New Component of the Vacuolar Class C-Vps Complex Couples Nucleotide Exchange on the Ypt7 GTPase to SNARE-dependent Docking and Fusion

Andrew E. Wurmser, Trey K. Sato, and Scott D. Emr
Division of Cellular and Molecular Medicine and Howard Hughes Medical Institute, University of California at San Diego, School of Medicine, La Jolla, California 92093

Abstract. The class C subset of vacuolar protein sorting (Vps) proteins (Vps11, Vps18, Vps16 and Vps33) assembles into a vacuole/prevacuole-associated complex. Here we demonstrate that the class C-Vps complex contains two additional proteins, Vps39 and Vps41. The COOH-terminal 148 amino acids of Vps39 direct its association with the class C-Vps complex by binding to Vps11. A previous study has shown that a large protein complex containing Vps39 and Vps41 functions as a downstream effector of the active, GTP-bound form of Ypt7, a rab GTPase required for the fusion of vesicular intermediates with the vacuole (Price, A., D. Seals, W. Wickner, and C. Ungermann. 2000. *J. Cell Biol.* 148:1231–1238). Here we present data that indicate that this complex also functions to stimulate nucleotide exchange on Ypt7. We show that Vps39 directly binds the GDP-bound and nucleotide-free forms of Ypt7 and that purified Vps39 stimulates nucleotide exchange on Ypt7. We propose that the class C-Vps complex both promotes Vps39-dependent nucleotide exchange on Ypt7 and, based on the work of Price et al., acts as a Ypt7 effector that tethers transport vesicles to the vacuole. Thus, the class C-Vps complex directs multiple reactions during the docking and fusion of vesicles with the vacuole, each of which contributes to the overall specificity and efficiency of this transport process.

Key words: Vps39/Vam6 • endosome • Vps41/Vam2 • rab • Vps11

Introduction

The vacuole, like the mammalian lysosome, is the primary site of hydrolase-mediated turnover of macromolecules in *Saccharomyces cerevisiae* (Klionsky et al., 1990). Many newly synthesized vacuolar proteins (e.g., hydrolases) require transport from the endoplasmic reticulum to the vacuole by the secretory pathway. At the trans-Golgi network, vacuolar proteins are diverted to endosomal compartments which ultimately fuse with the vacuole (Vida et al., 1993), a process regulated by >40 proteins designated vacuolar protein sorting (Vps) proteins. *vps* mutants have been divided into six major subgroups (classes A–F) based on distinct hydrolase missorting, vacuole morphology, and growth phenotypes (Raymond et al., 1992). Deletion of *VPS39*, a class B *VPS* gene, results in the cytoplasmic accumulation of endosomal compartments and vesicles which fail to fuse with the vacuole, fragmentation of the vacuole, and severe hydrolase missorting phenotypes (Raymond et al., 1992; Wada et al., 1992). Thus, although the precise function of Vps39 is unknown, it is likely that this protein is required for the fusion of endosomes and other types of transport intermediates with the vacuole.

Several other yeast mutants, in addition to *vps39*, exhibit class B *vps* phenotypes and many of these mutants have been found to impair endosome fusion with the vacuole. The relevant genes affected in these mutant strains have been identified through the *vps* and vacuole morphology (*vam*) genetic selections and found, in many cases, to encode homologues of proteins with well-established roles in vesicle-mediated trafficking. These proteins include Vam3, a vacuolar t-SNARE (target membrane soluble N-ethylmaleimide-sensitive fusion protein [NSF] attachment protein [SNAP] receptor), Vam7, a SNAP25 (synapse-associated protein of 25 kD)-like protein, and Ypt7/Vam4, an endosomal/vacuolar rab GTPase (Wada and Anraku, 1992; Wada et al., 1992, 1997; Wichmann et al., 1992). Vps39/Vam6 and Vps41/Vam2 are also in the class B group, but at present...
the specific function of these proteins is unknown (Nakamura et al., 1997). Given the apparent functional overlap of this group of proteins, it is possible that some of these proteins physically interact. In fact, data indicate that Vps39 complexes with Vps41 (Nakamura et al., 1997; Price et al., 2000). Furthermore, a large protein complex containing Vps39 and Vps41 has been demonstrated to associate with the Ypt7 rab GTPase in its active, GTP-bound form, suggesting that this protein complex functions as a downstream effector of Ypt7 (Price et al., 2000).

Like Vps39, class C Vps proteins are also required for the fusion of hydrolase-containing endosomes with the vacuole (Banta et al., 1990; Robinson et al., 1991; Rieder and Emr, 1997). Class C mutants accumulate fusion-incompetent endosomes and vesicles in the cytoplasm of the cell, lack vacuoles, and missort vacuolar hydrolases (Rieder and Emr, 1997). Class C Vps proteins, Vps18, Vps11, Vps16 and Vps33, are conserved in mammalian systems, Drosophila, and Caenorhabditis elegans and have been found to assemble into a membrane-associated protein complex (the C-Vps complex) which localizes to the vacuole and prevacuolar compartments (Rieder and Emr, 1997; Sevrioukov et al., 1999). Vps18 and Vps11 each contain RING domains and clathrin heavy chain repeats, protein motifs that are believed to mediate protein–protein interactions (Robinson et al., 1991; Rieder and Emr, 1997; Ybe et al., 1999). Vps33 encodes a Sec1 homologue, suggesting a potential role for the C-Vps complex in regulating the assembly of SNARE complexes (Banta et al., 1990). Consistent with this notion, mutants of the vacuolar t-SNARE, VAM3, both genetically and physically interact with components of the C-Vps complex (Darsow et al., 1997; Sato et al., 2000). The C-Vps complex has also been shown to physically associate with Vam3, promoting SNARE interactions between Vam3 and Vam7 (Sato et al., 2000). These findings suggest that the C-Vps complex acts cooperatively with Vam3 to regulate the fusion of vesicular transport intermediates with the vacuole.

Protein complexes that function at distinct stages of the secretory pathway, perhaps analogously to the C-Vps complex, have been identified. The Exocyst, a complex of seven proteins, functions as a multiprotein effector of the vesicular Sec4 rab GTPase, targeting Golgi-derived secretory vesicles with the plasma membrane (TerBush et al., 1996; Guo et al., 1999). The mammalian early endosome antigen 1 (EEA1) complex catalyzes nucleotide exchange on rab5, functions as a rab5 effector, and modulates SNARE activity in promoting early and late endosome fusion (Stenmark et al., 1995; Horiuchi et al., 1997; McBride et al., 1999; Simonsen et al., 1999). An analogous complex of proteins containing Vca1, the yeast homologue of EEA1, Pep12, an endosomal t-SNARE, Vps45, a Sec1 homologue, and the Vps21 rab GTPase functions in Golgi to endosome transport (Burd et al., 1997; Peterson et al., 1999). Transport protein particle (TRAPP) is a complex of nine proteins which functions upstream of SNAREs in the docking of ER-derived vesicles with the Golgi (Sacher et al., 1998). Thus, the Exocyst, EEA1, Vca1, and TRAPP complexes are proposed to be important determinants for the specificity of vesicle fusion sites at the appropriate target membrane.

