Proteins Needed for Vesicle Budding from the Golgi Complex Are also Required for the Docking Step of Homotypic Vacuole Fusion

Albert Price, William Wickner, and Christian Ungermann

Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755-3844

Abstract. Vam2p/Vps41p is known to be required for transport vesicles with vacuolar cargo to bud from the Golgi. Like other VAM-encoded proteins, which are needed for homotypic vacuole fusion, we now report that Vam2p and its associated protein Vam6p/Vps39p are needed on each vacuole partner for homotypic fusion. In vitro vacuole fusion occurs in successive steps of priming, docking, and membrane fusion. While priming does not require Vam2p or Vam6p, the functions of these two proteins cannot be fulfilled until priming has occurred, and each is required for the docking reaction which culminates in trans-SNARE pairing. Consistent with their dual function in Golgi vesicle budding and homotypic fusion of vacuoles, approximately half of the Vam2p and Vam6p of the cell are recovered from cell lysates with purified vacuoles.

Key words: Vps41/Vam2p • Vps39/Vam6p • priming • NSF/Sec18p • α-SNAP-Sec17p

Introduction

The fusion of cellular membranes during secretion, endocytosis, and organelle inheritance is essential for cellular compartmentation. Highly conserved proteins are involved in fusion events throughout the cell and across species. These include soluble NSF attachment protein receptors (SNAREs), the ATP-driven chaperone NSF/Sec18p and its partner proteins α-SNAP/Sec17p, and a large family of Rab GTPases. The pairing in trans of cognate v- (vesicle) and t- (target membrane) SNAREs is a central event in docking membranes before fusion (Rothman, 1994; Hay and Scheller, 1997). This pairing is regulated by the prior priming action of NSF/Sec18p and SNAP/Sec17p, which prepares the SNAREs for docking, and by Rab GTPases (Mayer and Wickner, 1997; Novick and Zerial, 1997). The relationship of the structurally diverse Rab effectors and tethering factors (Pfeffer, 1996; TerBush et al., 1996; Cao et al., 1998; McBride et al., 1999) to docking is currently studied for several trafficking reactions.

We have studied the homotypic fusion of yeast vacuoles. It occurs in the ordered stages of priming, docking, and bilayer fusion. During priming, individual vacuoles are prepared for interaction with other vacuoles. The starting vacuoles contain a cis complex of SNAREs bound together on the same vacuole. This cis-SNARE complex includes a t-SNARE (Vam3p), v-SNAREs (Nyv1p, Vti1p, and Ykt6p), an s-SNARE (Vam7p, which is a homologue of the synaptic SNAP-23/25 protein), an α-SNAP (Sec17p; Haaas et al., 1995; Haaas and Wickner, 1996; Nichols et al., 1997; Ungermann and Wickner, 1998; Ungermann et al., 1999a), as well as a novel chaperone (LMA1; Barlowe, 1997; Xu et al., 1998). Priming involves the disassembly of the cis-SNARE complex, the activation of the t-SNARE, and the association of SNARE proteins from apposed vacuoles, forming a trans-SNARE complex. trans-SNARE pairing triggers the release of luminal calcium to interact with calmodulin and mediate downstream events that lead to fusion (Peters and Mayer, 1998). These events include the release of LMA1 from the t-SNARE, which is regulated by a phosphatase-kinase pair (Xu et al., 1998).

Mutations in the genes encoding many of the above pro-
proteins, which were shown biochemically to catalyze each stage of the fusion reaction, cause severe vacuole fragmentation in the intact cell, presumably reflecting a failure of vacuole fusion. Type II vacuole morphology (vam) mutants were obtained in a nonselective screen for just such a highly fragmented vacuole morphology. Since Vam3p, Vam4p/Ypt7p, and Vam7p are each involved in the reaction, we examined whether Vam2p and Vam6p are also required. These proteins are required for normal vacuole morphology (Wada et al., 1992; Nakamura et al., 1997; Zheng et al., 1998), for protein sorting to the vacuole (Raymond et al., 1992; Cowles et al., 1997; Rehling et al., 1999) and for cytosol-to-vacuole protein targeting (Harding et al., 1995). Though Vam2p and Vam6p are reported to be localized to the vacuole (Wada et al., 1992) and to be associated in a large complex (Nakamura et al., 1997), Vps41p/Vam2p also fulfills a specific role in transport vesicle budding from the Golgi (Rehling et al., 1999) and for the formation of stable trans-SNARE pairs.

