Stringent 3Q·1R Composition of the SNARE 0-Layer Can Be Bypassed for Fusion by Compensatory SNARE Mutation or by Lipid Bilayer Modification* [S]

Rutilio A. Fratti, Kevin M. Collins, Christopher M. Hickey, and William Wickner

From the Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755

SNARE proteins form bundles of four α-helical SNARE domains with conserved polar amino acids, 3Q and 1R, at the “0-layer” of the bundle. Previous studies have confirmed the importance of 3Q·1R for fusion but have not shown whether it regulates SNARE complex assembly or the downstream functions of assembled SNAREs. Yeast vacuole fusion requires regulatory lipids (ergosterol, phosphoinositides, and diacylglycerol), the Rab Ypt7p, the Rab-effector complex HOPS, and 4 SNAREs: the Q-SNAREs Vti1p, Vam3p, and Vam7p and the R-SNARE Nyv1p. We now report that alterations in the 0-layer SNAREs: the Q-SNAREs Vti1p, Vam3p, and Vam7p and the R-SNARE Nyv1p. From the Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755.

SNARE function as regulated by the 0-layer is intimately coupled to the lipids, which must rearrange for fusion.

SNARE proteins form bundles of four α-helical SNARE domains with conserved polar amino acids, 3Q and 1R, at the “0-layer” of the bundle. Previous studies have confirmed the importance of 3Q·1R for fusion but have not shown whether it regulates SNARE complex assembly or the downstream functions of assembled SNAREs. Yeast vacuole fusion requires regulatory lipids (ergosterol, phosphoinositides, and diacylglycerol), the Rab Ypt7p, the Rab-effector complex HOPS, and 4 SNAREs: the Q-SNAREs Vti1p, Vam3p, and Vam7p and the R-SNARE Nyv1p. We now report that alterations in the 0-layer SNAREs: the Q-SNAREs Vti1p, Vam3p, and Vam7p and the R-SNARE Nyv1p. From the Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755.
**Vacuole SNARE 0-Layer**

ring,” which surrounds the boundary membrane (22–25). Vacuole SNAREs form *trans*-complexes between the apposed organelles (17). Ypt7p and HOPS are needed for SNARE complex assembly, and HOPS is an integral part of the assembled SNARE complex (26). Fusion occurs around the vertex ring, joining the outside membrane from each vacuole to form the larger, fused organelle and joining the two apposed boundary membrane discs to yield a luminal vesicle (22, 27).

Vacuoles have four SNAREs; their roles have been studied in the organelle-based fusion reaction (26, 28) and in liposome-based studies (29). Vam3p, Vam7p, and Vit1p are Q-SNAREs, which constitute the t-SNARE, and the R-SNARE Nyv1p can serve as the v-SNARE. Three of these have C-terminal *trans*-membrane anchors, but Vam7p has no apolar membrane anchor. Whereas most purified SNAREs require detergent for their solubility, and thus cannot be directly added to purified organelles without causing lysis, recombinant Vam7p is water-soluble without detergent and supports vacuole fusion (30).

Although SNAREs are central to fusion, it remains unclear how they act. SNAREs may apply torque to membranes (31, 32), destabilize bilayers through poorly fitting TM domains (33, 34), destabilize bilayers directly coupled to the lipid bilayer properties. Modifies the lipid bilayer, suggesting that 0-layer function is additive in the absence or presence of equimolar mixtures of soluble His6-Vam3p, MBP-Vit1p, and GST-Vam7p. Mixtures contained either wild-type Vam7p, Vam7pY42A (Y42A), Vam7pG283R (Q283R), or Vam7pYAQR (YA/QR).

**ExPERIMENTAL PROCEDURES**

**Strains**—The strains used for fusion assays were BJ3505 (MATα pep4::HIS3 prb1::Δ1.68 his3::200 lys2::801 trp1::101 (gal3) ura3::52 gal2 can1) (38) and DKY6281 (MATα leu2–3 leu 2–112 3gal2::52 his3::Δ200 trp1::101 lys2::801) (16). BJ3505 5′-GCTGTTAGACATCGAGAAGTTTCTTTATATTACCCAGGATCCGGC-3′ containing a BamHI site.