Here we present evidence indicating that the C-Vps complex regulates the fusion of transport intermediates with the vacuole by an unexpected mechanism. We find that the C-Vps complex also contains Vps39 and Vps41. Disruption of Vps39 interactions with the C-Vps complex mislocalizes Vps39 from membrane-enriched subcellular fractions to the soluble/cytosolic fraction and causes severe hydrolase missorting defects. This suggests that localization of Vps39 to the endosome/vacuole is essential for vacuolar protein transport. A protein complex containing Vps39 and Vps41 has been shown to interact with the GTP-bound form of the Ypt7 rab GTPase and to function as a downstream effector of Ypt7 (Price et al., 2000). Our findings indicate that the Vps39/Vps41-containing C-Vps complex also functions to stimulate nucleotide exchange on Ypt7. We show that Vps39 preferentially interacts with GDP-bound and nucleotide-free Ypt7, but not with other rab GTPases. Moreover, purified Vps39 stimulates GDP/GTP exchange on Ypt7 in vitro, indicating that Vps39 functions as a Ypt7 nucleotide exchange factor. Therefore, our evidence reveals a role for the C-Vps complex in stimulating nucleotide exchange on Ypt7.

**Materials and Methods**

**Strains**

For a full list of strains used in this study, please see Table I.

**Plasmids and DNA Methods**

Standard methods using restriction and modifying enzymes (Roche) were used in carrying out DNA ligations, bacterial minipreps, and bacterial and yeast transformations (Rieder and Emr, 1997). DNA fragments were purified using QIAquick Gel Extraction (QIAGEN). Protein A fusions of VPS39 and vps39 mutants were constructed by removing the VPS39 stop codon and engineering in its place an SphI site. An SphI–KpnI fragment encoding doubly mutated protein A was then ligated with VPS39 resulting in a COOH-terminal protein A–Vps39 fusion whose expression is driven by the endogenous VPS39 promoter. ypt7 point mutants and vps39 truncation mutants (for two-hybrid assays) as well as GST-Vps21 were produced by PCR using primers engineered to contain convenient restriction sites. The cloning of multicopy VPS41 (pWS26), VPS11, VPS18, VPS16, and VPS33 as well as protein A–Vps16, pmP139, and pmP41 have been described (Cowles et al., 1997; Rieder and Emr, 1997; Sato et al., 2000). Vps39-1 and ypt7-38 were generated through random PCR mutagenesis (amino acids 1–1,049 of Vps39 or 74–208 of Ypt7) using Taq polymerase (PerkinElmer) and were isolated using a colorimetric plate assay for secreted carboxypeptidase Y (CPY) (Burd et al., 1997).

**Vacuolar Protein Sorting**

CPY, alkaline phosphatase (ALP), and aminopeptidase I (API) maturation assays were carried out in whole cells (see Fig. 2 A and Fig. 5, A and B) or spheroplasts (see Fig. 5 A) by pulse-labeling with 10 μCi of Express [35S] protein labeling mix (Dupont) per OD 660 unit of cells, chasing for 0–120 min, and processing cells for immunoprecipitation with antisera specific to CPY, ALP, or API (Klionsky et al., 1992) before SDS-PAGE, as described (Rieder and Emr, 1997). For temperature shift experiments, cells were shifted from 26°C to 38°C 10 min before labeling.

**Subcellular Fractionation**

21 OD 600 units of spheroplasts were pulse-labeled with 420 μCi of Express [35S] (20 min), chased for 1 h, and immunoprecipitated for Vps39, as described (Rieder and Emr, 1997). Antiserum specific to amino acids 315–316 of Vps39 was raised by standard methods (Darsow et al., 1997).

**Purification of Protein A Fusions**

14 OD 600 units of spheroplasts expressing protein A fusions were pulse-labeled with 420 μCi of Express [35S] for 20 min and, as above, chased for 1 h. Spheroplasts were Dounce homogenized in 1.5 ml of PBS plus protease inhibitors, centrifuged at 325 g for 5 min and 100,000 × g for 30 min to clear lysates of unbroken cells and separate P 70/S100 fractions, respec-

The Journal of Cell Biology, Volume 151, 2000

552
The $F_{\text{rea}}$ was resuspended and incubated in 0.5 ml of PBS plus 250 mM NaCl and 0.5% IGEPA. CA-630 (NP-40; Sigma-Aldrich) for 5 min, after which 0.5 ml of PBS containing 0.5% NP-40 was added. The $F_{\text{rea}}$ extract was then centrifuged at 13,000 g, and both the $F_{\text{rea}}$ and $F_{\text{out}}$ plus 0.5% NP-40 were incubated with 10 µl of IgG-coupled sepharose (Amerham Pharmacia Biotech) for 60 min. The IgG-sepharose was washed three times with 1 ml of PBS plus 0.5% NP-40 and directly resolved by SDS-PAGE or denatured for 10 min at 95$^\circ$C in 50 µl of 50 mM Tris, pH 7.5, 1 mM EDTA, 1% SDS (see Fig. 3) and immunoprecipitated with monoclonal anti-HA antibody (Roche) or polyclonal antiserum (Figs. 4 A and 7). Unlabeled spheroplasts were treated as described (Babst et al., 1997). For Western blot analysis with monoclonal anti-HA antibody (Roche) or polyclonal antiserum (Figs. 4 A and 7 A), unlabeld spheroplasts were assayed as described above.

