

# Vesicle Formation at the Plasma Membrane and Trans-Golgi Network: The Same but Different

Mark A. McNiven\* and Heather M. Thompson

An elaborate vesicle transport system supports the active exchange of membranes and protein cargo between the plasma membrane and the trans-Golgi network. Many observations suggest that highly conserved mechanisms are used in vesicle formation and scission. Such similarity is found both at the level of the receptor-ligand sequestration process that uses clathrin and associated polymeric and monomeric adaptor proteins, and in the machinery used to deform and vesiculate lipid membranes.

The plasma membrane (PM) and the Golgi apparatus exhibit a yin and yang relationship centered on the sorting, packaging, vesiculation, and transport of membranes and protein cargo, either into (the PM) or out of (the Golgi apparatus) the cell. Rather than being antagonistic in function, these sister sites actually represent a “membrane continuum” that shares and exchanges a related functional machinery to support its complex tasks (Fig. 1 and table S1). Situated along this continuum are sorting stations, including several types of sorting and recycling endosomal compartments on one side and the trans-Golgi network (TGN) on the other. All of these compartments are in continuous communication with each other and share many similar structures and components. Here we focus on the similarities, while also indicating some differences, of this sorting and transport continuum.

## Similarities in Protein-Sorting Mechanisms at the PM and TGN

The selective sequestration and packaging of protein cargo are key functions performed at both the PM and TGN. Clathrin and its associated coat proteins provide the central scaffold on which many of these processes are organized and are very similar at both sites (Fig. 1 and table S1). Although most studies have focused on clathrin-coated vesicle (CCV) formation at the PM during endocytosis, marked similarities have been noted at the TGN (1–3).

Of the clathrin-associated accessory proteins, the heterotetrameric adaptor proteins (APs), as well as the more recently identified monomeric clathrin-associated sorting proteins, provide the “first line” of cargo-sequestration specificity on a widely distributed clathrin cage (4–6). Four AP complexes have been identified, and

they all exhibit a similar organization, consisting of two large subunits ( $\gamma/\beta 1$ ,  $\alpha/\beta 2$ ,  $\delta/\beta 3$ , and  $\epsilon/\beta 4$ ), a medium subunit ( $\mu 1-4$ ), and a small subunit ( $\sigma 1-4$ ). Despite the modest identity among analogous subunits (20 to 40%), they appear to be structurally similar and to assemble into their respective AP complexes in a similar manner. The AP complexes do, however, display differences in cellular localization patterns and mediate distinct vesicle-formation processes. AP-1, AP-3, and AP-4 are generally believed to function at the TGN and/or endosomes, whereas AP-2 functions at the PM (7). Both functional and structural studies have firmly established a role for AP-2 in protein sorting and CCV formation at the PM; in comparison, the roles of the other AP complexes in vesicle formation are somewhat less clear (8, 9).

AP-mediated protein sorting depends on the recognition of sorting motifs that are present in the cytosolic domains of transmembrane proteins (7, 10). There are at least three classes of sorting motifs recognized by AP complexes. The Asn-Pro-X-Tyr motif, which is found in the cytosolic tails of the insulin receptor, epidermal growth factor (EGF) receptor, and low-density lipoprotein receptor, appears to be recognized by AP-2 at the PM; however, this motif also interacts with non-AP-2 clathrin adaptors to mediate internalization (4). Another tyrosine-based motif, Tyr-X-X- $\emptyset$  (where  $\emptyset$  is a bulky hydrophobic amino acid), found in transmembrane proteins such as the transferrin receptor and mannose-6-phosphate receptor, is recognized by the  $\mu$  subunit of all four AP complexes, and this motif can thus mediate receptor cargo sorting at the PM, TGN, and endosomes. A third motif, [Asp-Glu]X-X-X-Leu[Leu-Ile] ([DE]XXXL[LI]), is dileucine-based and resides in the cytosolic tails of proteins targeted to endosomal and lysosomal compartments. In addition, this motif has been implicated in basolateral targeting in polarized epithelial cells. Dileucine-based motifs are recognized by AP-1, AP-2, and AP-3; however, each AP complex exhibits

distinct preferences for certain [DE]XXXL[LI] motifs. The affinity of interactions between the AP complexes and a specific binding motif might differ, depending on the context of the motif within the protein, the membrane-organelle environment, and the phosphorylation state of the AP complex. Thus, a nascent receptor may exhibit a distinct affinity for one AP complex when leaving the TGN but demonstrate other affinities for different AP complexes when being internalized from the PM or recycled from endosomes.