FIGURE 1. Equivalent function of wild-type and mutant Vam7p in inhibitory 3Q complexes. Standard fusion reactions (“Experimental Procedures”) were incubated for 90 min in the absence or presence of equimolar mixtures of soluble His6-Vam3p, MBP-Vit1p, and GST-Vam7p. Mixtures contained either wild-type Vam7p, Vam7pY42A (Y42A), Vam7pG283R (Q283R), or Vam7pYAQR (YA/QR).

**Reagents**—Reagents were dissolved in PS buffer (20 mM PIPES-KOH, pH 6.8, 200 mM sorbitol). Myristoylated alanine-rich C kinase substrate-effector domain (KKKKKRFSFKSK-LSNGFSFKKKKK, Keck Center, Yale) was dissolved at 10 μM in PS buffer. Anti-Vam3p F10 (24), anti-Vps33p (21), and anti-Sec17p (40) were previously described. His6-Sec18p was prepared as described (40). His6-Sec1p was further purified by gel filtration (30). Gdi1p (41), His6-Gyp1–46p (24), and IB2 (42) were prepared as described.

**Recombinant Vam7p**—The VAM7 open reading frame, excluding the first methionine, was amplified by BY4742 chromosomal DNA using forward primer 5′-GGGCGATCC-CCAGCTTTCTGATGGGAAA-3′ with a BamHI restriction site (bold), and reverse primer 5′-CGCGAATTCTCAAGGACCGAAGAGTTTCTTTATATTACCCAGGATCCGGC-3′ with an EcoRI site. Digested PCR product was ligated to EcoRI/BamHI-linearized DNA.

**Experimental procedures**

**Strains**—The strains used for fusion assays were BJ3505 (MATα pep4::HIS3 prb1::Δ1.68 his3::200 lys2::801 trp1::101 (gal3) 3gal2::52 his3::Δ200 trp1::101 lys2::801) (16). BJ3505 5′-GCTGTTAGACATCGAGAAGTTTCTTTATATTACCCAGGATCCGGC-3′ containing a BamHI site. The product was subcloned into pRS406 using HindIII and BamHI sites, generating the plasmid pRS406-pNYV1. The NYV1 3′-untranslated region containing the terminator was amplified from DKA6281 genomic DNA with forward primer 5′-TCCCTCTAGATGAAACGCTTTAATGTATGTAT-3′ and reverse primer 5′-GGGCGATCC-CCAGCTTTCTGATGGGAAA-3′ with a SacI site. The resulting PCR product was subcloned into pRS406-pNYV1 using XbaI and SacII sites, generating pRS406-pNYV1-NYV1. QuikChange mutagenesis (38) was used to generate pRS406-pNYV1Y42A/Q283R, using the forward primer 5′-AACATCGACAATCTTCTTGGAGCAACAGAAGAGTTTCTTTATATTACCCAGGATCCGGC-3′ and reverse primer 5′-CAACAAATAGAACAAATCTTCTTGGAGCAACAGAAGAGTTTCTTTATATTACCCAGGATCCGGC-3′. Fusion reactions were performed using BJ3505 NYV1 Δ and DKA6281 NYV1 Δ, respectively, with pRS406-pNYV1Y42A/Q283R and standard lithium acetate methods and plated on complete synthetic media lacking uracil.
pET42a(+) to generate pET42a-GST-Vam7p. Sequenced plasmid was transformed into *Escherichia coli* Rosetta-2 (DE3) pLysS (Novagen) and grown on Luria broth with kanamycin and chloramphenicol. Using pET42a-GST-VAM7 as a template, QuikChange mutagenesis was used to create VAM7 Y42A, VAM7 Q283R, and VAM7 Y42A/Q283R. Forward primer 5′-AACAAGGCGCCTTTACAAGGAGATCCGAGGTGTTTTGGAAACTGAAG-3′ and reverse primer 5′-CTTCAGTTTCCAAAAACCGATGCCGCTTTTGAAGGCCTTTTGTAAAGGCGCTTGTT-3′ were used to make VAM7 Y42A. To make VAM7 Q283R we used 5′-GAGATGAGAGGAGCTGCAAACGAGAATGAGCTACTTACAGCT-3′ and 5′-AAGTGTGTTAGAATGCTCATTCGCCGTGTGAGCTCTGTCCTATCTC-3′.

