Vma22p Is a Novel Endoplasmic Reticulum-associated Protein Required for Assembly of the Yeast Vacuolar H⁺-ATPase Complex*

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The Saccharomyces cerevisiae vacuolar H⁺-ATPase (V-ATPase) is a multi-subunit complex that can be structurally and functionally divided into peripheral (Vₜ) and integral membrane (V₀) sectors. The vma22Δ mutation was isolated in a screen for mutants defective in V-ATPase function. vma22Δ cells contain no V-ATPase activity due to a failure to assemble the enzyme complex; Vₜ subunits accumulate in the cytosol, and the V₀ 100-kDa subunit is rapidly degraded. Turnover of the 100-kDa integral membrane protein was found to occur in the endoplasmic reticulum (ER) of vma22Δ cells. The product of the VMA22 gene, Vma22p, is a 21-kDa hydrophilic protein that is not a subunit of the V-ATPase but rather is associated with ER membranes. The association of Vma22p with ER membranes was perturbed by mutations in VMA12, a gene that encodes an ER membrane protein (Vma12p) that is also required for V-ATPase assembly. These results indicate that Vma22p, along with Vma21p and Vma12p, form a set of ER proteins required for V-ATPase assembly.

The acidification of many organelles including the Golgi, vacuoles, endosomes, and clathrin-coated vesicles is generated via a vacuolar-type proton-translocating ATPase (V-ATPase). The V-ATPase function, which is generated by V-ATPases is used in cellular processes such as protein sorting, receptor-mediated endocytosis, andzymogen activation (Melman et al., 1986).

The yeast V-ATPase is similar in structure and function to V-ATPases from other fungi, plants, and animals (Uchida et al., 1985; Kane et al., 1989). All are multi-subunit complexes that, in a manner similar to the well-characterized FₐFₒ-ATPase, can be divided structurally and functionally into Vₜ and V₀ sectors. The yeast V-ATPase Vₜ sector consists of subunits peripherally associated with the membrane and constitutes the catalytic and regulatory functions of the enzyme complex. The V₀ portion of the enzyme contains integral membrane subunits and forms a pore through which protons are translocated into an organelle (Kane and Stevens, 1992).

Biochemical and genetic analyses have demonstrated that the yeast V-ATPase complex comprises at least ten polypeptides ranging in molecular mass from 100 to 14 kDa. The genes encoding the 100-kDa (VPH1, Manolson et al. (1992)), 69-kDa (VMA1/TFP1, Shih et al. (1988); Hirata et al. (1990)), 60-kDa (VMA2/VAT2, Nelson et al. (1989); Yamashiro et al. (1990)), 54-kDa (VMA13, Ho et al. (1993b)), 42-kDa (VMA5, Beltran et al. (1992); Ho et al. (1993a)), 36-kDa (VMA6, Bauerle et al. (1993)), 32-kDa (VMA8, Nelson et al. (1995); Graham et al. (1995)), 27-kDa (VMA4, Fourny (1990)), 14-kDa (VMA7, Nelson et al. (1994); Graham et al. (1994)), and two hydrophobic polypeptides of 17-kDa (VMA3, Nelson and Nelson (1989); VMA11, Umemoto et al. (1991)) have been cloned and sequenced.

Disruption of genes (vma, vacuolar membrane ATPase) encoding V-ATPase subunits (with the exception of VPH1) gives rise to a characteristic set of phenotypes (Vma⁻) associated with the loss of enzyme function. These include slow growth, an inability to grow on media buffered to a neutral pH, sensitivity to high Ca²⁺, and a Pet⁻ phenotype. In addition, vma ade2 double mutant colonies are white instead of the red color typical of ade2 colonies. These phenotypes have been used to design screens for mutations in genes encoding structural subunits of the V-ATPase complex as well as factors required for the assembly/stability but which are not subunits of the final enzyme complex (Ho et al., 1993a; Ohya et al., 1991).