**Two-Hybrid Analysis**

In Figs. 4 D and 6 B, interactions were assessed based on the histidine-independent growth of HF7C (CLONTECH Laboratories, Inc.). Interactions were quantified (see Figs. 4 C, 6 A, and 7 B) using the PCY2 strain as described (Rehling et al., 1999). The prenylation motif within ypt7 two-hybrid constructs was mutated (ypt7[R268C, C206]) of the nonredundant protein database suggest that homologues of Vps39 exist in Homo sapiens, Schizosaccharomyces pombe, and Caenorhabditis elegans. A multiple alignment using Clustal indicated that the COOH-terminal half of Vps39 (amino acids 596–1,021) is 28, 25, and 22% identical and 45, 40, and 38% similar to the COOH terminus of the H. sapiens, S. pombe, and C. elegans open reading frames (ORFs), respectively. Thus, these ORFs may reveal functionally conserved protein motifs, in addition to the CHCR, within the COOH terminus of Vps39. For instance, two regions adjacent to the CHCR that we designate domain I (amino acids 596–715) and domain II (amino acids 905–1,021) are encoded by the conserved region of Vps39 and complemented with vps39 cells. A multiple alignment using Clustal indicated that the COOH-terminal half of Vps39 (amino acids 596–1,021) is 28, 25, and 22% identical and 45, 40, and 38% similar to the COOH terminus of the H. sapiens, S. pombe, and C. elegans open reading frames (ORFs), respectively. Thus, these ORFs may reveal functionally conserved protein motifs, in addition to the CHCR, within the COOH terminus of Vps39. For instance, two regions adjacent to the CHCR that we designate domain I (amino acids 596–715) and domain II (amino acids 905–1,021) are encoded by the conserved region of Vps39 (Fig. 1, A and B). We hypothesized that domain I and domain II may be essential to Vps39 function. 

**Results**

Vps39 Contains Three Highly Conserved Subdomains That Are Essential for Vps39 Function

Vps39 encodes a hydrophilic, 1,049–amino acid protein that lacks a potential signal sequence or membrane-spanning region. Amino acids 733–893 of Vps39 encode a single clathrin heavy chain repeat (CHCR), an α-helical–rich protein motif that is iterated seven times in clathrin heavy chain and believed to mediate protein–protein interactions required for clathrin coat formation (Ybe et al., 1999). The single CHCR found within Vps39 is also likely to function as a protein-binding domain, although the identity of its ligand is unknown.

BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/) of the nonredundant protein database suggest that homologues of Vps39 exist in Homo sapiens, Schizosaccharomyces pombe, and Caenorhabditis elegans. A multiple alignment using Clustal indicated that the COOH-terminal half of Vps39 (amino acids 596–1,021) is 28, 25, and 22% identical and 45, 40, and 38% similar to the COOH terminus of the H. sapiens, S. pombe, and C. elegans open reading frames (ORFs), respectively. Thus, these ORFs may reveal functionally conserved protein motifs, in addition to the CHCR, within the COOH terminus of Vps39. For instance, two regions adjacent to the CHCR that we designate domain I (amino acids 596–715) and domain II (amino acids 905–1,021) are encoded by the conserved region of Vps39 (Fig. 1, A and B). We hypothesized that domain I and domain II may be essential to Vps39 function.
The morphology, despite the fact that these vps39 mutants were stably expressed (data not shown). Thus, domain I, the CHCR, and domain II are important to Vps39 function.

We also tested whether deletion of any one of these subdomains is sufficient to change the subcellular fractionation of Vps39. vps39D cells expressing single-copy levels of either VPS39, vps39D, or vps39D were converted to spheroplasts and pulse-labeled with [35S]methionine. Cell lysates were centrifuged at 13,000 g to yield a low-speed pellet (P13) and supernatant (S13). The S13 was centrifuged at 100,000 g to isolate the high-speed pellet (P100) and supernatant (S100). P13, S100, and P100 fractions were immunoprecipitated for Vps39. A significant portion of the total pool of wild-type Vps39 associated with the pelletable fractions (10% P13, 40% S100, 50% P100) (Fig. 2 B). Although mutation of domain I or the CHCR does not dramatically affect Vps39 localization (data not shown), deletion of domain II shifts Vps39DII to the soluble fraction (0% P13, 95% S100, 5% P100) (Fig. 2 B). Thus, it is likely that domain II plays an important role in localizing Vps39 to the pelletable fractions, which are enriched for cellular membranes and membrane-binding proteins, whereas domain I and the CHCR carry out a distinct aspect(s) of Vps39 function.

**Vps39 Is a Component of a Protein Complex Containing Multiple Vps Proteins**

One possibility raised by these results is that domain II anchors Vps39 to the pelletable cell fractions by mediating interactions with another pelletable protein(s). This possibility is especially likely since Vps39 has been isolated in a large protein complex (Nakamura et al., 1997; Price et al., 2000). Therefore, we affinity-purified a functional protein A–tagged Vps39 (protein A–Vps39) from P13 and P100 fractions and assayed for proteins which coprecipitate in a Vps39-dependent manner.

vps39Δ cells expressing single-copy protein A or protein A–VPS39 was pulse-labeled with [35S]methionine. Lysates of these strains were centrifuged at 100,000 g to generate the total cellular particulate fraction (Ptotal) that contains the proteins and/or membranes of both the P13 and P100 fractions. Detergent/NaCl extracts of the Ptotal were incubated with IgG-coupled sepharose. Analysis of the IgG-sepharose by SDS-PAGE and fluorography revealed multiple proteins that coprecipitated with protein A–Vps39 (indicated by asterisks), but not protein A alone, even though lysates of each strain contained ~5.6 × 10^8 cpm (Fig. 3, lanes 1 and 2). The apparent molecular masses of several proteins that coprecipitated with protein A–Vps39 correspond with Vps proteins as follows: Vps41 (120 kD), Vps11 (115 kD), Vps18 (100 kD), Vps16 (90 kD), and Vps33 (75 kD). Proteins copurifying with protein A and protein A–Vps39 were therefore subjected to denaturation and immunoprecipitation using antibodies specific

**Figure 1.** Multiple protein motifs exist within Vps39. (A) A BLAST search of the nonredundant protein database revealed homologues of Vps39 from H. sapiens, S. pombe, and C. elegans. (B) A multiple alignment (Clustal) of full-length Vps39 with these ORFs indicated that the COOH-terminus of Vps39 (amino acids 596–1,021) is 28, 25, and 22% identical and 45, 40, and 38% similar to the COOH terminal regions of the H. sapiens, S. pombe, and C. elegans ORFs, respectively. The conserved region of Vps39 contains two regions adjacent to the CHCR which we designate domain I and domain II.

**Figure 2.** Domain I and domain II are essential for Vps39 function. vps39Δ cells expressing the indicated single-copy protein A–VPS39 construct were labeled for 10 min with [35S]methionine and chased with excess unlabeled methionine for 30 min at 30°C. (A) Labeled lysates of these cells were immunoprecipitated for CPY or ALP and the resulting immunoprecipitates were analyzed by SDS-PAGE and fluorography. m, Mature; pro, precursor ALP. (B) Subcellular localization of these strains were centrifuged at 13,000 g to yield the low-speed pellet (P13), high-speed pellet (P100), and supernatant (S100). Vps39 was immunoprecipitated from P13, S100, and P100 fractions and analyzed by SDS-PAGE and fluorography. WT, Wild-type.
Component of the C-vps Complex

Domain II Mediates Vps39 Interactions with Vps11, a Component of the C-vps Complex

As described above, deletion of domain II shifted Vps39 from P13 and P100 subcellular fractions to the soluble fraction (S100) and resulted in strong CPY and ALP missorting defects (Fig. 2, A and B). This raises the possibility that domain II mediates Vps39 association with the C-Vps complex to potentially anchor Vps39 in the pelletable cell fraction. Therefore, we tested the relative capacity of wild-type Vps39 and Vps39ΔII to interact with the C-Vps complex.

