The vacuolar membrane H⁺-ATPase of the yeast Saccharomyces cerevisiae is a multisubunit enzyme complex composed of an integral membrane Vₒ sector, and a peripherally associated V₁ sector. Deletion of one of several structural genes for vacuolar H⁺-ATPase subunits was previously demonstrated to prevent proper assembly of the remaining V₁ subunits onto the vacuolar membrane (Kane, P. M., Kuehn, M. C., Howald-Stevenson, T. H., et al., 1992). J. Biol. Chem. 267, 447-454). A genetic screen was designed to identify new genes whose products were essential for the synthesis, assembly, and/or function of the yeast vacuolar H⁺-ATPase. Mutants were identified based on phenotypes associated with vacuolar membrane H⁺-ATPase loss of function (uma), including an inability to grow on media buffered at neutral pH. Representatives in five complementation groups were identified, including four novel mutants uma5, uma21, uma22, and uma23, all of which were defective in vacuolar ATPase enzyme activity. We report here the characterization of two genes, VMA4 and VMA5, that encode peripheral subunits of the vacuolar H⁺-ATPase. We determined that VMA5 encodes the 42-kDa subunit of the vacuolar H⁺-ATPase. The VMA4 gene, originally described by Foury (Foury, F., 1990) J. Biol. Chem. 265, 18565-18568), was determined to encode the 27-kDa subunit of the purified yeast vacuolar H⁺-ATPase. Characterization of the uma5 and uma4 mutants revealed that the 42- and 27-kDa subunits are essential for the assembly of the peripheral membrane portion of the H⁺-ATPase onto the vacuolar membrane.

The yeast vacuole is an acidic organelle that is functionally equivalent to the mammalian lysosome and the plant tonoplast (Pedersen and Carafoli, 1987; Raymond et al., 1992). The vacuole plays an important role in amino acid and calcium homeostasis (Klionsky et al., 1990). In addition, the vacuole contains a wide variety of proteases and other degradative enzymes (Jones, 1984).

The vacuolar membrane H⁺-ATPase is a multisubunit en-

* This work was supported by Grant GM38066 from the National Institutes of Health and an American Cancer Society Faculty Research Award (to T. H. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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ATPase complex. However, it is not yet known how many subunits actually compose the final form of this enzyme. In addition, it is likely that many additional gene products play a role in assembly and/or function of this multisubunit enzyme in the yeast vacuole.

We designed a genetic screen to identify mutant strains that exhibited uma phenotypes in order to elucidate the subunit composition of the yeast vacular H\textsuperscript+-ATPase. Using this genetic screen, we hoped to identify mutations in genes that were specifically required for vacuolar membrane H\textsuperscript+-ATPase function and/or assembly of this multisubunit enzyme complex. This paper describes the isolation and characterization of mutant strains identified by the uma mutant screen. We report the cloning of the gene, VMA5, that complements one of these mutations, as well as the subsequent characterization of the VMA5 gene product. In addition, the VMA4 gene product, which was originally described by Fourn\textsuperscript{(1990)}, is more fully characterized in this paper.

**EXPERIMENTAL PROCEDURES**

*Materials—* Enzymes for recombinant DNA methods were purchased from New England Biolabs, Bethesda Research Laboratories, Promega Biotec, or Boehringer Mannheim. All other chemicals were described by Kane et al. (1992).

**Strains and Culture Conditions**—The genotypes of the yeast strains used are as follows: SEY6211a, MATa leu2-3,112 urd-52 his3-A200 ade6 lacZ YPH500, MATa, urd-52 lys2-801 ade2-101 trpl-A63 his3-A200 ade6galZ YPH500, MATa, urd-3-2 lys2-801 ade6-101 trop-1/d3 his3-A200 leu2-3112 (Sikorski and Hieter, 1989). SF838-1Da was used to generate a number of isogenic strains disrupted in various vacuolar genes.

