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Vma21p Is a Yeast Membrane Protein Retained in the Endoplasmic Reticulum by a Di-lysine Motif and Is Required for the Assembly of the Vacuolar H^+-ATPase Complex

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The yeast vacuolar proton-translocating ATPase (V-ATPase) is a multisubunit complex comprised of peripheral membrane subunits involved in ATP hydrolysis and integral membrane subunits involved in proton pumping. The yeast *vma21* mutant was isolated from a screen to identify mutants defective in V-ATPase function. *vma21* mutants fail to assemble the V-ATPase complex onto the vacuolar membrane: peripheral subunits accumulate in the cytosol and the 100-kDa integral membrane subunit is rapidly degraded. The product of the *VMA21* gene (Vma21p) is an 8.5-kDa integral membrane protein that is not a subunit of the purified V-ATPase complex but instead resides in the endoplasmic reticulum. Vma21p contains a dilysine motif at the carboxy terminus, and mutation of these lysine residues abolishes retention in the endoplasmic reticulum and results in delivery of Vma21p to the vacuole, the default compartment for yeast membrane proteins. Our findings suggest that Vma21p is required for assembly of the integral membrane sector of the V-ATPase in the endoplasmic reticulum and that the unassembled 100-kDa integral membrane subunit present in Δ*vma21* cells is rapidly degraded by nonvacuolar proteases.

INTRODUCTION

The vacuolar-type proton-translocating ATPase (V-ATPase) is a multisubunit enzyme found in the membranes of many acidified organelles, including lysosomes, vacuoles, endosomes, the Golgi apparatus, and clathrin-coated vesicles. Acidification and/or the electrochemical gradient generated by these V-ATPases play important roles in such cellular processes as receptor-mediated endocytosis, protein sorting, and zymogen activation (Mellman *et al.*, 1986).

The yeast V-ATPase multisubunit complex closely resembles those from other fungi, plants, and animal cells (Uchida *et al.*, 1985; Kane *et al.*, 1989). In a manner analogous to the F_1F_0 ATPase complex, the yeast enzyme can be structurally and functionally divided into catalytic (V_1) and integral membrane (V_0) portions. The V_1 sector of the enzyme consists of hydrophilic subunits and is responsible for ATP hydrolysis, whereas the V_0 sector of the enzyme contains hydrophobic subunits and constitutes a proton-translocating pore (Kane and Stevens, 1992).

Biochemical analyses of the yeast V-ATPase indicated that it consists of at least eight polypeptides of 100, 69, 60, 42, 36, 32, 27, and 17 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*) (Nelson *et al.*, 1989; Yamashiro *et al.*, 1990), 42 kDa (*VMA5*) (Beltran *et al.*, 1992; Ho *et al.*, 1993a), 36 kDa (*VMA6*) (Bauerle *et al.*, 1993), 27 kDa (*VMA4*) (Foury, 1990), and two hydrophobic polypeptides of 17 kDa (*VMA3* [Nelson and Nelson, 1989] and *VMA11* [Umemoto *et al.*, 1991]) have been cloned and sequenced.

Disruption of the genes encoding various subunits of the V-ATPase complex gives a characteristic set of phenotypes (Vma^-) associated with loss of enzyme activity. These include slow growth, an inability to grow on media that is buffered to a neutral pH, sensitivity to Ca^{2+} ions, and a petite phenotype (inability to grow on nonfermentable carbon sources). Also, *vma ade2* double mutants fail to accumulate the characteristic red adenine biosynthetic pathway intermediate and therefore these colonies appear white instead of red.

Genetic screens based on Vma$^-$ phenotypes have recently identified new genes encoding not only additional subunits of the yeast V-ATPase but also factors required for the assembly/targeting/stability of the V-ATPase. Examples of these new genes include *VMA13*, which encodes a 54-kDa subunit of the V-ATPase (Ho *et al.*, 1993b), and *VMA12*, which encodes a 25-kDa protein that is required for V-ATPase assembly but is not itself a subunit of the V-ATPase enzyme complex (Hirata *et al.*, 1993).

Assembly of the V-ATPase complex onto the vacuolar membrane has been studied in mutants disrupted for either V_1 or V_0 subunit encoding genes. Disruption of V_1 sector genes results in the failure of all V_1 subunits to associate with the vacuolar membrane and their stable accumulation in the cytoplasm; however, V_0 subunits are transported to and stable in the vacuolar membrane (Kane *et al.*, 1992; Ho *et al.*, 1993a). Disruption of genes encoding components of the V_0 sector results not only in the failure to assemble the V_1 subunits onto the vacuolar membrane but also in the loss of V_0 membrane subunits (Kane *et al.*, 1992; Bauerle *et al.*, 1993).

In this study we describe the characterization of the *VMA21* gene, which was originally identified from a screen for Vma$^-$ mutants in *Saccharomyces cerevisiae*. *VMA21* has been found to encode a small membrane protein that resides in the endoplasmic reticulum (ER) and is required for assembly of the V-ATPase complex. The *VMA21* encoded protein, Vma21p, is retained in the ER due to its carboxy-terminal di-lysine motif, and ER retention is critical for Vma21p function.

MATERIALS AND METHODS

Strains, Media, and Microbiological Techniques

Yeast strains used in this study are listed in Table 1. The *pep4-3* allele of SF838-1D was converted to *PEP4* by transformation with a linearized *PEP4* fragment and Pep$^+$ colonies selected by the N-acetyl-phenylalanine-β-napthyl-ester assay (Wolf and Fink, 1975). The resulting yeast strain, SNY28, is otherwise isogenic to SF838-1D. Strain KHY5 was constructed as follows: strain SF838-1D was transformed with *Msc* I digested pKH30 and the presence of *VMA21::HA* confirmed by Western blot analysis. Loop out of wild-type *VMA21* was then selected on 5-fluoroorotic acid (Boeke *et al.*, 1984) and again the presence of *VMA21::HA* was confirmed by Western analysis. The same procedure was used to create KHY8 (SF838-1D transformed with *Msc* I digested pKH34). Yeast extract peptone dextrose pH 5.0 and pH 7.5 media used for growth of Vma$^-$ strains were prepared as described by Yamashiro *et al.* (1990). Low adenine containing synthetic dextrose media used for cloning *VMA21* was prepared as described by Cooper *et al.* (1993). Other standard yeast media and genetic manipulations were performed as described by Sherman *et al.* (1986).

Plasmid Construction and DNA Sequencing

Plasmids used in this study are listed in Table 2. Plasmids derived from pB8 capable of complementing *vma21-1* were constructed as follows (see Figure 1A). Plasmid pB8 was digested with *Eco*RI and religated; the resulting plasmid (pKH1) contains a 3.5-kilobase (kb) yeast DNA insert. Plasmid pB8 was digested with *Hind*III and religated; the resulting plasmid (pKH2) contains a 2.9-kb yeast DNA insert. Plasmid pKH3 was constructed by digesting pB8 with *Pvu* II and *Sal* I and inserting the 2.2-kb fragment into *Sma* I and *Sal* I digested pRS315 (Sikorski and Heiter, 1989). A 1.6-kb *Eco*RV-*Sal* I fragment from pB8 was excised and inserted into *Sma* I and *Sal* I digested pRS315 to create pKH4. pKH5 and pKH6 contain a 1.6-kb *Eco*RV-*Sal* I inserted as a *Bam*HI-*Sal* I fragment into pBluescript II SK$^+$ or pBluescript II KS$^+$ (Stratagene, La Jolla, CA) for sequencing purposes. pKH14 was constructed by digesting pKH4 with *Bam*HI and *Sac* I and inserting the 540-base pair (bp) fragment into pRS316 (Sikorski and Heiter, 1989).