**RESULTS**

Because Vam7p has no apolar membrane anchor, it can be produced in bacteria, purified in the absence of detergent, and added to *in vitro* fusion assays. To assess the role of the Vam7p C-terminal SNARE domain, we mutated its zero-layer glutamine to arginine (Q283R) to disrupt the 3Q:1R ratio. As a first test of this mutant Vam7p, we mixed bacterially expressed GST-Vam7, cultures were grown in 1 liter of Terrific broth at 37 °C to an *A*<sub>600</sub> of 0.6. Cells were induced with 500 μM isopropyl-β-D-galactopyranoside at 37 °C for 4 h, collected by centrifugation, and washed with lysis buffer (50 mM Tris-Cl, pH 8, 500 mM NaCl, 1 mM dithiothreitol, 2 mM EGTA, 1 mM EDTA) with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μM Pefabloc-SC, 1 μM leupeptin, and 5 μM pepstatin). Cells were lysed by 3 passages through a French pressure cell. Lysates were incubated with GSH-Sepharose 4B resin equilibrated with lysis buffer (16 h, 4 °C, nutating). Resin was washed with 20 column volumes of lysis buffer. GST-Vam7p was eluted with lysis buffer containing 10 mM reduced glutathione. Proteins were dialyzed against PS buffer with 125 mM KCl and 5 mM MgCl<sub>2</sub>.

**Vacuole Isolation and in Vitro Fusion Assay**—Vacuoles were isolated from the yeast strains BJ3505 (38) and DKY6281 (16). Fusion reactions (30 μl) contained 3 μg of BJ3505 vacuoles with inactive pro-Pho8p (pro-alkaline phosphatase) and lacking the protease Pep4p, 3 μg of DKY6281 vacuoles containing Pep4p but lacking Pho8p, standard fusion reaction buffer (125 mM KCl, 5 mM MgCl<sub>2</sub>, 20 mM PIPES-KOH, pH 6.8, 200 mM sorbitol), ATP regenerating system (1 mM ATP, 40 mM creatine phosphate, 0.1 mg/ml creatine kinase), 10 μM coenzyme A (18), and 930 nM I<sub>2</sub>B (inhibitor of protease B). After 90 min at 27 °C, Pho8p activity was assayed in 250 mM Tris-Cl, pH 8.5, 0.4% Triton X-100, 10 mM MgCl<sub>2</sub>, 1 mM *p*-nitrophenyl phosphate. One unit of fusion is 1 μmol of *p*-nitrophenolate produced per min per μg of BJ3505 vacuoles. *p*-Nitrophenolate absorbance was measured at 400 nm.

**FIGURE 2. Regulation of fusion by the 0-layer of Vam7p and Nyv1p.** Fusion assays were performed using vacuoles harvested from yeast strains BJ3505 and DKY6281 (A–C), which are wild-type for SNAREs but permit assay of fusion (“Experimental Procedures”) or from their isogenic strains RFY1 and RFY2 harboring Nyv1p<sup>R192Q</sup> (D–F). Vacuoles were incubated in the absence or presence of added recombinant wild-type or mutant Vam7p at the indicated concentrations. Vam7p was present from the start of the fusion incubation. Reactions were under standard conditions, as under “Experimental Procedures” (A and D) or α-Sec17 bypass (B and E) or no ATP bypass conditions (C and F) as described in (30). Fusion was measured by alkaline phosphatase activity and expressed in units.
recombinant Vam7p or Vam7pQ283R with the recombinant soluble cytoplasmic domains of Vti1p and Vam3p prior to introducing vacuoles and assaying vacuole fusion. The mixed soluble domains of the three Q-SNAREs interact directly with Nyv1p to form nonfunctional SNARE bundles and block vacuole fusion (43). Although this inhibition requires all three of the Q-SNARE-soluble domains, it is simpler than the physiological SNARE complex assembly, because it bypasses regulation by Ypt7p (43). The inhibitory effect of the mixed soluble domains of the t-SNAREs was undiminished by violation of the 3Q/H185281R rule or by another mutation, which we characterize in detail elsewhere,5 that disrupts the affinity of Vam7p for phosphatidylinositol 3-phosphate (Fig. 1). Because Vam7pQ283R can still interact with other SNAREs to regulate fusion, it warranted further characterization.

