

Dartmouth College

Dartmouth Digital Commons

Dartmouth Scholarship

Faculty Work

8-7-2007

Assays of Vacuole Fusion Resolve the Stages of Docking, Lipid Mixing, and Content Mixing

Youngsoo Jun
Dartmouth College

William Wickner
Dartmouth College

Follow this and additional works at: <https://digitalcommons.dartmouth.edu/facoa>



Part of the [Medical Biochemistry Commons](#)

Dartmouth Digital Commons Citation

Jun, Youngsoo and Wickner, William, "Assays of Vacuole Fusion Resolve the Stages of Docking, Lipid Mixing, and Content Mixing" (2007). *Dartmouth Scholarship*. 1432.
<https://digitalcommons.dartmouth.edu/facoa/1432>

This Article is brought to you for free and open access by the Faculty Work at Dartmouth Digital Commons. It has been accepted for inclusion in Dartmouth Scholarship by an authorized administrator of Dartmouth Digital Commons. For more information, please contact dartmouthdigitalcommons@groups.dartmouth.edu.

Assays of vacuole fusion resolve the stages of docking, lipid mixing, and content mixing

Youngsoo Jun and William Wickner*

Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755

Edited by Peter Novick, Yale University, New Haven, CT, and accepted by the Editorial Board June 30, 2007 (received for review February 1, 2007)

Membrane fusion entails organelle docking and subsequent mixing of membrane bilayers and luminal compartments. We now present an *in vitro* assay of fusion, using yeast vacuoles bearing domains of either Fos or Jun fused to complementary halves of β -lactamase. Upon fusion, these proteins associate to yield β -lactamase activity. This assay complements the standard fusion assay (activation of pro-Pho8p in protease-deficient vacuoles by proteases from *pho8 Δ* vacuoles). Both the β -lactamase and pro-Pho8p activation assays of fusion show the same long kinetic delay between SNARE pairing and luminal compartment mixing. Lipid-mixing occurs rapidly after SNARE pairing but well before aqueous compartment mixing. These results support a model in which SNARE pairing leads to rapid hemifusion, followed by slow further lipid rearrangement and aqueous compartment mixing.

GTP γ S | hemifusion | membrane fusion | SNARE | yeast vacuole

Yeast vacuole homotypic fusion occurs in several stages. During priming, Sec18p (NSF) and Sec17p disassemble *cis*-SNARE complexes, releasing Sec17p and the soluble SNARE Vam7p. Vacuole tethering is mediated by the Ypt7p Rab GTPase and its effector complex HOPS. Fusion proteins and regulatory lipids (diacylglycerol, phosphoinositides, and ergosterol) are concentrated at a ring-shaped microdomain, the “vertex ring,” surrounding the apposed membranes of docked vacuoles. During docking, the released Vam7p rebinds to the vacuole through its affinities for phosphatidylinositol 3-phosphate (1) and HOPS (2) and participates in the formation of *trans*-SNARE complexes between apposed vacuolar membranes. Bilayer fusion around the vertex ring permits lipid and aqueous compartment mixing. It has been proposed that membrane fusion may require a lipidic “hemifusion” intermediate, where the closely apposed membrane leaflets are fused and mix, whereas the distal leaflets and aqueous compartments remain distinct. Hemifusion has been directly observed with influenza HA (3–5), either wild-type or with a GPI-anchor in place of its transmembrane domain (TMD), in a minimal SNARE-driven liposome fusion system (6, 7) with low levels of wild-type SNARE proteins or with mutant SNARE proteins with partial TMDs that span only one bilayer leaflet (6). Hemifusion was also observed in cell–cell fusion mediated by GPI-anchored SNARE proteins expressed on the outer surface of the plasma membrane (8), suggesting that all SNARE-dependent fusion might proceed by means of hemifusion. A hemifusion intermediate was proposed for yeast vacuole fusion, based on the finding that GTP γ S allows lipid mixing while blocking the fusion-dependent activation of pro-Pho8p (9). The standard *in vitro* assay of vacuole fusion measures the proteolytic activation of the inactive pro-Pho8p (alkaline phosphatase or ALP) by vacuolar proteases upon content mixing between two populations of vacuoles. One limitation of this assay is its sensitivity to reagents that inhibit the activation of ALP. We have therefore developed an *in vitro* assay of yeast vacuole fusion in which content mixing between two vacuole populations is measured by the reconstitution of active *Escherichia coli* TEM-1 β -lactamase from two complementary fragments of the enzyme, each contributed from distinct vacuole populations. This fusion assay, coupled with a lipid mixing assay,

has revealed that docking and lipid mixing are completed long before luminal contents mix. The kinetic gap between lipid and content mixing suggests that vacuoles fuse via a hemifusion intermediate, although we have not directly observed hemifusion structures.

