Leading Edge
Essay

Cell

Membrane Curvature in Synaptic Vesicle Fusion and Beyond

Harvey T. McMahon,[1,*] Michael M. Kozlov,[2,*] and Sascha Martens[1,3,*]
[1]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB0 2QH, UK
[2]Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, 69978 Tel Aviv, Israel
[3]Max F. Perutz Laboratories, University of Vienna, Dr. Bohr-Gasse 9, A-1030 Vienna, Austria
*Correspondence: hmm@mrc-lmb.cam.ac.uk (H.T.M.), michk@post.tau.ac.il (M.M.K.), sascha.martens@univie.ac.at (S.M.)
DOI 10.1016/j.cell.2010.02.017

Recent evidence suggests that the Ca^{2+}-sensors synaptotagmin-1 and Doc2b deform synaptic membranes during synaptic vesicle exocytosis. We discuss how local curvature generated by these and other proteins may stimulate membrane fusion and discuss the potential implications of these findings for other cellular fusion events.

Introduction

Insulating membranes that create compartments surround the cell and its organelles. Information is exchanged between compartments by means of vesicular and tubular carriers, which must fuse with the recipient membrane compartment to deposit content and membrane components. One of the most studied systems for cellular membrane fusion is the Ca^{2+}-dependent fusion of synaptic vesicles and granules with the plasma membrane in neurons and chromaffin cells, respectively. In these events, the fusion process is triggered by a rise in the intracellular Ca^{2+} concentration and a specialized fusion machinery, comprising the SNARE proteins, synaptotagmins, Doc2 proteins, and a variety of other proteins (Chernomordik and Kozlov, 2008; Groffen et al., 2010; Martens and McMahon, 2008; Sudhof, 2004) . It has recently been suggested that synaptotagmin-1 and Doc2b trigger vesicle fusion by the Ca^{2+}-dependent induction of membrane curvature (Groffen et al., 2010; Hui et al., 2009; Martens et al., 2007; Martens and McMahon, 2008). This Essay lays out the evidence that membrane fusion is ultimately triggered by extreme membrane curvature. We make hypotheses concerning the mechanism by which curvature can be generated and collate evidence for proteins that produce this curvature-induced stress and strain. Although much of the recent work in this area has focused on synaptotagmin-1 and synaptic vesicles, we propose that many other proteins in diverse biological contexts can contribute to membrane destabilization induced by curvature, not necessarily in a Ca^{2+}-dependent manner.

Curvature and C2 Domains

Synaptogmin-1 and Doc2b play a central role alongside SNARE proteins in the Ca^{2+}-dependent fusion of synaptic vesicles with the plasma membrane (Fernández-Chacón et al., 2001; Geppert et al., 1994; Sudhof, 2004; Groffen et al., 2010). The C2 domains of synaptotagmin-1 and Doc2b induce a high degree of curvature on liposomes in a Ca^{2+}-dependent manner (17 nm diameter relative to the already highly curved synaptic vesicles [diameter ~42 nm] or the flat plasma membrane) (Martens et al., 2007,Groffen et al., 2010). Furthermore, the induction of curvature promotes lipid mixing, an indicator of fusion, in an in vitro assay of SNARE-dependent fusion and dense-core granule exocytosis in neuronal PC12 cells (Lynch et al., 2008). These findings are consistent with a mechanistic function for synaptotagmin-1 and Doc2b in membrane fusion. The recent report by Hui et al. (2009) provides additional lines of evidence that bending of the target membrane by synaptotagmin-1 facilitates the fusion of synaptic vesicles.