Vps39ΔII cells expressing protein A or protein A fusions of VPS39, vps39ΔII, or vps39ΔI were transformed with a single-copy plasmid encoding either hemagglutinin-tagged VPS11 (HA-VPS11, pMP139) or VPS18 (HA-VPS18, pMP141). Ptotal and S100 fractions of these strains were incubated with IgG-coupled sepharose to purify protein A–Vps39 fusions, as described above. Western blot analysis indicated that wild-type Vps39 and Vps39ΔI derived from the membrane-associated fraction (Ptotal), but not the soluble fraction (S100), interacted with C-Vps complex components Vps11 and Vps18 (Fig. 4 A, data not shown), suggesting that the C-Vps complex is enriched in the Ptotal even though soluble pools of Vps11 and Vps18 exist (Rieder and Emr, 1997). Vps39ΔII failed to interact with Vps11 or Vps18 (Fig. 4 A, data not shown). Thus, domain II, which appears to be an important localization determinant for Vps39, is also required for the association of Vps39 with C-Vps complex components Vps11 and Vps18. It is possible that deletion of domain II detaches Vps39 from the C-Vps complex, but otherwise leaves the C-Vps complex intact. Alternatively, inclusion of Vps39 in the C-Vps complex may be required for overall complex assembly.

It has previously been proposed that Vps39 and Vps41 interact, although it has not been determined whether this association is direct (Nakamura et al., 1997). Two approaches were undertaken to test this. First, protein A–Vps39 was affinity purified from [35S]methionine-labeled strains cooverexpressing protein A–VPS39 and either VPS41, VPS11, VPS18, VPS16, or VPS33. Multicopy vectors lead to ~15-fold overexpression of these genes (data not shown), allowing us to study the capacity of Vps39 to directly interact with Vps41, Vps11, Vps18, Vps16, or Vps33 in the relative absence of other proteins. When only protein A–Vps39 is overproduced and purified from the soluble (S100) fraction using IgG-sepharose, as described, IgG-sepharose binds only protein A–Vps39. Cooverexpressing both protein A–VPS39 and VPS11 led to the isolation of protein A–Vps39 and another protein which comigrates with immunoprecipitated Vps11, indicating that Vps39 and Vps11 directly interact (Fig. 4 B, lanes 1 and 3). Protein A itself did not bind overproduced Vps11 (lane 2). In contrast, Vps39ΔII exhibited a marked decrease in Vps11 binding, even though Vps39ΔII was expressed and bound to IgG-sepharose, whereas Vps39ΔI and Vps39ΔCHCR each interacted with Vps11 (Fig. 4 B, lanes 4–6). The soluble (S100) pools of overexpressed Vps18, Vps16, Vps33, and Vps41 failed to interact with overexpressed protein A–Vps39 under identical conditions (lanes 7–10), despite the fact that these proteins were all readily detectable by immunoprecipitation of identically prepared soluble (S100) fractions (data not shown).

The two-hybrid system revealed that coexpression of bait VPS11 and prey VPS39 resulted in 2.8 U of β-galactosidase activity, indicative of an interaction between the Vps39 and Vps11 (Fig. 4 C). Neither bait VPS39 nor prey VPS11 constructs produced significant background (<0.2 U). Bait vps39ΔII was expressed, but failed to generate a detectable two-hybrid interaction with prey VPS11 (Fig. 4 D). Moreover, coexpression of a bait vector containing do-
main II alone (amino acids 901–1,049) with prey VPS11, but not empty prey vector, exhibited a two-hybrid interaction, suggesting that domain II is sufficient to bind Vps11 (Fig. 4 D). Although Vps18, Vps16, Vps33, and Vps41 were produced as Gal4 fusions (data not shown), these proteins did not exhibit two-hybrid interactions with Vps39 (Fig. 4 C). Thus, domain II mediates Vps39 association with the pelletable cell fraction, the C-Vps complex, and the C-Vps complex component, Vps11, suggesting that incorporation of Vps39 into the C-Vps complex is required for Vps39 localization to the pelletable cell fractions.

The rab GTPase, Ypt7, Functionally Interacts with Vps39

Although domain II is required for Vps39 association with Vps11, deletion of domain I does not appear to eliminate this interaction, suggesting that domain I has a distinct function. This notion raises the possibility that Vps39 may interact with additional Vps proteins which are essential for the docking or fusion of transport vesicles with the vacuole such as Vam7, a SNAP25 protein, Vam3, a vacuolar t-SNARE, or Ypt7, a vacuolar and prevacuolar rab GTPase (Wichmann et al., 1992; Darsow et al., 1997; Sato et al., 1998). To determine if a genetic relationship exists between vps39 and either ypt7, vam7, or vam3, temperature-sensitive vps39 and ypt7 mutants were generated.

The entire coding region of VPS39 and the region of YPT7 that encodes amino acids 74–208 were subjected to random PCR–mediated mutagenesis leading to the selection of mutants that secreted the vacuolar hydrolase, CPY, at 38°C, but not at 26°C, using a colorimetric plate assay (see Materials and Methods). 5,000 vps39 and 10,000 ypt7 candidates were screened, leading to the isolation of vps39-1 and ypt7-38. To characterize these alleles, we monitored the vacuolar delivery of CPY, ALP, and API along the three known vacuolar transport pathways (Klionsky et al., 1992; Cowles et al., 1997) in both vps39Δ cells expressing vps39-1 (vps39Δ1) and WSY99 cells (ypt7Δ) expressing ypt7-38 (ypt7Δ1) from single-copy plasmids.

