VMA12 Encodes a Yeast Endoplasmic Reticulum Protein Required for Vacuolar H\(^+\)-ATPase Assembly*

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The Saccharomyces cerevisiae vacuolar membrane proton-translocating ATPase (V-ATPase) can be divided into a peripheral membrane complex (V\(_1\)) containing at least eight polypeptides of 69, 60, 54, 42, 32, 27, 14, and 13 kDa, and an integral membrane complex (V\(_0\)) containing at least five polypeptides of 100, 36, 23, 17, and 16 kDa. Other yeast genes have been identified that are required for V-ATPase assembly but whose protein products do not co-purify with the enzyme complex. One such gene, VMA12, encodes a 25-kDa protein (Vma12p) that is predicted to contain two membrane-spanning domains. Biochemical analysis has revealed that Vma12p behaves as an integral membrane protein with both the N and C termini oriented toward the cytosol, and this protein immunolocalizes to the endoplasmic reticulum (ER). In cells lacking Vma12p (vma12\(\Delta\)), the 100-kDa subunit of the V\(_0\) complex (which contains six to eight putative membrane-spanning domains) was rapidly degraded (t\(_{1/2}\) ~ 30 min). Protease protection assays revealed that the 100-kDa subunit was inserted/translocated correctly into the ER membrane of vma12\(\Delta\) cells. These data indicate that Vma12p functions in the ER after the insertion of V\(_0\) subunits into the ER membrane. We propose that Vma12p functions directly in the assembly of the V\(_0\) subunits into a complex in the ER, and that assembly is required for the stability of the V\(_0\) subunits and their transport as a complex out of this compartment.

The yeast vacuolar proton-translocating ATPase (V-ATPase)\(^1\) belongs to a family of multisubunit vacuolar-type ATPases found in all eukaryotic cells (1, 2). V-ATPases acidify the lumen of many organelles, including vacuoles, lysosomes, endosomes, the Golgi apparatus, as well as clathrin-coated and secretory vesicles (2). V-ATPases play a role in many cellular processes, such as zymogen activation, receptor-mediated endocytosis, and protein sorting (3). The proton gradient generated by the yeast V-ATPase drives the transport of ions, basic amino acids, metabolites, and storage sugars into the vacuole (4–6).

The yeast V-ATPase is similar in both structure and subunit composition to the F\(_{1}\)F\(_{0}\)-ATP synthase of mitochondria. The yeast vacuolar proton-translocating ATPase (V-ATPase) can be divided into a peripheral membrane complex (V\(_1\)) containing ATP hydrolytic and regulatory domains and an integral membrane complex (V\(_0\)) housing the proton pore (7–8). Biochemical and genetic analyses have demonstrated that the yeast V-ATPase contains at least 13 subunits. V\(_1\) subunits that have been identified include hydrophilic polypeptides of 69, 60, 54, 42, 32, 27, 14, and 13 kDa (2, 9). The V\(_0\) complex includes integral membrane proteins of 100, 23, 17, and 16 kDa and a hydrophilic protein of 36 kDa (10–12). Yeast cells contain two isoforms of the 100-kDa V\(_0\) subunit, encoded by the VPH1 and STV1 genes (Vph1p, Ref. 10; Stv1p, Ref. 13).

Vma12p is required for V-ATPase function but is not part of the V-ATPase complex (22). Yeast cells lacking Vma12p (vma12\(\Delta\) cells) are phenotypically indistinguishable from two other vma mutants, vma21A and vma22A (19, 23). Vma21p and Vma22p are endoplasmic reticulum (ER) proteins required for V-ATPase function, yet these polypeptides are not part of the V-ATPase complex (24–25). vma12\(\Delta\), vma21\(\Delta\), and vma22\(\Delta\) mutant cells behave just like cells lacking a V\(_0\) subunit (11, 22, 24–25). Vma12p, Vma21p, and Vma22p may function as an assembly complex at the level of the ER, but we presently cannot rule out an earlier function for these proteins, such as translocation or insertion of V\(_0\) polypeptides into the ER membrane.