Studies of the assembly of the V-ATPase in cells mutant in either V₀ or Vₜ subunit genes have indicated that assembly requires the coordination of integral membrane subunits and peripheral subunits. Disruption of a gene encoding any single Vₜ peripheral subunit of the V-ATPase complex leads to a failure of the Vₜ sector to assemble on the vacuolar membrane and the stable accumulation of remaining Vₜ subunits in the cytosol. The V₀ sector of the enzyme, however, is transported to and stable in the vacuolar membrane in the absence of an assembled Vₜ sector (Doherty and Kane, 1993; Ho et al., 1993a). The absence of a V₀ sector subunit leads to the destabilization/degradation of the remaining V₀ polypeptides as well as a failure of Vₜ subunits to associate with the vacuolar membrane (Kane et al., 1992; Bauerle et al., 1993).

Recent studies have characterized the VMA12 and VMA21 genes, which are involved in V-ATPase assembly but whose products (Vma12p and Vma21p) are not subunits of the final vacuolar enzyme complex (Hirata et al., 1993; Hill and Stevens, 1994). Vma21p is a membrane protein of the endoplasmic reticulum (ER), and mutations in VMA21 lead to a rapid degradation of the 100-kDa V₀ polypeptide. A model has been proposed in which assembly of the V-ATPase complex is initiated in the ER, and failure to do so results in the rapid turnover of the 100-kDa integral membrane polypeptide within the ER (Hill and Stevens, 1994).

In this work, we describe the characterization of the VMA22 gene and its product, Vma22p. Similar to Vma21p, Vma22p is a non-subunit assembly factor that is associated with the ER. Loss of Vma22p function leads to a rapid and specifically ER-based degradation of the 100-kDa V₀ polypeptide and failure of the V-ATPase complex to assemble.
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**EXPERIMENTAL PROCEDURES**

**Plasmid Construction and DNA Sequencing—Plasmids used in this study are listed in Table I. Plasmids derived from plKH221 were constructed as described by Sherman et al. (1986).**

**Strains, Media, and Microbiological Techniques—Yeast strains used in this study are listed in Table I. Strains KHY26 and SF838–1D are isogenic except at the PEP4 locus. SNY28, KHY13, and KHY34 are isogenic except at the VMA22 locus. SF838–1D, KHY34, and KHY39 are isogenic except at the VMA12 locus. Yeast strain MBY10–7A was a gift from T. Nakano. Yeast extract peptone dextrose buffered to pH 5.0 or 7.5 was prepared as described by Nothwehr et al. (1995). Monoclonal antibodies specific for the 15-kD and 38-kD subunits of V-ATPase were described by Hill and Stevens (1994). Data base searches were performed using the BLAST service of the National Center for Biotechnology Information (Altschul et al., 1990). Sequence analysis was performed using the GCG analysis software available from the University of Wisconsin.

Antibodies, Protein Preparation, and Western Blotting—To generate rabbit polyclonal antibodies against Vma22p, the following DNA constructs were prepared. A BglII site was introduced via in vitro mutagenesis (Kunkel et al., 1987) near the N terminus of VMA22 (in pKH2212) (base pairs 43 and 44 of the VMA22 open reading frame) changingbp GC → AT and thus amino acid Glu15 → Leu to create pKH2214. pKH2214 was then digested with BglII, and the ends were blunted with Klenow enzyme. The plasmid was digested with XbaI, and the 760-bp fragment inserted into Smal and XbaI cut pXP25-5 (Roberts et al., 1992) to create pKH2218 (Fig. 1A). Induction of Escherichia coli carrying plasmid pKH2218 with isopropyl-β-d-thiogalactopyranoside produced a 21-kD fusion protein that was purified and used to inject New Zealand White rabbits for antibody elicitation (Vaitukaitis, 1981). Ananti-murine recognizing Vma22p was affinity purified against protein produced from pKH2224 using the method of Roberts et al. (1991). Plasmid pKH2224 was created by inserting the BglII (blunted)-XbaI fragment described above into XmnI- and Xbal-digested pMAL-c2 (New England Biolabs). Upon induction with isopropyl-β-d-thiogalactopyranoside, E. coli cells expressing pKH2224 produced a ~63-kDa Vma22p-MBP fusion protein.