Stage of homotypic vacuole fusion and for the formation of approximately half of the Vam2p and Vam6p of the cell copurified with vacuoles, and that it is required for the docking stage of homotypic vacuole fusion and for the formation of stable trans-SNARE pairs.

Materials and Methods

Yeast Strains and Genetic Manipulations

Saccharomyces cerevisiae strains BJ 3505 (MATa pep4::HIS3 prb1-11.6 R H113 lys2-208 trpl-D101 ura3-52 gal2 can) and D KY 6281 (MATa leu2-3 leu2-112 ura3-D102 100 lys2-801 suc2-D9 pho8::TRP1) were used. The VAM2 and VAM6 genes were disrupted in S. cerevisiae BJ 3505 by recombination with PCR-generated cassettes containing 5' and 3' homologous regions of either VAM2 or VAM6 and the URA3 gene of S. cerevisiae. The PCR primers contained 40 bases of identity to the regions flanking the open reading frame (VAM6 5' primer sequence: 5' TGT CTA TGG TTA TGG TGA TGT TAT A C 3'; VAM2 5' primer sequence: 5' AAT ACC GGA TCC (VAM2 primer sequence 5' GGG CTA TAT GCC TGT CTC TTC ATG GGA AAG AAT (5' of the PEP4 gene or the PHO8 gene). Fusion of these vacuoles exposes pro-alkaline phosphatase from one vacuole population to its activating protease from the other. A nitrocellulose filter was washed with 1 M NaCl and 0.1 M Tris-HCl (pH 7.5) to remove any remaining vacuoles. The filter was then incubated in 0.1 M Tris-HCl (pH 7.5) containing 0.1% SDS, 0.1% Triton X-100, and 5% glycerol. The resulting solution was centrifuged at 10,000 g for 5 min to remove any remaining vacuoles. The supernatant was then transferred to a new tube and the remaining vacuoles were analyzed by SDS-PAGE immunoblotting (Harlow and Lane, 1988) with antibodies to Vam2p and Vam6p.

Biochemical Reagents

All inhibitors and antibodies were dissolved in or dialyzed into PBS buffer (20 mM Pipes-KOH, pH 6.8, 200 mM sorbitol) unless stated otherwise.

Antibody Production and Purification

A nitrocellulose filter was washed with 1 M NaCl and 0.1 M Tris HCl (pH 7.5) to remove any remaining vacuoles. The filter was then incubated in 0.1 M Tris-HCl (pH 7.5) containing 0.1% SDS, 0.1% Triton X-100, and 5% glycerol. The resulting solution was centrifuged at 10,000 g for 5 min to remove any remaining vacuoles. The supernatant was then transferred to a new tube and the remaining vacuoles were analyzed by SDS-PAGE immunoblotting (Harlow and Lane, 1988) with antibodies to Vam2p and Vam6p.

Results

Vam2p and Vam6p Are Required on both Partner Vacuoles for Fusion

Vam2p and Vam6p are fused to green fluorescent protein, are localized to the vacuole in vivo (Wada et al., 1992) and, yet, Vam2p is also found associated with A P-3 coat adapter subunits on the Golgi and is required there for vesicle budding (Rehling et al., 1999). To determine whether the proportion of Vam2p and Vam6p recovered with the vacuoles was consistent with vacuolar and/or nonvacuolar localizations, we purified vacuoles from cell lysates and compared the recovery of Vam2p and Vam6p with that of Pho8p, an established vacuolar marker protein. We find that 45% of the Vam2p and 54% of the Vam6p are recovered in our purified vacuoles, after correction for the loss of 23% of the vacuolar marker Pho8p during isolation (Fig. 1). The presence of Vam2p and Vam6p in this fraction is not due to contaminating Golgi, as <5% of the late Golgi marker Kex2p is recovered with the vacuoles (Fig. 1). Thus, half of the Vam2p and Vam6p is localized to the vacuole while the rest is elsewhere in the cell, in accordance with both Wada et al. (1992) and Rehling et al. (1999).