How might synaptotagmin-1 and Doc2b induce membrane curvature in a Ca^{2+}-dependent manner? The main functional modules of synaptotagmin-1, Doc2b, and other family members are two C-terminal C2 domains (C2A and C2B) (Martens and McMahon, 2008; Sudhof, 2004), which bind Ca^{2+} ions in negatively charged pockets. Ca^{2+}-binding reverses the net negative charge, enabling the binding and insertion of these regions of the C2 domains into the target membrane (Martens and McMahon, 2008). This insertion penetrates one leaflet of the membrane to approximately the depth of lipid glycerol backbones (Figure 1) (Chapman, 2008; Herrick et al., 2006). It has been shown that the insertion of amphipathic helices into monolayers to the level of the glycerol backbones of lipids (the region of maximum rigidity) leads to induction of membrane curvature (Campelo et al., 2008; Ford et al., 2002; Gallop et al., 2006; Kweon et al., 2006; Lee et al., 2005) (Figure 1A). This is because an inserted helix expands the area occupied by the lipid polar heads but not the acyl chains resulting in a void below the helix that must be filled by tilted/splayed acyl chains (Figure 1). This tilting is propagated through neighboring lipids and induces local bending of the monolayer harboring the insertion. Due to monolayer coupling, the other monolayer follows, and thus the entire bilayer is bent. Hence, C2 domains might work similarly, inserting into the monolayer to a depth that induces maximal local curvature. In addition, the inserting regions of an individual C2 domain will occupy a somewhat larger footprint than, for example, the amphipathic helix of epsin1 in the membrane (Ford et al., 2002) (Figure 1). Thus the close positioning of two C2 domains in synaptotagmins and Doc2s appears to be perfectly adapted to cause the maximal possible local curvature.