Results

The standard *in vitro* vacuole fusion assay requires vacuoles from two strains. One lacks the proteases that activate vacuolar enzymes and contains only the catalytically inactive form of Pho8p (proALP). The other strain has vacuolar proteases but is deleted for the *PHO8* gene. Fusion allows the proteases to gain access to the inactive proALP and form active ALP. This assay has the intrinsic limitation of sensitivity to any reagent that inhibits ALP activity, the formation of active ALP, or vacuolar proteases. We have therefore developed a complementary fusion (content mixing) assay that does not require ALP or proteases. This assay, based on the studies of Blau and colleagues (10), uses vacuoles from two yeast strains (Fig. 1A). One contains a chimeric protein of three parts: a fragment of carboxypeptidase Y (CPY) specifying vacuole targeting (11), the α 197 fragment of the *E. coli* TEM-1 β -lactamase, and the leucine zipper helix from c-Jun (α -Jun). The other carries a fusion protein of the same domain of CPY, the ω 198 fragment of β -lactamase, and the leucine zipper helix from c-Fos (Fos- ω). Although neither population of vacuoles [supporting information (SI) Fig. 5A] has β -lactamase activity (Fig. 1B, diamonds and squares), fusion allows luminal protein mixing and rapid heterodimerization of Fos and Jun helices, which brings the α 197 and ω 198 fragments together to reconstitute β -lactamase activity (Fig. 1B, circles). This assay (hereafter termed the β -lactamase assay) complements the ALP fusion assay: (i) No orthologs of β -lactamase have been found in eukaryotes, including *Saccharomyces cerevisiae*, and thus the assay may avoid background activities. (ii) Yeast vacuolar proteases can be released by lysis during *in vitro* incubations and interfere with biochemical analysis. The β -lactamase assay uses only protease-deficient vacuoles. (iii) Although phosphorylation and proteolysis can regulate trafficking, phosphatase or protease inhibitors may inhibit the ALP assay itself. The β -lactamase fusion assay allows facile study of the roles of phosphorylation or proteolysis in vacuole fusion. The β -lactamase and ALP assays measure luminal compartment

Author contributions: Y.J. and W.W. designed research; Y.J. performed research; Y.J. and W.W. analyzed data; and Y.J. and W.W. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. P.N. is a guest editor invited by the Editorial Board.

Abbreviations: MED, myristoylated alanine-rich C-kinase substrate (MARCKS) effector domain; CPY, carboxypeptidase Y; ALP, alkaline phosphatase; HOPS, homotypic vacuole fusion and vacuole protein sorting.

*To whom correspondence should be addressed at: Department of Biochemistry, Dartmouth Medical School, 7200 Vail Building, Hanover, NH 03755-3844. E-mail: bill.wickner@dartmouth.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0700970104/DC1.