In the analysis reported here, Vma12p has been found to be an integral membrane protein that resides in the ER. Yeast cells lacking Vma12p (vma12\(\Delta\)) rapidly degrade the 100-kDa V\(_0\) subunit (Vph1p) at a rate that is independent of vacuolar proteases or secretory traffic exiting the ER. Vph1p is localized to the ER membrane in vma12\(\Delta\) cells, where it is translocated and inserted normally. These results indicate that Vma12p functions in assembly of the V\(_0\) membrane complex after V\(_0\) subunits are inserted into the ER membrane.

**EXPERIMENTAL PROCEDURES**

Strain Construction and Culture Conditions—Table I lists the strains of Saccharomyces cerevisiae used in this study. All strains were grown as described previously (11) and transformed using standard techniques (26). The wild type strain, SF838-1D, was transformed with Xhol-SpeI-digested pMJ4 and a "Leu" Vma colony was selected to create DJY62 (vma12\(\Delta\):LEU2, pep4\(\Delta\)). DJY62 was transformed with a linearized PE4 fragment, and a Pep" colony was selected (27) to create DJY63 (vma12\(\Delta\):LEU2, PE4).

SF838-1D and SF838-9D were gene converted to VMA12::HA3 by loop-in of Mun1-linearized pDJ19 and loop-out of VMA12 by passage.

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‡ The abbreviations used are: V-ATPase, vacuolar proton-translocating adenosine triphosphatase; ER, endoplasmic reticulum; 5-FOA, 5-fluoroorotic acid; PAGE, polyacrylamide gel electrophoresis; CPY, carboxypeptidase Y; WT, wild type; FITC, fluorescein isothiocyanate; IIF, indirect immunofluorescence; HA, hemagglutinin.
over 5-FOA to create DJY69 and DJY58. These strains were mated to create DJY60, which was transformed with a galactose-inducible HO plasmid to effect mating type switching. A halo assay was performed (28) to identify a/a and a/A diploids (DJY71 and DJY72), which were mated to form the tetraploid VMA12::HA3 strain (DJY70). SF838-1D was gene converted to VMA12::HA3 by passage over 5-FOA to create DJY68. To construct a vma12Δ sec12–4 ts strain, yeast strain MBY10–7A (sec12–4 ts) was transformed with pRSY679 (URA3 SEC12; Ref. 29) and then disrupted as described above. The SEC12 plasmid was removed by passage over 5-FOA to create DJY91 (vma12Δ sec12–4). Plasmid Construction—Plasmids used in this study are listed in Table II. A 1.2-kilobase pair KpnI-SacI fragment encoding VMA12 was cloned into pRS316 (30) and YEpl52 (31) to create pDJ1 and pDJ2. A LEU2 knockout plasmid of VMA12 was created by digesting pPH430 (22) with EcoRI and BclI, blunting the ends with Klenow (New England Biolabs) and inserting a 2.2-kilobase pair HpaI fragment encoding LEU2 (pDJ4, vma12Δ LEU2).

Monoclonal antibodies used in this study were as follows. Antibodies 10D7 and 7B1, used interchangeably or in combination, were used at 1:1000 to detect Vph1p as described previously (18). Antibody 13D11 was used at 1:1000 to detect Vma23p as described previously (18). The antibody to Dpm1p (clone 5C5-A7) that was generated against a TrpE-Dpm1p fusion protein provided by Peter Orlean (University of Illinois, Urbana-Champaign) was used at 1:500. Monoclonal antibodies specific for Vph1p (1D7-7B2), Vma23p (1D11-2D2), and Dpm1p (5C5-A7) were purchased from Molecular Probes, Inc. (Eugene, OR). Polyclonal antibodies used in this study were as follows. Affinity-purified antibodies against Vma12p, the HA epitope, phosphoglycerol kinase, Kar2p, Sec63p, and Vph1p were used at 1:1000, 1:10,000, 1:20,000, 1:200,000, and 1:5000, respectively (12, 22, 24, 35–36).