Whole cell protein extracts and vacuolar membrane fractions were prepared as described (Hill and Stevens, 1994). Vacuolar membrane fractions were extracted with chloroform/methanol as described by Ho et al. (1993b). Monoclonal antibodies recognizing the 100- (10D7, 7B1), 69- (881), 60- (13D11), and 42-kDa (7A2) V-ATPase subunits were described by Kane et al. (1992). Monoclonal antibodies specific for the 100-, 69-, and 60-kDa V-ATPase subunits and Dpm1p were from Molecular Probes Inc. Polyclonal antibodies directed against the 100-, 54-, and 27-kDa V-ATPase subunits were purified and used as described by Hill and Stevens (1994). Polyclonal antibody directed against phosphoglycerol kinase was used as described by Ndhwehr et al. (1995). Monoclonal antibodies specific for the ER membrane protein, Dpm1p, were used as described by Piper et al. (1994). Secondary antibodies conjugated to horseradish peroxidase were purchased from Amersham. Immunoblots were detected using chemiluminescent detection (DuPont NEN).

Immunoprecipitation—35S-Express label was purchased from DuPont NEN. Immunoprecipitation of Vph1p was performed as described.
Cloning the VMA22 Gene—The vma22-1 allele (KHY28) was isolated in a screen for mutants that were pH-sensitive and formed white colonies in the SEY6211 (ade2) strain, which normally forms red colonies (Ho et al., 1993a). KHY28 cells also displayed other phenotypes that are characteristic of vma mutants such as slow growth and a failure to grow on non-fermentable carbon sources (Petr) or on media containing 100 mM CaCl2 (Cis). KHY28 cells also failed to accumulate the fluorescent weak base quinacrine in their vacuoles, indicating the organelle was not acidified.

VMA22 was cloned by complementation of the pH and color defects of vma22-1 cells. Strain KHY28 was transformed with a plasmid library (Rose et al., 1987). 1 of 2500 Ura+ transformants was red, able to grow on media buffered to pH 7.5, and complementation was plasmid dependent. Plasmid pKH2201 was recovered and found to contain a yeast genomic DNA insert of 8.5 kb. Various subclones of pKH2201 (pKH2203, pKH2204, pKH2206, pKH2207, pKH2208, pKH2209, and pKH2211) demonstrated that a 1-kb SnaBl-SpeI fragment (pKH2211, Fig. 1A) was sufficient to fully complement vma22-1.

The nucleotide sequence of the 1-kb complementing fragment in pKH2211 was determined for both strands (Genbank accession number U24501). One long open reading frame (VMA22) of 543 bp was found predicting a protein (Vma22p) of 181 amino acids with a molecular mass of 21 kDa (Fig. 1B). Data base searches showed that VMA22 was identical to the sequence designated YHR060w in the CPR2-ERG7 intergenic region of chromosome VIII recently identified by the yeast genome project. No significant homology to other sequences in the data base was found. Rabbit antiserum generated using an E. coli expressed Vma22p fusion protein (codons 16-181 of Vma22p) was used to detect Vma22p in yeast cells. Anti-Vma22p antibodies recognized a single band of 21 kDa in wild-type cells, and this 21-kDa protein was absent in vma22Δ strains (KHY38 and KHY13) (Fig. 1C).

Strains carrying a null allele of vma22Δ (Fig. 1A) were constructed by transforming SFF838–1D and SNY28 with either the vma22Δ::LEU2 disruption fragment contained within pKH2213 (KHY34 and KHY13) or the vma22Δ::URA3 fragment (KHY38 and KHY34). The structure of the disruption was confirmed by PCR amplification of the disrupted VMA22 open reading frame using oligonucleotides complementary to sequences flanking VMA22 (see “Experimental Procedures”). A strain carrying a marked allele of VMA22 (VMA22::URA3) was crossed with KHY28 (vma22-1), the diploid sporulated, dissected, and the tetrad progeny examined. All tetrads examined showed 2 Vma-2 Vma+ segregation indicating co-segregation and, taken together with complementation analysis, demonstrating that the cloned gene was VMA22.