© 2007 by The National Academy of Sciences of the USA

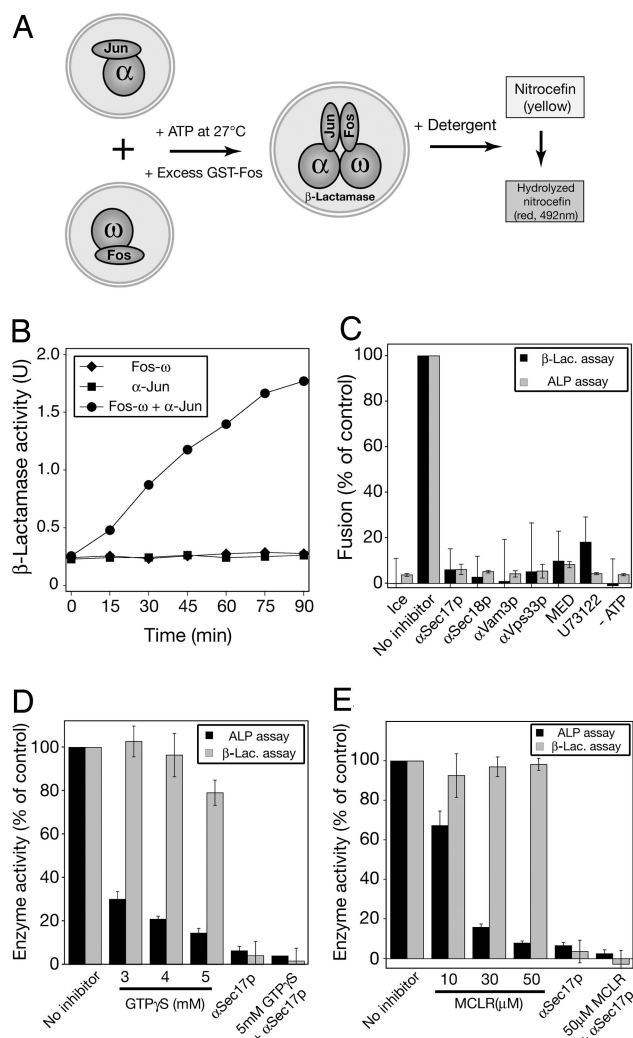


Fig. 1. A vacuole fusion assay. (A) An *in vitro* vacuole fusion assay based on complementary reconstitution of β -lactamase. (B) Fusion-dependent β -lactamase activity formation over the 90-min time course. BJ3505-Fos- ω vacuoles, BJ3505- α -Jun vacuoles, or both were incubated at 27°C in fusion reactions. A portion of reactions was transferred to ice at indicated times and assayed for β -lactamase activity. One unit (U) of the β -lactamase activity is defined as 1 nmol of hydrolyzed nitrocefin per min from 12 μ g of vacuole protein. (C) Comparison between the β -lactamase (black bars) and ALP (gray bars) fusion assays. Fusion reactions (see *Materials and Methods*) were incubated for 90 min on ice or at 27°C with indicated inhibitors. One reaction lacked ATP. Values were normalized to the reactions without inhibitors (3.78 ± 0.65 units for the ALP assay; 1.76 ± 0.45 units for the β -lactamase assay). Data represent mean \pm SEM ($n = 3$). Inhibitors concentrations: affinity-purified anti-Sec17p (153 nM), affinity-purified anti-Sec18p (142 nM), anti-Vam3p IgG (444 nM), affinity-purified anti-Vps33p (155 nM), MED (10 μ M), and U73122 (60 μ M). (D and E) The β -lactamase fusion assay is less sensitive to microcystin-LR (MCLR) and GTP γ S than the ALP assay. The β -lactamase (gray bars) and the ALP (black bars) fusion assays were performed in the presence of increasing concentrations of GTP γ S (D) or MCLR (E) and antibodies to Sec17p where indicated. Values were normalized to the reactions without inhibitors (4.6 ± 0.38 units for the ALP assay; 1.74 ± 0.27 units for the β -lactamase assay). Data represent mean \pm SEM ($n = 3$).

mixing; each will be sensitive to inhibitors of fusion or of the maturation or catalytic activity of its respective reporter enzyme.

To determine whether the β -lactamase assay monitors Rab- and SNARE-dependent vacuole fusion, we compared the effects of known fusion inhibitors on the β -lactamase and ALP assays (Fig. 1C). Expression of the vacuole-targeted α -Jun or Fos- ω