## Variations of a Common Sorting Machine

There is substantial homology between the protein-sequestration and -sorting machinery at the PM and TGN. Superimposed on this conserved process are several layers of regulation and targeting that provide specificity. Because clathrin, adaptor proteins, and many additional linker proteins are widely used, what variations then might provide distinction between the two sites? It appears that different lipids and phosphoinositides (11–13); small guanosine triphosphatases (GTPases) (14, 15); and a second family of adaptors, the GGAs [Golgi-localized,  $\gamma$ -ear-containing, adenosine diphosphate ribosylation factor (ARF)-binding proteins] (16, 17), work together toward this end.

The classes of lipids that make up the lipid environments at the PM and TGN are similar in many respects, containing cholesterol, sphingolipids, and phosphoinositides (11–13, 18, 19). In addition, lipid microdomains or rafts are present at both sites [for site differences, see (20)]. Lipid rafts are thought to mediate clathrin-independent vesicle formation and have been proposed to participate in sorting at the TGN in polarized epithelial cells for the transport of apically localized proteins (8, 21). Thus, although the ratio of these lipids may differ to some degree at the two sites, with cholesterol and sphingolipids being more enriched at the PM than the TGN, the participation of membrane microdomains is maintained.

The use of inositol phospholipids during vesicle formation also seems to be conserved; however, phosphoinositides containing different combinations of phospho-modifications on the inositol ring appear to be preferentially generated at certain membranous sites, where they can then participate in distinct vesicle-trafficking pathways (11, 12). Whereas phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) is more prominent at the PM and binds to AP-2, phosphatidylinositol 4-monophosphate (PI4P) is more prominent at the TGN and binds to AP-1. The delegation of PI4P to the Golgi and PIP<sub>2</sub> to the cell surface is less tidy than it may first appear, because PIP<sub>2</sub> has been implicated not only in clathrin-dependent and -independent vesicle formation from the PM but also in the early formation of the phagocytic cup, Golgi intracisternal transport, and “comet”-based vesicle formation from the TGN. Thus, the same phosphoinositide appears to be

Department of Biochemistry and Molecular Biology and the Miles and Shirley Fiterman Center for Digestive Diseases, Mayo Clinic College of Medicine, Rochester, MN 55905, USA.

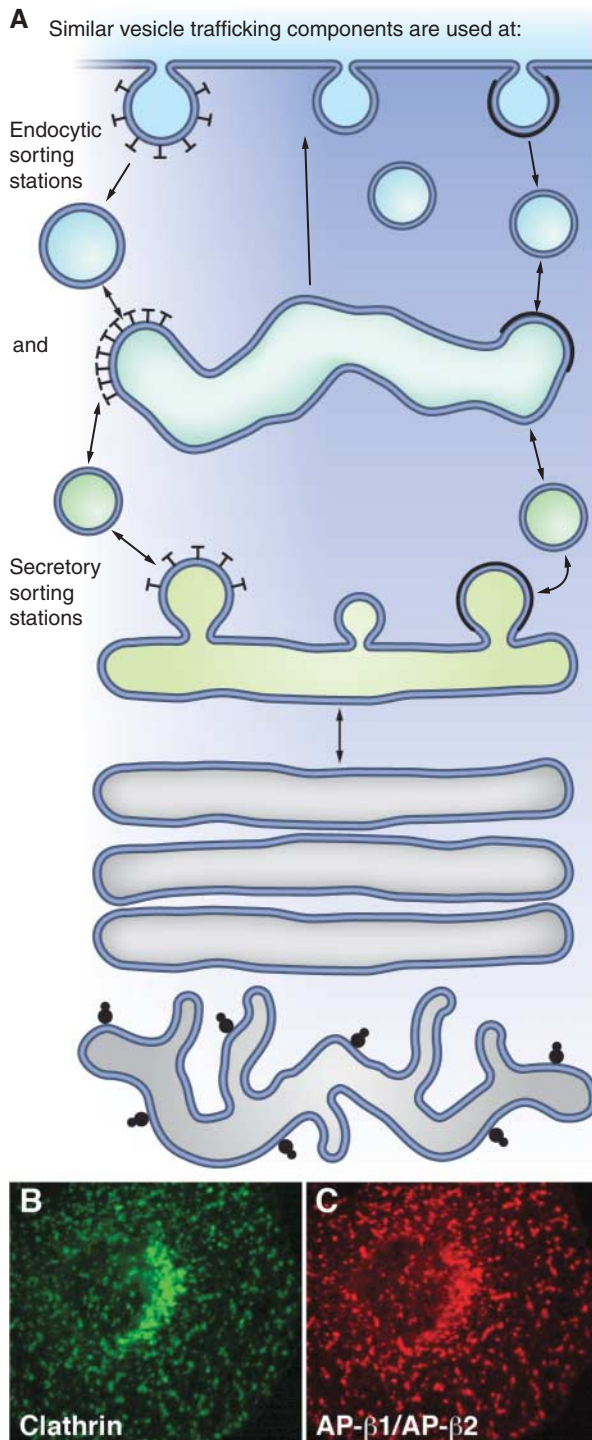
\*To whom correspondence should be addressed. E-mail: mcniven.mark@mayo.edu