vps39Δ1 and ypt7Δ1 cells were grown at 26°C, converted to spheroplasts, and pulse-labeled with [35S]methionine at either 26°C or 38°C. Both the intracellular (I) and extracellular (E) fractions were subjected to immunoprecipitation with an antibody specific for either CPY or ALP. Although vps39Δ1 and ypt7Δ1 cells maintained at 26°C were able to mature CPY and ALP, indicating that both hydrolases were delivered to the vacuole, the vps39Δ1 and ypt7Δ1 strains shifted to 38°C for 10 min before labeling accumulated intracellular pools of precursor CPY (p2CPY) and precursor ALP (proALP) (Fig. 5 A, lanes 3, 5, 9, and 11; data not shown). Only small amounts (<5%) of the cellular pool of p2CPY were secreted upon inactivation of Vps39 or Ypt7 (lanes 10 and 12). An uncharacterized form of CPY (i.e., not p1CPY) which migrates faster than p2CPY was also present (lanes 9 and 11), possibly because p2CPY accumulates in endosomes and is aberrantly processed in ypt7Δ1 cells under these conditions. In contrast, SEY6210 (wild-type) cells exhibited the mature forms of CPY and ALP at both 26°C and 38°C (lanes 1 and 7). Similarly, after a 10-min shift to 38°C, vps39Δ1 and ypt7Δ1 cells failed to mature API, even after the 120-min chase point, while wild-type cells converted API from the 60-kD precursor form (prAPI) to the 50-kD mature form (mAPI) by
between strains that there is a strong synthetic genetic interaction (Wurmser et al., 1997; Rieder and Emr, 1997; Sato et al., 1998).

Figure 5. Ypt7 functionally interacts with vps39. Wild-type (WT), vps39tsf, or ypt7tsf strains were grown overnight at 26°C and pulse-labeled with [35S]methionine for 10 min at 26°C or 38°C. (A) After a 30-min chase, labeled wild-type, vps39tsf, or ypt7tsf spheroplasts were separated into intracellular (I) and extracellular (E) fractions. CPY immunoprecipitates of I and E were resolved by SDS-PAGE and fluorography, revealing CPY maturation defects in the vps39tsf and ypt7tsf at 38°C. API immunoprecipitates derived from labeled wild-type, vps39tsf, and ypt7tsf cells were analyzed by SDS-PAGE and fluorography, revealing defects in API maturation in the ypt7tsf, but not wild-type cells at 38°C. (B) CPY immunoprecipitates derived from the indicated strains were analyzed by SDS-PAGE and fluorography, revealing a strong genetic interaction with vps39tsf/ypt7tsf but not other double mutants at 26°C. m. Mature; p2, precursor CPY; pr, precursor API.

90 min (Fig. 5 A). At 26°C, wild-type, vps39tsf, and ypt7tsf cells matured API with similar kinetics (data not shown). Consistent with previous CPY and ALP sorting results using vps39 and ypt7 null mutants (Wichmann et al., 1992; Nakamura et al., 1997), these findings indicate that Vps39 and Ypt7 play important roles in the transport of newly synthesized hydrolase precursors along the CPY, ALP, and API/autophagic trafficking pathways. Thus, the sorting defects of the vps39tsf and ypt7tsf strains are characteristic of a specific subset of vps mutants that mediate the docking and fusion of vesicular compartments with the vacuole (Darsow et al., 1997; Rieder and Emr, 1997; Sato et al., 1998).

To determine if vps39 genetically interacts with ypt7, a vps39tsf/ypt7tsf double-mutant strain was generated and assayed for CPY missorting defects at 26°C. Although wild-type, vps39tsf, and ypt7tsf cells converted CPY to its mature form, strikingly, AWY2 (vps39tsf/ypt7tsf) double-mutant cells exhibited >95% p2-precursor CPY at 26°C, demonstrating that there is a strong synthetic genetic interaction between vps39tsf and ypt7tsf (Fig. 5 B). Similar results were observed when ALP maturation was assayed (data not shown). In contrast, TKSY80 (vps39tsf/vam7tsf), TKSY20 (vps39tsf/vam3tsf), TKSY74 (ypt7tsf/vam7tsf), and ypt7tsf/vam3tsf double-mutant strains each exhibited >95% mature CPY (Fig. 5 B, data not shown). This genetic evidence raises the intriguing possibility that Vps39 and the Ypt7 rab GTPase may physically associate.

Figure 6. Ypt7 physically interacts with Vps39. Physical interactions between Vps39 and Ypt7 were tested for by two-hybrid assay. No significant background resulted from YPT7 bait plasmid or VPS39 prey plasmids; however, when YPT7 bait was cotransformed with VPS39 prey, cellular 255-galactosidase activity of ~4 U was detected (Fig. 6 A). Interactions were also detected between bait VPS39 and prey YPT7 (data not shown). Combining prey VPS39 with bait VPS21, the yeast rab5 GTPase (Horazdovsky et al., 1994; Singer-Kruger et al., 1994), or bait RAS1 resulted in <0.25 U of 255-galactosidase activity. Ypt7 did not exhibit interactions with Vps11, Vps18, Vps41, or Vam7 by the two-hybrid assay (data not shown), suggesting that the Vps39 interaction with Ypt7 is specific.

Although the Ypt7–Vps39 interaction did not require the CHCR or domain II of Vps39, Ypt7 failed to interact with Vps39 lacking significant portions of domain I (Vps39Δ1/ΔCHCR/ΔII) or with Vps39 truncations lacking amino acids 1–368 (Fig. 6 B), despite the fact that these mutants were expressed (data not shown). Therefore, Ypt7 associates with Vps39 in a manner which requires domain I and additional NH2-terminal regions of Vps39, but not the CHCR or domain II. This establishes that the requirements for Vps39–Ypt7 interactions (e.g., domain I) are distinct from those of Vps39–Vps11 interactions (i.e., domain II).

Vps39 Stimulates Nucleotide Exchange on Ypt7

Our evidence indicates that association of Vps39 with the C-Vps complex component, Vps11, requires domain II of Vps39 (Fig. 4) whereas interactions between Ypt7 and Vps39 do not (Fig. 6 B). This evidence suggests that Ypt7 binds Vps39 independent of the other members of the C-Vps complex. To further test this, protein A–Vps39 was overexpressed and purified from the S100 fraction of SEY6211e (vps11Δ) cells using IgG-Sepharose and assayed for nucleotide-dependent binding to Ypt7 in vitro. GST–Ypt7 and GST–Vps21 were expressed in bacteria, purified using glutathione–Sepharose, and eluted off the beads. The purified GTPases were then preloaded with either GTPγS or GDP and incubated with IgG-Sepharose–immobilized Vps39. Western blot analysis revealed that Vps39 preferentially bound GDP-Ypt7, but not GTPγS–Ypt7 (Fig. 7 A, lanes 1 and 2). No significant binding of Ypt7 to protein A alone occurred (lanes 3 and 4), nor did Vps21 nonspecifically associate with Vps39 or protein A (lanes 5–8). It has been found that a large protein complex containing Vps39 and Vps41 associates with the GTP-
bound form of Ypt7 (Price et al., 2000). Our data show that purified Vps39 binds Ypt7 in its GDP-bound form, but not in its GTP-bound form, suggesting that a protein other than Vps39 mediates Ypt7-GTP interactions with the Vps39 and Vps41 complex.