helical domains *per se* did not affect fusion, because vacuoles from BJ3505- α -Jun or BJ3505-Fos- ω fused with DKY6281 vacuoles just as well as BJ3505 vacuoles (data not shown). Fusion in both assays requires ATP and was blocked by priming inhibitors such as antibodies to Vam3p or Vps33p, and lipid modifiers such as the MED peptide that binds to phosphoinositides and the PLC inhibitor U73122. Thus the β -lactamase assay measures authentic vacuole fusion. However, microcystin-LR (MCLR) and the nonhydrolyzable GTP analogue GTP γ S, previously reported as fusion reaction inhibitors (12, 13), showed different inhibitory effects (Fig. 1D and E). The β -lactamase assay was less sensitive to MCLR or GTP γ S than the ALP assay. Neither MCLR nor GTP γ S alone increases β -lactamase activity in a fusion-independent manner, as the development of β -lactamase activity seen with 5 mM GTP γ S or 50 μ M MCLR is fully sensitive to antibodies to Sec17p. The formation of β -lactamase activity in mixed detergent lysates of BJ3505- α -Jun or BJ3505-Fos- ω vacuoles is little affected by 5 mM GTP γ S or 100 μ M MCLR (see SI Fig. 5C). MCLR prevents the development of active ALP in mixed detergent lysates of BJ3505 and DKY6281 vacuoles (SI Fig. 6A, triangles) even though it permits substantial proteolytic formation of ALP from pro-ALP with intact vacuoles, a hallmark of fusion (SI Fig. 6B, lane 6). Thus, MCLR has greater potency to inhibit the ALP assay than to prevent *in vitro* vacuole fusion. Because higher concentrations of GTP γ S showed some inhibition of the β -lactamase assay (Fig. 1D), we further explored GTP γ S inhibition. Vacuoles from BJ3505 or DKY6281 were solubilized in Triton X-100, incubated separately in the absence or presence of 5 mM GTP γ S on ice, and after 20 min, mixed and incubated at 27°C. GTP γ S (5 mM) partially (< 50%) blocked the proteolytic cleavage of pro-ALP in detergent (SI Fig. 6B, lane 5 vs. lane 4) and completely prevented the development of ALP activity (SI Fig. 6A, squares). The slow rate of pro-ALP maturation in this experiment likely reflects the large dilution of the proteases upon lysis as compared with their high concentrations within intact vacuoles. Thus GTP γ S inhibits pro-ALP cleavage or ALP activation more than it inhibits fusion *per se*. To further explore this, fusion reactions with intact vacuoles were incubated for 90 min under low Mg²⁺ conditions, where the ALP assay is more sensitive to GTP γ S, and were assayed for ALP activity (SI Fig. 6D) or for the proteolytic maturation of pro-ALP by immunoblotting (SI Fig. 6C and D). High concentrations of GTP γ S prevent the proteolytic maturation of pro-ALP. Although 2 mM GTP γ S reduced the proteolytic maturation of pro-ALP by only 15%, the ALP activity was reduced by 80% (SI Fig. 6D). These data show that low concentrations of GTP γ S can inhibit the ALP assay by preventing the development of the active form of the enzyme; they do not distinguish whether higher concentrations inhibit fusion or merely block the proteolytic maturation of pro-Pho8p. We therefore examined the effects of GTP γ S by measuring the fusion-dependent size increase of vacuoles (Fig. 2). Vacuoles were incubated on ice or at 27°C, without inhibitor or with GTP γ S or Fab fragments of antibody to Sec18p, and were then labeled with the lipophilic dye MDY-64 and observed by fluorescence microscopy. Although many large vacuoles were formed in a standard reaction (Fig. 2B), no large vacuoles were formed on ice (Fig. 2A) or in reactions with antibodies to Sec18p (Fig. 2C). As many large vacuoles were seen in a reaction with 4 mM GTP γ S (Fig. 2D) as in the reaction without inhibitor (Fig. 2B), although the development of ALP activity was 80% inhibited (Fig. 2G). The fusion-dependent size increase of vacuoles in the presence of 4 mM GTP γ S is indistinguishable from that of the reaction without inhibitors (Fig. 2F). Vacuole clustering and fusion began to be inhibited by 5 mM GTP γ S (Fig. 2E). Large vacuoles are a particularly clear sign of vacuole fusion (Fig. 2F). Whereas 86 vacuoles acquired >60 μ m² surface area through fusion in a