Fusion Activity of Vam7Q283R Protein—Although the other, integral-membrane SNAREs are present on isolated vacuoles in the unpaired state as well as in SNARE complexes, all the Vam7p on purified vacuoles appears to be in cis-SNARE complexes (26). In standard fusion reactions with ATP, the disassembly of these complexes by Sec18p and Sec17p provides the Vam7p for trans-SNARE complex formation. For this reason, the requirement for Sec18p/Sec17p and for ATP can be bypassed by added Vam7p (30). We first tested the effects of adding recombinant wild-type and mutant Vam7p to our standard fusion reaction, which does not required exogenously added Vam7p. The addition of either wild-type Vam7p, Vam7pQ283R, or other mutants had little effect on fusion (Fig. 2A). Recombinant wild-type Vam7p can “bypass” the need for priming, the disassembly of cis-SNARE complexes, by forming complexes with the unpaired vacuolar SNAREs (30). In bypass fusion assays, priming is blocked by either the absence of ATP or, in the presence of ATP, by antibody to Sec17p. Although wild-type Vam7p supported bypass fusion (circles, Fig. 2, B and C), Vam7pQ283R did not support the bypass fusion of wild-type vacuoles (triangles, Fig. 2, B and C). These assays allow direct determination of whether Vam7pQ283R can compete with wild-type Vam7p. Even when Vam7p and Vam7pQ283R were pre-mixed prior to addition to vacuoles, a 100-fold excess of Vam7pQ283R only gave modest inhibition of Vam7p-supported fusion (Fig. 3). In these studies, Vam7pQ283R was added at up to micromolar levels, a large excess over the endogenous Vam7p (30). Because Vam7pQ283R binds to vacuoles as well as wild-type Vam7p (data not shown) but does not support fusion (Fig.

5 Fratti, R. A., and Wickner, W. (2007) J. Biol. Chem. 282, 13133–13138.

FIGURE 3. Vacuoles discriminate between Vam7p and Vam7pQ283R according to the 0-layer. Vacuoles blocked for priming with α-Sec17 antibody or by ATP omission (30) were incubated with indicated amounts of premixed wild-type Vam7p and Vam7pQ283R. Reactions were incubated for 90 min, and fusion was measured by alkaline phosphatase activity. Data represent mean fusion ± S.E. (n = 3).
Vacuole SNARE 0-Layer

2, B and C), vacuoles show exquisite discrimination for Vam7p with the normal Gln residue at the 0-layer position.

Vam7pQ283R Stimulates the Fusion of Vacuoles Bearing Nyv1pR192Q—To test whether Vam7pQ283R is merely denatured or could under some circumstances form functional SNARE complexes, we replaced the 0-layer arginine of Nyv1p with a glutamine and purified vacuoles from yeast harboring Nyv1pR192Q. In a standard fusion reaction with ATP but without added Vam7p, vacuoles bearing Nyv1pR192Q were unable to fuse (Fig. 2D), suggesting that four Q-SNAREs are not readily primed (44). In standard fusion reactions, the same Vam7pQ283R that was inert with vacuoles bearing the wild-type Nyv1p supported the fusion of Nyv1pR192Q vacuoles with a K_m of ~50 nM (Fig. 2D). Surprisingly, wild-type Vam7p also promoted fusion of Nyv1pR192Q with a similar K_m, albeit only ~60% as well as Vam7pQ283R. Thus, as seen in other studies (45), the 3Q-1R rule is not inviolate; fusion can occur with four Q-SNAREs. When priming was blocked in the presence of ATP by the addition of anti-Sec17p, Vam7pQ283R or wild-type Vam7p supported fusion (Fig. 2E), as seen under standard fusion conditions (Fig. 2D).