**VMA5 Cloning, Subcloning, and Gene Disruption**—The VMA5 gene was originally isolated on a yeast genomic DNA-containing YEP24 plasmid pSEL1 (Lillie and Brown, 1992). A partial sequence of this plasmid insert showed an open reading frame that had similarity to the cDNA encoding the 39-kDa subunit of the mitochondrial ATPase complex. Using this open reading frame, we cloned the 5'-end of the cDNA encoding the 39-kDa subunit from the plasmid insert showed an open reading frame that had similarity to the cDNA encoding the 39-kDa subunit of the mitochondrial ATPase complex. We then transformed wild-type YPH500 yeast cells expressing the 39-kDa subunit with pSEL1. Double stranded DNA sequencing using Taq DNA polymerase was done according to the manufacturer's recommendations (Promega). The VMA5 gene was sequenced on both strands over the entire MluI to SpeI fragment of pSEL1. Double stranded DNA sequencing using Taq DNA polymerase was done according to the manufacturer's recommendations (Promega). The VMA5 gene was sequenced on both strands over the entire MluI to SpeI fragment of pSEL1.

**Sequence Determinations**—The chain termination method of Sanger et al. (1977) was used to determine the DNA sequence of the VMA5 gene. The VMA5 gene was sequenced on both strands over the entire MluI to SpeI fragment of pSEL1. Double stranded DNA sequencing using Taq DNA polymerase was done according to the manufacturer's recommendations (Promega). The VMA5 gene was sequenced on both strands over the entire MluI to SpeI fragment of pSEL1.

**Sequence Determinations**—The chain termination method of Sanger et al. (1977) was used to determine the DNA sequence of the VMA5 gene. The VMA5 gene was sequenced on both strands over the entire MluI to SpeI fragment of pSEL1. Double stranded DNA sequencing using Taq DNA polymerase was done according to the manufacturer's recommendations (Promega). The VMA5 gene was sequenced on both strands over the entire MluI to SpeI fragment of pSEL1.
The uma genetic screen was initiated by mutagenizing SEY6211a haploid cells with UV light. Mutagenized cells were plated on YEPD media buffered to pH 5.0. Colonies were grown at 30 °C and the red pigment allowed to accumulate. Colonies that were white were picked and tested for growth on YEPD media buffered to pH 7.5. Colonies that were unable to grow on pH 7.5 buffered YEPD plates, but grew relatively well on media buffered to pH 5.0, were tested for quinacrine accumulation in the vacuole. Mutants that did not accumulate quinacrine, and in addition had all of the above mutant phenotypes, were designated uma mutants.

Of the 146,000 colonies originally screened, 18 exhibited the Vma− phenotypes. Each of the mutants was crossed to strains containing tfp1, uma2, uma3, uma4, uma11, uma12 and uma13 disruption alleles. Thirteen of these original mutants failed to complement a strain containing the tfp1 disruption allele. TFP1 encodes the 69-kDa vacuolar H+-ATPase subunit (Hirata et al., 1990; Kane et al., 1990). The other five mutants complemented all of the uma disruption strains. These mutants were backcrossed to SEY6211a and the resulting heterozygotes sporulated and dissected. The Vma− phenotype segregated 2:2 in all tetrads examined (data not shown). Vma− backcrosses of each of the five mutants were crossed against each other, and analysis revealed that the five mutants defined four new complementation groups (Table I).

Characterization of the uma Mutants—Whole cell protein extracts were prepared from representative strains of each of the newly isolated uma complementation groups. These extracts were analyzed using monoclonal antibodies against the 100-, 69-, and 60-kDa subunits of the vacuolar H+-ATPase in order to determine whether the synthesis or stability of these polypeptides was perturbed in these mutant strains (Kane et al., 1989; 1992). In addition, we analyzed the whole cell protein extracts with monoclonal antibodies directed against the 42-kDa polypeptide, which was predicted to be a subunit of the vacuolar H+-ATPase based on biochemical data (Kane et al., 1992). The results of this analysis are shown in Fig. 1. Whole cell extracts prepared from one of these mutants, uma5, appeared to be devoid of the 42-kDa putative subunit as determined by this analysis (Fig. 1, lane 2). The synthesis of the remaining subunits that were monitored in these mutant cells appeared not to be altered as compared to wild type strains. uma21, uma22, and uma23 cell extracts contained wild type levels of all subunits monitored (Fig. 1, lanes 3–5). Interestingly, mutants in two complementation groups, uma21 and uma22, showed decreased levels of the 100-kDa polypeptide in protein extracts from whole cells. The full characterization of the uma21 and uma22 mutants will be reported elsewhere. Together, these results suggested that the synthesis of a putative subunit was affected in uma5, uma21, and uma22 mutants. The 42-kDa polypeptide detected in the whole cell extracts of ula23 appeared to be devoid of the 42-kDa putative subunit.