Deletion of the *VMA21* open reading frame (ORF) was created in the following way. pB8 was digested with *Pvu* II and *Sal* I and the 2.2-kb fragment inserted into *Sma* I and *Sal* I digested pBluescript II KS$^+$ to create pKH9. pKH9 was then digested with *Pst* I and *Eco*RV, the ends were blunted with T4 DNA polymerase, and a 2-kb *Hpa* I fragment carrying *LEU2* was inserted to create pKH10B. pKH10B contains *LEU2* in the opposite orientation to *VMA21* and was digested with *Xba* I before transformation.

Plasmids used for determining which ORF was *VMA21* were made as follows. pKH19 was derived from the site-directed mutagenesis (Kunkel *et al.*, 1987) of pKH14 to destroy the initiating methionine of the 48 aa ORF. Base pair 400 in the 540-bp complementing region (Figure 2A) was mutagenized from A to T and successful clones identified by introduction of a *Dra* I site. pKH14 was also mutagenized to destroy the 77 aa ORF; base pair 109 of the 540-bp complementing region was changed from G to C (pKH15), which introduced a *Bgl* II site. pKH15 was then digested with *Bgl* II, and the site was filled in with Klenow and religated to produce plasmid pKH16.

pKH22 is a derivative of pKH14 in which a *Bgl* II site has been introduced starting at bp 207 of the *VMA21* ORF. This changes amino acid G_{72} to L_{72} and results in a fully functional Vma21p. Insertion of the 9 amino acid hemagglutinin (HA) epitope tag was constructed by site-directed mutagenesis of pKH22 to create pKH28. pKH28 was digested with *Hind*III and *Sac* I and the 540-bp fragment inserted into *Hind*III and *Sac* I digested pRS306 to create pKH30. pKH30 was digested with *Msc* I before integration into SF838-1D.

Amino acids K_{74} and K_{75} of Vma21p were changed to Q_{74} and Q_{75} by site-directed mutagenesis of pKH28 to create pKH31. Successfully mutagenized clones were chosen by introduction of a *Dra* I site and confirmed by sequencing. To create pKH34, the 540-bp *Hind*III-*Sac* I fragment from pKH31 was inserted into *Hind*III and *Sac* I digested pRS306. pKH34 was digested with *Msc* I before integration into SF838-1D. The allele of *VMA21* in which lysine 74 and 75 have been changed to glutamine residues was designated *vma21-10::HA*.

The Vph1p antigen-producing plasmid, pKH102, was constructed by digesting pVIP1-82 (Manolson *et al.*, 1992) with *Dra* I and *Pfl*MI, isolating and blunt ending a 1.13-kb fragment and inserting it into *Xmn* I digested pMAL-c2 (New England Biolabs, Beverly, MA) in which a *Nhe* I nonsense linker (New England Biolabs) had been inserted at the *Hind*III site (blunted). pKH102 contains codons 1–369 of *VPH1* fused in-frame to the maltose-binding protein.

The nucleotide sequence of the *VMA21* gene was determined by sequence analysis of the 1.6-kb *Eco*RV-*Sal* I genomic fragment from pB8 in either pKH5 or pKH6. Nested deletions using *Exo*III (Erase-A-Base, Promega, Madison, WI) were generated and sequenced by the dideoxy method of Sanger *et al.* (1977). Database 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 sequence analysis software available from the University of Wisconsin (Madison, WI).

Epitope Tagging of VMA21

Epitope tagging of *VMA21* was performed using site-directed mutagenesis to introduce a nine amino acid epitope (YPYDVPDYA) from influenza virus hemaglutinin protein HA1 (Wilson *et al.*, 1984). A synthetic oligonucleotide encoding the HA epitope and flanking bases complementary to *VMA21* sequence was used in mutagenesis of

Table 1. Yeast strains used in this study

Strain	Genotype	Source or Reference
SEY6211	*MATa ade2-101 ura3-52 leu2-3,112 his3-Δ200 trp1-901 suc2-Δ9*	Robinson *et al.* (1988)
SF838-1D	*MAT ura3-52 leu2-3,112 his4-519 ade6 pep4-3*	Rothman and Stevens (1986)
SNY28	*MATα ura3-52 leu2-3,112 his4-519 ade6*	This work
CYY7	*MATa/α leu2-3,112/leu2-3,112 ura3-52/ura3-52 his4-519/HIS4 ade6/ADE6 suc2-Δ9/SUC2*	Yamashiro *et al.* (1990)
KHY1	*MATa ade2-101 ura3-52 leu2-3,112 his3-Δ200 trp1-901 suc2-Δ9 vma21-1*	This work
KHY2	*MATa ade2-101 ura3-52 leu2-3,112 his3-Δ200 trp1-901 suc2-Δ9 Δvma21::LEU2*	This work
KHY3	*MATα ura3-52 leu2-3,112 his4-519 ade6 pep4-3 Δvma21::LEU2*	This work
KHY4	*MATα ura3-52 leu2-3,112 his4-519 ade6 Δvma21::LEU2*	This work
KHY5	*MATα ura3-52 leu2-3,112 his4-519 ade6 pep4-3 VMA21::HA*	This work
KHY6	*MATa/α leu2-3,112/leu2-3,112 ura3-52/ura3-52 his4-519/HIS4 ade6/ADE6 suc2-Δ9/SUC2 Δvma21::LEU2/VMA21*	This work
KHY8	*MATα ura3-52 leu2-3,112 his4-519 ade6 pep4-3 vma21-10::HA*	This work

SNY28 and SF838-1D are isogenic except the *pep4-3* allele was gene converted to *PEP4*. SF838-1D, KHY3, KHY5, and KHY8 are isogenic except at the *VMA21* locus. SNY28 and KHY4 are isogenic except at the *VMA21* locus. SEY6211, KHY1, and KHY2 are isogenic except at the *VMA21* locus. CYY7 and KHY6 are isogenic except at the *VMA21* locus.

pKH22. Successful insertion was determined by screening plasmid DNAs for the introduction of a *Nde* I site from within the HA encoding sequence and was confirmed by DNA sequencing.

Southern Blotting Analysis

Disruption of the *VMA21* locus was confirmed by Southern blot analysis. Genomic DNA was prepared from strains SF383-1D, KHY2, KHY3, and KHY6 as described by Hoffman and Winston (1987), digested with *Eco*RI and *Xba* I, separated by gel electrophoresis, and blotted onto nitrocellulose membrane. pKH9 was labeled by nick translation using a nonradioactive labeling kit (Bethesda Research Laboratories, Gaithersburg, MA) and used as a probe. The 3-kb wild-type fragment detected in SF838-1D strains was separated into 3.2- and 1.64-kb fragments by the presence of *LEU2* at the *VMA21* locus in disrupted strains.

Protein Preparation, Antibodies, and Western Blotting

Whole cell vacuolar vesicle samples were prepared as described by Kane *et al.* (1992) with the exception of whole cell protein preparations for detecting the 100-kDa subunit, which were prepared as described by Manolson *et al.* (1992).