RESULTS

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Phenotypes of vma22Δ Cells—vma22Δ cells displayed phenotypes that were characteristic of other vma strains and identical to the vma22-1 mutant. vma22Δ cells (KHY38) contained no V-ATPase activity. Vacular membranes purified from wild-type cells were assayed for V-ATPase activity and were found to contain a V-ATPase specific activity of 7.0 units per mol of ATP hydrolyzed/min/mg protein. Western blot analyses of V-ATPase sub-units in wild-type cells (SNY28) and vma22Δ (KHY38) cells are shown in Fig. 2A. Immunoblotting of whole cell protein extracts indicated that the level of the 100-kDa V-ATPase subunit (Vph1p) was substantially lowered in vma22Δ cells compared to wild-type cells. However, the steady state levels of other subunits (69, 60, 54, 42, 36, and 27 kDa) appeared comparable between wild-type and vma22Δ cells.

Western blot analyses of vacuolar membrane fractions prepared from wild-type and vma22Δ cells indicated that although Vc subunits appeared to be produced at wild-type levels in vma22Δ cells, they failed to associate with the vacuolar membrane (Fig. 2A). In addition, indirect immunofluorescence of the 60-kDa subunit of the Vc sector in vma22Δ cells showed a cystolic pattern instead of the vacuolar membrane-staining pattern seen in wild-type cells (data not shown). Immunoblots also indicated that while a low level of the 100-kDa V-ATPase subunit was present in vma22Δ cells, no 100-kDa polypeptide was present on the vacuolar membrane (Fig. 2A). Chloroform/methanol treatment of vacuolar membranes has been shown to extract the 17-kDa proteolipid (Vma3p/Vma11p) component of the Vc sector of the V-ATPase, which can then be detected by SDS-PAGE and silver staining (Ho et al., 1993b). The 17-kDa
proteolipid was substantially reduced in vma22Δ cells in contrast with the proteolipid extracted from wild-type vacuolar membranes (Fig. 2B). In summary, immunoblot results indicate that Vph1p (100 kDa) and Vma3p/Vma11p (17 kDa), constituents of the V₀ integral membrane sector of the V-ATPase enzyme, are destabilized in vma22Δ cells. Subunits that comprise the V₁ sector of the enzyme (69, 60, 54, 42, 36, and 27 kDa) appeared to be present at wild-type levels and were stable in vma22Δ cells but were unable to associate with the vacuolar membrane. These results fully accounted for the lack of V-

ATPase activity seen in vma22Δ cells, since this enzyme complex was unassembled.

The low level of the 100-kDa subunit (Vph1p) seen in immunoblots from vma22Δ cells led us to investigate further the nature of this defect. We performed a kinetic analysis of the synthesis and turnover of Vph1p in wild-type and vma22Δ cells. We also investigated the effect of the PEP4 gene status on the turnover and stability of Vph1p. PEP4 is a critical protease in the activation cascade of vacuolar hydrolases (Ammerer et al., 1986; Woolford et al., 1986). Any effect of a mutation in the PEP4 gene (pep4–3) on the half-life of Vph1p would implicate vacuolar proteases in the turnover of this protein.

Wild-type and vma22Δ cellswereradiolabeled with [35S]methionine and chased by the addition of non-radiolabeled methionine. At various chase times, samples were immunoprecipitated with anti-Vph1p antibodies and analyzed by SDS-PAGE and fluorography. In wild-type cells (SNY28), Vph1p was very stable with a half-life of \( \frac{1}{2} \) 500 min (Fig. 3, lanes 1–4). The
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**Table III**

| Strain       | Relevant genotype | t_{1/2} Vph1p at 36°C |
|--------------|-------------------|-----------------------|
| SNY28        | Wild type         | >400                  |
| KHY13        | vma22Δ            | 23                    |
| KHY9         | vma22Δ sec12-4    | 30                    |
| MBY10-7A     | sec12-4           | 400                   |
| KHY22        | vma21Δ            | 28                    |
| KHY20        | vma21Δ sec12-4    | 20                    |