We have identified four novel uma complementation groups (uma5, uma21, uma22, and uma23) using a mutant screen to identify gene products essential for the activity of the vacuolar H+-ATPase in yeast. Members of each group had substantially decreased levels of vacuolar H+-ATPase activity as compared to wild type strains. In addition, three of the four uma complementation groups were altered in the levels of known or putative vacuolar H+-ATPase subunits. These newly identified uma mutants appear to have mutations in genes whose products are directly or indirectly responsible for the normal activity of the vacuolar membrane H+-ATPase in yeast.

Cloning of the VMA5 Gene—We were interested in determining whether the genes identified by the uma genetic screen actually encode subunits of the vacuolar H+-ATPase. It was also possible that these gene products indirectly lead to loss of vacuolar H+-ATPase activity. In order to begin to resolve these issues, the gene that complemented the uma5 mutant phenotypes was cloned and the encoded protein characterized. SEY6211a uma5 was transformed with a plasmid (pSEL1) containing a portion of the yeast genome in the YEp24 multicopy vector. This insert has been partially sequenced by Lillie and Brown (1992) (identified as a gene adjacent to a multicopy suppressor of a myo2 mutation) and exhibits amino acid similarity to the cDNA encoding the 41-kDa subunit C of the bovine chromaffin granule vacuolar H+-ATPase (Lillie and Brown, 1992; Nelson et al., 1990).
**Characterization of vma Mutants**

**vma5** mutant colonies transformed with pSEL1 were able to grow on YEPD media buffered at pH 7.5, suggesting that the vma5 mutant phenotype was fully complemented. In addition, these transformed colonies were red, indicating that they were accumulating the adenine biosynthetic intermediate in their vacuoles (Foury, 1990). We identified the minimum region required for complementation (Fig. 2) by showing that this region complemented the vma5 mutant phenotypes when carried on a centromere-containing (CEN) single copy plasmid (pMH14).

**The VMA5 Gene Encodes the 42-kDa Subunit of the Vacuolar H^+-ATPase**—The DNA sequence was determined for both strands of the complementing region, and an open reading frame of 373 amino acids was identified (Fig. 3). We designated this gene VMA5. The predicted molecular mass of the putative VMA5 polypeptide is 42,283 daltons. The sequence predicts a hydrophilic polypeptide that lacks both a signal sequence and membrane-spanning domain. Data bank analysis showed that the VMA5 gene has 37.5% amino acid identity with subunit C of the bovine chromaffin granule vacuolar H^+-ATPase (Nelson et al., 1990). The sequence encoded by the VMA5 gene was found to be identical to the recently reported sequence of subunit C of yeast vacuolar H^+-ATPase (Beltran et al., 1992). In addition, the predicted Vma5p sequence contained internal peptides that were derived from the 42-kDa polypeptide that copurified with the vacuolar H^+-ATPase enzyme (Fig. 3). Therefore, we concluded that the VMA5 gene encodes the 42-kDa subunit of the yeast vacuolar H^+-ATPase.

**Disruption of the VMA5 Gene**—To determine whether the phenotype of the vma5 mutant strain coincided with loss of the VMA5 gene function, the chromosomal VMA5 allele was replaced by the vma5A::LEU2 disruption allele (Fig. 2). The vma5A::LEU2 gene was transformed into SEY6211a, SF838-1Da, and SF838-5α strains and Leu^+ prototrophs selected. Disruption of the VMA5 gene in SF838-1Da was confirmed by Southern blot analysis (data not shown).