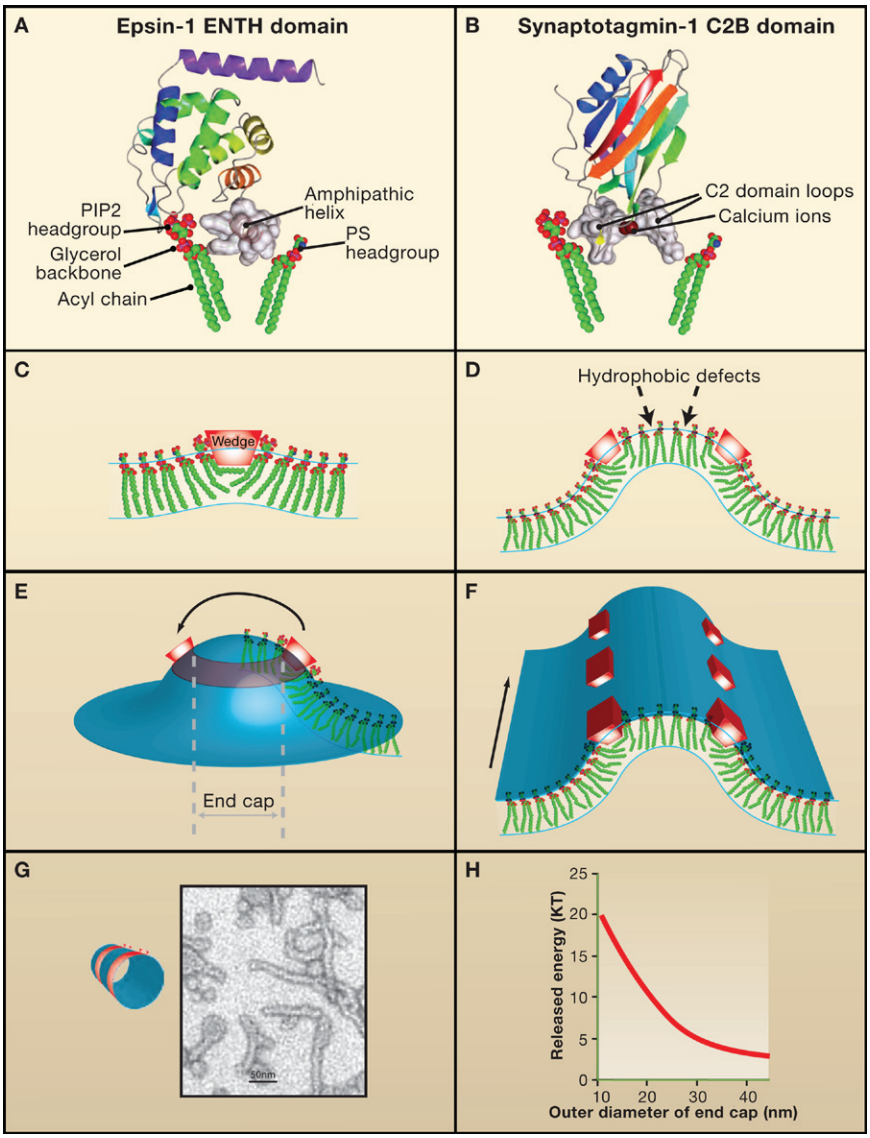

Figure 1. Models of Membrane Bending and Fusion

(A) An amphipathic helix, such as found in epsin or endophilin, is shown to insert into the most rigid part of a membrane leaflet to a depth approaching the lipid glycerol backbones. The adjacent hydrocarbon chains tilt and splay to fill the resulting void and drive the generation of local curvature. The picture illustrates the insertion of helix-0 from epsin-1 (PDB: 1h0a) (Ford et al., 2002), and the lipid and structure are to scale.

(B) Membrane-inserting C2 domains, illustrated by the synaptotagmin-1 C2B domain (PDB: 1uow), occupy a similar position in the membrane to amphipathic helices but are more bulky than a four-turn helix (taking into account the complete volume) and because of this are likely to be more effective at generating curvature.

(C) Response of the surrounding lipids to a wedge-like hydrophobic insertion (amphipathic helix or C2 domain). Lipid tilting could result in an increased membrane curvature that is quickly dissipated on either side of the insertion.

(D) Two insertions, when tethered close to each other, could result in high curvature between the wedges. High curvature in this region is not stabilized by insertions and because of this would likely give rise to local instability and transient "hydrophobic-defects," where the hydrophobic phase of the membrane is exposed.

(E) Propagation of an insertion in a circular direction (as in the SNARE organization of vesicle fusion) would result in a region of high curvature that we refer to as the "end cap."

(F) Propagation of insertions in a longitudinal direction would result in a ridge of high curvature, and if also propagated in a circular direction could create a membrane tubule.

(G) Propagation of insertions in both circular and longitudinal directions would create membrane tubules. Tubules created by insertions of synaptotamin-1 C2AB domains (see panel) and Doc2b C2AB domains have end caps with diameters of 17 nm (Martens et al., 2007; Groffen et al., 2010).

(H) Theoretical calculation of the dependence of energy released by formation of the hemifusion stalk on the curvature of the end cap.

Models are drawn in CCP4-MG freely available from http://www.ysbl.york.ac.uk/~ccp4mg/.

Each C2 domain, and in fact any other insertion, will cause only a localized curvature, but the effects of insertions are additive. Therefore, large-scale deformations of the membrane, such as seen for tubulated liposomes, can be achieved by a sufficiently high density of C2 domains on the membrane. In fact, we estimated that in order to induce and stabilize a tubule with a diameter of 17 nm (external surface of the outer monolayer), less then 30% of the membrane surface area has to be covered by C2 domains (Martens et al., 2007). Given that the C2 domains within a dimer are coupled, the dimer has a higher avidity for the membrane than two separate C2 monomers. As a result, at a given overall C2 domain concentration, more dimeric protein will be membrane bound compared to the monomeric C2 domains. This may be the reason why we observed a much more efficient induction of membrane curvature for a synaptotagmin-1 fragment containing both the C2A and C2B domains (Martens et al., 2007). Also, given a high enough concentration, individual C2 domains should also be able to induce curvature, as has recently been shown for isolated C2B domains (Hui et al., 2009). It is also possible that the C2A and C2B domains bind to opposing membranes. Thus, the C2A domain may bind the vesicle/granule membrane, while the C2B domain may bind the plasma membrane (Stein et al., 2007). In this orientation, the C2 domains may induce curvature in both membranes destined to fuse. There are a number of proteins with three or more C2 domains (Martens and McMahon, 2008). In principle, this could lead to a greater local concentration of C2 domains and thus the ability to respond to a wider variety of calcium concentrations and also could result in more local curvature with fewer molecules.