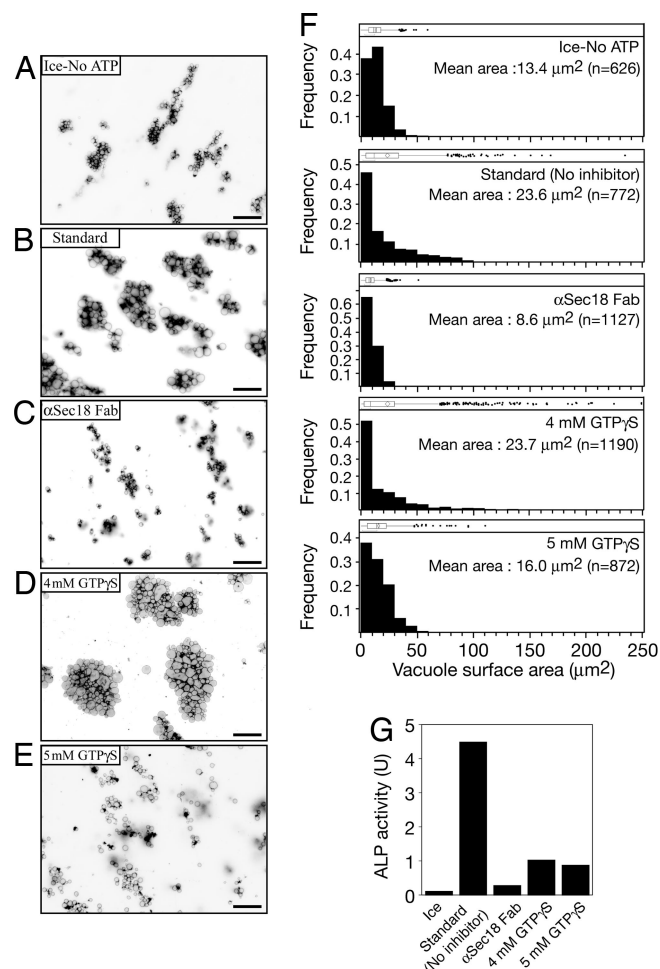


Fig. 2. GTP γ S allows fusion but prevents pro-ALP activation. Fusion reactions were performed under standard conditions but with 10 μ g of vacuoles. After 90 min, aliquots were stained with 3.3 μ M MDY-64 for fluorescent microscopy (A–F) or assayed for ALP activity (G). Images were acquired and analyzed by using ImageJ 1.36b and JMP 5.0.1a (SAS Institute). Vacuole diameters were measured from micrographs taken from random fields, and the surface areas of individual vacuoles were plotted as distribution histograms (F). A representative field for each condition is shown (Scale bars, 20 μ m).

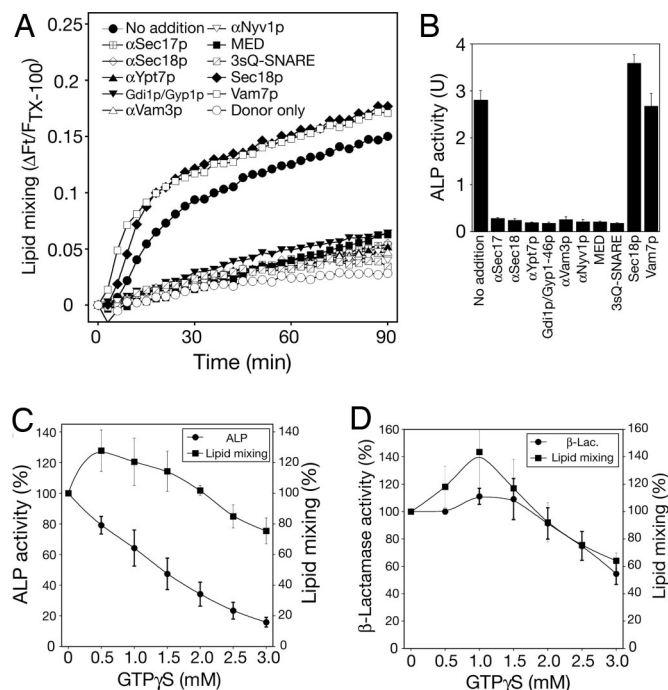


Fig. 3. The R18-dequenching lipid mixing assay. (A) Vacuoles were labeled with R18, mixed with unlabeled vacuoles in lipid mixing assay reactions containing anti-Sec17p (154 nM), anti-Sec18p (142 nM), anti-Ypt7p (167 nM), Gdi1p (1.2 μ M)/Gyp1–46p (5 μ M), anti-Vam3p (444 nM), anti-Nyv1p (261 nM), MED (10 μ M), 3sQ-SNARE (2 μ M), recombinant Sec18p (63 nM), rVam7p (40 nM), or buffer only (No addition), and lipid mixing was monitored at 27°C. One reaction did not receive unlabeled acceptor vacuoles (open circles). (B) After 90 min, ALP activity was measured. (C and D) Vacuoles isolated from BJ3505-Fos- ω were labeled with R18, reisolated, and added to lipid mixing assay reactions containing unlabeled DKY6281 vacuoles (C) or unlabeled vacuoles from BJ3505- α -Jun (D). Lipid mixing (squares) was monitored by R18 dequenching under low Mg^{2+} condition at 27°C for 90 min with the indicated concentrations of GTP- γ S. After 90 min, the reactions were assayed for ALP activity (C; circles) or β -lactamase activity (D; circles). Values obtained without inhibitors [2.31 ± 0.46 units for the ALP assay coupled with the R18 assay ($\Delta F_{90min}/F_{TX100} = 0.102 \pm 0.047$); 1.53 ± 0.07 units for the β -lactamase assay coupled with the R18 assay ($\Delta F_{90min}/F_{TX100} = 0.201 \pm 0.023$)] were used as the 100% reference. Data represent mean \pm SEM ($n = 3$).