Monoclonal antibodies recognizing the 100-kDa (7B1, 10D7), 69-kDa (8B1), 60-kDa (13D11), and 42-kDa (7A2) subunits of the V-ATPase were used as described by Kane *et al.* (1992). Affinity purified polyclonal antisera recognizing Vma13p was used as described by Ho *et al.* (1993b). Polyclonal antisera recognizing Vma6p was used as described by Bauerle *et al.* (1993). Affinity purified polyclonal antisera recognizing Vma4p was used as described by Ho *et al.* (1993a). *Escherichia coli* strains containing pKH102 were used to produce Vph1p antigen (aa 1–369) as previously described (Roberts *et al.*, 1989). The antigen was injected into New Zealand white rabbits as described (Vaitukaitis, 1981) and the resulting serum used for immunoprecipitation.

Epitope tagged Vma21p was detected using HA monoclonal antibody 12CA5 (Babco Inc., Berkeley, CA) and was used at a 1:50–1:100 dilution for Western blotting.

Immunoprecipitation and Carbonate Extraction

^{35}S-Express label was purchased from New England Nuclear/Dupont (Wilmington, DE). Immunoprecipitations were performed essentially as described by Roberts *et al.* (1992) with minor modifications. For immunoprecipitation of Vph1p, after radiolabeling, spheroplasted cells were lysed in 0.6% sodium dodecyl sulfate (SDS) and heated to 62°C for 5 min.

Strains KHY5 and KHY3 (carrying plasmid pKH31) were spheroplasted, the spheroplasts treated with 100 mM Na_2CO_3 (pH 11.5), and a portion fractionated into pellet (membrane) and supernatant (soluble) fractions as described by Roberts *et al.* (1989). These total, membrane, and soluble fractions were then subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot analysis. Sodium carbonate fractionation of strains SNY28 and KHY4 was performed as follows. Strains were radiolabeled with ^{35}S-Express label, spheroplasted, and the spheroplasts lysed in 10 mM sodium phosphate (pH 7.0) and 2 mM EDTA. The spheroplast lysate was added to an equal volume of 0.2 M Na_2CO_3 (pH 11.5), vortexed, and incubated on ice for 30 min. A portion was removed, SDS was added to 0.6%, the sample heated before dilution, and Vph1p was immunoprecipitated (total fraction). The remaining spheroplast lysate in 0.1 M Na_2CO_3 was subjected to centrifugation at 100 000 × *g* for 30 min. The supernatant fraction was removed, SDS added to 0.6%, the sample heated, and treated for immunoprecipitation of Vph1p (soluble fraction). The pellet fraction was solubilized in 10 mM tris(hydroxymethyl)aminomethane/2% SDS, heated, and treated for immunoprecipitation of Vph1p (membrane fraction). The Vph1p immunoprecipitates from the total, soluble, and membrane fractions were separated by SDS-PAGE and visualized by autoradiography.

Fluorescence Microscopy

Cells were fixed and stained for immunofluorescence as described by Roberts *et al.* (1991). For HA epitope detection, fixed cells were treated with 1.5% SDS for 3 min and probed with a 1:250 dilution of 12CA5 anti-HA monoclonal antibody. For detection of the 60-kDa subunit of the V-ATPase, monoclonal antibody 13D11 culture supernatant was used at a 1:10 dilution. Affinity purified, adsorbed anti-alkaline phosphatase antiserum was used as described previously (Raymond *et al.*, 1992). Monoclonal anti-Pma1p supernatant was provided by

Table 2. Plasmids used in this study

Plasmid	Description
pB8	4.8-kb yeast genomic DNA insert in YCp50
pKH1	3.5-kb yeast genomic DNA insert in YCp50
pKH2	2.9-kb yeast genomic DNA insert in YCp50
pKH3	2.2-kb yeast genomic DNA insert in pRS315
pKH4	1.6-kb yeast genomic DNA insert in pRS315
pKH5	1.6-kb DNA fragment containing *VMA21* in pBluescript II KS$^+$
pKH6	1.6-kb DNA fragment containing *VMA21* in pBluescript II SK$^+$
pKH9	2.2-kb DNA fragment containing *VMA21* in pBluescript II KS$^+$
pKH10B	Δ*vma21::LEU2* disruption construct in pBluescript II KS$^+$
pKH14	540-bp fragment containing *VMA21* in pRS316
pKH15	pKH14 with a *Bgl* II site introduced by mutagenesis
pKH16	pKH15 cut *Bgl* II, blunted ended, and religated
pKH19	pKH14 with a *Dra* I site introduced by mutagenesis
pKH22	pKH14 with a *Bgl* II site introduced by mutagenesis
pKH28	pKH22 with HA epitope introduced by mutagenesis
pKH30	*VMA21::HA* containing DNA fragment in pRS306
pKH31	pKH28 with KK → QQ introduced by mutagenesis
pKH34	DNA fragment containing *vma21-10::HA* in pRS306
pKH102	*Dra* I-*Pfl* MI DNA fragment containing *VPH1* in pMAL-c2 + STOP

Dr. John Teem and was used at a 1:10 dilution. 4,6-Diamidino-2-phenylindole (DAPI) staining, slide preparation, and photography were carried out as described by Roberts *et al.* (1989). Quinacrine staining of cells was carried out as described by Roberts *et al.* (1991).

Preparation of Vacuolar Vesicles

Vacuolar membranes were purified from SF838-1D (wild-type), KHY3 (Δ*vma21*), KHY5 (*VMA21::HA*), and KHY8 (*vma21-10::HA*) essentially as described previously (Roberts *et al.*, 1991). Alterations included incubating H_2O washed cells in 50 mM glycine/2 mM dithiothreitol (pH 10) for 30 min at 30°C before pelleting and resuspending in 1.2 M sorbitol for spheroplasting. Spheroplasts were also washed three times in 1.2 M sorbitol before lysis. Measurements of V-ATPase activities were carried out as described by Roberts *et al.* (1991).

RESULTS

Cloning of the VMA21 Gene

The *vma21-1* mutant was isolated in a screen for mutants that were pH sensitive and that failed to produce red colonies in an SEY6211 *ade2* genetic background (Ho *et al.*, 1993a). The *VMA21* gene was cloned by complementation of these phenotypes. Strain KHY1 was transformed with a plasmid library (Rose *et al.*, 1987) and three of the resulting 6000 Ura$^+$ transformants formed red colonies and were able to grow on media buffered to pH 7.5. The complementing phenotype was plasmid dependent. Plasmids pB8 (4.8-kb insert), pB9 (9.5-kb insert), and pB18 (13-kb insert) were recovered, and restriction endonuclease analyses indicated that all contained a common 4.8-kb insert (Figure 1A). Plasmid pB8 was chosen for further analysis.

Various subclones of the 4.8-kb yeast DNA insert in pB8 (plasmids pKH1, pKH2, pKH3, pKH4, and pKH14; all centromere-based plasmids) indicated that a 540-bp *Eco*RV-*Sac* I fragment was sufficient for complementation (Figure 1A).