In addition to binding to membranes, C2 domains and in particular the C2B domain of synaptotagmin-1 bind to the SNARE proteins, SNAP25 and syntaxin, either in their dimeric state or in complex with synaptobrevin (Chapman, 2008). As the SNARE complex is formed at the future fusion site, this interaction will result in concentration of the synaptotagmin-1 C2 domains, and thus a relatively high surface density of membrane-

inserting C2 domains can be locally achieved. The surface at the opposite end of the C2B domain to its membrane insertion loops has critical arginine residues that may interact with the vesicle membrane (Xue et al., 2008) and might help (alongside SNARE zippering) to reorientate the C2 domain during membrane deformation.

How does high curvature promote fusion? If enough insertions are concentrated around the fusion site (for example synaptotagmins and Doc2s will both be concentrated by binding to SNARE complexes), then a buckle-like structure is predicted to form between the insertions (Figures 1D, 1E, and 2C). The curvature on the top of this structure will dramatically reduce the energy barrier for fusion. Transmembrane proteins are not predicted to occupy the dimpled end-cap. Equally, the synaptotagmin C2 domains are unlikely to occupy the dimple, as they repel each other in order to minimize overall free energy, but yet are restrained locally by the SNARE proteins to which they are bound. Thus, the end cap membrane is highly curved despite the absence of insertions and thus the lipids are under stress. This stress is partially relived during lipid rearrangements accompanying the fusion process, which reduces the overall energy cost of the reaction (Figures 1H and 2).

More specifically, at the first stage, the lipids in the outer monolayer of the end cap reorient to form the hemifusion stalk, the initial structure formed when the apposed monolayers from each bilayer merge (Chernomordik and Kozlov, 2008). This reorientation releases some of the outer-monolayer stress, promoting the stalk formation. Given that the lipids in the end cap inner monolayer are also bent, their reorientation to form the fusion pore releases the next portion of the end cap stress, facilitating formation of the fusion pore. Thus, the curvature stress promotes the formation of the hemifusion intermediate as well as the opening of the fusion pore (Figure 2). In fact, in many biological fusion events, the presence of curvature stress may couple hemifusion and fusion pore opening such that the hemifusion intermediate does not exist for any biologically relevant period of time. There may even be a role for curvature stress beyond fusion pore opening extending into a limited dilation/expansion of the fusion pore. This would be the case if the curvature stress is not fully released by opening of the fusion pore and is consistent with the finding that synaptotagmin-1 promotes the expansion of the fusion pore (Lynch et al., 2008). Furthermore, the end cap may define the point of fusion (where many SNARE complexes and bound synaptotagmins participate to define a single fusion point on which many SNAREs can cooperate in synchrony) and may thus allow for nonleaky fusion, an essential requirement for cellular fusion events.