essential in multiple distinct vesicle-formation processes at the PM and the Golgi. The lipid environment, therefore, seems to be similar in some aspects between these two sites, although the exact lipid composition of vesicles (formed from the PM and TGN) most likely exhibits differences. Although they are important, lipids alone do not provide for complete specificity; some additional identity is provided by small GTPases.

The ARF family of small GTPases plays an important role in regulating vesicle formation in the secretory as well as endocytic pathways (22). When active in the GTP-bound state, ARF1 recruits the Golgi-associated adaptor AP-1 to the TGN. Thus, TGN targeting of AP-1 is achieved by interaction with both the Golgi-enriched PI4P and active ARF1. A similar dependence on ARF family members for CCV formation also exists at the PM in some instances. For example, the ARF family member ARF6, which resides primarily at the PM, participates in clathrin-mediated endocytosis in polarized epithelial cells and interacts with the PM adaptor AP-2 to modulate CCV formation (23). A general function for ARF6 in CCV formation, however, is not present in all cell types.

At the TGN, another family of adaptor proteins, the GGAs, functions along with the AP-based sorting system (16, 17). Like AP-1, the recruitment of GGAs to the Golgi is dependent on ARF1 (24). In addition, GGAs may function along with AP-1 in the generation of a subset of CCVs targeted to lysosomes. In this regard, it is noteworthy that the GGAs mediate the sorting of lysosome-targeted proteins through binding to dileucine motifs distinct from those recognized by AP-1. Whereas AP-1 binds to [DE]XXXL[L] motifs, GGAs bind to Asp-X-X-Leu-Leu motifs.

The GGAs also contain a protein domain that binds ubiquitin (25, 26). The ubiquitin modification appears to be used as a mechanism to initiate the sorting of nascent proteins at the TGN that are to be targeted to the vacuole in yeast (27, 28). Although such a mechanism has not been demonstrated in mammalian cells, ubiquitin modification is used by the endocytic machinery at the PM to sort endocytosed proteins to endosomes and subsequently lysosomes for degradation (27, 29, 30). Thus, ubiquitin sorting signals may be used at both vesicle-formation sites but are recognized by different ubiquitin-binding proteins: GGAs at the TGN and adaptor proteins, such as Eps15 and Epsin, at the



**Fig. 1.** The PM and Golgi apparatus as sister sites that communicate via a tubular-vesicular membrane continuum. (A) Vesicle trafficking between the PM (blue) and TGN (green) is shown as a continuum indicated by the shift between blue and green and designed to represent the communication of membranes and vesiculation machinery between the two sister sites. The "T" symbols and solid black lines along the outside of vesicles and organelles represent clathrin and nonclathrin coats, respectively. The gray coloration of the endoplasmic reticulum, cis-Golgi, and medial-Golgi is meant to indicate a sorting and vesiculation machinery distinct from that used by the TGN and PM. (B and C) Fluorescence micrograph of a cell costained for clathrin (B) and the  $\beta$  subunits of AP-1 and AP-2 (C), indicating similarities in coat-protein components present at the PM and TGN.