The vma5Δ::LEU2 disruption strains had phenotypes that were indistinguishable from the originally isolated vma5 mutant. Particularly, vacuolar vesicles derived from the vma5Δ::LEU2 mutant strains contained <1% of the wild type vacuolar H^+-ATPase activity (data not shown). Furthermore, Western analysis of whole cell protein extracts revealed that the 42-kDa subunit was absent from the strains containing the vma5 disruption allele (Fig. 4). The mutant phenotypes resulting from the vma5 gene disruption were fully complemented by the VMA5 gene carried on a CEN plasmid, pMH14. In addition, SF838-1Da vma5Δ::LEU2 cells transformed with plasmid pMH14 contained wild type levels of the 42-kDa polypeptide as determined by Western analysis of whole cell protein extracts (Fig. 4). The vma5Δ::LEU2 strain was crossed with the vma5 mutant strain isolated in our vma mutant screen, the diploid sporulated, and tetrads dissected. The Vma- phenotype segregated 4:0 in all 13 tetrads examined, indicating close linkage between the alleles and suggesting that the cloned gene was indeed VMA5. Tetrad progeny exhibited 2:2 segregation for all nutritional markers (data not shown). Together, these results indicate that a loss of the 42-kDa vacuolar H^+-ATPase subunit is responsible for the mutant phenotypes exhibited by the vma5 strain that was identified by the vma mutant screen.

**The VMA4 Gene Encodes the 27-kDa Vacular H^+-ATPase Subunit**—The VMA4 gene was recently cloned and predicted to encode a subunit of the yeast vacuolar H^+-ATPase (Foury, 1990). The sequence predicted that Vma4p is a hydrophilic polypeptide of 26.6 kDa. The internal peptide sequence LLSEEALPAIR, which was derived from the 27-kDa polypeptide that copurified with the vacuolar H^+-ATPase (Kane et al., 1989), is identical to a region (aa209-aa219) of Vma4p. A polyclonal antibody was generated against VMA4 protein expressed in E. coli. Immunoblots of whole cell protein extracts from wild type and vma4Δ::URA3 strains were probed using this Vma4p antibody. These immunoblots verified that Vma4p migrates at an apparent molecular mass of 27 kDa and that this protein was absent from the extracts derived from vma4Δ::URA3 strains, verifying that the antibody was specific for the VMA4 gene product (Fig. 5). We therefore concluded that the VMA4 gene encodes the 27-kDa subunit of the vacuolar H^+-ATPase.

**Fig. 2.** Restriction maps of VMA5 complementing region and uma5 disruption allele. A, the open box represents the reading frame of the VMA5 gene, and the arrow beneath the box indicates the direction of translation. Restriction enzymes: E, EcoRI; H, HindIII; M, MluI; P, SphI; R, EcoRV; S, SalI. B, depiction of the uma5Δ::LEU2 allele construction as described under "Experimental Procedures." Open boxes represent the uninterrupted VMA5 gene, and the shaded box shows the inserted LEU2 gene with the direction of translation indicated by the arrow.

**Fig. 3.** Amino acid sequence of the VMA5 gene product. Predicted amino acid sequence of the VMA5 gene as determined by DNA sequence (plain type) as it aligns with internal peptide sequences derived from the 42-kDa subunit of the vacuolar H^+-ATPase (bold type). Sequences were determined as described under "Experimental Procedures."

**Fig. 4.** The VMA5 gene on a CEN plasmid complements the uma5Δ::LEU2 mutation. Whole cell protein extracts were prepared from SF838-1Da wild type, uma5Δ::LEU2, or uma5Δ::LEU2 transformed with the VMA5 gene on a CEN containing plasmid (pMH14). Protein extract equivalent to 2 x 10^7 cells was loaded for each sample, and the polypeptides separated by SDS-PAGE on a 10% acrylamide gel. Immunoblots of the extracts were probed with monoclonal antibody specific for the 42-kDa vacuolar H^+-ATPase subunit. Vma5p
of the yeast vacuolar H\(^+\)-ATPase.

**Synthesis and Assembly of the Vacuolar H\(^+\)-ATPase in uma5\(\Delta\) and uma4\(\Delta\) Mutants**—Yeast strains carrying a null allele of either the VMA5 or VMA4 gene were devoid of vacuolar H\(^+\)-ATPase activity (see above; Foury (1990)). We were interested in determining the levels of the remaining vacuolar H\(^+\)-ATPase subunits in the VMA5 and VMA4 disruption strains. In addition, we were interested in determining the degree of assembly of the vacuolar H\(^+\)-ATPase in these mutant strains.