To investigate the functions of Vam2p and Vam6p, we have employed an in vitro assay of vacuole fusion. Vacuoles are purified from two S. cerevisiae strains bearing deletions in either the PEP4 gene or the PHO8 gene. Fusion of these vacuoles exposes pro-alkaline phosphatase from one vacuole population to its activating protease from the other. A nitrocellulose filter was washed with 1 M NaCl and 0.1 M Tris-HCl (pH 7.5) to remove any remaining vacuoles. The filter was then incubated in 0.1 M Tris-HCl (pH 7.5) containing 0.1% SDS, 0.1% Triton X-100, and 5% glycerol. The resulting solution was centrifuged at 10,000 g for 5 min to remove any remaining vacuoles. The supernatant was then transferred to a new tube and the remaining vacuoles were analyzed by SDS-PAGE immunoblotting (Harlow and Lane, 1988) with antibodies to Vam2p and Vam6p.

Figure 1. Vam2p and Vam6p are associated with purified vacuoles. A BJ 3505 wild-type culture was processed for vacuole purification (Haaas, 1995). A nitrocellulose filter was washed with 1 M NaCl and 0.1 M Tris-HCl (pH 7.5) to remove any remaining vacuoles. The filter was then incubated in 0.1 M Tris-HCl (pH 7.5) containing 0.1% SDS, 0.1% Triton X-100, and 5% glycerol. The resulting solution was centrifuged at 10,000 g for 5 min to remove any remaining vacuoles. The supernatant was then transferred to a new tube and the remaining vacuoles were analyzed by SDS-PAGE immunoblotting (Harlow and Lane, 1988) with antibodies to Vam2p and Vam6p.

The Journal of Cell Biology, Volume 148, 2000
other, resulting in the production of enzymatically active alkaline phosphatase, which is assayed spectrophotometrically. The \textit{VAM2} and \textit{VAM6} genes were deleted in one of our vacuole fusion tester strains, BJ3505. Although mutations in \textit{VAM2} and \textit{VAM6} affect the rate of trafficking of proteins to the vacuole in vivo (Nakamura et al., 1997; Radisky et al., 1997), the steady state levels of the vacuolar marker proteins Pho8p, CPY, and Vma1p are unaffected in our purified vacuole preparations (Fig. 2 A). Thus, whereas these vacuoles are smaller than wild type (Nakamura et al., 1997) and are recovered with somewhat lower yield, they contain the normal complement of vacuolar proteins. However, when normal amounts (assayed by Bradford protein determination) of vacuoles derived from BJ3505 vam2Δ or vam6Δ strains are combined with wild-type DKY 6281 vacuoles.

\textbf{VAM2 and VAM6 Are Required for Docking Sec18-primed Vacuoles}

Previous studies of vacuole fusion in vitro have defined three distinct reaction stages. First, priming requires the action of Sec17p and Sec18p (Mayer et al., 1996). Second, docking requires primed vacuoles, v- and t-SNAREs, and Ypt7p (Mayer and Wickner, 1997; Ungermann and Wickner, 1998). Third, fusion no longer requires trans-SNARE pairs (Ungermann et al., 1998) but needs calmodulin (Peters and Mayer, 1998) and protein phosphatase 1 (Peters et al., 1999). To determine the stages where Vam2p and Vam6p act, aliquots were removed from vacuole fusion reactions at various times and mixed with buffer or inhibitors, or placed on ice, and the incubation was continued until all samples had been incubated for 90 min (Fig. 4). In contrast to the inhibition by anti-Sec18p (closed squares), which is only seen at early reaction times, resistance to anti-Vam2p (crosses) and anti-Vam6p (closed triangles) is achieved with intermediate kinetics that correspond to the docking stage inhibitors Gdi1p (open triangles) or the antibody to the t-SNARE Vam3p (diamonds; Mayer and Wickner, 1997; Ungermann et al., 1998). Resistance to anti-Vam2p and anti-Vam6p is achieved before fusion itself (open squares), suggesting that these factors are not needed for the fusion stage of the reaction. These proteins are also not involved in priming, since antibodies to...
Vam2p and Vam6p have no effect on Sec17p release from the vacuoles (data not shown), indicating that these components are not involved in priming (Mayer et al., 1996). To further investigate the role of Vam2p and Vam6p in docking, fusion reactions without inhibitors were started with the partner vacuoles in separate tubes. At various times, aliquots of each preincubated vacuole population were combined with inhibitors and with each other, and the reaction was continued for 70 min (Fig. 5). Resistance to anti-Sec18p (closed squares) is achieved early, confirming that the priming subreaction can occur when vacuoles are separated (Mayer et al., 1996). However, as with an inhibitor of Ypt7p (open triangles), resistance to anti-Vam2p (crosses) and anti-Vam6p (closed triangles) is never achieved when the vacuole partners are in separate tubes. Therefore, the action of Vam2p and Vam6p requires contact between vacuole fusion partners and indicates a role in docking.