*The radioactivity of Vph1p samples visualized by immunoprecipitation were quantified using an AMBIS radioanalytic imaging system (AMBIS Systems, San Diego) and then plotted against time to estimate the half-life of Vph1p.*

In contrast, vma22Δ cells showed a rapid turnover of Vph1p at the non-permissive temperature (Fig. 4A, lanes 6–10), with a half-life of ~23 min (Table III). The rapid degradation of Vph1p seen in vma22Δ cells was not stabilized by...
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before delivery and activation to the mature enzyme in the vacuole (Klionsky and Emr, 1989). Pulse-chase immunoprecipitation of alkaline phosphatase from wild-type (SNY28) and vma22A (KYH38) cells showed that this vacuolar membrane protein was correctly translocated into the secretory pathway, targeted to the vacuole, and matured normally in vma22Δ cells (Fig. 5B).

Subcellular Localization of Vma22p—The amino acid sequence of Vma22p predicted a hydrophilic protein containing neither an ER signal sequence nor a transmembrane domain, suggesting that Vma22p might be a cytosolic protein. To determine the subcellular localization of Vma22p, we immunolocalized the protein by indirect immunofluorescent staining with affinity-purified anti-Vma22p antibodies. Wild-type yeast cells showed a weak ER staining pattern for Vma22p. However, wild-type yeast cells producing approximately twice the normal level of Vma22p (due to the presence of a low copy, centromere-based plasmid carrying VMA22) (pKH2211) exhibited a perinuclear staining pattern, as well as some cytosolic staining (Fig. 6). The immunofluorescent staining for Vma22p was specific since vma22Δ cells showed a very faint, nonspecific, cytosolic staining pattern (data not shown). The perinuclear staining pattern seen for Vma22p is typical of ER proteins and has been observed for ER proteins such as Vma21p (Hill and Stevens, 1994), Kar2p (yeast BiP) (Rose et al., 1989), and Egerp1 (Tachibana and Stevens, 1992). Interestingly, yeast cells overproducing Vma22p (due to the presence of a multicopy plasmid carrying the VMA22 gene) exhibited a bright cytosolic as well as an ER staining pattern, suggesting that the association of Vma22p with the ER is saturable (data not shown).

Association of Vma22p with the ER membrane might result from an interaction between Vma22p and an ER membrane protein. Two intriguing candidate proteins are Vma12p and Vma21p. Yeast cells lacking either of these two proteins (vma21 (Hill and Stevens, 1994) or vma12Δ) have been found to rapidly degrade Vph1p. Vma21p (Hill and Stevens, 1994) and Vma12p (Hirata et al., 1993) are ER membrane proteins necessary for V-ATPase assembly and activity. We investigated whether the subcellular fractionation profile of Vma22p could be altered by mutations in either the VMA21 or VMA12 gene. SNY28 (wild-type), KYH4 (vma21Δ), and DJY63 (vma12Δ) cells were spheroplasted and lysed, and the lysates were subjected to differential centrifugation to generate lysate, P13, S100, and P100 fractions. Horazdovsky and Emr (1993) have demonstrated that fractions generated by this procedure separate soluble cytosolic proteins (S100) from Golgi (P100) and plasma membrane/ER/vacuolar (P13) proteins. Lysate, P13, S100, and P100 fractions were separated by SDS-PAGE, and immunobots were probed to detect phosphoglycerol kinase, a soluble cytosolic protein, Dpm1p, an ER transmembrane protein (Orlean et al., 1988), and Vma22p. In wild-type cells, Vma22p was detected in the P13 membrane fraction (Fig. 7), consistent with an ER localization as shown by indirect immunofluorescence experiments. While the P13 fraction contains both ER and vacuolar membranes, additional experiments with purified vacuolar membranes revealed that Vma22p is not associated with this organelle (data not shown). As expected, phosphoglycerol kinase and Dpm1p fractionated with the cytosol and P13 membranes, respectively, in these preparations. The fractionation profile of Vma22p was unaltered in vma21Δ cells (data not shown). However, when fractions prepared from vma12Δ cells were probed with anti-Vma22p antibodies, Vma22p was now detected in the S100 fraction with little detectable signal in the P13 membrane fraction. The fraction-

introduction of the sec12-4 temperature-sensitive allele (Fig. 4A, lanes 11-15), with the half-life of Vph1p in these double mutant cells still only ~30 min. Together, these data clearly indicate that the degradation of Vph1p in vma22Δ cells is occurring in the ER compartment of the secretory pathway.