In the absence of ATP (Fig. 2F), Vam7pQ283R promoted fusion of Nyv1pR192Q vacuoles whereas wild-type Vam7p did not, just as vacuoles bearing wild-type Nyv1p could only undergo bypass fusion with wild-type Vam7p (Fig. 2C). However, the concentration of Vam7pQ283R needed for fusion of Nyv1pR192Q vacuoles was ~100 times greater than with wild-type Vam7p and Nyv1p (Fig. 2, F versus C). It may take far more energy to form SNARE bundles containing both Vam7pQ283R and Nyv1pR192Q than with wild-type Vam7p and Nyv1p, even though both have 3Q-1R 0-layers. This is consistent with energy being needed to distort the SNARE α-helical backbone at the 0-layer to accommodate the altered positions of bulky arginine versus the less bulky glutamine, or with “proofreading” by a SNARE-bound factor such as HOPS. Once formed, these 3Q-1R complexes have the same capacity to promote fusion. A detailed examination of the specific protein and lipid requirements for fusion supported by each form of Vam7p, with wild-type vacuoles or Nyv1pR192Q vacuoles, is presented in the supplemental Fig. S1.

Physical Interactions of Vam7p with SNAREs and HOPS—Because Vam7p mutant proteins bind to vacuoles in a manner indistinguishable from wild-type Vam7p yet differentially support fusion, we examined their capacities to form protein complexes with SNAREs and HOPS. For this study, vacuole fusion was blocked by antibody to Sec17p for 15 min, secondary inhibitors were added, and, after 5 min, the priming block was rescued by the addition of Vam7p (Fig. 4A). As reported (26), wild-type Vam7p relieves the anti-Sec17p block to allow fusion (Fig. 4B, filled bars) and enters into complexes with SNARES and HOPS (Fig. 4C, left panel). These interactions were inhibited by antibodies to Vam3p or Vps33p. Strikingly, Vam7pQ283R, although it does not support the fusion of wild-type vacuoles (Figs. 2B, 2C, and 4B, open symbols), forms stable and isolable HOPS-SNARE complexes (Fig. 4C) which contained Nyv1p and are thus 2Q-2R. The formation of this complex is also inhibited by antibody to Vam3p or to the HOPS subunit Vps33p, the SM protein of the vacuole.

Bypass of the 2Q-2R Block—Because Vam7pQ283R binds to vacuoles and enters SNARE complexes as readily as wild-type Vam7p, but with little or no consequent fusion, we sought conditions that might activate fusion in this 2Q-2R system when priming was blocked by either antibody to Sec17p or by the omission of ATP. Bypass fusion with Vam7pQ283R in the absence of ATP was restored by the addition of the phospholipase C inhibitor U73122, but restoration was not seen in the presence of ATP (Fig. 5A). Strikingly, bypass fusion with Vam7pQ283R in the presence of ATP, when priming was blocked by antibody to Sec17p, was restored by the addition of chlorpromazine (Fig. 5B), and this restoration required ATP. In each case, the restored fusion required the normal fusion pathway, because it was blocked by characterized inhibitors that target SNAREs, Ypt7p, and HOPS. Even though bypass fusion with wild-type Vam7p is unaffected by ATP (30), ATP directly regulates the ability of U73122 or chlorpromazine to restore 2Q-2R fusion. To further examine this bypass, we characterized the effects of chlorpromazine on both the physical associations of added Vam7pQ283R as well as on the functional restoration of fusion for the same samples (Fig. 6). Chlorpromazine had no measurable effect on the binding of Vam7pQ283R to vacuoles (Fig. 6B, top panel). Chlorpromazine had only a small effect on the association of vacuole-bound Vam7pQ283R with HOPS or with other SNAREs (Fig.