PM. Eps15 was first identified in a search for EGF receptor substrates (31) and functions in CCV formation at the PM (32). Epsin, on the other hand, was identified as a result of its binding to Eps15 through Asn-Pro-Phe motifs present in the C terminus of Epsin and Eps15 homology domains present in the N terminus of Eps15 (33). In addition to binding to each other, both Eps15 and Epsin also bind to AP-2 (33–35) and contain ubiquitin interacting motifs (UIMs) (25). These UIMs mediate the binding of Eps15 and Epsin to ubiquitinated EGF receptors after ligand stimulation and are also necessary for the ubiquitination of the proteins themselves. Because Eps15 and Epsin interact with each other, with the components of the CCV-formation machinery, and with ubiquitinated EGF receptors, these attributes may provide a means to sequester and sort activated EGF receptors for internalization and degradation (29, 30). In addition to functioning at the PM, Eps15 has also been localized to the TGN, where it binds to AP-1 through the  $\gamma$ -adapin appendage domain (36). Thus, Eps15 and GGAs, in conjunction with AP-1, might mediate the sorting of ubiquitinated cargo at the TGN.

### Lipid-Membrane Binding, Bending, and Pinching

Membrane curvature and severing at the PM and TGN after cargo sequestration are essential aspects of vesicle formation and are dependent on the cumulative contribution of lipids, proteins, and lipid-protein interactions (37–39). As for the coat-cargo sorting scaffold, the vesiculation machinery is highly redundant at both sites and uses many of the same types of lipid-binding scaffold proteins and classes of lipid-modifying enzymes (Fig. 2 and table S1). Many of these lipid-binding proteins contain one of two motifs that are present from yeast to mammals: the Epsin N-terminal homology/AP180 N-terminal homology (ENTH/ANTH) domain (40–42) and the Bin-amphiphysin-Rvs161/167p (BAR) domain (43, 44).

As the name implies, the ENTH domain was originally identified as a protein module present in Epsin. Subsequently, the ANTH domain was identified in the brain-specific AP180 protein, which mediates CCV formation. A non-neuronal homolog of AP180, termed clathrin-assembly lymphoid myeloid leukemia protein, that contains an ANTH domain and is involved in CCV formation also exists. Both the ENTH and ANTH domains bind to inositol phos-

pholipids, exhibiting a preference for PIP<sub>2</sub>, but through somewhat different mechanisms (41, 42). Outside of the ENTH/ANTH domains, these proteins are divergent, but all contain motifs supportive of a role in endocytic CCV formation (42). Although the mechanism by which ANTH

domain-containing proteins deform membranes is not totally clear, more information is known about ENTH proteins. The ENTH domain of Epsin is thought to bind to PM regions rich in PIP<sub>2</sub> and, through membrane insertion, to support membrane deformation in synergy with other effector proteins, such as AP-2 and clathrin (39, 45).

Some proteins containing ENTH/ANTH domains support clathrin-mediated endocytosis, whereas other ENTH/ANTH domain-containing proteins, such as Epsin-related (EpsinR) and HIP1/HIP1-related (HIP1R), are involved in CCV formation at the TGN or TGN and PM, respectively (40, 42, 46). EpsinR, also known as Enthoprotin and Clint, has a clathrin-binding motif, like Epsin; but rather than an AP-2-binding motif, EpsinR contains an AP-1/GGA2-binding motif and exhibits a slight preference for the TGN-enriched PI4P, making this modified Epsin well suited for TGN function. HIP1/HIP1R, on the other hand, is involved in CCV formation (both at the PM and TGN) and provides a link between CCV formation and actin dynamics (47).