The nucleotide dependency of the interaction between Ypt7 and Vps39 was also assessed by the two-hybrid assay. Although cells coexpressing bait YPT7 and prey VPS39 vectors yielded ~4 U of β-galactosidase activity, ypt7Q68L, a stably expressed mutant predicted to shift Ypt7 into the GTP-bound state (Der et al., 1986), exhibited a less potent interaction with Vps39 (~3 U, Fig. 7 B), ypt7 effector domain mutants, ypt7T40A and ypt7I41S, are predicted to impair the interactions of Ypt7 with effector or GTPase activating proteins (GAPs), but not guanine-nucleotide exchange factors (GEFs) or guanine-nucleotide dissociation inhibitors (GDIs; Becker et al., 1991). Consistent with this prediction, ypt7T40A and ypt7I41S, although stably expressed, resulted in strong CPY and ALP missorting defects as well as fragmented vacuoles, the morphology typical of class B vps mutants (data not shown). Vps39 bound both Ypt7T40A and Ypt7I41S by the two-hybrid assay (~13 U), exhibiting stronger interactions than Vps39 and wild-type Ypt7. Perhaps, the effector mutants were unable to bind an endogenous effector/GAP, thus increasing the pool of Ypt7 available for interactions with Vps39.

ypt7D129A, which is expected to remain in the nucleotide-free form in vivo due to a severalfold lower affinity for guanine-nucleotides (Weijland et al., 1994), enhanced Ypt7 binding to Vps39 approximately eightfold (31 U, Fig. 7 B). Unfortunately, Ypt7T22N, predicted to be in the
C-Vps Complex Activates the Ypt7 rab GTPase

Wurmser et al.

The C-Vps complex (Vps18, Vps11, Vps16, Vps33) is conserved in mammalian cells, *Drosophila*, and *C. elegans* (Sevrioukov et al., 1999). The vacuolar and prevacuolar C-Vps complexes regulate the assembly of Vam3 and/or Vam7 SNARE complexes, an event required for the fusion of hydrolase-containing transport intermediates with the vacuole (Darsow et al., 1997; Rieder and Emr, 1997; Sato et al., 2000). Here, we find that the C-Vps complex also contains Vps39 and Vps41. Failure to incorporate Vps39 into the C-Vps complex mislocalizes Vps39 from membrane-enriched cell fractions to cytosolic fractions and results in severe vacuolar protein sorting defects, suggesting that Vps39 is essential to C-Vps complex function.

**Discussion**

The C-Vps complex (Vps18, Vps11, Vps16, Vps33) is conserved in mammalian cells, *Drosophila*, and *C. elegans* (Sevrioukov et al., 1999). The vacuolar and prevacuolar C-Vps complex regulates the assembly of Vam3 and/or Vam7 SNARE complexes, an event required for the fusion of hydrolase-containing transport intermediates with the vacuole (Darsow et al., 1997; Rieder and Emr, 1997; Sato et al., 2000). Here, we find that the C-Vps complex also contains Vps39 and Vps41. Failure to incorporate Vps39 into the C-Vps complex mislocalizes Vps39 from membrane-enriched cell fractions to cytosolic fractions and results in severe vacuolar protein sorting defects, suggesting that Vps39 is essential to C-Vps complex function.
Thus, domain I and NH₂-terminal sequences of Vps39 define a novel rab-binding motif.

**Function of the C-Vps Complex**

Ypt7 localizes to and functions on both donor (e.g., endosome) and acceptor (vacuole) compartments, consistent with a proposed symmetrical requirement for rabs in membrane fusion events (Haas et al., 1995; Barbieri et al., 1998). This suggests that a mechanism exists to localize Vps39 to Ypt7-containing membranes. However, the targeting of Vps39 to the membranes does not depend on interactions with Ypt7, as Vps39ΔII bound Ypt7 but failed to associate with the particulate cell fractions. Vps11, Vps18, Vps16, and Vps33 (class C Vps proteins) form a prevacuolar and vacuolar protein complex required, like Vps39, for the fusion of CPY, ALP, and API transport intermediates with the vacuole (Rieder and Emr, 1997). Affinity purification of Vps39- and Vps16-associated proteins revealed that Vps39 and Vps41 are additional components of this complex (Fig. 3). This is consistent with observations indicating that Vps39 is part of a 38 S protein complex which contains Vps41 (Nakamura et al., 1997; Price et al., 2000). Association of Vps39 with the C-Vps complex occurs in the membrane-enriched pelletable fractions; the soluble/cytosolic pool of Vps39 does not appear to bind the C-Vps complex. Thus, incorporation of Vps39 into the C-Vps complex may serve to localize and/or activate Vps39 such that it triggers nucleotide exchange on Ypt7 in the context of a fully assembled C-Vps complex. Although assembly of Vps39 into the C-Vps complex could be essential for multiple aspects of C-Vps complex function, this could be the underlying basis for the strong CPY and ALP missorting defects resulting from deletion of domain II (Fig. 2 A).

Recent observations by the Wickner lab have resulted in the identification of the C-Vps complex—referred to as homotypic fusion and vacuole protein sorting (HOPS)—as a downstream effector of GTP-bound Ypt7 (Seals et al., 2000). The activated Ypt7 rab GTPase is believed to tether acceptor (i.e., vacuole) and donor (e.g., endosome) membrane compartments, facilitating v- and t-SNARE pairing and hence, membrane fusion (Ungermann et al., 1998). This suggests that the C-Vps complex not only activates Ypt7 through Vps39, but also may act as a Ypt7 effector through an as yet undefined protein partner(s). Our two-hybrid assay did not reveal individual interactions between GTP-bound Ypt7 and components of the C-Vps complex (i.e., Vps18, Vps11, Vps16, Vps39, and Vps41). Perhaps this result indicates that a fully assembled C-Vps complex is required to form a binding surface for activated Ypt7, ensuring that docking/fusion does not proceed until all components of the C-Vps and SNARE complexes are present.