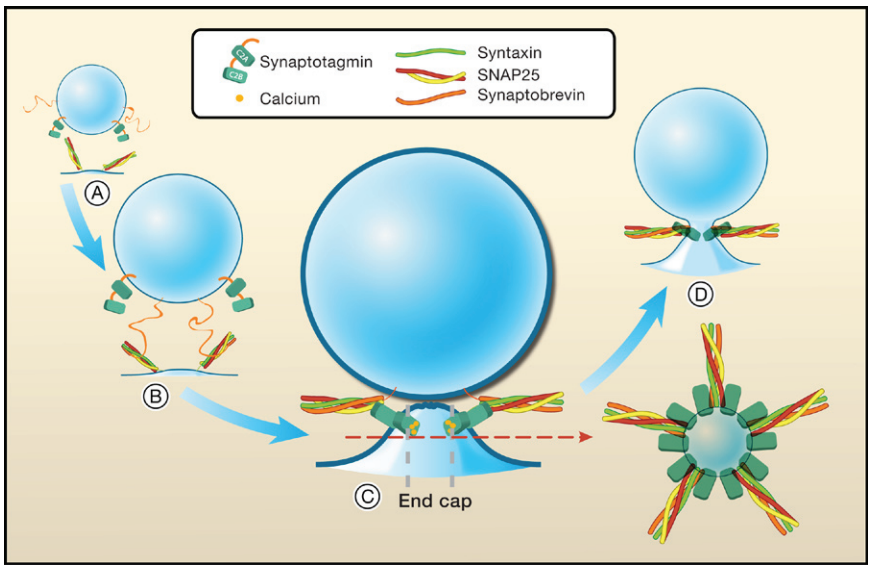

Figure 2. Membrane Buckling by C2 Domains as a Trigger for Fusion
As the vesicle approaches the membrane in this model, the vesicular SNARE component binds to its SNARE counterparts on the target membrane, resulting in the formation of a complex that pulls the two membranes into close apposition (steps A and B). The C2 domains of synaptotagmin bind to the SNARE complex, potentially helping to complete their zippering into a continuous helix. The C2 domains also insert into the target membrane in a Ca^{2+}-dependent manner, resulting in membrane buckling and an unstable membrane region optimally localized for fusion (jagged membrane in step C). As the fusion pore opens, the C2 domains would still be localized to the neck, where they might promote the early stages of fusion pore opening (step D).

There are a plethora of C2 domain-containing proteins implicated in synaptic vesicle fusion. Although this might reflect the exquisite Ca^{2+} dependence of synaptic vesicle fusion, it seems unlikely that all of these are proteins directly involved in fusion—some are likely to be both positive and negative regulators of the process. Promotion of positive curvature at the site of fusion (via SNARE protein interactions for example) would promote fusion whereas C2 domains that do not insert into the membrane but are localized to the SNAREs may inhibit fusion.

Although C2 domain proteins can trigger membrane stress and are prevalent in the nervous system, this does not mean that they are the only effectors of curvature. It is possible (and indeed likely) that SNAREs or other proteins involved in the process might also promote the same types of changes in curvature.

Curvature and SNARE Proteins

SNARE proteins play a key role in many membrane fusion events (contributing directionality, energy, and specificity), and we believe will contribute to high curvature intermediates both directly and indirectly (for example via localizing the C2 domains of synaptotagmins) (Figure 2). Each of the two membranes being fused contributes at least one SNARE motif (helix)-containing transmembrane anchored protein to a very stable SNARE complex of four helices that tether apposed membranes together. In vitro, these proteins alone can catalyze lipid mixing in an assay for liposome fusion and so are thought to be the "minimal" fusion machinery (Weber et al., 1998). The SNARE helices are separated from the membrane by

linker regions, but a recent study provides evidence that a continuous helical bundle can form right up to the transmembrane domain through this linker region and into the transmembrane helices, leaving no flexible domain between the SNARE helices and the membrane. This suggests that the force of SNARE complex formation may be transmitted right into the membrane (Stein et al., 2009) in the form of a bending stress that makes the membranes bulge toward each other promoting fusion. The fact that the proteins can form such a conformation implies that the SNARE proteins contribute to forcing the membranes through their high curvature intermediate. The extent to which the SNAREs pull the fusing membranes together is currently unclear as the rigidity of the linker between the SNARE motif and the transmembrane domain remains unknown. Regardless, the curvature induced by the formation of the continuous SNARE complex will act in the same direction as the Ca^{2+}-dependent curvature induced by C2 domains. It is possible that the linker region of the SNAREs by itself is not rigid enough to bend the membrane and that the accompanying curvature induced by synaptotagmin-1 and Doc2b enables the SNARE complex to extend into the membrane and create a bulge in the latter. Moreover, Ca^{2+}-dependent binding of synaptotagmin to SNAREs may stabilize this conformation.

Are SNAREs the Minimal Fusion Machinery?