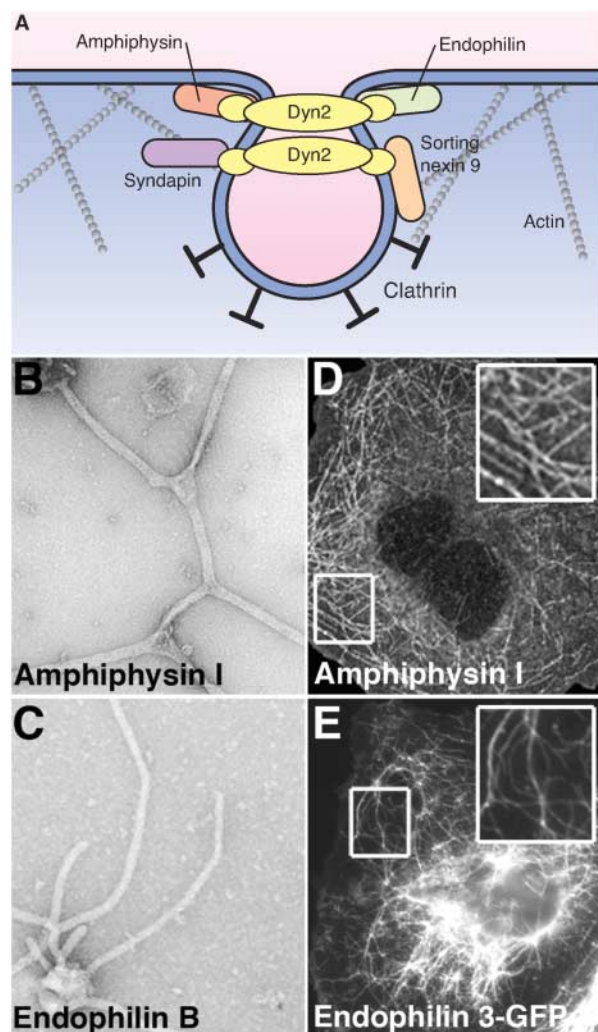
A second recently identified protein domain with membrane-binding properties is the BAR domain (43, 44). This domain is present in many proteins with roles in membrane dynamics, including membrane tubulation and ruffling. In its simplest form, the BAR domain functions as a membrane curvature-sensing module, meaning that the exact curvature of a membrane affects the binding of proteins with this type of BAR domain (44). However, some proteins contain an N-BAR domain, where an unstructured amphipathic helix is also present N-terminal to the BAR domain. The presence of this amphipathic helix in addition to the BAR domain seems to allow these proteins to both sense and induce membrane curvature, presumably toward vesicle formation and scission. Amphiphysin, which contains an N-BAR domain, is able to bind and tubulate membranes both *in vitro* and *in vivo* (Fig. 2, B and D) and is involved in CCV formation during endocytosis. Amphiphysin may function at late stages of vesicle formation, when a

membrane tubule or neck would be generated just before severing of the vesicle from its donor membrane (37). Other proteins containing BAR domains have been identified that also play a role in endocytosis, including endophilin and sorting nexin 9 (43, 44, 48–50). At the TGN, variants of amphiphysin and endophilin are present and exhibit functions independent of endocytosis (51–53). Sorting nexin 9, in contrast, has been demonstrated to bind to both the  $\alpha$  subunit of AP-2 (48, 49, 54) and the  $\gamma$  subunit of AP-1 (49, 55). Thus, a series of related ENTH/ANTH and BAR domain-containing proteins with membrane-deforming properties has been superimposed on the clathrin-adaptor sorting machinery to initiate the tubulation and vesiculation of sequestered cargo from both the PM and the TGN.