Vaccuolar Membrane Preparation—V-ATPase Activity Assays, and Expression of Vacular Membranes by Chaotropic Agents—Vaccuolar membranes were prepared and V-ATPase activity assays performed as described previously (18). Vaccuolar membranes were treated with either 0.5% Triton X-100, 100 mM Na3C03, pH 11.5, or 0.5% NaCl essentially as described by Bauerle et al. (11).

Subcellular Fractionation—Cells were spheroplasted and lysed according to Feldheim et al. (37), except that instead of homogenization, cells were allowed to lyse osmotically on ice for 20 min before the removal of unlysed cells. Lysates were then subjected to subcellular fractionation as described in Horazdovsky and Emr (38). Briefly, lysates were separated into pellet and supernatant fractions after centrifugation at 13,000 × g for 1 h. The supernatant fraction was then subjected to centrifugation at 100,000 × g to generate pellet and supernatant fractions.

Fluorescence Microscopy—Fluorescence microscopy was carried out as described previously (39). Affinity-purified antibodies recognizing Vph1p and Eug1p were used at 1:20 and 1:50, respectively. Mouse antibody 12CA5 recognizing the HA epitope (Babco, Inc., Berkeley, CA) was preadsorbed to SF838-1D cells and used at 1:20 dilution.

Proteolysis of Microsomal Membrane Fractions—Cells were spheroplasted and lysed as described for subcellular fractionation (37). Microsomes were collected at 30,000 × g for 1 h and proteolyzed using either 0.1 mg/ml trypsin plus 0.1 mg/ml protease K as described previously (36) or 0.004 mg/ml subtilisin Carlsberg.

Immunoepitope—EXPL35S35S label was purchased from NEN Life Science Products. Immunoprecipitation of radiolabeled Vph1p and CPY was performed as described previously (24). Briefly, after radiolabeling, spheroplasted cells were lysed in 0.6% SDS plus protease inhibitors and denatured for 5 min at 62°C (Vph1p) or at 100°C (CPY) before dilution and immunoprecipitation with polyclonal anti-Vph1p sera or monoclonal anti-CPY sera.

Table I

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| SF838-1D | MATα ura3-52 leu2-3,112 his4-519 ade6 pep4-3 | (55) |
| SNT28   | MATα ura3-52 leu2-3,112 his4-519 ade6 | (24) |
| DJY62   | MATα ura3-52 leu2-3,112 his4-519 ade6 pep4-3 vma12αΔ::LEU2 | This work |
| DJY63   | MATα ura3-52 leu2-3,112 his4-519 ade6 vma12Δ::LEU2 | This work |
| SF838-9D | MATα ura3-52 leu2-3,112 his4-519 leu2 pep4-3 | This work |
| R211    | MATα ura3-52 leu2-3,112 his4-519 ade6 pep4-3 vma12αΔ::LEU2 | This work |

*a* Diploid and tetraploid strains are isogenic for mutations listed singly.

Table II

| Plasmid name | Description |
|--------------|-------------|
| pRH430      | VMA12 in pBluescript KS+ |
| pDJ4        | VMA12 gene on CEN-URA3 plasmid |
| pDJ2        | VMA12 gene on 2μ-URA3 plasmid |
| pDJ4        | vma12::LEU2 disruption construct in pBluescript KS+ |
| pDJ10       | VMA12::HA1 gene on CEN-URA3 plasmid |
| pDJ12       | VMA12::HA3 gene on CEN-URA3 plasmid |
| pDJ14       | VMA12::HA1 gene on 2μ-URA3 plasmid |
| pDJ16       | VMA12::HA1 gene on 2μ-URA3 plasmid |
| pDJ17       | VMA12::HA1 gene on integrating-URA3 plasmid |
| pDJ19       | VMA12::HA3 gene on integrating-URA3 plasmid |
| pRSY679     | Centromere-based URA3 SEC12 |