We had previously determined that Vph1p was rapidly degraded in another vma mutant, vma21 (Hill and Stevens, 1994). We have also found that the degradation of Vph1p in vma21Δ cells (Hill and Stevens, 1994) occurs in the ER. KHY22 cells (vma21Δ, half-life ~28 min) and KHY71 cells (vma21Δ sec12-4, half-life ~20 min) showed approximately the same rate of degradation of Vph1p at the restrictive temperature (Fig. 4B and Table I).

Effects of vma22Δ on Other Membrane Proteins—To investigate whether the vma22Δ mutation had any effect on other membrane proteins transiting the secretory pathway, we examined the subcellular localization of the multiple membrane-spanning plasma membrane ATPase, Pma1p (Serrano et al., 1986), in wild-type and vma22Δ cells by indirect immunofluorescence. The immunolocalization of Pma1p was indistinguishable between wild-type (SNY28) and vma22Δ cells (KYH38) (Fig. 5A) and indicated that Vma22p was not required for the intracellular targeting of this protein. We also examined the processing of the vacuolar membrane protein, alkaline phosphatase, which transits the secretory pathway as a precursor...
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The specific defect in vma22Δ cells appears to be at the level of assembly of the V-ATPase complex. Peripherally associated V\(_1\) subunits were stable in vma22Δ cells but were unable to associate with the vacuolar membrane and instead accumulated in the cytosol. V\(_0\) subunits appeared to be destabilized in vma22Δ cells, and the 100-kDa subunit, Vph1p, was not found on the vacuolar membrane. The small amount of proteolipid found in chloroform/methanol extracts from vma22Δ vacuolar membranes could reflect a stable but unassembled vacuolar portion of the V\(_0\) sector or merely represent the contamination of vacuolar membrane preparations with membranes from other organelles.

The low level of Vph1p in vma22Δ cells was the result of destabilization of the protein. In kinetic analyses, Vph1p was rapidly degraded in vma22Δ cells with a half-life of \(~40\) min, approximately 8% of that observed in wild-type cells. Degradation of Vph1p was independent of the vacuolar hydrolase, Pep4p, indicating that the increased turnover of Vph1p seen in vma22Δ cells was likely occurring in an organelle other than the vacuole.

To define the subcellular organelle in which Vph1p was degraded in vma22Δ cells, we performed our kinetic analyses in sec12–4 vma22Δ double mutant cells, which accumulate the ER compartment at the non-permissive temperature. The continued degradation of Vph1p in these cells at the permissive or non-permissive temperature showed clearly that degradation of Vph1p occurred in the ER. We also confirmed that the rapid turnover of Vph1p seen in vma21Δ cells (Hill and Stevens, 1994) also occurred specifically in the ER.

Other membrane proteins of the secretory pathway appeared to be unaffected by the vma22Δ mutation. Pma1p was present at the plasma membrane of both wild-type and vma22Δ cells, as revealed by immunofluorescence experiments. Also, the vacuolar membrane protein, alkaline phosphatase, was transported to and matured in the vacuole similarly in wild-type and vma22Δ cells. These results suggest that the vma22Δ mutation may be specific in its effects on the assembly of the V-ATPase complex.