Homotypic vacuole fusion requires the early action of Sec18p to prime the vacuoles for subsequent docking (Mayer et al., 1996; Mayer and Wickner, 1997). To determine whether priming is a prerequisite for the action of Vam2p and Vam6p, a two-stage experiment was performed in which the activity of Sec18p was inhibited by anti-Sec18p antibody in the first incubation. After 30 min, a time which otherwise suffices for the acquisition of resistance to Gdi1p or to antibodies to Vam3p, Vam2p, or Vam6p (Fig. 4), Sec18p activity was restored by the addition of Sec18 protein, and the reaction was continued in the presence or absence of inhibitors. Fusion is restored by the addition of Sec18p in the second incubation (Fig. 6, lane 2). However, as with the docking inhibitors Gdi1p and anti-Vam3p, the reaction remains fully sensitive to anti-Vam2p and anti-Vam6p (Fig. 6, lanes 3–6). Thus, Sec18p must act before Vam2p and Vam6p can fulfill their functions.

We have previously reported that docking can be divided into the stages of Ypt7p-dependent tethering, followed by trans-SNARE pairing. These stages are distinguished by the addition of an excess of Sec18p to vacuole fusion reactions, which allows Ypt7p to complete its function while preventing stable trans-SNARE pairing (Ungermann et al., 1998). To examine the role of Vam2/6p in docking, vacuoles were incubated under standard fusion conditions for 20 min, but with an excess of Sec18p, and an

Figure 4. Kinetics of acquisition of resistance to Gdi1p and antibodies to Vam3p, Vam2p, and Vam6p. Aliquots (30 μl) were removed from 10× scale fusion reactions at the indicated times and added to control buffer on ice, buffer at 27°C, Gdi1p (64 μg/ml), or antibodies against Sec18p, Vam3p, Vam2p, or Vam6p at 27°C. Fusion reactions were incubated for a total of 90 min at 27°C before being assayed for alkaline phosphatase activity.

Figure 5. Completion of Vam2p and Vam6p function requires vacuole docking. 10× scale reactions with vacuoles from either BJ 3505 or DKY 6281 were incubated at 27°C in the presence of ATP. At the indicated times, 15-μl aliquots were removed from each reaction and combined in the presence of control buffer on ice, buffer at 27°C, Gdi1p, antibody to Vam3p, Vam2p, or Vam6p at 27°C, incubated for 70 min at 27°C, and analyzed for alkaline phosphatase activity.

Figure 6. The action of Sec18p must precede that of Vam2p and Vam6p. A 10× scale fusion reaction with affinity-purified antibody against Sec18p was incubated at 27°C for 30 min. The vacuoles were reisolated and resuspended in the original volume of reaction buffer containing 25 μg/ml Sec18p and divided into 30-μl aliquots. Where indicated, Gdi1p or antibodies against Vam3p, Vam2p, or Vam6p were added. The incubations were continued at 27°C for 90 min and analyzed for alkaline phosphatase activity. The background activity of 0.22 U (a sample transferred to ice after the first 30-min incubation) was subtracted.
Figure 7. Vam2/6p continues to function after Ypt7p. Standard fusion reactions were supplemented with cytosol and excess Sec18p (3.8 μg) to prevent SNARE pairing and incubated on ice (lane 1) or at 27°C for 20 min. After 20 min, one reaction was placed on ice (lane 2), the others received inhibitors or antibodies and were placed on ice for 3 min. Reactions were diluted with 500 μL PS buffer. Vacuoles were sedimented (for 5 min, 10,000 g, at 4°C) and resuspended in the original volume of reaction buffer (without cytosol and Sec18p). The indicated inhibitors were added and the reaction continued for 70 min at 27°C. Fusion activity was measured and background activity (0.25 U) was subtracted.