In order to determine the level of synthesis and stability of vacuolar H\(^+\)-ATPase subunits in uma5\(\Delta\) and uma4\(\Delta\) strains, whole cell protein extracts were prepared from SF838-1\(\Delta\) wild type cells and from SF838-1\(\Delta\) strains disrupted in the 42-kDa subunit gene (uma5\(\Delta\)), the 27-kDa subunit gene (uma4\(\Delta\)), or the 69-kDa subunit gene (tfpl\(\Delta\)). Immunoblots of these whole cell extracts were probed using antibodies that recognize the 100-, 60-, 42-, and 27-kDa vacuolar H\(^+\)-ATPase subunits. As expected, these results showed that the 69-kDa subunit was absent from the tfpl\(\Delta\) strain (Hirata et al., 1992) (Fig. 5). In addition, the 42-kDa subunit was absent from uma5\(\Delta\) cells and the 27-kDa subunit was absent from uma4\(\Delta\) cells. However, disruption of any of these subunit genes appeared to have no effect on the synthesis or stability of the remaining vacuolar H\(^+\)-ATPase subunits in whole cell protein extracts (Fig. 5).

Vacuolar vesicles were prepared from tfpl\(\Delta\), uma5\(\Delta\), and uma4\(\Delta\) mutant strains to determine whether or not the H\(^+\)-ATPase subunits synthesized were assembled onto the vacuolar membrane. The resulting immunoblots are shown in Fig. 6. Vacuolar membranes prepared from wild type cells contained all of the vacuolar H\(^+\)-ATPase subunits, characteristic of the fully assembled enzyme. However, vacuolar membranes prepared from the mutants were largely deficient in the 69-, 60-, 42-, or 27-kDa polypeptides, whereas a significant portion of the 100-kDa subunit (and/or the 75-kDa breakdown product) (Kane et al., 1992) was still present on the vacuolar membrane in all three mutant strains. The results obtained for the tfpl\(\Delta\) mutants are consistent with those previously reported (Kane et al., 1992). Together, these data show that the enzyme is not assembled on the vacuolar membrane in the tfpl\(\Delta\), uma5\(\Delta\), or uma4\(\Delta\) strains.

Indirect immunofluorescence microscopy was used in a second procedure to analyze the extent of H\(^+\)-ATPase assembly onto the vacuolar membrane in tfpl\(\Delta\), uma5\(\Delta\), and uma4\(\Delta\) mutant cells. SF838-1\(\Delta\) wild type (WT), tfpl\(\Delta\)::LEU2 (tfpl\(\Delta\)), uma5\(\Delta\)::LEU2 (uma5\(\Delta\)), and uma4\(\Delta\)::URA3 (uma4\(\Delta\)) cells were fixed and then stained with monoclonal antibodies that recognize either the 60- (a) or the 100-kDa (b) subunit of the vacuolar H\(^+\)-ATPase. Identical fields are shown that were viewed under Nomarski optics (A) or fluorescein fluorescence optics (B).
with the vacuolar membrane in \textit{uma}5\textDelta strains. Together, these results demonstrated that the peripheral membrane 60-kDa \textit{H}\textsuperscript{+}-ATPase subunit was largely dissociated from the vacuolar membrane in \textit{tpf1}\textDelta, \textit{uma}5\textDelta, and \textit{uma}4\textDelta mutant cells.

The localization of the integral membrane subunits of the vacuolar \textit{H}\textsuperscript{+}-ATPase, represented by the 100-kDa subunit, was also analyzed using indirect immunofluorescence microscopy (Fig. 7b). Monoclonal antibodies against the 100-kDa subunit recognize an epitope that is masked in cells containing the wild type enzyme (Kane et al., 1992). However, in the \textit{tpf1}\textDelta, \textit{uma}5\textDelta, and \textit{uma}4\textDelta mutant cells, the 100-kDa epitope was unmasked. In these mutant cells, the 100-kDa subunit was clearly visible on the vacuolar membrane. This result demonstrated that the 100-kDa subunit remained associated with the vacuolar membrane and was stable in \textit{tpf1}\textDelta, \textit{uma}5\textDelta, and \textit{uma}4\textDelta mutant cells. The results obtained for the \textit{tpf1}\textDelta mutants are consistent with those previously reported (Kane et al., 1992). These data clearly show that the 100-kDa integral membrane \textit{H}\textsuperscript{+}-ATPase subunit is stable in the vacuolar membrane in yeast cells lacking either the 27- or 42-kDa peripheral membrane vacuolar \textit{H}\textsuperscript{+}-ATPase subunits.