![FIGURE 5. Vacuole 2Q-2R fusion, with Vam7pQ283R and vacuoles bearing wild-type Nyv1p, can be restored by U73122 or chlorpromazine. Bypass fusion (30) was assayed either without ATP or with ATP present but in the presence of antibody to Sec17p. Fusion reactions were incubated for 10 min, and inhibitors were added and incubated for 5 min before addition of 1.6 μM U73122 (A) or 150 μM chlorpromazine (CPZ) (B). U73122 and chlorpromazine were allowed to act for 5 min before addition of 100 nM Vam7pQ283R. Reactions were incubated for an additional 70 min, and then assayed for fusion. Fusion inhibitors were 120 nM affinity-purified α-Nvy1p, 353 nM α-Vam3p IgG, 133 nM affinity-purified α-Ypt7p, 32 nM affinity-purified α-Vps33p, 2.8 μM GDP dissociation inhibitor protein (GDI), and 11.4 μM Gyp1–46. Data represent mean fusion ± S.E. (n = 3).](image-url)
Vacuole SNARE 0-Layer

**A**

\[ \alpha\text{-Sec17} \rightarrow \pm \text{Inhibitors} \rightarrow \pm \text{CPZ} \rightarrow \pm \text{Vam7p} \rightarrow [\text{GST pulldown}] \]

\[ 0' - 10' - 15' - 20' - / / - 90' \]

With vacuoles bearing the wild-type R-SNARE Nyv1p, Vam7pQ283R is inactive for fusion, yet forms an HOPS-SNARE complex of 2Q-2R composition. Vacuoles bearing Nyv1pR192Q can fuse when given either Vam7pQ283R, restoring the 3Q-1R composition of the 0-layer, or wild-type Vam7p, yielding a 4Q 0-layer. These findings are in accord with studies showing that 4Q SNARE complexes are functional for exocytosis (46) or endoplasmic reticulum to Golgi traffic (45), whereas 2Q-2R complexes are at least somewhat defective (45). Studies with recombinant neuronal SNAREs have shown that the syntaxin 0-layer Gln is essential for NSF factor and \( \alpha\text{-SNAP} \)-mediated SNARE bundle disassembly (44), and this may account for some of the loss of fusion that accompanies mutation of the Vam3p 0-layer Gln to Arg in an earlier study (47). However, diminished disassembly of cis-SNARE complexes is not a factor in our current studies in which the action of Sec18p(NSF)/Sec17p(\( \alpha\text{-SNAP} \)) is blocked, by antibody or by the absence of ATP.

**DISCUSSION**

What is the role of the 0-layer in vacuole fusion? Our studies show that the composition of glutamine and arginine residues in the 0-layer and their precise spatial distribution control the energy needed to assemble functional SNARE complexes, because normal bypass fusion has a \( K_m \) for Vam7p of only 3–8 nM, whereas far higher concentrations of mutant or wild-type Vam7p are needed for 4Q fusion (Fig. 2). Once formed, SNARE complexes with 2Q-2R are stable but inactive, yet fusion can be restored when the membrane is perturbed by the intercalating amphiphile chlorpromazine or by the phospholipase C inhibitor U73122. These may modulate bilayer properties such that a “weakened” 2Q-2R complex is still capable of driving fusion.

Chlorpromazine is an amphipathic molecule that partitions into the negatively curved inner leaflets of membrane bilayers. Once intercalated into membranes, chlorpromazine alters the physical properties of bilayers. Upon insertion into inner leaflets, chlorpromazine deforms membranes to induce cupping (48) and can alter lateral diffusion and membrane tension (48). In doing so, chlorpromazine reduces relaxation times of membrane tethers by lowering thresholds for remodeling. This was seen in force measurement experiments using optical tweezers to pull membranes (49). Membrane remodeling by chlorpromazine may also alter phosphoinositide metabolism by activating both phosphoinositide kinases and phospholipase C (50). Each of these actions of chlorpromazine may lower the threshold for the bilayer rearrangements of fusion, permitting 2Q-2R SNARE complexes to function. Similarly, U73122 inhibits vacuolar phospholipase C activities (51) and thereby alters the ratio of two vacuolar lipids that are crucial for fusion, phosphatidylinositol 4,5-bisphosphate and diacylglycerol. Restoration of 2Q-2R fusion by chlorpromazine or U73122 is regulated by the presence or absence of ATP; one of the effects of ATP in this restoration may be to support phosphoinositide synthesis.