Additional biochemical and genetic evidence suggests that the C-Vps complex interacts with unpaired Vam3 (vacuolar t-SNARE), possibly by preventing nonproductive binding of Vam3 with the pool of its partner v-SNARE, Vti1, present in the same membrane (vacuole) (Sato et al., 2000). By this mechanism, the C-Vps complex facilitates the formation of productive trans-SNARE interactions between endosomal Vti1 and vacuolar Vam3, allowing for the selective recognition and efficient fusion of vacuolar and prevacuolar compartments. Thus, the C-Vps complex carries out at least three functions essential for membrane fusion: (1) stimulates nucleotide exchange on Ypt7; (2) functions as a downstream effector of GTP-
bound Ypt7; and (3) preserves a pool of Vam3 in an unpaired/activated state in order to promote trans-SNARE interactions. The multifunctional role of the C-Vps complex in rab-mediated tethering and SNARE-mediated fusion is depicted in Fig. 8. According to this model, Vps39 associates with the C-Vps complex, localizing Vps39 to Ypt7 on prevacuolar cargo vesicles and the vacuole. This enables Vps39 to convert inactive GDP-Ypt7 to its active GTP-bound state (Fig. 8, step 1). During the initial stages of the fusion reaction, Vam3 is maintained in an unpaired state through interactions with the C-Vps complex. After activation of Ypt7, GTP-bound Ypt7 associates with an effector protein(s) contained within the C-Vps complex (step 2) (Price et al., 2000). Since Ypt7 and the C-Vps complex localize to both prevacuolar vesicles and the vacuole (Haas et al., 1995; Rieder and Emr, 1997), this interaction is likely to tether these compartments, bringing Vti1 and Vam3 into close proximity. At step 3, the C-Vps complex releases Vam3, allowing Vti1/Vam3/Vam7 trans-SNARE complexes to form and drive membrane fusion. Thus, the C-Vps complex orchestrates at least three distinct interactions that ensure the high degree of specificity and efficiency required during endosome/vacuole fusion.

The proteins which constitute the C-Vps complex, although not conserved with the components of other protein complexes with recognized roles in vesicular trafficking, functionally overlap with these complexes. Similar to the C-Vps complex, the EEA1 complex, required for the fusion of early and late endosomes in mammalian systems, contains the exchange factor (Rabex5) and effectors (Rabaptin5, EEA1) of rab5 and modulates SNARE activity through NSF and EEA1 (Stenmark et al., 1995: Horiuchi et al., 1997; McBride et al., 1999; Simonsen et al., 1999).

Vam1, the yeast homologue of EEA1, integrates signals from GTP-bound Vps21 and the Sec1 homologue, Vps45, in the regulation of SNARE-mediated fusion of Golgi-derived vesicles with the prevacuolar endosome (Burd et al., 1997; Peterson et al., 1999). Furthermore, the Exocyst, a protein complex required for the docking of secretory vesicles with the plasma membrane, functions as a multiprotein effector of the Sec4 rab GTPase (Guo et al., 1999). However, unlike the C-Vps complex, the Sec4 exchange factor (Sec2) is not included in the Exocyst (TerBush et al., 1996; Walch-Solimena et al., 1997). Thus, the C-Vps complex, the EEA1/Vac1 regulatory complexes, and the Exocyst each appear to function by regulating the activity of ubiquitously conserved elements of the membrane trafficking apparatus (rabs and SNAREs). It will be interesting to determine whether this emerging theme applies to other large protein complexes required for membrane fusion such as TRAPP, which plays an essential role in endoplasmic reticulum to Golgi transport (Sacher et al., 1998).

We thank William Wickner for helpful discussions; Eric Marcussen and William Snyder for strains and reagents; and Mike Peterson for contributions to protein A purifications.

A.E. Wurmser and T.K. Sato are members of the Biomedical Sciences Graduate Program and supported by the Program Project Grant, National Institutes of Health grant CA58689. S.D. Emr is an investigator of the Howard Hughes Medical Institute.

Submitted: 7 August 2000 Revised: 15 September 2000 Accepted: 20 September 2000

References

Babst, M., T.K. Sato, L.M. Banta, and S.D. Emr. 1997. Endosomal transport function in yeast requires a novel AAA-type AtPase, Vps4sp. EMBO (Eur. Mol. Biol. Organ.) J. 16:1820–1831.

Banta, L.M., J.A. Vida, P.K. Herman, and S.D. Emr. 1990. Characterization of yeast Vps33p, a protein required for vacuolar protein sorting and vacuole biogenesis. Mol. Cell. Biol. 10:4638–4649.

Barnes, M.A., S. Hoffenberg, R. Roberts, A. Mukhopadhyay, A. Pomrehn, B.F. Horacek, and P.D. Stahl. 1998. Evidence for a symmetrical requirement for Rab5-GTP in in vitro endosome-endosome fusion. J. Biol. Chem. 273: 25850–25855.

Becker, J.T. Tan, H.H. Trepte, and D. Gallwitz. 1991. Mutational analysis of the putative effector domain of the GTP-binding Ypt1 protein in yeast suggests specific regulation by a novel GAP activity. EMBO (Eur. Mol. Biol. Organ.) J. 10:785–792.

Burd, C.G., M. Peterson, C.R. Cowles, and S.D. Emr. 1997. A novel Sec18p NSF-dependent complex required for Golgi-to-endosome transport in yeast. Mol. Biol. Cell. 8:1089–1104.

Cowles, C.R., W.B. Snyder, C.G. Burd, and S.D. Emr. 1997. Novel Golgi to vacuole delivery pathway in yeast: identification of a sorting determinant and required transport component. EMBO (Eur. Mol. Biol. Organ.) J. 16:2769–2782.

Darsow, T., S.E. Rieder, and S.D. Emr. 1997. A multifunctional syntaxin homolog, Vam3p, essential for autologous and biosynthetic protein transport to the vacuole. J. Cell Biol. 138:517–529.

Der, C.J., T. Finkel, and G.M. Cooper. 1986. Biological and biochemical properties of human rasH genes mutated at codon 61. Cell, 44:167–176.

Feig, L.A., and G.M. Cooper. 1988. Inhibition of NIH 3T3 cell proliferation by a mutant ras protein with preferential affinity for GDP. Mol. Cell. Biol. 8:3235–3243.

Guo, W., D. Roth, C. Walch-Solimena, and P. Novick. 1999. The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. EMBO (Eur. Mol. Biol. Organ.) J. 18:1071–1080.

Haas, A., D. Schegmann, T. Lazar, D. Gallwitz, and W. Wickner. 1995. The GTPase Ypt6p of Saccharomyces cerevisiae is required on both partner vacuoles for the homotypic fusion step of vacuole inheritance. EMBO (Eur. Mol. Biol. Organ.) J. 14:5258–5270.

Horazdovsky, B.F., G.R. Busch, and S.D. Emr. 1994. VPS21 encodes a rab5-like GTP binding protein that is required for the sorting of yeast vacuolar proteins. EMBO (Eur. Mol. Biol. Organ.) J. 13:1297–1309.