See “Experimental Procedures” for complete description of the plasmids.
degrees. Treatment with 100 mM Na2CO3, pH 11.5, selectively solubilizes peripheral membrane proteins to variable hydropathy plot of Vma12p predicts two membrane-spanning domains, indicating that it resides either in the ER or plasma membrane. The ER resident protein Dpm1p (41) also exhibited a 2-fold enrichment in the vacuolar fraction (Fig. 2). Thus, based on the low level of enrichment, Vma12p is likely to be present in membranes that contaminate the vacuolar membrane.

Although low levels of Vma12p were found previously to fractionate with vascular membranes (22), it was unclear whether this resulted from the presence of contaminating membranes. To determine whether Vma12p is a vascular membrane protein, the extent of enrichment of Vma12p in vascular membranes was quantified (Fig. 2). Proteins from crude extracts and vascular membranes were analyzed by quantitative Western blotting (see "Experimental Procedures"). As expected, the V-ATPase subunit Vph1p was enriched 20–40-fold with either plasmid-borne or integrated versions of VMA12::HA1 and VMA12::HA3 (see "Experimental Procedures"). Plasmid-borne VMA12::HA1 and VMA12::HA3 fully complemented the growth defects of the vma12Δ mutation, and vascular membranes isolated from these strains showed wild type levels of vascular ATPase activity (data not shown). When IIF experiments were performed with either plasmid-borne or integrated versions of VMA12::HA1, staining of neither Vma12p-HA1 nor Vma12p-HA3 was sufficient to immunolocalize Vma12p. A tetraploid VMA12::HA3 strain was then constructed (DJY70), because increasing the ploidy of the strain had allowed Antebi and Fink (42) to immunolocalize Pmr1p-HA to Golgi membranes. In DJY70 cells, Vma12p-HA3 exhibited a perinuclear staining pattern typical of ER proteins, as revealed by staining DNA with 4',6'-diamidino-2-phenylindole (Fig. 4A). In the same cells, Eug1p, a known ER protein (43), also exhibited perinuclear immunofluorescent staining (Fig. 4B). These data indicate that Vma12p is an ER membrane protein.

Subcellular Localization of Vma12p-VMA12 cells were subjected to differential centrifugation to determine the subcellular location of Vma12p. The fractionation method of Horazdovsky and Emr (38) separates Golgi (P100), endosomal (P100), and cytosolic (S100) proteins from ER, vascular, and plasma membranes (P13) proteins. In this assay, the cytosolic protein phosphoglycerate kinase was found in the S100 fraction as expected (Fig. 3). The ER resident protein Dpm1p was found exclusively in the P13 fraction as expected. Vma12p was also found exclusively in the P13 fraction, suggesting that, inasmuch as Vma12p was not enriched in vascular membranes, it resides either in the ER or plasma membrane.

Vma12p was next immunolocalized by indirect immunofluorescence (IIF) (39). Initial attempts at IIF using anti-Vma12p antibodies were unsuccessful due to low levels of Vma12p expression. To immunolocalize Vma12p, we introduced the hemagglutinin epitope tag (HA) (33) at two different positions in Vma12p to create VMA12::HA1 and VMA12::HA3 (see "Experimental Procedures"). Plasmid-borne VMA12::HA1 and VMA12::HA3 fully complemented the growth defects of the vma12Δ mutation, and vascular membranes isolated from these strains showed wild type levels of vascular ATPase activity (data not shown). When IIF experiments were performed with either plasmid-borne or integrated versions of VMA12::HA1, staining of neither Vma12p-HA1 nor Vma12p-HA3 was sufficient to immunolocalize Vma12p. A tetraploid VMA12::HA3 strain was then constructed (DJY70), because increasing the ploidy of the strain had allowed Antebi and Fink (42) to immunolocalize Pmr1p-HA to Golgi membranes. In DJY70 cells, Vma12p-HA3 exhibited a perinuclear staining pattern typical of ER proteins, as revealed by staining DNA with 4',6'-diamidino-2-phenylindole (Fig. 4A). In the same cells, Eug1p, a known ER protein (43), also exhibited perinuclear immunofluorescent staining (Fig. 4B). These data indicate that Vma12p is an ER membrane protein.