**SNAREs may promote membrane fusion by several means:**

1) They may provide physical stress on the bilayer, which could lower the activation energy for lipid rearrangements for fusion

2) They may promote membrane fusion by several means:

   a) They may provide physical stress on the bilayer, which could lower the activation energy for lipid rearrangements for fusion

   b) They may promote membrane fusion by several means:
Vacuole SNARE 0-Layer

REFERENCES

1. Jahn, R., and Scheller, R. H. (2006) Nat. Rev. Mol. Cell Biol. 7, 631–643
2. Sutton, R. B., Fasshauer, D., Jahn, R., and Brunger, A. T. (1998) Nature 395, 347–353
3. Fasshauer, D., Eliason, W. K., Brunger, A. T., and Jahn, R. (1998) Biochemistry 37, 10354–10362
4. Söllner, T., Bennett, M. K., Whiteheart, S. W., Scheller, R. H., and Rothman, J. E. (1993) Cell 75, 409–418
5. Weber, T., Zemelman, B. V., McNew, J. A., Westermann, B., Gmachl, M., Parlati, F., Söllner, T. H., and Rothman, J. E. (1998) Cell 92, 759–772
6. Nickel, W., Weber, T., McNew, J. A., Parlati, F., Söllner, T. H., and Rothman, J. E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12571–12576
7. Dennison, S. M., Bowen, M. E., Brunger, A. T., and Lentz, B. R. (2006) Biophys. J. 90, 1661–1675
8. Chen, X., Arac, D., Wang, T. M., Gilpin, C. J., Zimmerberg, J., and Rizo, J. (2006) Biophys. J. 90, 2062–2074
9. McNew, J. A., Parlati, F., Fukuda, R., Johnston, R. J., Paz, K., Paument, F., Söllner, T. H., and Rothman, J. E. (2000) Nature 407, 153–159
10. Tucker, W. C., Weber, T., and Chapman, E. R. (2004) Science 304, 435–438
11. Scott, B. L., Van Komen, J. S., Irshad, H., Liu, S., Wilson, K. A., and McNew, J. A. (2004) J. Cell Biol. 167, 75–85
12. Bhalla, A., Chicka, M. C., Tucker, W. C., and Chapman, E. R. (2006) Nat. Struct. Mol. Biol. 13, 323–330
13. Nichols, B. J., Ungermann, C., Pelham, H. R., Wickner, W. T., and Haas, A. (1997) Nature 387, 199–202
14. Conradt, B., Shaw, J., Vida, T., Emm, S., and Wickner, W. (1992) J. Cell Biol. 119, 1469–1479
15. Bone, N., Millar, J. B., Toda, T., and Armstrong, J. (1998) Curr. Biol. 8, 135–144
16. Haas, A., Conradt, B., and Wickner, W. (1994) J. Cell Biol. 126, 87–97
17. Ungermann, C., Nichols, B. J., Pelham, H. R., and Wickner, W. (1998) J. Cell Biol. 140, 61–69
18. Haas, A., Schegmann, D., Lazard, T., Gallwitz, D., and Wickner, W. (1995) EMBO J. 14, 5258–5270
19. Stroep, C., Collins, K. M., Fratti, R. A., and Wickner, W. (2006) EMBO J. 25, 1579–1589
20. Sato, T. K., Rehling, P., Peterson, M. R., and Emm, S. D. (2000) Mol. Cell 6, 661–671
21. Seals, D. F., Etzien, G., Margolis, N., Wickner, W. T., and Price, A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9402–9407
22. Wang, L., Seeley, E. S., Wickner, W., and Merz, A. J. (2002) Cell 108, 357–369
23. Etzien, G., Wang, L., Thomgren, N., and Wickner, W. (2002) J. Cell Biol. 158, 669–679
24. Wang, L., Merz, A. J., Collins, K. M., and Wickner, W. (2003) J. Cell Biol. 160, 365–374