Sequencing of VMA21

The sequence of the 540-bp complementing DNA fragment was determined for both strands (Genbank accession no. UO9329). Two small ORFs were found that could encode proteins of 48 and 77 amino acids (Figure 1B). Several approaches were taken to determine which of the ORFs corresponded to *VMA21*. First, the initiating methionine of the 48 amino acid ORF was altered by mutagenesis. The resulting plasmid (pKH19) carrying this modified allele fully complemented the *vma21-1* mutation. Second, the 77 amino acid ORF was mutagenized such that a frameshift mutation was introduced after codon 4, and a plasmid containing this allele

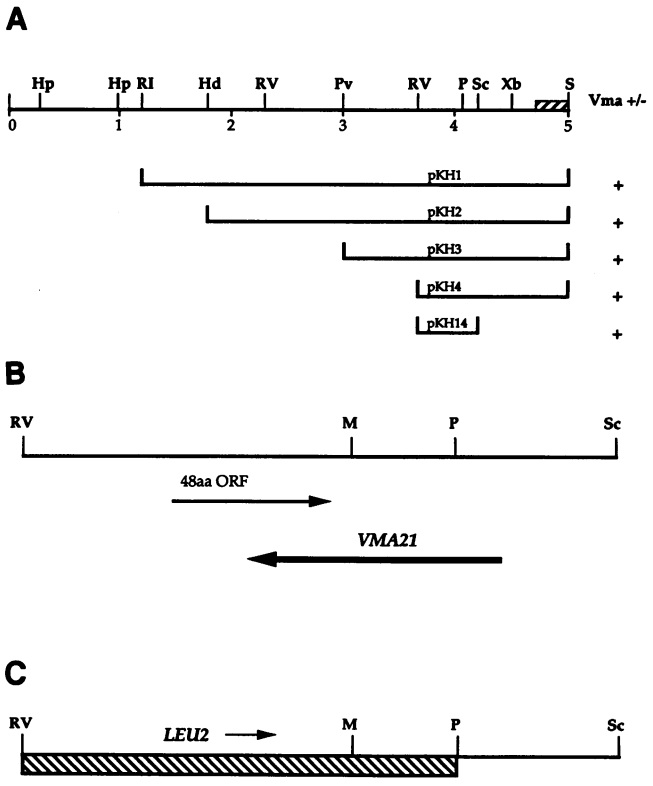

Figure 1. Structure of the genomic fragment containing *VMA21* and the Δ*vma21::LEU2* construct. (A) The ~5-kb of yeast DNA insert containing *VMA21* and various subclones complementing *vma21-1* is shown. A solid line indicates yeast insert DNA, a hatched box indicates YCp50 DNA. (B) Two ORFs found within 540-bp complementing region are shown. Arrows indicate the direction of each ORF. (C) Structure of Δ*vma21::LEU2* disruption construct. A hatched box indicates insertion of the *LEU2* gene. Restriction endonucleases are as follows: Hd, *Hin*dIII; Hp, *Hpa* I; M, *Msc* I; P, *Pst* I; Pv, *Pvu*II; RI, *Eco*RI; RV, *Eco*RV; and Xb, *Xba* I.

(pKH16) no longer complemented *vma21-1*. The 77 amino acid ORF was therefore designated *VMA21*.

The sequence of the *VMA21* ORF (Figure 2A) predicts a protein (Vma21p) of 8.5 kDa, with hydrophobic stretches sufficient to span a membrane twice. Database searches revealed homology between Vma21p and the UncI protein of the *E. coli* ATPase operon (Walker *et al.*, 1984). The two proteins share 33% identity over 52 amino acids (Figure 2B); however, the function of the UncI protein is unknown. When the Vma21p amino acid sequence was randomized (using the GCG Shuffle program) and compared to UncI, identity dropped to 21%, indicating that the observed homology between the proteins was not merely due to a similarity in amino acid composition.

The carboxy-terminal four residues of Vma21p are predicted to be KKED. The carboxy-terminal cytoplasmically exposed KKXX motif has been reported to be an ER retention motif for integral membrane proteins in mammalian cells (Jackson *et al.*, 1990, 1993).

Disruption of VMA21

Strains carrying a null allele of *VMA21* were constructed by transforming SF838-1D, SNY28, SEY6211, and the diploid CYY7 with *Xba* I digested pKH10B (Δ*vma21::LEU2*; Figure 1C) to create KHY3, KHY4, KHY2, and KHY6, respectively. Leu^+ KHY6 transformants were sporulated, dissected, and the haploid progeny examined for Leu^+ and pH sensitivity. In all tetrads examined, spores segregated 2 Leu^+ Vma^- : 2 Leu^- Vma^+. Vma^- Leu^+ spores had phenotypes identical to *vma21-1* cells. The structure of the Δ*vma21* disruption was confirmed by Southern blot analysis (see MATERIALS AND METHODS). A strain carrying an integrated copy of the cloned *VMA21* (*VMA21::URA3*) gene was crossed with KHY1 (*vma21-1*), the diploid sporulated, dissected, and the spore progeny examined. All (20 out of 20) tetrads showed 2 Vma^+ Ura^+ : 2 Vma^- Ura^- segregation, indicating close linkage and that the cloned gene was *VMA21*.

Phenotypes of vma21 Null Mutants

Δ*vma21* null mutant cells had phenotypes that were characteristic of Vma^- strains, including an inability to grow on media buffered to pH 7.5, sensitivity to high concentrations of Ca^{2+} in the medium, an inability to grow on glycerol as a carbon source, and a slow growth phenotype even under optimal growth conditions (YEPD, pH 5.0). As has been found for all other *vma* mutants (Kane and Stevens, 1992), Δ*vma21* cells did not accumulate the weakly basic fluorescent dye quinacrine in their vacuoles, indicating that the vacuoles were not acidified. Vacuolar membranes isolated from Δ*vma21* cells also had no detectable ATPase activity (Table 3).

A

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                                SacI
1                          GAGCTCTCACTATATATT
19  ACTAACCTGCAAGCTAAAATGAACGCAATACTGGAAAGC
58  AATAGTAGAAACTAACCAAGACTTCAAAAGAATCAAATA

97  ATG GCT GTA GAT GTT CCT CGT GCG GTG ATT
 1   M   A   V   D   V   P   R   A   V   I

127 AAT AAA CTT ATG CTG TTT ACT GCA GCG ATG
 11  N   K   L   M   L   F   T   A   A   M

157 GTG GTA CTG CCC GTA CTC ACT TTT TTC ATT
 21  V   V   L   P   V   L   T   F   F   I

187 ATT CAG CAA TTT ACG CCA AAT ACC TTA ATT
 31  I   Q   Q   F   T   P   N   T   L   I

217 AGT GGA GGT TTA GCT GCT GCA ATG GCC AAT
 41  S   G   G   L   A   A   A   M   A   N

247 GTT GTT CTA ATC GTT TAC ATT GTT GTA GCG
 51  V   V   L   I   V   Y   I   V   V   A

277 TTC CGC GAG GAT ACT GAA GAT CAC AAA GTT
 61  F   R   E   D   T   E   D   H   K   V
                                           ⇑
307 GAT GGT AAT AAA AAG GAA GAC TGA TTG AGT
 71  D   G   N   K   K   E   D   *

337 GTATATGTTGCTAGAAGAGAATATACAAGAAAAATAGTA
376 GAGGCTAAATAAAAAAAAAGTAGCATTAAATGTTATGGA
415 TGATTGGGATATATATGCAAATAACCCAATCTTACATAT
454 GTTTCTTTCAAATATATGAGTATATATAACGATTAATTG
493 TTTTTTTTTATTGGGTTTGTTGATTTTTCCAACGCACCA
532 TATGTGATATC
        EcoRV
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B

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                    10        20        30
Vma21p        MAVDVPRAVINKLMLFTAAMVVLPVLTFFII
                |:|:| :|||:|    :|: : | | :
UncI        MKNVMSVSLVSRNVARKLLLVQLLVVIASGLLFSLK
                10        20        30

                40        50        60
Vma21p      QQFTPNTLISGGLAAAMSNVVLIVYIVVAFREDTD
            ::|   : ||||||: ::||:::::
UncI        DPFWGVSAISGGLAVFLPNVLFMIFAWRQHTPAK
              40        50        60        70
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Figure 2. Nucleotide and predicted protein sequence of *VMA21* and homology with UncI. (A) Nucleotide and deduced amino acid sequence (GenBank accession No. UO9329). Amino acid residues are given in the single letter code. An arrow indicates the insertion point for the HA epitope. (B) Protein sequence alignment between Vma21p and UncI. A vertical line indicates identity between residues; double dots indicate conservative residue changes.