Although vma22Δ cells displayed phenotypes typical of other vma mutations, including those in subunit structural genes, Vma22p was found not to be a subunit of the V-ATPase complex. Vma22p was de-enriched in vacuolar membrane fractions and did not co-purify with the V-ATPase. The immunofluorescent labeling pattern of Vma22p showed both an ER and some cytosolic localization. The ER membrane localization of Vma22p is similar to that for Vma21p (Hill and Stevens, 1994) and is suggestive of an ER-based set of proteins required for V-ATPase expression. Although Vma22p is predicted to be a hydrophilic protein, both indirect immunofluorescence and

**DISCUSSION**

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Although vma22Δ cells displayed phenotypes typical of other vma mutations, including those in subunit structural genes, Vma22p was found not to be a subunit of the V-ATPase complex. Vma22p was de-enriched in vacuolar membrane fractions and did not co-purify with the V-ATPase. The immunofluorescent labeling pattern of Vma22p showed both an ER and some cytosolic localization. The ER membrane localization of Vma22p is similar to that for Vma21p (Hill and Stevens, 1994) and is suggestive of an ER-based set of proteins required for V-ATPase expression. Although Vma22p is predicted to be a hydrophilic protein, both indirect immunofluorescence and
subcellular fractionation experiments indicate that Vma22p is associated with the ER membrane.

The effects of the vma22Δ mutation on the V-ATPase complex described here are similar to those reported for vma21Δ (Hill and Stevens, 1994) and vma12Δ (Hirata et al., 1993) mutants. The 100-kDa polypeptide is rapidly degraded in vma21Δ cells, and in this work we have shown that, as in vma22Δ cells, Vph1p turnover occurs in the ER. Like Vma22p, Vma21p is also an ER protein, and its ER localization is determined by a C-terminal di-lysine motif (Hill and Stevens, 1994). Vma21p, a 25-kDa membrane protein, also resides in the ER. In vma12Δ cells, Vma22p did not fractionate with membranes as in wild-type cells but rather with soluble cytosolic proteins such as phosphoglycerol kinase. It therefore appears likely that ER membrane association of Vma22p is mediated through an interaction with Vma12p. Although we did not find any perturbation of Vma22p localization in vma21Δ cells, it will be necessary to extend our analysis of potential interactions between all combinations of Vma21p, Vma12p, and Vma22p. Our results suggest that this set of proteins is necessary for the assembly of the V0 sector of the V-ATPase complex in the ER. In the future, we will attempt to establish whether Vma21p/Vma22p/Vma12p physically interact. Because we have a large number of other vma mutants to screen for phenotypes similar to those of vma21Δ/vma22Δ/vma12Δ, we may yet identify additional proteins required for V-ATPase assembly.

What could the function be of Vma22p/Vma12p/Vma21p in V-ATPase assembly? We have not ruled out a role for these proteins in the correct insertion of the 100-kDa integral membrane protein into the ER. As yet we have no data relating to the topology of the V0 sector polypeptides in either wild-type or vma mutant cells. While it is too early to be confident, we favor an alternative model in which the Vma22p/Vma21p/Vma12p proteins act as chaperones, allowing the proper assembly of the V0 subunits after their insertion into the ER membrane.

The requirement for additional ER proteins for folding, insertion into the ER, or oligomerization has been observed for membrane proteins other that the 100-kDa V-ATPase subunit. Shp3p, an ER protein, is required for the exit of amino acid permeases from the ER in yeast (Ljungdahl et al., 1992). Shp3p is proposed to act either as a chaperone-like role in the folding of permeases or possibly as a permease-specific component of the ER translocation machinery. In Drosophila, mutations in the cyclophilin homologue ninaA result in a failure to transport two related opsin proteins from the ER of photoreceptor cells (Colley et al., 1991). Finally, the influenza virus HA membrane protein requires BIP for trimerization (Gething et al., 1986), and this oligomerization is required for exit from the ER (Copeland et al., 1988).

In summary, we have identified a protein, Vma22p, which is required for the ER assembly of the V0 sector of the V-ATPase complex. Failure to assemble the V0 sector leads to rapid degradation of the 100-kDa polypeptide in the ER and failure to assemble the V-ATPase enzyme complex. Vma22p, along with Vma21p and Vma12p, may form an ER assembly complex specific for the V-ATPase enzyme.

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