**DISCUSSION**

Deletion of any one of the yeast vacuolar \textit{H}\textsuperscript{+}-ATPase structural genes disrupts both the function and composition of the enzyme complex (Yamashiro et al., 1990). Yeast cells that are devoid of vacuolar membrane \textit{H}\textsuperscript{+}-ATPase (\textit{uma}) activity possess distinct phenotypes including the inability to grow on media buffered at neutral pH. A genetic screen was designed based on several of these \textit{uma} phenotypes in order to fully elucidate the subunit composition of the vacuolar \textit{H}\textsuperscript{+}-ATPase, and to identify nonsubunit proteins required for vacuolar \textit{H}\textsuperscript{+}-ATPase function. This screen resulted in the identification of four novel \textit{uma} mutants, \textit{uma}5, \textit{uma}21, \textit{uma}22, and \textit{uma}23, whose wild type gene products are essential for normal activity of the vacuolar \textit{H}\textsuperscript{+}-ATPase. It is clear that we have not saturated the screening process as we did not isolate mutations in the \textit{VMA2}, \textit{VMA3}, or \textit{VMA4} genes, although we did isolate mutations in \textit{TFP1} 13 times. This result may reflect differential sensitivity of \textit{uma} genes to ultraviolet radiation. Nevertheless, subsequent screenings for \textit{uma} mutants will be necessary to fully elucidate the subunit composition of this multisubunit enzyme complex.

Our results show that the protein encoded by \textit{VMA5} is the 42-kDa subunit of the vacuolar \textit{H}\textsuperscript{+}-ATPase originally described by Kane et al. (1989). The \textit{VMA5} gene was deleted (\textit{uma}5\textDelta) from the yeast genome and the phenotypes were analyzed. The phenotypes of the \textit{uma}5\textDelta strains were indistinguishable from those exhibited by the originally isolated \textit{uma}5 mutant. The 373 amino acid \textit{VMA5} gene product is predicted to be a hydrophilic polypeptide and does not contain a signal sequence or a membrane spanning domain. While this work was in progress, Beltran et al. (1992) reported the sequence of the "subunit C" of the yeast vacuolar \textit{H}\textsuperscript{+}-ATPase. The \textit{VMA5} gene is identical to the subunit C gene.

The \textit{VMA4} gene, which was originally cloned by Foury (1990), was determined to encode the 27-kDa subunit of the yeast vacuolar \textit{H}\textsuperscript{+}-ATPase that was originally described by Kane et al. (1989). Yeast strains containing disruption alleles of the \textit{VMA4} gene displayed Vma\textsuperscript{-} mutant phenotypes. As expected, \textit{uma}4\textDelta strains completely lacked the 27-kDa vacuolar \textit{H}\textsuperscript{+}-ATPase subunit.

We were interested in determining whether the biosynthesis, localization, and assembly of the remaining vacuolar \textit{H}\textsuperscript{+}-ATPase subunits was altered in cells lacking either the 42- (\textit{uma}5\textDelta) or 27-kDa (\textit{uma}4\textDelta) polypeptides. \textit{uma}5\textDelta and \textit{uma}4\textDelta cells contained wild type levels of all the remaining vacuolar \textit{H}\textsuperscript{+}-ATPase subunits in the whole cell protein extracts. However, vacuolar vesicles prepared from either mutant strain were largely devoid (\textless;10\% of the total) of any of the peripherally associated subunits (collectively referred to as the VI sector of the enzyme) of the \textit{H}\textsuperscript{+}-ATPase complex. The VI portion of the enzyme is predicted to be composed of hydrophilic subunits that associate peripherally with the cytoplasmic face of the vacuolar membrane, and is responsible for the catalytic activity of the enzyme. VI was proposed to consist of at least three subunits the 69-, 60-, and 42-kDa polypeptides (Kane et al., 1992). We have demonstrated that none of the components of the VI portion of the enzyme is properly assembled onto the vacuolar membrane in \textit{uma}5\textDelta and \textit{uma}4\textDelta strains. In addition, vacuolar vesicles from \textit{uma}4\textDelta and \textit{tpf1}\textDelta cells were largely devoid of the 27-kDa polypeptide. We therefore conclude that the 27-kDa subunit is also a component of the VI sector of the yeast vacuolar \textit{H}\textsuperscript{+}-ATPase.