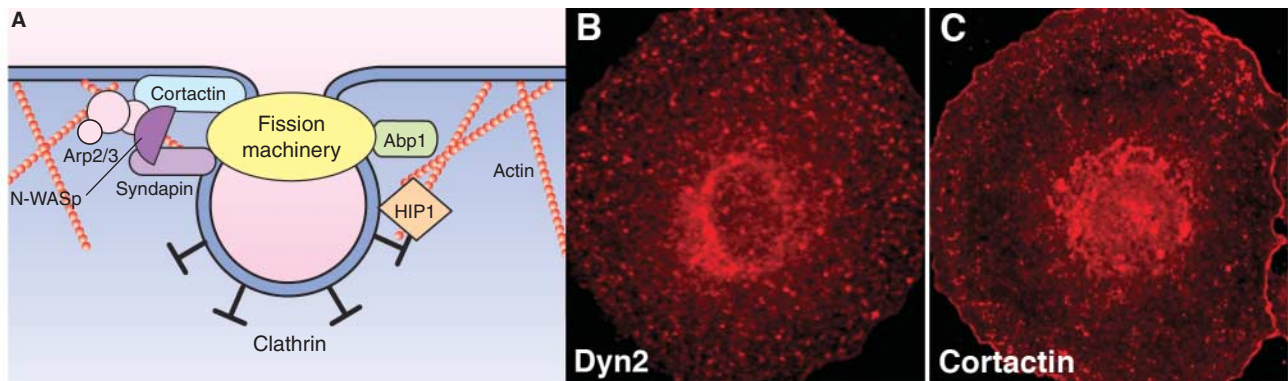
### Vesicle Scission

The actin cytoskeleton, in tandem with an extended family of membrane-tubulating and -severing proteins, may provide the final mechanical action to liberate nascent vesicles from both sister sites (Fig. 3 and table S1) (46, 47, 56–61). Several actin-binding proteins, some of which also have lipid-binding properties, participate in the act of deforming both the PM and TGN donor membranes in concert with the large GTPase dynamin (Dyn) (56–59, 61, 62). Dyn is well known for its role as a molecular pinchase that assembles as an oligomeric complex in the form of spirals on lipid tubules and at the neck of nascent vesicle buds to help mediate the scission process upon GTP hydrolysis (63–65). Once thought to function exclusively in clathrin-mediated endocytosis at the PM, Dyn has since been found to mediate multiple forms of vesicle formation and to function at many cellular organelles, including the TGN (66). Conserved Dyn-independent fission mechanisms also exist at the PM and TGN, one example of which is vesicle formation mediated by C-terminal binding protein 3/brefeldin A-ribosylated substrate (CtBP3/BARS) (67). In epithelial cells, CtBP3/BARS mediates the Dyn-independent fission of basolateral transport carriers from the TGN and fluid-phase endocytosis; however, the exact mechanism of fission is unclear.

A subset of actin-regulatory proteins bind the proline-rich domain (PRD) of Dyn and also have membrane-deforming properties (56, 57). These proteins contain a Fes/CIP4 homology (FCH) domain adjacent to a region that shares some homology with the C-terminal half of the BAR domain; thus, this domain was termed F-BAR for FCH and BAR (57) and EFC for extended FC (56). As for BAR domain-containing proteins, F-BAR/EFC domain-containing proteins exhibit membrane tubulation activity both *in vitro* and *in vivo*. Further, many of these proteins bind to Dyn through a Src-homology 3 (SH3) domain-PRD-based interaction to support membrane tubulation and vesiculation (57). Just as some BAR domain-containing proteins are



**Fig. 2.** Common membrane-tubulating proteins at the PM and TGN. (A) Similar protein players are used to bind and deform lipids at both the PM and TGN. Along with conventional clathrin coats and adaptors, a variety of BAR and F-BAR/EFC domain-containing proteins bind and deform membranes into tubules, in preparation for vesiculation in concert with the large GTPase Dyn and the actin cytoskeleton. (B and C) Electron micrographs of negative-stained liposomes tubulated *in vitro* by the addition of purified BAR domain-containing proteins present at the PM [amphiphysin I in (B)] and TGN [endophilin B in (C)]. (D and E) Fluorescence micrographs indicating the tubulating action of the BAR domain-containing proteins amphiphysin I (D) and endophilin 3 (E) within the confines of living cells upon their overexpression. Insets show higher magnifications of the boxed regions, emphasizing the massive membrane tubulation induced by these proteins. Different family members of both of these tubulating proteins have been localized to either the PM or TGN. [(B) and (C) are reproduced from (52) with permission from The Rockefeller University Press; (D) is reproduced from (44) with permission from the American Association for the Advancement of Science; and (E) is reproduced from (57) with permission from Elsevier.]



**Fig. 3.** The vesicle fission machinery and an associated actin cytoskeleton act in concert to liberate nascent vesicles from both the PM and TGN. **(A)** A complex actin cytoskeletal network centered around proteins involved in membrane fission functions together with the clathrin-based sorting and budding

machinery to complete the process of cargo sequestration, vesicle formation, and membrane scission. **(B and C)** Fluorescence micrographs of cells stained for Dyn2 **(B)** and cortactin **(C)** as examples of components of the fission machinery and actin cytoskeleton that are present at both the PM and TGN.

present at the PM and TGN, the F-BAR/EFC domain-containing protein syndapin, also called PACSIN, has been implicated in vesicle formation from both the PM and TGN (61, 68). Thus, these studies implicate a synergistic action among Dyn, actin, and F-BAR/EFC domain-containing proteins during vesicle formation from both of these sister sites.

Vesicle formation at either the PM or TGN might include a dynamic structural scaffold on which the membrane can be tubulated and vesiculated by a protein complex containing several actin- and lipid-binding proteins. Thermodynamically, this scission process is no small task. Thus, it is not surprising that a tag team approach that uses multiple structural and lipid-deforming proteins, as well as force-generating enzymes, would be required to work together in concert to liberate nascent vesicles from a stable membrane bilayer.