Table 3. V-ATPase activities in *VMA21* strains

Strain	Genotype	Vma21p expressed	V-ATPase specific activity*	Wild-type activity (%)
SF838-1D	*VMA21*	Vma21p	1.21	100
KHY3	*Δvma21::LEU2*	—	0.01	0.8
KHY5	*VMA21::HA*	Vma21p-HA	1.30	107
KHY8	*vma21-10::HA*	Vma21p-HA-QQ	0.40	33

* V-ATPase specific activity is expressed as μmol ATP hydrolyzed/min/mg of vacuolar vesicle protein.

To address the basis of the V-ATPase defect in *Δvma21* cells, we examined levels of the V-ATPase subunits in these cells. The results of Western blot analyses are shown in Figure 3. The 100-kDa integral membrane V-ATPase subunit was present at reduced levels in whole cell protein extracts from *Δvma21* cells as compared to wild-type cells, whereas other subunits (69, 60, 54, 42, 36, and 27 kDa) were present at wild-type levels (Figure 3A).

Western analysis of vacuolar membrane fractions isolated from *Δvma21* (KHY3) and wild-type (SF838-1D) cells was performed to analyze whether the V-ATPase was assembled onto the vacuolar membrane in *Δvma21* cells. Results showed that the peripheral (V_1) subunits, although present at wild-type levels in *Δvma21* cells, were unable to assemble onto the vacuolar membrane (Figure 3B). Also, the residual 100-kDa V_0 polypeptide that remained in *Δvma21* cells did not fractionate with vacuolar membranes.

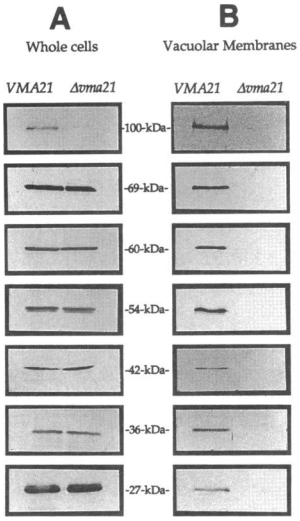

Figure 3. Assembly of the V-ATPase in wild-type and *Δvma21* cells. (A) Whole cell extracts were prepared from wild-type and *Δvma21* cells. Protein extracts (10–20 μg) were loaded onto SDS-PAGE gels, and the resulting blots were probed to detect the 100-, 69-, 60-, 54-, 42-, 36-, and 27-kDa V-ATPase polypeptides. (B) Vacuolar vesicles were prepared from wild-type and *Δvma21* cells. Protein extracts (~4 μg) were loaded onto SDS-PAGE gels and the resulting blots used to detect the 100-, 69-, 60-, 54-, 42-, 36-, and 27-kDa V-ATPase subunits.

We were interested in examining the basis for the decreased steady state levels of the 100-kDa subunit (Vph1p) in *Δvma21* cells. A kinetic analysis of the synthesis and turnover of Vph1p in *Δvma21* and wild-type cells was performed to determine whether the observed defect was posttranslational in nature. Wild-type (SNY28) and *Δvma21* (KHY4) cells were radiolabeled with ^{35}S-methionine and chased for various times before labeled Vph1p was immunoprecipitated and analyzed by SDS-PAGE. In wild-type cells the level of Vph1p remained stable over the chase period of 80 min ($t_{1/2}$ >80 min; Figure 4A, lanes 1–4), whereas Vph1p was rapidly degraded in *Δvma21* cells ($t_{1/2}$ ~25 min; Figure 4A, lanes 5–8). We also examined whether the short half-life for Vph1p in *Δvma21* cells could be increased by mutation of the vacuolar proteinase A gene, *PEP4*, which encodes the critical protease in the activation cascade of vacuolar proteases (Ammerer *et al.*, 1986; Woolford *et al.*, 1986). However, the introduction of the *pep4-3* mutation into *Δvma21* cells (KHY3) had no effect on the rapid degradation of Vph1p (Figure 4B, lanes 5–8) as compared with wild-type cells (SF838-1D) (Figure 4B, lanes 1–4). These results indicate that turnover of the 100-kDa V-ATPase subunit in *Δvma21* cells occurs via an Pep4p-independent mechanism and therefore most likely in a compartment other than the vacuole.

In a preliminary attempt to further elucidate the fate of Vph1p in *Δvma21* cells, we examined whether the Vph1p synthesized in *Δvma21* cells behaved as an in-

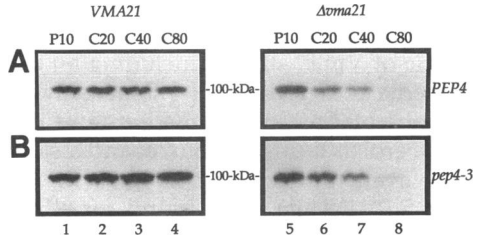

Figure 4. Immunoprecipitation of Vph1p from wild-type and *Δvma21* cells and the effects of *PEP4*. Wild-type (A and B, lanes 1–4) and *Δvma21* cells (A and B, lanes 5–8) were pulse labeled (P) with ^{35}S-Express label for 10 min and chased (C) for various times before labeled Vph1p was immunoprecipitated and analyzed by SDS-PAGE and autoradiography. (A) *PEP4* cells. (B) *pep4-3* mutants.

tegral membrane protein. High pH sodium carbonate extraction of membranes from ^{35}S-labeled KHY3 and SF838-1D cells are shown in Figure 6,C and D. As expected for this highly hydrophobic polypeptide (Manolson *et al.*, 1992), in both wild-type and Δ*vma21* cells Vph1p (100-kDa and the 75-kDa proteolytic breakdown product) (Kane *et al.*, 1992) fractionated entirely with the membrane pellet after carbonate extraction.

Effects of Δvma21 on Other Membrane Proteins

To examine whether other membrane proteins were destabilized or mislocalized in Δ*vma21* cells, we determined the localization of alkaline phosphatase, a vacuolar integral membrane protein (Klionsky and Emr, 1989), and Pma1p, the highly hydrophobic yeast plasma membrane ATPase (Serrano *et al.*, 1986). The indirect immunofluorescence staining patterns for alkaline phosphatase and Pma1p were indistinguishable for wild-type and Δ*vma21* cells (Figure 5A), indicating that the Δ*vma21* mutation does not affect the localization of these membrane proteins. Another vacuolar integral membrane protein, dipeptidyl aminopeptidase B (Roberts *et al.*, 1989), was also present at wild-type levels in whole cell extracts from Δ*vma21* cells and was equally enriched in vacuolar membrane fractions isolated from Δ*vma21* and wild-type cells.