Topology of Vma12p in the ER Membrane—It had been shown previously that Vma12p was required for the membrane association of the hydrophilic ER membrane protein Vma22p (25). In the absence of Vma12p, Vma22p is found in the cytosol, suggesting that a portion of Vma12p is exposed to the cytosol and responsible for Vma22p’s membrane association. The Kyte and Doolittle hydropathy plot for Vma12p predicts that the N-terminal ~60% and the C-terminal ~20% are hydrophilic. To identify cytosolic portions of Vma12p with which Vma22p might interact, microsomes from yeast cells expressing either Vma12p-HA1 or Vma12p-HA3 (tagged in the C- and N-terminal domains, respectively) were subjected to partial proteolysis.
Fig. 4. Immunolocalization of Vma12p. A, tetraploid VMA12::HA3 (DJY70) cells were fixed, spheroplasted, and stained with monoclonal anti-HA epitope (12CA5) antibody. Cells were viewed using Nomarski microscopy for cell morphology and by epifluorescence microscopy for nuclear and Vma12p staining. Signal from the anti-HA antibody was amplified using a goat anti-mouse, mouse anti-goat, FITC-conjugated goat anti-mouse “sandwich.” B, DJY70 cells were treated and stained as in A, but in addition were also stained with polyclonal anti-Eug1p antibodies to determine the staining pattern of a known ER protein.

The Role of Vma12p in V-ATPase Assembly

Digestion products were generated, separated by SDS-PAGE, and probed for marker proteins and Vma12p-HA. Kar2p, a soluble luminal protein of the ER (44), was not digested under these conditions (Fig. 5A, lanes 1–4), demonstrating that the membranes remained intact throughout the incubation (37). Sec63p, an integral membrane protein of the ER, was cleaved on its cytosolic C-terminal tail to a slightly faster migrating species (Fig. 5A, lanes 5–8), indicating that proteins found in the microsomal membranes were both accessible to proteases and in their proper orientation (37). Vma12p-HA1 was degraded to a faster migrating species, which could only be detected for 15 s using an antibody against the HA epitope (Fig. 5B, lanes 1–4), demonstrating that the C terminus of Vma12p-HA1 was readily accessible to proteases and therefore located on the cytosolic face of the ER membrane. The N terminus of Vma12p-HA3 was also readily accessible to proteases (data not shown), indicating that it is also located on the cytosolic face of the membrane. Analysis with anti-Vma12p antibodies revealed that two Vma12p-HA1 proteolysis products could be detected after 10 min of treatment (Fig. 5B, lane 8). Neither product contained the HA epitope (compare lane 8 to lane 4), supporting the argument that the epitope had been removed from these Vma12p-HA1 proteolysis products (lane 8). Sensitivity of the HA tags to proteolysis supports the model (Fig. 5C) that the N and C termini of Vma12p are on the cytosolic face of the ER membrane.