Discussion

Vam2p and Vam6p are components of a multifunctional complex that is required both for protein trafficking to the vacuole (Raymond et al., 1992; Harding et al., 1995; Cowles et al., 1997; Nakamura et al., 1997) as well as for homotypic vacuole–vacuole fusion. Surprisingly, recent findings have suggested a novel function for Vam2p (Price et al., accompanying manuscript) are required for the docking stage of homotypic vacuole fusion. The fragmented vacuole phenotype seen in vam2 and vam6 mutants is identical to that seen in mutants of the small G protein Vam4/Ypt7p and the vacuolar t-SNARE (Wada et al., 1992; Haas et al., 1995; Nakamura et al., 1997; Nichols et al., 1997). Each of these proteins has been shown biochemically to act at the docking stage of the homotypic vacuole fusion reaction. Vam2p and Vam6p localize to the vacuole in vivo (Nakamura et al., 1997); additional localization to the Golgi or Golgi-derived vesicles might have been difficult to visualize. Vam2p and Vam6p presumably use different receptors at the Golgi and vacuole, which is consistent with the findings that vacuolar Ypt7p is required for optimal steady state association of Vam2p and Vam6p at the vacuole (Price et al., 2000 [this issue]) and, yet, normal budding of trafficking vesicles from the Golgi (Rehling et al., 1999) may not require Ypt7p. We have shown that approximately half of the Vam2p and Vam6p copurify with vacuoles (when compared with the vacuole marker protein Pho8p), whereas Kex2p, a trans-Golgi marker protein, does not (Fig. 1). In the accompanying manuscript (Price et al., 2000 [this issue]), we demonstrate that both Vam2p and Vam6p are physically and functionally associated with factors specifically required for homotypic vacuole fusion. Except for the study
SNARE proteins nucleate COP I/II budding from the yeast ER, there is little precedent for such an unusual dual function in both vesicle budding and membrane docking. This raises the fascinating possibility that V am26p may couple these processes in vivo. Further work will be required to test this idea.

Since many of the same proteins are needed for heterotypic trafficking to the vacuole and for homotypic vacuole fusion, the recovery of organelles with vacuole density from lysates of vam2Δ, vamΔ, vam3Δ, and ypt7Δ strains, and the demonstration that these organelles contain vacuole marker proteins at approximately normal specific activities, raises a question of their identity and origins. They may be small, but rather normal, vacuoles that have obtained most of their proteins by slow, or even bypass, pathways and are small because of defective homotypic fusion. Alternatively, they may represent an earlier organelle on the Golgi to vacuole pathways that has expanded because of accumulated vacuole proteins until it acquires certain vacuole characteristics such as density. Further studies are needed to resolve this yet, in either case, assay of the capacity of this organelle to fuse with vacuoles from wild-type cells provides one measure of the role of the missing protein in fusion pathways. Other complementary assays are inhibition of the homotypic fusion of wild-type vacuoles by the relevant antibody and, as detailed for V am2p and V am6p in the accompanying manuscript (Price et al., 2000), direct demonstration of physical and functional associations with other catalysts of vacuole fusion such as Y pt7p and SNA REs.

How general is the yeast vacuole and the role of V am2p and V am6p in its fusion as a model for priming and docking in other vesicle trafficking pathways? Yeast vacuoles require priming before docking (Mayer and Wickner, 1997) and, in this regard, might be thought to differ, at least superficially, from other trafficking reactions. For example, the priming of SNA REs by NSF and SNA P is not needed for docking at the neural synapse in Drosophila (Littleton et al., 1998) and Cao et al. (1998) have shown that the docking of ER-derived vesicles at the Golgi requires U so1p but not the priming of SNA REs by Sec18p. However, we suggest that there is a fundamental unity in these three systems. Vacuole tethering is mediated by Y pt7p, but it is not mediated by the pairing of SNA REs in trans (Ungermann et al., 1998). Vacuoles from a vam3Δ strain, in which the SNA REs are not associated in cis with each other (Ungermann and Wickner, 1998), can also tether without priming (Ungermann et al., 1998), as in the studies of Cao et al. (1998). Furthermore, since synaptic transmission is sensitive to some proteolytic toxins that only cleave unpaired SNA REs (Otto et al., 1997) and attachment of vesicles to the presynaptic membrane is unaffected by SNA RE mutants (Broadie et al., 1995), the morphologically docked state at the synapse may correspond to tethering but not to complete trans-SNA RE pairing. We propose that the disassembly of v- and t-SNA REs after heterotypic fusion and the sorting of the v- and t-SNA REs at the start of v-SNA RE retrograde traffic may spatially disconnect the priming activity of Sec18p/NSF from docking, whereas these events are necessarily linked in homotypic fusion reactions.