### Future Directions

The similarities between the clathrin-based vesiculation machinery at both the PM and TGN are strong. Although differences do exist, it is clear that a central theme of vesicle formation has evolved within the continuum of the two related but distinct sorting stations. With the rapid and overwhelming identification of many previously unrecognized adaptors, lipid-binding and -modifying enzymes, and cytoskeletal components, the task is now to define how all of these players interact with each other within the confines of a living cell, how they are regulated during the endocytic and secretory processes, and how they malfunction during human disease.

### References and Notes

- S. A. Mousavi, L. Malerod, T. Berg, R. Kjekens, *Biochem. J.* **377**, 1 (2004).
- F. M. Brodsky, C. Y. Chen, C. Kneuhl, M. C. Towler, D. E. Wakeham, *Annu. Rev. Cell Dev. Biol.* **17**, 517 (2001).
- L. M. Traub, *Biochim. Biophys. Acta* **1744**, 415 (2005).
- M. S. Robinson, *Trends Cell Biol.* **14**, 167 (2004).
- M. A. Edeling, C. Smith, D. J. Owen, *Nat. Rev. Mol. Cell Biol.* **7**, 32 (2006).
- T. J. Brett, L. M. Traub, *Curr. Opin. Cell Biol.* **18**, 395 (2006).
- J. S. Bonifacino, L. M. Traub, *Annu. Rev. Biochem.* **72**, 395 (2003).
- E. Rodriguez-Boulant, G. Kreitzer, A. Musch, *Nat. Rev. Mol. Cell Biol.* **6**, 233 (2005).
- M. Boehm, J. S. Bonifacino, *Gene* **286**, 175 (2002).
- D. J. Owen, B. M. Collins, P. R. Evans, *Annu. Rev. Cell Dev. Biol.* **20**, 153 (2004).
- M. A. De Matteis, A. Godi, *Nat. Cell Biol.* **6**, 487 (2004).
- C. P. Downes, A. Gray, J. M. Lucocq, *Trends Cell Biol.* **15**, 259 (2005).
- J. C. Holthuis, G. van Meer, K. Huitema, *Mol. Membr. Biol.* **20**, 231 (2003).
- R. Behnia, S. Munro, *Nature* **438**, 597 (2005).
- S. Ellis, H. Mellor, *Trends Cell Biol.* **10**, 85 (2000).
- J. S. Bonifacino, *Nat. Rev. Mol. Cell Biol.* **5**, 23 (2004).
- I. Hinners, S. A. Tooze, *J. Cell Sci.* **116**, 763 (2003).
- L. Liscum, N. J. Munn, *Biochim. Biophys. Acta* **1438**, 19 (1999).
- G. van Meer, J. C. Holthuis, *Biochim. Biophys. Acta* **1486**, 145 (2000).
- I. Gkantiras et al., *Mol. Biol. Cell* **12**, 1819 (2001).
- J. B. Helms, C. Zurzolo, *Traffic* **5**, 247 (2004).
- C. D'Souza-Schorey, P. Chavrier, *Nat. Rev. Mol. Cell Biol.* **7**, 347 (2006).
- O. Paleotti et al., *J. Biol. Chem.* **280**, 21661 (2005).
- J. G. Donaldson, A. Honda, R. Weigert, *Biochim. Biophys. Acta* **1744**, 364 (2005).
- L. Hicke, H. L. Schubert, C. P. Hill, *Nat. Rev. Mol. Cell Biol.* **6**, 610 (2005).
- R. Puertollano, J. S. Bonifacino, *Nat. Cell Biol.* **6**, 244 (2004).
- L. Hicke, R. Dunn, *Annu. Rev. Cell Dev. Biol.* **19**, 141 (2003).
- D. J. Katzmann, G. Odorizzi, S. D. Emr, *Nat. Rev. Mol. Cell Biol.* **3**, 893 (2002).
- K. Haglund, P. P. Di Fiore, I. Dikic, *Trends Biochem. Sci.* **28**, 598 (2003).
- P. P. Di Fiore, S. Polo, K. Hofmann, *Nat. Rev. Mol. Cell Biol.* **4**, 491 (2003).