standard reaction of 772 scored (11%), and the same proportion, 118 of 1,190 scored (10%), acquired this size during incubation with 4 mM GTP γ S, none acquired this size among either the 626 scored that had been incubated on ice without ATP or from the 1,127 scored that had been incubated with anti-Sec18 Fab. Thus, although high concentrations of GTP γ S can inhibit fusion, GTP γ S inhibition of the formation of active ALP is not a reliable indicator of fusion inhibition.

To assay lipid mixing between fusing vacuoles, vacuoles were labeled with self-quenching concentrations of octadecyl rhodamine B (R18). Fusion of R18-labeled donor vacuoles with unlabeled acceptor vacuoles permits dequenching through dilution of R18, as reported for rhodamine-phosphatidylethanolamine (PE) labeled vacuoles (9, 14). The increase in R18 fluorescence required unlabeled acceptor vacuoles (Fig. 3*A*, filled vs. open circles), was accelerated by recombinant Vam7p (open squares) or Sec18p (filled diamonds) and was blocked by priming inhibitors such as antibodies to Sec17p or Sec18p, Rab inhibitors including antibodies to Ypt7p and Gdi1p/Gyp1-46p, SNARE inhibitors such as a mixture of three soluble domains of vacuolar Q-SNAREs (15) and antibodies to Vam3p or Nvy1p or the phosphoinositide ligand MED, all of which inhibited the

fusion in these same incubations (Fig. 3*B*). R18 has been extensively used in HA protein-mediated hemifusion studies (3–5, 16, 17). Because R18-dequenching during vacuole fusion requires unlabeled acceptor vacuoles, is sensitive to known fusion inhibitors, and can be stimulated by known fusion factors, it represents the authentic fusion pathway rather than R18 translocation (18) or fusion-independent R18 transfer. We compared the two content mixing assays, each coupled with the R18 dequenching assay, in the presence of GTP γ S (Fig. 3*C* and *D*). Low concentrations of GTP γ S allowed or even stimulated lipid mixing (Fig. 3*C*, squares) while preventing the development of active ALP (circles), as reported (9). In contrast, when we assayed lipid and content mixing simultaneously by incubating R18-labeled BJ3505 vacuoles that contain Fos- ω with unlabeled BJ3505 vacuoles that contain α -Jun (Fig. 3*D*), GTP γ S showed only modest and similar inhibition of both lipid mixing and content mixing. Differences in dequenching between these experiments (Fig. 3*C* and *D*) may reflect that vacuoles from DKY6281 are only present in the ALP assay. We therefore sought other means of resolving the late stages of fusion.

To examine late reaction stages, docking can be synchronized. A portion of the membrane-anchored vacuole SNAREs is unpaired and ready to pair in trans, whereas Vam7p, which lacks

Fos- ω or BJ3505- α -Jun (13). Standard β -lactamase fusion reactions (60 μ l) contained 6 μ g of vacuoles containing the fusion protein Fos- ω , 6 μ g of vacuoles with α -Jun, the same reaction buffer, ATP, creatine phosphate, creatine kinase, and CoA as for the ALP fusion assay, and 11 μ M recombinant GST-Fos protein (to reduce any background signal due to lysis). After 90 min at 27°C, the reactions were transferred to ice, 140 μ l of ice-cold nitrocefin developing buffer [100 mM NaPi (pH 7.0)/150 μ M nitrocefin/0.2% (vol/vol) Triton X-100] was added, and the mixture was placed in a 96-well clear plate with flat bottom (Corning Life Sciences, Corning, NY). Nitrocefin hydrolysis was measured by absorbance at 492 nm every 15 seconds for 5–10 min at 30°C with a VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA); the absorbance increased linearly during this time interval, and the slope was determined. A blank reference well contained 150 μ l from a sample containing 6 μ g of Fos- ω vacuole, 6 μ g of α -Jun vacuole, 11 μ M recombinant GST-Fos protein, and 140 μ l of nitrocefin developing buffer in 200 μ l. One fusion unit of the β -lactamase fusion assay is defined as 1 nmol of hydrolyzed nitrocefin per minute from 12 μ g of vacuole protein. One hundred nmol of hydrolyzed nitrocefin equals 0.53 of Δ OD₄₉₂ (31).