The docking process is now known to require an unexpected variety of factors. These include t- and v-SNA REs on apposed vacuoles (Nichols et al., 1997), the Rab-like Y pt7p on both vacuoles (Haas et al., 1995), vacuolar phosphatidylinositol 4,5-bisphosphate (Mayer et al., 2000), vacuole acidification (Ungermann et al., 1999b), and now V am2p and V am6p. The relationships between these factors are not clear. However, in an accompanying paper, we show that a complex that contains V am2p and V am6p functions as an effector complex for Y pt7p, linking membrane priming to trans-SNA RE pairing. Our finding (Fig. 7) that V am2p is still needed even after Y pt7p has completed its function suggests that V am2p might function between Y pt7p-mediated tethering and trans-SNA RE pairing. This result is consistent with the finding that V am2p is needed for trans-SNA RE pairing (Fig. 8), but further studies will be needed to test this hypothesis.

We thank Dr. Y. oh Wada (University of Tokyo, Tokyo, Japan) for providing the HA–V am6 plasmid. We thank Drs. Charles Barlowe, Jerry Eichler, Gary Karp, Daniel Klausky, Darren Seals, Zuoyu Xu, and especially Dr. Tom Rapoport for helpful discussions, and Dr. Scott Emr (University of California, San Diego, San Diego, CA) for communicating unpublished results. We thank Li Wang for sharing preliminary results.

This research was supported by a grant from the National Institute of General Medical Sciences. C. Ungermann was supported by a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft (DFG).

Submitted: 18 January 2000
Revised: 14 February 2000
Accepted: 15 February 2000

References

A usubel, F., R. B rent, R. E. K ingston, D.D. M ore, and J.G. Seidman. 1995. Short Protocols in Molecular Biology. J. A. Smith and K. Struhl, editors. Third edition. John Wiley & Sons, Inc., New York.

Barlowe, C. 1997. Coupled ER to Golgi transport reconstituted with purified cytosolic proteins. J. Cell Biol. 139:1097–1108.

Broadie, K., A. Prokop, H. J. Bellin, C.J. O’Kane, K.L. Schulze, and S.T. Sweeney. 1995. Syntaxin and synaptobrevin function downstream of vesicle docking in Drosophila. Neuron. 15:663–673.

Cao, X., N. Ballew, and C. Barlowe. 1998. Initial docking of ER-derived vesicles requires U so1p and Y pt1 but is independent of SNA RE proteins. EMBO (Eur. Mol. Biol. Organ.) J. 17:2156–2165.

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.

Haas, A. 1995. A quantitative assay to measure homotypic vacuole fusion in vitro. Methods Cell Sci. 17:283–294.

Haas, A., and W. Wickner. 1996. Homotypic vacuole fusion requires Sec17p (yeast α-SNAP) and Sec18p (yeast NSF). EMBO (Eur. Mol. Biol. Organ.) J. 15:3296–3305.

Haas, A., D. Scheglmann, T. Lazar, D. Galwitz, and W. Wickner. 1995. The GT Pase Y pt7p 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.

Harding, T.M., K.A. Morano, S.V. Scott, and D.J. Klionsky. 1995. Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. J. Cell Biol. 131:591–602.

Harlow, E., and O. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Hartl, U.F., A. Blobel, and H. Walter. 1999. The molecular chaperones for protein folding in eukaryotic cells: current concepts and future perspectives. Cell 97:265–269.

Hartl, U.F., and H. Walter. 2002. Molecular Chaperones in ER Stress and Disease. Annual Review of Cell and Developmental Biology.

Hartley, J., and K. Rout. 2003. The mammalian endoplasmic reticulum stress response. Annual Review of Cell and Developmental Biology. 19:427–462.

Haas, A., D. Scheglmann, T. Lazar, D. Galwitz, and W. Wickner. 1995. The GT Pase Y pt7p 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.

Harding, T.M., K.A. Morano, S.V. Scott, and D.J. Klionsky. 1995. Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. J. Cell Biol. 131:591–602.

Harlow, E., and O. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Hay, J.C., and R.H. Scheller. 1997. SNA REs and NSF in targeted membrane fusion. Curr. Opin. Cell Biol. 9:505–512.