- F. Fazioli, L. Minichiello, B. Matoskova, W. T. Wong, P. P. Di Fiore, *Mol. Cell Biol.* **13**, 5814 (1993).
- A. Benmerah, V. Poupon, N. Cerf-Bennussan, A. Dautry-Varsat, *J. Biol. Chem.* **275**, 3288 (2000).
- H. Chen et al., *Nature* **394**, 793 (1998).
- G. Iannolo et al., *Cancer Res.* **57**, 240 (1997).
- A. Benmerah, B. Begue, A. Dautry-Varsat, N. Cerf-Bennussan, *J. Biol. Chem.* **271**, 12111 (1996).
- H. M. Kent, H. T. McMahon, P. R. Evans, A. Benmerah, D. J. Owen, *Structure* **10**, 1139 (2002).
- H. T. McMahon, J. L. Gallop, *Nature* **438**, 590 (2005).
- M. A. De Matteis, A. Godi, *Biochim. Biophys. Acta* **1666**, 264 (2004).
- J. Zimmerberg, M. M. Kozlov, *Nat. Rev. Mol. Cell Biol.* **7**, 9 (2006).
- M. C. Duncan, G. S. Payne, *Trends Cell Biol.* **13**, 211 (2003).
- P. De Camilli et al., *FEBS Lett.* **513**, 11 (2002).
- V. Legendre-Guillemin, S. Wasiaik, N. K. Hussain, A. Angers, P. S. McPherson, *J. Cell Sci.* **117**, 9 (2004).
- B. Habermann, *EMBO Rep.* **5**, 250 (2004).
- B. J. Peter et al., *Science* **303**, 495 (2004).
- R. V. Stahelin et al., *J. Biol. Chem.* **278**, 28993 (2003).
- S. Carreno, A. E. Engqvist-Goldstein, C. X. Zhang, K. L. McDonald, D. G. Drubin, *J. Cell Biol.* **165**, 781 (2004).
- A. E. Engqvist-Goldstein et al., *Mol. Biol. Cell* **15**, 1666 (2004).
- R. Lundmark, S. R. Carlsson, *J. Biol. Chem.* **278**, 46772 (2003).
- F. Soulet, D. Yasar, M. Leonard, S. L. Schmid, *Mol. Biol. Cell* **16**, 2058 (2005).
- R. Lundmark, S. R. Carlsson, *J. Biol. Chem.* **279**, 42694 (2004).
- P. Sarret et al., *J. Biol. Chem.* **279**, 8029 (2004).
- K. Farsad et al., *J. Cell Biol.* **155**, 193 (2001).
- J. Modregger, A. A. Schmidt, B. Ritter, W. B. Huttner, M. Plomann, *J. Biol. Chem.* **278**, 4160 (2003).
- R. Lundmark, S. R. Carlsson, *Biochem. J.* **362**, 597 (2002).
- J. Hirst, A. Motley, K. Harasaki, S. Y. Peak Chew, M. S. Robinson, *Mol. Biol. Cell* **14**, 625 (2003).
- K. Tsujita et al., *J. Cell Biol.* **172**, 269 (2006).
- T. Itoh et al., *Dev. Cell* **9**, 791 (2005).
- H. Cao et al., *Mol. Cell Biol.* **23**, 2162 (2003).
- H. Cao et al., *Nat. Cell Biol.* **7**, 483 (2005).
- A. E. Engqvist-Goldstein, D. G. Drubin, *Annu. Rev. Cell Dev. Biol.* **19**, 287 (2003).
- M. M. Kessels, J. Dong, W. Leibig, P. Westermann, B. Qualmann, *J. Cell Sci.* **119**, 1504 (2006).
- M. M. Kessels, A. E. Engqvist-Goldstein, D. G. Drubin, B. Qualmann, *J. Cell Biol.* **153**, 351 (2001).
- J. E. Hinshaw, *Annu. Rev. Cell Dev. Biol.* **16**, 483 (2000).
- D. Danino, K. H. Moon, J. E. Hinshaw, *J. Struct. Biol.* **147**, 259 (2004).
- A. Roux, K. Uyhazi, A. Frost, P. De Camilli, *Nature* **441**, 528 (2006).
- M. A. McNiven, H. Cao, K. R. Pitts, Y. Yoon, *Trends Biochem. Sci.* **25**, 115 (2000).
- M. Bonazzi et al., *Nat. Cell Biol.* **7**, 570 (2005).
- M. M. Kessels, B. Qualmann, *J. Cell Sci.* **117**, 3077 (2004).

### Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5793/1591/DC1

Table S1

References

10.1126/science.1118133