Lipid Mixing Assay Using Octadecyl Rhodamine B (R18) Dequenching. Freshly prepared BJ3505 vacuoles (300 μ g) were suspended in 400 μ l of PS buffer containing 150 μ M R18 in an amber microcentrifuge tube. After mixing gently with a wide-bore pipette tip, the mixture was incubated (10 min, 4°C) with rocking. PS buffer (600 μ l) with 15% (wt/vol) Ficoll was added, and the suspension was transferred to a Polyallomer tube (11 \times 60 mm) (Beckman, Fullerton, CA), overlaid with 1.2 ml of PS buffer with 8% (wt/vol) Ficoll, 1.2 ml of PS buffer with 4% (wt/vol) Ficoll, and 0.5 ml of PS buffer without Ficoll, centrifuged [20 min, 4°C, 105,200 \times g, SW 60 Ti (Beckman)], and labeled vacuoles were recovered from the 0%/4%-Ficoll interface by using a wide-bore pipette tip. Standard lipid mixing assays (180 μ l) contained 4 μ g of R18-labeled vacuoles, 32 μ g of unlabeled vacuoles, and the same reaction buffer, ATP, creatine phosphate, creatine kinase, and CoA as in fusion assays. Reaction mixtures (150 μ l) were transferred to a 96-well flat-bottom assay plate with nonbinding surface (Corning). Fluorescence change was measured with a SpectraMax GeminiXPS fluorescent plate reader (Molecular Devices) at 27°C, 560-nm excitation and 590-nm emission as

described (9) with minor modifications. Measurements were taken every 2–3 min for 60–90 min, yielding fluorescence values at the onset (F_0) and during the reaction (F_t). The final 10 measurements of a sample containing 0.33% (vol/vol) Triton X-100 was averaged and used as a value for the fluorescence after infinite dilution (F_{TX100}). The relative total fluorescence change $\Delta F_t/F_{TX100} = (F_t - F_0)/F_{TX100}$ was calculated. Fusion-dependent fluorescence change was calculated by subtracting fusion-independent fluorescence change values represented by a sample containing only labeled donor vacuoles from total fluorescence change values.

Assay of trans-SNARE Complexes. Assays of trans-SNARE complexes were performed as described (23) with modifications. Briefly, standard trans-SNARE and fusion assays (16 \times) contained 48 μ g of vacuoles isolated from the BJ3505-CBP-Vam3p *nyv1* Δ yeast strain and 48 μ g of vacuoles isolated from DKY6281 yeast strain. After 60 min, reactions were placed on ice (5 min). From each 16 \times reaction, 30 μ l was withdrawn to assay Pho8p maturation, and the rest (450 μ l) was centrifuged (11,000 \times g, 5 min, 4°C). The supernatant was removed, and the sedimented vacuoles were overlaid with ice-cold solubilization buffer [200 μ l of 20 mM TrisCl (pH 7.5)/150 mM NaCl/1 mM MgCl₂/0.5% Nonidet P-40 Alternative (Calbiochem)/10% glycerol/1 \times protease inhibitor mixture (0.46 μ g/ml leupeptin/3.5 μ g/ml pepstatin/2.4 μ g/ml pefabloc-SC/1 mM PMSF], resuspended on ice, and solubilization buffer was added to a final volume of 600 μ l. The extracts were mixed on a nutator rocker (20 min, 4°C), and the detergent insoluble material was removed by centrifugation (16,000 \times g, 20 min, 4°C). Ten percent of the extract was removed for a total sample, and the remaining extract was brought to 2 mM CaCl₂. The CBP-Vam3p was recovered with calmodulin affinity beads (50 μ l per 600 μ l of extract; Stratagene, La Jolla, CA) on a nutator at 4°C overnight. Beads were collected by brief centrifugation (4,000 \times g, 2 min, 4°C) and suspended five times with the solubilization buffer, followed by bead sedimentation. Bound proteins were eluted by boiling beads in SDS sample buffer containing 5 mM EGTA (94°C, 5 min) for SDS/PAGE analysis and immunoblotting.