A more quantitative assessment of the targeting and stability of the vacuolar membrane protein alkaline phosphatase in Δ*vma21* was obtained by a pulse-chase immunoprecipitation analysis of the processing of the precursor form of this enzyme by vacuolar proteases (Klionsky and Emr, 1989). Immunoprecipitation of alkaline phosphatase from wild-type (SNY28) and Δ*vma21* (KHY4) cells showed that alkaline phosphatase processing and stability were not altered by the *vma21* mutation (Figure 5B). This result indicates that this vacuolar membrane protein is transported to the vacuole efficiently and is stable in Δ*vma21* vacuoles.

Vma21p Is an ER Membrane Protein

To allow detection of Vma21p, we tagged the protein with the nine amino acid influenza hemaglutinin HA1 epitope (HA) introduced via mutagenesis into *VMA21* (Figure 2A) to create *VMA21::HA*. The centromere-based plasmid pKH28, containing *VMA21::HA*, fully complemented the growth defect and pH sensitivity of Δ*vma21* (KHY3) cells. In addition, immunolocalization of the 60-kDa V-ATPase subunit to the vacuolar membrane in Δ*vma21* cells carrying pKH28 further demonstrated that the V-ATPase complex was fully assembled (Kane *et al.*, 1992). Finally, vacuolar membranes isolated from KHY5 cells had an ATPase specific activity similar to that of wild-type cells (Table 3). These results indicate that by all criteria the HA-tagged Vma21p was fully functional.

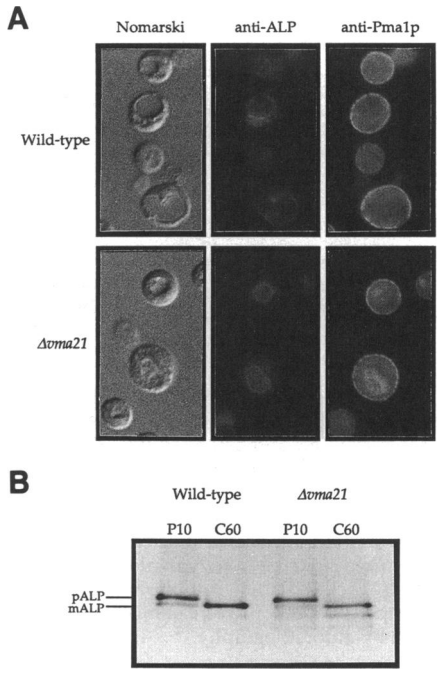

Figure 5. Alkaline phosphatase and Pma1p localization and processing in wild-type and Δ*vma21* cells. (A) Wild-type and KHY3 (Δ*vma21*) cells were fixed, spheroplasted, and stained with anti-alkaline phosphatase (anti-ALP) and anti-plasma membrane ATPase (anti-Pma1p) antibodies. Cells were viewed by epifluorescence microscopy using a filter set specific for fluorescein to observe anti-Pma1p staining or rhodamine to observe anti-ALP staining. (B) Wild-type and KHY4 (Δ*vma21*) cells were pulse labeled (P) for 10 min and chased (C) for 60 min before labeled alkaline phosphatase was immunoprecipitated and analyzed by SDS-PAGE and autoradiography. mALP refers to Pep4p processed mature alkaline phosphatase, whereas pALP refers to the unprocessed precursor protein.

The sequence of Vma21p predicts a very hydrophobic integral membrane protein. This was confirmed by carbonate extraction (pH 11.5) of membranes prepared from Δ*vma21* cells carrying pKH28 (Figure 6A). Vma21p-HA exclusively fractionated with the membrane pellet even after carbonate treatment, which solubilizes all but integral membrane proteins (Fujiki *et al.*, 1982). A soluble protein, phosphoglycerol kinase, and an integral membrane protein, dipeptidyl aminopeptidase B, fractionated as expected in these preparations (Roberts *et al.*, 1989; Bauerle *et al.*, 1993).

In mammalian cells, the carboxy-terminal di-lysine motif (KKXX) has been shown to function in retention of a number of integral membrane proteins in the ER (Jackson *et al.*, 1993). However, it is not known whether yeast ER membrane proteins use the same or similar motifs for retention. To test whether Vma21p is actually localized to the ER membrane, we carried out indirect immunofluorescence of cells expressing the functional epitope-tagged *VMA21::HA* allele. KHY3 cells expressing Vma21p-HA exhibited bright perinuclear (as defined

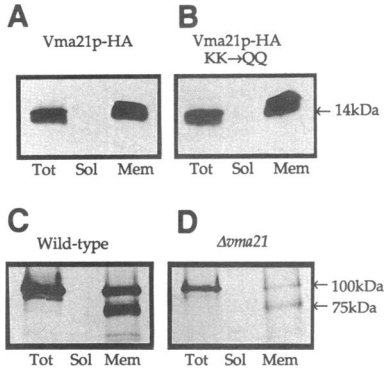

Figure 6. Membrane association of Vma21p and Vph1p. KHY3 cells expressing either Vma21p-HA (A) or Vma21p-HA-QQ (B) were converted to spheroplasts, lysed, and diluted in 100 mM sodium carbonate (pH 11.5). A portion of the total (Tot) was saved and the remainder separated by centrifugation into soluble (Sol) and membrane pellet (Mem) fractions. Fractions were separated by SDS-PAGE and immunoblotted with anti-HA antibodies to detect Vma21p. Radiolabeled Vph1p was immunoprecipitated from high pH carbonate treated wild-type (C) and Δ*vma21* cells (D) total, soluble, and membrane fractions. The immunoprecipitated proteins were subjected to SDS-PAGE and Vph1p visualized by autoradiography.

by DAPI staining) and cell peripheral staining, whereas the strain transformed with a control plasmid, pKH14 (*VMA21*), showed no specific staining (Figure 7). The observed staining pattern of Vma21p-HA is indistinguishable from that observed for the ER integral membrane protein, Sec62p (Deshaies and Schekman, 1990), and to the ER lumenal proteins, Kar2p (Rose *et al.*, 1989) and Eug1p (Tachibana and Stevens, 1992). Vma21p-HA appears therefore to be a membrane protein resident in the ER.

Vma21p Is Retained by the Carboxy-Terminal Di-lysine Motif

To determine whether the carboxy-terminal lysine residues (KKED) were functioning in the retention of Vma21p in the ER, we mutagenized both lysine residues and examined the intracellular location of the mutant Vma21p. pKH28 was mutagenized to change lysines 74 and 75 to glutamine residues (*vma21-10::HA*), and the resulting plasmid (pKH31) was transformed into Δ*vma21* cells (KHY3). The mutant Vma21p-HA made in cells carrying pKH31 (Vma21p-HA-QQ) was present at the same level as that of wild-type Vma21p-HA and remained membrane bound after carbonate extraction (Figure 6B). Indirect immunofluorescence using the HA antibody to localize Vma21p-HA-QQ revealed that this protein was no longer detected on the ER membrane but was now localized to the vacuolar membrane (Figure 8). Vacuoles were easily identified as depressions by Nomarski optics, and vacuolar membranes were identified by immunolocalization of the vacuolar membrane protein alkaline phosphatase. As seen in Figure 8, Vma21p-HA-QQ indirect immunofluorescence staining completely co-localized with that of alkaline phosphatase, thus demonstrating that Vma21p-HA-QQ resides in the vacuolar membrane.

