometry of 4, whereas according to the same model the V-ATPase would have a H+/ATP stoichiometry of 2.

Vma3p- and Vma11p-like proteolipids have been identified in a number of species including *S. cerevisiae* (14, 30), Caenorhabditis elegans (36), Manduca sexta (37), Drosophila melanogaster (38), Schizosaccharomyces pombe (39), Mus musculus (40), and Homo sapiens (41). Table III illustrates the conserved nature of these proteins between species. However, to date only a single gene encoding a subunit c-like proteolipid has been identified in mouse (40) and humans (41), although a number of other pseudogenes have been reported (42). Comparing the sequence identities of both the mouse and human subunit c against Vma3p (c)- and Vma11p (c')-like proteins, identified in *S. cerevisiae*, *S. pombe*, *C. elegans*, and *D. melanogaster*, suggests that both human and mouse forms of subunit c are more closely related to Vma3p than Vma11p. The human form of Vma3p (h-16.5 kDa) is 63% identical to *S. cerevisiae* Vma3p and 58% identical to *S. cerevisiae* Vma11p. The mouse Vma3p (m-16.5 kDa) is 68% identical to *S. cerevisiae* Vma3p and also 58% identical to *S. cerevisiae* Vma11p (Table II). Based on the presence of Vma11p (c') homologues in *S. cerevisiae*, *C. elegans*, and *D. melanogaster*, we predict that further investigation will reveal Vma11p (c') homologues in the mouse and human genomes.

We have demonstrated that every V0 domain of the yeast V-ATPase contains Vma16p, and to date this proteolipid has been identified in *S. cerevisiae* (18), *C. elegans* (36), mouse, and human (43). The yeast subunit Vma16p sequence shares 56% identity with the *S. pombe* sequence and 47% identity with the human Vma16p (Table III). The *C. elegans* Vma16p shares 64% sequence identity with the human Vma16p and 52% identity with *S. cerevisiae* Vma16p. In addition, the mouse Vma16p is reported to share 96% identity with the human homologue, illustrating the highly conserved nature of this proteolipid between species. Vma16p is larger than Vma3p or Vma11p and may form five transmembrane domains due to a 50-amino acid extension at its amino terminus. This first membrane-spanning helix of Vma16p (shown in Fig. 3B) may fill a portion of the space at the center of the proteolipid ring. Comparing the sequences of all Vma16p proteolipids identified to date has revealed that all Vma16p-like proteolipids contain two buried conserved acidic residues within transmembrane regions. These are located within putative helices 3 and 5 (Fig. 3B), assuming five transmembrane helices. However, in yeast only

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the acidic residue located within the third transmembrane domain is required for V-ATPase activity and function (18). Thus, the V0 domain can be modeled as a hexamer with four copies of Vma3p and a single copy of each of Vma11p and Vma16p (Fig. 3B).

Structural difference within the membrane domain proteolipid components of the V-ATPase and F-ATPase complexes is one explanation for the unique properties of these two complexes. The fact that the V-ATPase functions in vivo to hydrolyze ATP may be primarily due to the presence of three essential but different proteolipid species all operating cooperatively within each V-ATPase complex.

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**TABLE III**
Comparison of amino acid identity of proteolipid isoforms between different species

| Vma3p proteolipids | m16.5 kDa | h16.5 kDa | Ce16.5 kDa | Dm16.5 kDa | Sp16.5 kDa | Sc16.5 kDa |
|---------------------|----------|----------|------------|------------|------------|------------|
| M. musculus (m)     | 100      | 91       | 83         | 73         | 71         | 68         |
| H. sapiens (h)      | 100      | 78       | 72         | 64         | 68         |            |
| C. elegans (Ce)     | 100      | 72       | 68         |            |            |            |
| D. melanogaster (Dm)| 100      | 79       | 62         |            |            |            |
| S. pombe (Sp)       |          |          |            |            | 100        | 62         |
| S. cerevisiae (Sc)  |          |          |            |            |            | 100        |

| Vma11p proteolipids | Ce17 kDa | Dm17 kDa | Sp17 kDa | Sc17 kDa |
|---------------------|----------|----------|----------|----------|
| C. elegans (Ce)     | 100      | 59       | 46       | 49       |
| D. melanogaster (Dm)| 100      | 62       |          | 60       |
| S. pombe (Sp)       |          | 100      | 59       |          |
| S. cerevisiae (Sc)  |          |          | 100      |          |

| Vma16p proteolipids | h22 kDa | Ce22 kDa | Sp22 kDa | Sc22 kDa |
|---------------------|----------|----------|----------|----------|
| H. sapiens (h)      | 100      | 64       | 48       | 47       |
| C. elegans (Ce)     | 100      | 43       |          | 52       |
| S. pombe (Sp)       |          | 100      |          | 56       |
| S. cerevisiae (Sc)  |          |          | 100      |          |

**FIG. 3.** Model of the yeast V-ATPase V0 domain based on similarities to the F0 domain of the E. coli F-ATP synthase. A, model illustrating the organization of proteolipids within the yeast V-ATPase V0 domain, spanning the vacuolar membrane. B, a model for the V0 domain of the V-ATPase illustrating the number and organization of proteolipids required to form a ring-like structure that is similar to the F0 arrangement of 12 copies of subunit c forming a ring of 24 transmembrane a-helices. Helices lining the center of the pore in both models are drawn smaller due to the high number of amino acids with short side chain lengths (Ala/Gly). The 1st transmembrane helix of Vma16p is shown in this model, residing in the center of the proteolipid ring.
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