We thank Dr. Helen Blau for reagents, Drs. Charles Barlowe and Ta Yuan Chang for suggestions, and Naomi Thorngren for excellent technical assistance. This work was supported by a grant from the National Institute of General Medical Sciences.

- Cheever ML, Sato TK, de Beer T, Kutateladze TG, Emr SD, Overduin M (2001) *Nat Cell Biol* 3:613–618.
- Stroupe C, Collins KM, Fratti RA, Wickner W (2006) *EMBO J* 25:1579–1589.
- Kemble GW, Danieli T, White JM (1994) *Cell* 76:383–391.
- Melikyan GB, White JM, Cohen FS (1995) *J Cell Biol* 131:679–691.
- Chernomordik LV, Frolov VA, Leikina E, Bronk P, Zimmerberg J (1998) *J Cell Biol* 140:1369–1382.
- Xu Y, Zhang F, Su Z, McNew JA, Shin YK (2005) *Nat Struct Mol Biol* 12:417–422.
- Yoon TY, Okumus B, Zhang F, Shin YK, Ha T (2006) *Proc Natl Acad Sci USA* 103:19731–19736.
- Giraud CG, Hu C, You D, Slovic AM, Mosharov EV, Sulzer D, Melia TJ, Rothman JE (2005) *J Cell Biol* 170:249–260.
- Reese C, Heise F, Mayer A (2005) *Nature* 436:410–414.
- Wehrman T, Kleaveland B, Her JH, Balint RF, Blau HM (2002) *Proc Natl Acad Sci USA* 99:3469–3474.
- Johnson LM, Bankaitis VA, Emr SD (1987) *Cell* 48:875–885.
- Conradt B, Haas A, Wickner W (1994) *J Cell Biol* 126:99–110.
- Haas A, Conradt B, Wickner W (1994) *J Cell Biol* 126:87–97.
- Reese C, Mayer A (2005) *J Cell Biol* 171:981–990.
- Jun Y, Thorngren N, Starai VJ, Fratti RA, Collins K, Wickner W (2006) *EMBO J* 25:5260–5269.
- Chernomordik LV, Leikina E, Frolov V, Bronk P, Zimmerberg J (1997) *J Cell Biol* 136:81–93.
- Melikyan GB, Brenner SA, Ok DC, Cohen FS (1997) *J Cell Biol* 136:995–1005.
- Melikyan GB, Deriy BN, Ok DC, Cohen FS (1996) *Biophys J* 71:2680–2691.
- Collins KM, Thorngren NL, Fratti RA, Wickner WT (2005) *EMBO J* 24:1775–1786.
- Merz AJ, Wickner WT (2004) *Proc Natl Acad Sci USA* 101:11548–11553.
- Merz AJ, Wickner WT (2004) *J Cell Biol* 164:195–206.
- Thorngren N, Collins KM, Fratti RA, Wickner W, Merz AJ (2004) *EMBO J* 23:2765–2776.
- Collins KM, Wickner WT (2007) *Proc Natl Acad Sci USA* 104:8755–8760.
- Klionsky DJ, Emr SD (1989) *EMBO J* 8:2241–2250.
- Eitzen G, Will E, Gallwitz D, Haas A, Wickner W (2000) *EMBO J* 19:6713–6720.
- Fratti RA, Jun Y, Merz AJ, Margolis N, Wickner W (2004) *J Cell Biol* 167:1087–1098.
- Wang L, Seeley ES, Wickner W, Merz AJ (2002) *Cell* 108:357–369.
- Cho SJ, Kelly M, Rognien KT, Cho JA, Horber JK, Jena BP (2002) *Biophys J* 83:2522–2527.
- Han X, Wang CT, Bai J, Chapman ER, Jackson MB (2004) *Science* 304:289–292.
- Jones EW (2002) *Methods Enzymol* 351:127–150.
- O'Callaghan CH, Morris A, Kirby SM, Shingler AH (1972) *Antimicrob Agents Chemother* 1:283–288.