Stability of Vph1p in vma12Δ Cells—To investigate the function of Vma12p, we monitored the fate of the 100-kDa V0 subunit (Vph1p) in vma12Δ mutant cells. Hirata et al. (22) reported that Vph1p was present in crude extracts of vma12Δ cells at 5–10-fold reduced levels relative to wild type cells. To investigate a potential role for Vma12p in the stability of Vph1p, a kinetic analysis was performed by immunoprecipitation of Vph1p from lysates of radiolabeled wild type and vma12Δ cells. The half-life of Vph1p was calculated from data derived from an AMBIS™ quantitation of signal intensity present at each chase time point (Fig. 6). Vph1p is a long-lived protein in wild type cells with a half-life of >4.5 h (Fig. 6A, lanes 1–4). In vma12Δ cells, however, Vph1p exhibited a half-life of 25 min (Fig. 6A, lanes 5–12). Vph1p was not stabilized significantly in vma12Δ cells lacking vacuolar proteases (pep4Δ–) (compare either vma12Δ plot to the WT (VMA12) plot, Fig. 6C), suggesting that Vph1p is degraded in a nonvacular compartment.

The results above suggest that Vph1p is turned over before reaching the vacuole. To investigate this possibility, sec12–4 mutant cells, which are incapable of budding ER-derived transport vesicles at the restrictive temperature (48), were used to determine if Vph1p turnover requires exit from the ER. Vph1p was not stabilized at the restrictive temperature (data not shown), suggesting that Vph1p is degraded in the ER of vma12Δ cells.

To test whether the residual Vph1p in vma12Δ mutant cells resided in the ER, Vph1p was immunolocalized by IIF in wild type and vma12Δ cells. Vph1p was immunolocalized to vacuolar membranes in wild type cells and in yeast cells lacking a V1 subunit (vma3Δ) as reported previously (18, 46). In strains lacking either a V1 subunit (vma3Δ) or Vma12p (vma12Δ), however, Vph1p localized to the ER (Fig. 7, right two sets of columns), as revealed by staining that encircled the nuclear 4′,6′-diamidino-2-phenylindole staining. These results indicate that Vph1p becomes a short-lived ER membrane protein when the V0 complex cannot properly as-
semble due to the loss of either another V0 subunit or Vma12p.

Translocation of Vph1p in vma12Δ Cells—One model for Vma12p function consistent with the experimental results is that Vma12p functions as an assembly factor in the ER membrane for the V-ATPase V0 complex. A second model is that Vma12p plays a specialized role in translocation/insertion of Vph1p into the ER membrane. To test the translocation model, sensitivity of Vph1p to exogenous proteases was monitored in microsomes from wild type and vma12Δ yeast cells. Vph1p is a polytopic membrane protein (Fig. 8A) for which partially translocated or untranslocated protein would be expected to be differentially sensitive to exogenous proteases as compared with wild type protein. Microsomes from wild type and vma12Δ cells were subjected to partial proteolysis as described previously, except subtilisin Carlsberg was used at 0.004 mg/ml. A profile of Vph1p proteolytic products was generated, and blots were probed using a polyclonal antibody raised against the Vph1p N-terminal hydrophilic domain (24). Identical patterns consisting of ~75-, 55-, 20-, and 18-kDa Vph1p proteolytic fragments were generated from wild type and vma12Δ cells (Fig. 8B). Identical patterns were also observed when trypsin and protease K were used at 0.001 mg/ml each (data not shown). The microsomes remained intact throughout proteolysis, as demonstrated by the stability of Kar2p (Fig. 8C). Proteolysis of Vph1p from both wild type and vma12Δ cells to an identical fragment pattern argues that Vma12p is not required for the translocation/insertion of Vph1p into the ER membrane.

DISCUSSION

In this work, we have characterized Vma12p in an effort to elucidate its function in yeast cells. Vma12p behaved biochemically like an integral membrane protein. A combination of differential centrifugation and indirect immunofluorescence revealed that Vma12p resides in the ER membrane. Protease protection analysis revealed that the N and C termini of Vma12p are located on the cytosolic side of the ER membrane.