A long-standing question for cell biologists has been what is the identity of the "fusogen" for a given membrane fusion process. The assumption is that the cellular fusogen would, in analogy to the viral fusion proteins, be the sole molecule that ultimately causes the membranes to fuse. The best candidates for the cellular fusogens are the SNARE proteins, which have been shown to mediate lipid mixing in a membrane fusion assay using reconstituted liposomes (Weber et al., 1998) and between entire cells when the SNAREs are expressed on the cell surface (Hu et al., 2003). Although SNAREs are essential players during the fusion process in vitro and in vivo (Jahn and Scheller, 2006), it is also clear that in vivo there are many proteins that play key roles in synaptic vesicle fusion apart from the SNARE proteins (Rizo and Rosenmund, 2008). Previously, these proteins have been classified according to how they affect fusion kinetics, with some affecting the extent or number of fusion events, whereas others alter the kinetics of an individual fusion event. A plethora of proteins fall in the former category and SNARE proteins, and synaptotagmins fall in the latter.

Hence, the search for "the fusogen" may be in vain as the functions required for efficient, nonleaky fusion may be divided into several polypeptides. This has been elegantly shown for endosome fusion, where the presence of up to 17 proteins is required to achieve physiological rates of fusion (Ohya et al., 2009). The division of labor into several polypeptides is actually not surprising, given that membrane fusion requires several steps. This is equally true for Ca^{2+}-dependent and Ca^{2+}-independent fusion. Thus there must be tethering of the correct membranes into close apposition, a generation of high curvature to destabilize the membranes, and the input of energy and directionality into the system. Although evolution may have repeatedly used the same SNARE modules to achieve fusion of various membranes, the Ca^{2+}-dependent C2 module is largely confined to the nervous system and to a few other fusion events. In all these situations, we suggest that high curvature is an essential ingredient of fusion events, including viral fusion. Viral fusion proteins are often compared to SNARE proteins, and evidence indicates that viral fusion peptides also mediate high curvature (Chernomordik and Kozlov, 2008; Martens and McMahon, 2008).

Beyond the Synapse?

SNARE proteins are required for endosome-endosome fusion but are not sufficient (Ohya et al., 2009), and it will be interesting to dissect the lipid binding proteins that induce membrane curvature in this context. The tethering factor EEA1 is a good candidate, as it interacts with SNAREs and inserts into membranes (Brunecky et al., 2005; Simonsen et al., 1999). It should not be a requirement that local curvature is induced after tethering but might equally occur before tethering, assuming that the curvature can be stabilized. This could occur with oligomeric proteins that prefer or generate high curvatures. For example, during cell plate formation in plant root cells, dynamins tubulate membranes that subsequently fuse (Gu and Verma, 1997) and mitochondrial fusion requires tubulation proteins of the mitofusin family (Hales and Fuller, 1997). Here again, these proteins likely give rise to the high curvature and unstable caps where fusion is more likely to take place. In a more recent example, atlastins (dynamin-like proteins) are shown to act as fusion proteins for endoplasmic reticulum networks (Orso et al., 2009). In the case of synaptic vesicle fusion, the curvature intermediate is small and transient and will therefore not be visualized by light microscopy. In contrast, the occurrence of transient high curvature intermediates in vivo has been seen for the transfer of material between organelles (Bright et al., 2005).

Now that we appreciate that SNARE proteins are involved in scaffolding many different fusion events, the search must be refocused toward finding the proteins that induce curvature. Of equal urgency is to understand the fusion events for which there are no confirmed molecular players. In most cases the "fusion protein" may in fact be a complex or assembly of multiple proteins, and given that shallow membrane insertions promote fusion in vitro, it will be important to look out for amphipathic helices and other types of shallow insertions in the generation of curvature.

REFERENCES

Bright, N.A., Gratian, M.J., and Luzio, J.P. (2005). Curr. Biol. *15*, 360–365.

Brunecky, R., Lee, S., Rzepecki, P.W., Overduin, M., Prestwich, G.D., Kutateladze, A.G., and Kutateladze, T.G. (2005). Biochemistry *44*, 16064–16071.

Campelo, F., McMahon, H.T., and Kozlov, M.M. (2008). Biophys. J. *95*, 2325–2339.

Chapman, E.R. (2008). Annu. Rev. Biochem. *77*, 615–641.

Chernomordik, L.V., and Kozlov, M.M. (2008). Nat. Struct. Mol. Biol. *15*, 675–683.