25. Fratti, R. A., Jun, Y., Merz, A. J., Margolis, N., and Wickner, W. (2004) J. Cell Biol. 167, 1087–1098
26. Collins, K. M., Thomgren, N. L., Fratti, R. A., and Wickner, W. T. (2005) EMBO J. 24, 1775–1786
27. Merz, A. J., and Wickner, W. T. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 11548–11553
28. Ungermann, C., von Mollard, G. F., Jensen, O. N., Margolis, N., Stevens, T. H., and Wickner, W. (1999) J. Cell Biol. 145, 1435–1442
29. Fukuda, R., McNew, J. A., Weber, T., Parlati, F., Engel, T., Nickel, W., Rothman, J. E., and Söllner, T. H. (2000) Nature 407, 198–202
30. Thomgren, N., Collins, K. M., Fratti, R. A., Wickner, W., and Merz, A. J. (2004) EMBO J. 23, 2765–2776
31. Skehel, J. I., and Wiley, D. C. (1998) Cell 95, 871–874
32. McNew, J. A., Weber, T., Parlati, F., Johnston, R. J., Melia, T. J., Söllner, T. H., and Rothman, J. E. (2000) J. Cell Biol. 150, 105–117
33. Langosch, D., Crane, J. M., Brosig, B., Hellwig, A., Tamm, L. K., and Reed, J. (2001) J. Mol. Biol. 311, 709–721
34. Siegel, D. P., Cherezov, V., Greathouse, D. V., Koepp, R. E., 2nd, Killian, J. A., and Caffrey, M. (2006) Biophys. J. 90, 200–211
35. Han, X., Wang, C. T., Bai, J., Chapman, E. R., and Jackson, M. B. (2004) Science 304, 289–292
36. Cheever, M. L., Sato, T. K., de Beer, T., Kutateladze, T. G., Emr, S. D., and Overduin, M. (2001) Nat. Cell Biol. 3, 613–618
37. Fasshauer, D., Sutton, R. B., Brunger, A. T., and Jahn, R. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 15781–15786
38. Jones, E. W. (2002) Methods Enzymol. 351, 127–150
39. Wang, W., and Malcolm, B. A. (1999) BioTechniques 26, 680–682
40. Haas, A., and Wickner, W. (1996) EMBO J. 15, 3296–3305
41. Garrett, M. D., and Novick, P. J. (1995) Methods Enzymol. 257, 232–240
42. Slusarewicz, P., Xu, Z., Seefeld, K., Haas, A., and Wickner, W. T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5582–5587
43. Jun, Y., Thomgren, N., Starai, V. J., Fratti, R. A., Collins, K., and Wickner, W. (2006) EMBO J. 25, 5260–5269
44. Scales, S. J., Yoo, B. Y., and Scheller, R. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 14262–14267
45. Graf, C. T., Riedel, D., Schmitt, H. D., and Jahn, R. (2005) Mol. Biol. Cell 16, 2263–2274
46. Katz, L., and Brenwald, P. (2000) Mol. Biol. Cell 11, 3849–3858
47. Wang, Y., Dulubova, I. R., Jizo, J., and Sudhof, T. C. (2001) J. Biol. Chem. 276, 28598–28605
48. Oghalai, J. S., Zhao, H. B., Kutz, J. W., and Brownell, W. E. (2000) Science 287, 658–661
49. Murdoch, D. R., Ermilov, S. A., Spector, A. A., Popel, A. S., Brownell, W. E., and Anvari, B. (2005) Biophys. J. 89, 4090–4095
50. Raucher, D., and Sheetz, M. P. (2001) J. Cell Biol. 144, 3759–3766
51. Jun, Y., Fratti, R. A., and Wickner, W. (2004) J. Biol. Chem. 279, 53186–53195
52. Hofmann, M. W., Peplovska, K., Rohde, J., Poschner, B. C., Ungermann, C., and Langosch, D. (2006) J. Mol. Biol. 364, 1048–1060