Horiuchi, H., R. Lippe, H.M. McBride, M. Rubino, P. Woodman, H. Stenmark, V. Rybin, M. Wilm, K. Ashman, M. Mann, and M. Zerial. 1997. A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. Cell, 90:1149–1159.

Klionsky, D.J., P.K. Herman, and S.D. Emr. 1990. The fungal vacuole: composition, function, and biogenesis. Microbiol. Rev. 54:266–292.

Klionsky, D.J., R. Cuevas, and D.S. Yaver. 1992. Aminopeptidase I of Saccharomyces cerevisiae is localized to the vacuole independent of the secretory pathway. J. Cell Biol. 119:287–299.

McBride, H.M., V. Rybin, C. Murphy, A. Giner, R. Teasdale, and M. Zerial. 1999. Oligomeremic complexes link Rab5 effectors with NSF and drive membrane fusion. J. Biol. Chem. 274:11344–11349.

Peterson, M.R., C.G. Burd, and S.D. Emr. 1999. Vam1p coordinates Rab and phosphatidylinositol 3-kinase signaling in Vps45p-dependent vesicle docking/fusion at the endosome. Curr. Biol. 9:159–162.

Price, A., J. Sadows, W. Wickner, and C. Ungermann. 2000. The docking stage of yeast vacuole fusion requires the transfer of proteins from a cis-SNARE complex to a Rab/Ypt protein. J. Cell Biol. 148:1231–1238.

Raymond, C.K., I. Howald-Stevenson, C.A. Vater, and T.H. Stevens. 1998. TRAPP, a multispecificity syntaxin homolog, associates with the C-Vps complex to a Rab/Ypt protein. J. Cell Biol. 148:2307–2327.

Robinson, J.S., T.R. Graham, and S.D. Emr. 1991. A putative zinc finger protein, Vps18p, affects late Golgi functions required transport component. Mol. Biol. Cell, 2:346–353.

Sacher, M., Y. Jiang, J. Burrowan, A. Scarpa, J. Burston, L. Zhang, D. Schleich, J.R. Yates, 3rd, H. Aebi, and N. Fetrow. 1998. TRAPP, a highly conserved novel complex in the cis-Golgi that mediates vesicle docking and fusion. EMBO (Eur. Mol. Biol. Organ.) J. 17:2494–2503.

Sato, T.K., T. Darsow, and S.D. Emr. 1998. Vam7p, a SNAP-25-like molecule, and Vam3p, a syntaxin homolog, function together in yeast vacuolar protein trafficking. Mol. Cell. Biol. 18:5308–5319.
Sato, T.K., P. Rehling, M.R. Peterson, and S.D. Emr. 2000. Class C Vps protein complex regulates SNARE pairing and is required for vesicle docking/fusion. Mol. Cell. 6:661–667.
Seals, D.F., G. Eitzen, N. Margolis, W.T. Wickner, and A. Price. 2000. A Ypt/Rab effector complex containing the sec1 homolog vps33p is required for homotypic vacuole fusion. Proc. Natl. Acad. Sci. USA. 97:9402–9407.
Svrzoukov, E.A., J.P. He, N. Moghrabi, A. Sunio, and H. Kramer. 1999. A role for the deep orange and carnation eye color genes in lysosomal delivery in Drosophila. Mol. Cell. 4:479–486.
Simonsen, A., J.M. Gaullier, A. D’Arrigo, and H. Stenmark. 1999. The Rab5 effector EEA1 interacts directly with syntaxin-6. J. Biol. Chem. 274:28857–28860.
Singer-Kruger, B., H. Stenmark, A. Dusterhoft, P. Philipsen, J.S. Yoo, D. Gallwitz, and M. Zerial. 1994. Role of three rab5-like GTPases, Ypt51p, Ypt52p, and Ypt53p, in the endocytic and vacuolar protein sorting pathways of yeast. J. Cell Biol. 125:283–298.
Stenmark, H., G. Vitale, O. Ullrich, and M. Zerial. 1995. Rabaptin-5 is a direct effector of the small GTPase Rab5 in endocytic membrane fusion. Cell. 83: 423–432.
TerBush, D.R., T. Maurice, D. Roth, and P. Novick. 1996. The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO (Eur. Mol. Biol. Organ.) J. 15:6483–6494.
Ungerermann, C., K. Sato, and W. Wickner. 1998. Defining the functions of trans-SNARE pairs. Nature. 396:543–548.
Vida, T.A., G. Huyer, and S.D. Emr. 1993. Yeast vacuolar enzymes are sorted in the late Golgi complex and transported to the vacuole via a pre-vacuolar endosome-like compartment. J. Cell Biol. 121:1245–1256.
Wada, Y., and Y. Anraku. 1992. Genes directing vacuolar morphogenesis in Saccharomyces cerevisiae. I. VAM7, a gene for regulating morphogenic assembly of the vacuoles. J. Biol. Chem. 267:18671–18675.
Wada, Y., Y. Ohsumi, and Y. Anraku. 1992. Genes for directing vacuolar morphogenesis in Saccharomyces cerevisiae. I. Isolation and characterization of two classes of vam mutants. J. Biol. Chem. 267:18665–18670.
Wada, Y., N. Nakamura, Y. Ohsumi, and A. Hirata. 1997. Vam3p, a new member of syntaxin related protein, is required for vacuolar assembly in the yeast Saccharomyces cerevisiae. J. Cell Sci. 110:1299–1306.
Walch-Solimena, C., R.N. Collins, and P.J. Novick. 1997. Sec2p mediates nucleotide exchange on Sec3p and is involved in polarized delivery of post-Golgi vesicles. J. Cell Biol. 137:1495–1509.
Weijland, A., G. Parlato, and A. Parmeggiani. 1994. Elongation factor Tu D138N, a mutant with modified substrate specificity, as a tool to study energy consumption in protein biosynthesis. Biochemistry. 33:10711–10717.
Wichmann, H., L. Hengst, and D. Gallwitz. 1992. Endocytosis in yeast: evidence for the involvement of a small GTP-binding protein (Ypt7p). Cell. 71: 1131–1142.
Wurmser, A.E., and S.D. Emr. 1998. Phosphoinositide signaling and turnover: PtdIns(3)P, a regulator of membrane traffic, is transported to the vacuole and degraded by a process that requires luminal vacuolar hydrolase activities. EMBO (Eur. Mol. Biol. Organ.) J. 17:4930–4942.
Ybe, J.A., F.M. Brodsky, K. Hofmann, K. Lin, S.H. Liu, L. Chen, T.N. Earnest, R.J. Fletterick, and P.K. Hwang. 1999. Clathrin self-assembly is mediated by a tandemly repeated superhelix. Nature. 399:371-375.