Fernández-Chacón, R., Königstorfer, A., Gerber, S.H., García, J., Matos, M.F., Stevens, C.F., Brose, N., Rizo, J., Rosenmund, C., and Südhof, T.C.

(2001). Nature *410*, 41–49.

Ford, M.G., Mills, I.G., Peter, B.J., Vallis, Y., Praefcke, G.J., Evans, P.R., and McMahon, H.T. (2002). Nature *419*, 361–366.

Gallop, J.L., Jao, C.C., Kent, H.M., Butler, P.J., Evans, P.R., Langen, R., and McMahon, H.T. (2006). EMBO J. *25*, 2898–2910.

Geppert, M., Goda, Y., Hammer, R.E., Li, C., Rosahl, T.W., Stevens, C.F., and Südhof, T.C. (1994). Cell *79*, 717–727.

Groffen, A., Martens, S., Diez, R., Cornelisse, L., Lozovaya, N., de Jong, A., Goriounova, N., Habets, R., Takai, Y., Borst, G., et al. (2010). Science. Published online February 11, 2010. 10.1126/science.1183765.

Gu, X., and Verma, D.P. (1997). Plant Cell *9*, 157–169.

Hales, K.G., and Fuller, M.T. (1997). Cell *90*, 121–129.

Herrick, D.Z., Sterbling, S., Rasch, K.A., Hinderliter, A., and Cafiso, D.S. (2006). Biochemistry *45*, 9668–9674.

Hu, C., Ahmed, M., Melia, T.J., Söllner, T.H., Mayer, T., and Rothman, J.E. (2003). Science *300*, 1745–1749.

Hui, E., Johnson, C.P., Yao, J., Dunning, F.M., and Chapman, E.R. (2009). Cell *138*, 709–721.

Jahn, R., and Scheller, R.H. (2006). Nat. Rev. Mol. Cell Biol. *7*, 631–643.

Kweon, D.H., Shin, Y.K., Shin, J.Y., Lee, J.H., Lee, J.B., Seo, J.H., and Kim, Y.S. (2006). Mol. Cells *21*, 428–435.

Lee, M.C., Orci, L., Hamamoto, S., Futai, E., Ravazzola, M., and Schekman, R. (2005). Cell *122*, 605–617.

Lynch, K.L., Gerona, R.R., Kielar, D.M., Martens, S., McMahon, H.T., and Martin, T.F. (2008). Mol. Biol. Cell *19*, 5093–5103.

Martens, S., and McMahon, H.T. (2008). Nat. Rev. Mol. Cell Biol. *9*, 543–556.

Martens, S., Kozlov, M.M., and McMahon, H.T. (2007). Science *316*, 1205–1208.

Ohya, T., Miaczynska, M., Coskun, U., Lommer, B., Runge, A., Drechsel, D., Kalaidzidis, Y., and Zerial, M. (2009). Nature *459*, 1091–1097.

Orso, G., Pendin, D., Liu, S., Tosetto, J., Moss, T.J., Faust, J.E., Micaroni, M., Egorova, A., Martinuzzi, A., McNew, J.A., and Daga, A. (2009). Nature *460*, 978–983.

Rizo, J., and Rosenmund, C. (2008). Nat. Struct. Mol. Biol. *15*, 665–674.

Simonsen, A., Gaullier, J.-M., D'Arrigo, A., and Stenmark, H. (1999). J. Biol. Chem. *274*, 28857–28860.

Stein, A., Radhakrishnan, A., Riedel, D., Fasshauer, D., and Jahn, R. (2007). Nat. Struct. Mol. Biol. *14*, 904–911.

Stein, A., Weber, G., Wahl, M.C., and Jahn, R. (2009). Nature *460*, 525–528.

Sudhof, T.C. (2004). Annu. Rev. Neurosci. *27*, 509–547.

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.

Xue, M., Ma, C., Craig, T.K., Rosenmund, C., and Rizo, J. (2008). Nat. Struct. Mol. Biol. *15*, 1160–1168.