Littleton, J.T., E.R. Chapman, R. K. Reber, M.B. Getman, S.D. Carlson, and B. G anetzky. 1998. Temperature-sensitive paralytic mutations demonstrate that synaptic exocytosis requires SNA RE complex assembly and disassembly. Neuron. 21:401–413.

Mayer, A., and W. Wickner. 1997. Docking of yeast vacuoles is catalyzed by the Ras-like G T Pase Y pt7p after symmetric priming by Sec18p (NSF). J. Cell Biol. 136:307–317.

Mayer, A. W., and V. Wickner, and A. Haas. 1996. Sec18p (NSF)-driven release of Sec17p (α-SNAP) can precede docking and fusion of yeast vacuoles. Cell. 85:83–94.

Mayer, A., D. Scheglmann, S. Dove, A. G latz, W. Wickner, and A. Haas. 2000.
Phosphatidylinositol 4,5-bisphosphate regulates two steps of homotypic vacuole fusion. Mol. Cell Biol. In press.

McBride, H. M., V. Rybin, C. Murphy, A. Giner, R. Teasdale, and M. Zerial. 1999. Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. Cell. 98:377-386.

Nakamura, N., A. Hirata, Y. Ohsumi, and Y. Wada. 1997. Vam2/Vps41p and Vam6/Vps39p are components of a protein complex on the vacuolar membranes and involved in the vacuolar assembly in the yeast Saccharomyces cerevisiae. J. Biol. Chem. 272:11344-11349.

Nichols, B. J., C. Ungermann, H. R. B. Pelham, W. T. Wickner, and A. Haas. 1997. Homotypic vacuole fusion mediated by t- and v-SNAREs. Nature. 387:199-202.

Novick, P., and M. Zerial. 1997. The diversity of Rab proteins in vesicle transport. Curr. Opin. Cell Biol. 9:496-504.

Otto, H., P. I. Hanson, and R. Jahn. 1997. Assembly and disassembly of a ternary complex of synaptobrevin, syntaxin, and SNAP-25 in the membrane of synaptic vesicles. Proc. Natl. Acad. Sci. USA. 94:6197-6201.

Peters, C., and A. Mayer. 1998. Ca2+/calmodulin signals the completion of docking and triggers a late step of vacuole fusion. Nature. 396:575-580.

Raymond, C. K., C. J. Roberts, K. E. Moore, I. Howald, and T. H. Stevens. 1992. Biogenesis of the vacuole in Saccharomyces cerevisiae. Int. Rev. Cytol. 139:59-120.

Rehling, P., T. Darsow, D. J. Katzung, and S. D. Emr. 1999. Formation of AP-3 transport intermediates requires Vps41p function. Nat. Cell Biol. 1:346-353.

Rothman, J. E. 1994. Mechanisms of intracellular protein transport. Nature. 372:55-63.

Springer, S., and R. Schekman. 1998. Nucleation of COPII vesicular coat complex by endoplasmic reticulum to Golgi vesicle SNAREs. Science. 281:698-700.

TerBush, D. R., T. Maurice, D. Roth, and P. Novick. 1996. The exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMB0 (Eur. Mol. Biol. Organ.) J. 15:6483-6494.

Ungermann, C., and W. Wickner. 1998. Vam7p, a vacuolar SNAP-25 homolog, is required for SNARE complex integrity and vacuole docking and fusion. EMB0 (Eur. Mol. Biol. Organ.) J. 17:3269-3276.

Ungermann, C., K. Sato, and W. Wickner. 1998. Defining the functions of trans-SNARE pairs. Nature. 396:543-548.

Ungermann, C., G. Fischer von Mollard, O. N. Jensen, T. H. Stevens, and W. Wickner. 1999a. Three v-SNAREs and two t-SNAREs, present in a pentameric cis-SNARE complex on isolated vacuoles, are essential for homotypic vacuole fusion. J. Cell Biol. 145:1435-1442.

Ungermann, C. U., W. Wickner, and Z. Xu. 1999b. Vacuole acidification is required for trans-SNARE pairing, LMA1 release, and homotypic fusion. Proc. Natl. Acad. Sci. USA. 96:11194-11199.

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.

Zheng, B., J. N. Wu, W. Schober, D. E. Lewis, and T. Vida. 1998. Isolation of yeast mutants defective for localization of vacuolar vital dyes. Proc. Natl. Acad. Sci. USA. 95:11721-11726.