When pKH31 was introduced into Δ*vma21* cells, the mutant Vma21p produced, Vma21p-HA-QQ, only partially complemented the pH sensitivity and growth defects of Δ*vma21* cells. Yeast cells expressing Vma21p-

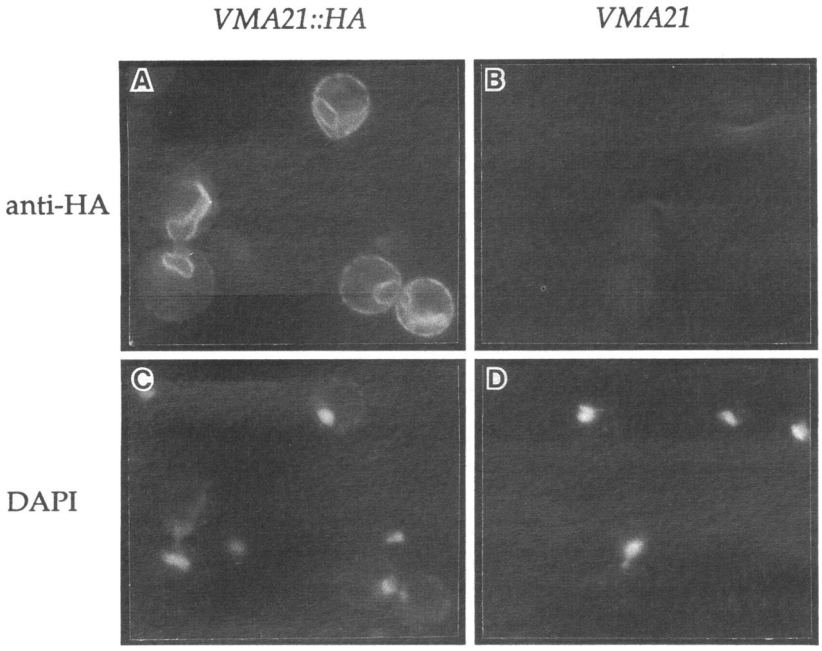

Figure 7. Immunolocalization of Vma21p-HA. KHY3 cells (Δ*vma21*) carrying either pKH28 (A and C) or pKH14 (B and D) were fixed, spheroplasted, and stained with anti-HA antibodies. Cells were viewed by epifluorescence microscopy using a filter set specific for fluorescein to observe anti-HA staining (A and B) or DAPI (C and D) to observe nuclear DNA.

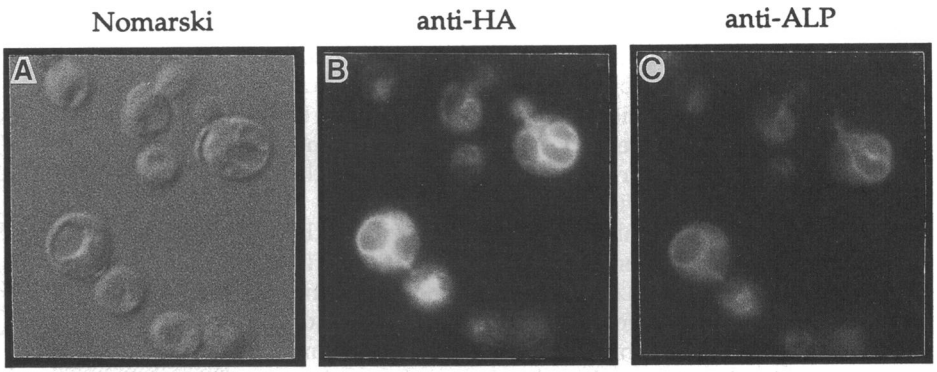

Figure 8. Immunolocalization of Vma21p-HA-QQ. KHY3 cells (*Δvma21*) carrying pKH31 were fixed, spheroplasted, and stained with anti-HA and anti-alkaline phosphatase (anti-ALP) antibodies. Cells were viewed by Nomarski optics (A), and epifluorescence microscopy using a filter set specific for fluorescein to observe anti-HA staining (B) or rhodamine to observe alkaline phosphatase staining (C).

HA-QQ grew more slowly (doubling time of ~420 min) in SD media buffered to pH 7.5 than cells expressing wild-type Vma21p (doubling time of ~180 min). Vacuolar membranes from cells (KHY8) expressing Vma21p-HA-QQ also had reduced levels of V-ATPase specific activity (~30% of wild-type; Table 3). Immunolocalization of the 60-kDa V-ATPase subunit in cells expressing Vma21p-HA-QQ revealed a large cytoplasmic pool of the 60-kDa V_1 sector component, further indicating that the V-ATPase complex was only partially assembled in these cells. Therefore, we conclude that Vma21p must reside in the ER membrane to be functional in V-ATPase assembly and that in cells expressing Vma21p-HA-QQ, the low level of V-ATPase activity (30%) and partial assembly of the V-ATPase complex reflects the low level of Vma21p-HA-QQ transiently residing in the ER membrane.

DISCUSSION

In this article we report the isolation of the *VMA21* gene, whose product Vma21p is essential for yeast to produce a functional V-ATPase complex. Our results also indicate that rather than being a subunit of the V-ATPase, Vma21p is an ER membrane protein required for the assembly of the V-ATPase enzyme complex.

Yeast cells lacking Vma21p display phenotypes characteristic of other *vma* strains, including pH sensitivity, Ca^{2+} ion sensitivity, and a respiratory deficiency. *Δvma21* cells are devoid of detectable V-ATPase activity and, as expected, do not contain acidified vacuoles. The lack of V-ATPase activity observed in *Δvma21* cells reflected the state of assembly of the V-ATPase complex. Subcellular fractionation as well as immunolocalization indicated that although V_1 subunits are synthesized at wild-type levels in *Δvma21* cells, these polypeptides fail to assemble onto the vacuolar membrane and now reside in the cytoplasm.

The 100-kDa V-ATPase subunit, a polytopic membrane protein component of the V_0 sector, was found to be present at greatly reduced levels in *Δvma21* cells. The low level of the 100-kDa polypeptide in *Δvma21* cells was due to its rapid turnover, and this increased rate of turnover was vacuolar-protease independent, suggesting that proteolysis may occur in the ER rather than the vacuole. The fate of the proteolipid components (Vma3p and Vma11p) (Ho *et al.*, 1993b) of the V_0 sector remains uncertain because of the lack of antibodies for these highly hydrophobic polypeptides. However, attempts to epitope tag these proteins are underway, and it will be interesting to assess the effect of the *vma21* mutation on the proteolipid components of the V_0 sector.

Membrane proteins other than those of the V-ATPase, alkaline phosphatase, the plasma membrane ATPase, and dipeptidyl aminopeptidase B were found to be present in normal levels in *Δvma21* cells. Experiments also indicated that a representative protein, alkaline phophatase, was orientated correctly in the vacuolar membrane and underwent normal Pep4p-dependent maturation in *Δvma21* cells. Together these results indicate that the *vma21* defect may be limited to the destabilization of the V_0 polypeptides of the V-ATPase and that the inability to assemble the V_0 sector results in the failure to assemble the V-ATPase enzyme complex.