The 100- and 17- kDa subunits are components of the integral membrane VI\textsubscript{O} portion of the vacuolar enzyme. The integral membrane vacuolar \textit{H}\textsuperscript{+}-ATPase subunits that constitute the VI\textsubscript{O} sector were stably assembled into the vacuolar membrane in \textit{uma}5\textDelta and \textit{uma}4\textDelta mutant strains. Therefore, it appeared that although the VI subunits of the vacuolar \textit{H}\textsuperscript{+}-ATPase were not properly assembled onto the vacuolar membrane in either the \textit{uma}5\textDelta or the \textit{uma}4\textDelta mutant strains, the VI\textsubscript{O} subunits of the enzyme (represented by the 100-kDa subunit) were properly targeted to and stable in the vacuolar membrane.

This paper describes a genetic screen that was designed to identify novel genes whose products are essential for the function of the vacuolar \textit{H}\textsuperscript{+}-ATPase in yeast. To date, 12 yeast genes have been described that are essential for the synthesis, assembly, and/or function of the vacuolar \textit{H}\textsuperscript{+}-ATPase. We identified four new yeast genes, \textit{uma}5, \textit{uma}21, \textit{uma}22, and \textit{uma}23, that are essential for vacuolar \textit{H}\textsuperscript{+}-ATPase activity. We subsequently characterized \textit{VMA5} and showed that the gene product is essential for the activity of the vacuolar \textit{H}\textsuperscript{+}-ATPase, and for the assembly of this multisubunit enzyme. Biochemical and immunolocalization data support the assignment of \textit{VMA4} as a component of the VI sector of the yeast vacuolar \textit{H}\textsuperscript{+}-ATPase.

The characterization of these \textit{VMA} genes enhances our understanding of the yeast vacuolar \textit{H}\textsuperscript{+}-ATPase enzyme complex. However, it is not clear how many genes are required for the function of the vacuolar \textit{H}\textsuperscript{+}-ATPase in yeast. To date, 12 yeast genes have been described that are essential for vacuolar \textit{H}\textsuperscript{+}-ATPase function, \textit{VPH1} (Manolson et al., 1992), \textit{VMA1} (\textit{TFP1}) (Shih et al., 1988; Hirata et al., 1990; Kane et al., 1990), \textit{VMA2} (\textit{VAT2}) (Nelson and Nelson, 1990; Yamashiro et al., 1990), \textit{VMA3} (Umemoto et al., 1990), \textit{VMA4} (Foury, 1990), \textit{VMA5} (Beltran et al., 1992; this work), \textit{VMA11} (Shih et al., 1990; Umemoto et al., 1991), \textit{VMA12} and \textit{VMA13} (Obya et al., 1991), \textit{VMA21}, \textit{VMA22}, and \textit{VMA23} (this work). The identification of additional \textit{uma} complementation groups will be necessary to fully elucidate the subunit composition of the vacuolar \textit{H}\textsuperscript{+}-ATPase and to determine the assembly process of this multisubunit enzyme on route to the vacuolar membrane.

Acknowledgments—We thank Sue Little and Susam Brown for sending us the pSELP plasmid containing the \textit{VMA4} gene. We thank Margaret Haughton for assistance in the isolation of \textit{uma} mutants and Cindy Bauerle for comments on the manuscript. We thank Yasushiro Anraku and his colleagues for \textit{uma}11, \textit{uma}12, and \textit{uma}13 disruption
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strains and for stimulating discussions throughout the course of this work. Also, we thank Patty Kane for many thoughtful conversations.

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