Hirata et al. (22) reported that Vph1p was present at reduced levels in extracts from vma12Δ cells. A kinetic analysis revealed that Vph1p was long-lived in wild type cells but was degraded rapidly in vma12Δ cells. Degradation in the vma12Δ cells was independent of the activity of vacuolar proteases, suggesting that Vph1p was degraded in a nonvacuolar location.
The Role of Vma12p in V-ATPase Assembly

A kinetic analysis was performed in vma12Δ sec12–4 cells, for which secretory traffic exiting the ER is blocked at the restrictive temperature. Vph1p degradation was unaffected by the sec12–4 mutation, suggesting that Vph1p was degraded in the ER of vma12Δ cells. Thus, Vma12p joins Vma21p and Vma22p as ER membrane proteins required for V-ATPase assembly (24, 25). Based on these results, it appears that Vph1p does not exit the ER when the V0 complex fails to assemble but instead is quickly degraded. In an assembly factor mutant or a mutant lacking a V0 subunit (vma3Δ), the low steady-state level of Vph1p is localized to the ER. These results support a function for Vma12p in the assembly of the membrane sector of the V-ATPase in the yeast ER.

One hypothesis is that Vma12p functions in the translocation/membrane insertion of subunits of the V-ATPase membrane sector. An alternative hypothesis is that Vma12p functions as a molecular chaperone for subunits of the V-ATPase membrane sector. A growing body of evidence supports certain ER proteins functioning as chaperones for specific families of proteins. NinaA, a cyclophilin homologue in Drosophila, is proposed to encode a chaperone for transport and/or folding of specific rhodopsins in photoreceptor cells (47–49). SHR3 in yeast is proposed to encode a chaperone specific to amino acid permeases destined for the plasma membrane (50–51). Our protease protection data revealed that Vph1p is equally sensitive to proteolysis in wild type and vma12Δ cells. Based on these data, the translocation, insertion, and folding of Vph1p are normal in vma12Δ cells and thus Vma12p does not function in these very early biosynthetic processes.

The model of Vph1p topology proposed in Fig. 8A is similar to the model recently proposed by Leng et al. (52), but differs on the topological assignment of Vph1p’s N terminus and on the number of transmembrane domains. Whereas Leng et al. (52) proposed that the N terminus of Vph1p is luminal, our data place the N-terminal domain of Vph1p in the cytosol. Leng et al. (52) also proposed that Vph1p contains seven membrane-spanning domains, placing the Vph1p C terminus on the cytosolic side of the membrane. Inasmuch as our preliminary results also place the Vph1p C terminus in the cytosol, we propose that Vph1p has either six or eight transmembrane domains.

All of the data available for Vma12p indicate that this protein plays a critical role in the assembly of the V-ATPase in the ER, after the V0 polypeptides have been inserted into the ER membrane. However, it is possible that Vma12p has additional roles in V-ATPase biosynthesis. For example, Vma12p could serve to load the assembled V-ATPase complex into ER-derived transport vesicles, as has been proposed for Shr3p (51). Vma12p might also escort the V-ATPase in ER-derived vesicles to the Golgi complex. Certain yeast ER proteins with the sequence -KKXX at their extreme C termini have been shown to exit the ER and be retrieved from an early Golgi compartment (24, 53–54). Vma21p, for example, has a functional -KKXX retention signal at its C terminus (24). Vma12p has a weak ER retention motif, -KITL, at its extreme C terminus, but it appears that this sequence does not function in Vma12p ER retention, inasmuch as addition of the HA epitope to the C terminus did not compromise function for Vma12p-HA1. If the three known V-ATPase assembly factors form an assembly complex in the ER, then Vma21p could serve to retrieve this complex from post-ER compartments. Vma22p, a peripheral ER membrane protein, was found to associate with the ER membrane in a Vma12p-dependent manner (25), and we predict that Vma22p interacts with a cytosolic domain of Vma12p. We are currently investigating in detail the interactions between Vma12p, Vma21p, and Vma22p and other proteins in the ER membrane.

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