Although *Δvma21* cells display phenotypes characteristic of cells disrupted for V-ATPase subunit structural genes, Vma21p was found not to be a subunit of the final complex but is in fact an ER membrane protein. The ER residency of Vma21p is conferred by the carboxy-terminal motif, KKED, and thus this motif functions in ER membrane protein retention in yeast as well as in mammalian cells. Whereas at least one other yeast ER membrane protein (Wbp1p) (te Heesen *et al.*, 1991) contains a carboxy-terminal KKXX motif, Vma21p is the first protein demonstrated to require this motif for retention in the yeast ER. Removal of this signal by mutagenesis resulted in the mislocalization of Vma21p to the vacuolar membrane, the default compartment for membrane proteins (Cooper and Bussey, 1992; Roberts *et al.*, 1992; Wilcox *et al.*, 1992). In animal cells the KKXX motif must be cytoplasmically exposed to function in ER retention, and thus it is highly likely that the carboxy-terminal domain of Vma21p is also orientated toward the cytoplasm. Vma21p contains two hydrophobic

stretches in the middle of the protein, each sufficient to span a membrane bilayer, as well as hydrophilic/charged amino- and carboxy-terminal regions. Thus, based on these considerations, we predict that Vma21p assumes a hairpin-like orientation in the ER membrane, with both amino- and carboxy-termini exposed to the cytosol, as illustrated in Figure 9.

How might an ER membrane protein function in the assembly of a vacuolar membrane protein complex? The V-ATPase is a complex enzyme containing both soluble hydrophilic subunits predicted to be synthesized on soluble cytoplasmic ribosomes as well as integral membrane subunits that are predicted to be synthesized on ER-bound ribosomes. There are at least three V-ATPase membrane subunits known in yeast, two 17-kDa polypeptides referred to as "proteolipids" (Vma3p and Vma11p), and the 100-kDa polypeptide (Vph1p). Upon insertion into the ER membrane, these three polypeptides presumably must be assembled into a V_0 membrane sector, and either in the ER or at some later stage of the secretory pathway the V_1 hydrophilic subunits must assemble to form the functional V-ATPase. Thus, we propose that Vma21p functions in the ER to aid in the assembly of the V_0 sector of the V-ATPase.

There are several lines of evidence indicating that the V_0 sector and probably the entire V-ATPase complex assembles in the ER. In this article we report that the 100-kDa component of the V_0 sector is rapidly degraded in V-ATPase assembly-defective mutant cells independent of the major vacuolar proteases, suggesting that this 100-kDa polypeptide may never exit the ER unless assembled into a V_0 complex. In yeast cells defective for assembly of just the V_1 hydrophilic V-ATPase sector (Kane *et al.*, 1992; Ho *et al.*, 1993a,b), the V_0 sector assembles, is transported to the vacuolar membrane, and is stable in that membrane. In addition, yeast cells lacking a 17-kDa component of the V-ATPase (*vma3*) (Kane *et al.*, 1992) fail to assemble a V_0 sector, and the 100-kDa V-ATPase subunit never reaches the vacuolar membrane. Finally, there is growing evidence from kinetic studies in both yeast cells (Doherty and Kane, 1993) and mammalian cells (Forgac, 1992) that the V-ATPase complex is assembled in the ER. Taken together, these data are most consistent with a model in which the V-ATPase V_0 sector subunits are assembled in the ER, and that failure to assemble this subcomplex results in the rapid turnover of its components in the ER.

Evidence that Vma21p may not act alone in the ER to aid in the assembly of the V-ATPase comes from studies of other *vma* mutants, in particular *vma12* (Hirata *et al.*, 1993). Like Vma21p, Vma12p is required for assembly of the V-ATPase yet is not itself a subunit. In fact, Vma12p is a 25-kDa membrane protein that is not localized to the vacuolar membrane, and recent data suggest that Vma12p resides in the ER membrane (Jackson and Stevens, unpublished data). We are currently testing the hypothesis that Vma21p and Vma12p

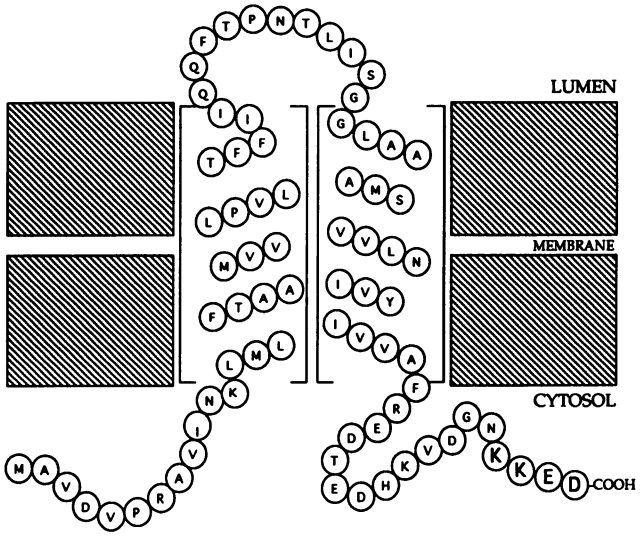

Figure 9. Schematic diagram of the predicted topology for Vma21p. The two hydrophobic stretches in Vma21p are predicted to span the membrane bilayer with both the amino- and carboxy-termini of the protein cytoplasmically exposed.

are part of a V-ATPase assembly complex localized in the ER membrane.

The role for a Vma21p assembly complex could be to interact with the newly translocated V_0 subunits and aid in their association and assembly. Alternatively, Vma21p could aid in the proper insertion of V_0 polypeptides, like the 100-kDa subunit into the ER membrane. At present, the data do not allow us to rule out one of these models; however, the finding that the short-lived 100-kDa V-ATPase subunit in Δ*vma21* cells is still intregrally associated with the membrane suggests that this polypeptide is correctly inserted into the ER membrane. Detailed topology studies of the 100-kDa V-ATPase subunit in wild-type and Δ*vma21* cells will be necessary to eliminate the membrane insertion model for Vma21p function.

A number of integral membrane proteins have been found to require additional proteins in the ER for either insertion, proper folding, or oligomerization. The influenza virus hemaglutinin (HA) protein is an integral membrane protein that requires the ER lumenal chaperone BiP for trimerization (Gething *et al.*, 1986), and this oligomerization appears to be required for exit of HA from the ER (Copeland *et al.*, 1988). Amino acid permeases in yeast have been shown to require the Shr3 protein for exit from the ER (Ljungdahl *et al.*, 1992), and Shr3p has been found to be an ER membrane protein. However, it is not yet clear whether Shr3p functions in insertion of the permeases into the ER membrane or whether some other step required for ER exit is affected. Finally, *Drosophila melanogaster ninA* mutants, which lack a functional ER isoform of cyclophilin, accumulate Rh1 opsin in the ER of photoreceptor cells

(Colley *et al.*, 1991), indicating that the NinaA protein is required for some process early in opsin biosynthesis that is crucial for ER exit.

In summary, Vma21p is an ER membrane protein required for assembly of the yeast V-ATPase. Vma21p function requires that it be retained in the ER membrane by its carboxy-terminal di-lysine motif. A model has been proposed in which Vma21p functions in assembly of the V_0 membrane sector in the ER and that the assembly of this subcomplex is required for the subsequent attachment of the hydrophilic V_1 catalytic subunits of the V-ATPase. As with many other multisubunit enzymes entering the secretory pathway, the V-ATPase may only exit the ER compartment once the assembly process is complete.

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