Membrane tethering in intracellular transport

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Studies of various membrane trafficking steps over the past year indicate that membranes are tethered together prior to the interaction of v-SNAREs and t-SNAREs across the membrane junction. The tethering proteins identified to date are quite large, being either fibrous proteins or multimeric protein complexes. The tethering factors employed at different steps are evolutionarily unrelated, yet their function seems to be closely tied to the more highly conserved Rab GTPases. Tethering factors may collaborate with Rabs and SNAREs to generate targeting specificity in the secretory pathway.

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Abbreviations

COP	coat protein
EEA1	early endosome antigen 1
EM	electron microscopy
ER	endoplasmic reticulum
GDI	guanine nucleotide dissociation inhibitor
NSF	*N*-ethylmaleimide-sensitive factor
SNAP	soluble NSF attachment protein
SNARE	SNAP receptor
TRAPP	transport protein particle

Introduction

Transit of cargo through the secretory pathway is mediated by membrane-bound carriers [1], which can take the form of vesicles or larger membrane tubules. To maintain an ordered flow through the pathway, pairs of membranes must specifically recognize one another and subsequently fuse. The determinants of this targeting specificity were for some time suspected to be members of the Rab GTPase family because distinct family members are arrayed on different membranous compartments (for review see [2]). This concept was called into question when it was found that a single chimeric Rab protein could function at two transport steps [3,4]. In a similar fashion, SNARE proteins, which are integral membrane proteins found predominantly on vesicles (v-SNAREs) or target membranes (t-SNAREs), were proposed to embody specificity [5]. This too has been debated because some SNAREs can function at several transport steps *in vivo* [6], some SNAREs have been found in multiple SNARE complexes by co-immunoprecipitation [7,8], and SNAREs that faithfully function at different transport steps *in vivo* can interact promiscuously *in vitro* [9•]. These findings indicate that neither Rabs, nor SNAREs, are the sole determinants of targeting specificity.

Table 1

Proteins potentially involved in tethering.

Transport step	Potential tethering factors	References
ER to Golgi	Uso1p, Sec35p, TRAPP	[14–16,17••,18, 19,20••,21••]
Intra-Golgi	Giantin/p115/ GM130/GRASP65	[25–32,33•, 34–36,37••,38,39••] [14,25–27,30,31]
Golgi to plasma membrane	Exocyst	[44–48,49••,50]
Vacuole	Rab GTPase?	[53••,57,58••,59]
Endosome	EEA1/Vac1p	[60–64,65•, 66•,67••,68,70•,71•]

The above considerations make it likely that some other component imparts specificity, or that specificity is generated by the collaboration of several factors with no individual component playing a dominant role. Tethering factors (Table 1), which are proteins that bind membranes together prior to SNARE interactions, are likely to assist in imparting targeting specificity. Although they are of critical importance, they cannot be the sole determinants of targeting specificity because they are soluble or peripheral membrane proteins and therefore must interact with integral membrane components with distinct localization patterns. In this review we briefly describe what is known about tethering factors used in different transport steps and conclude with a consideration of how the seemingly evolutionarily unrelated tethering factors might interact with the evolutionarily conserved 'core components' found at all steps. In the end, it may turn out that a collaborative effort between tethering factors and core components is required to generate the specificity inherent in membrane interactions. For definitions of some of the terms used in this review see Table 2.

Early evidence for tethering factors

For some time, it was thought that the SNAREs themselves mediated the initial contact of membranes that are destined to fuse [5]; however, several observations suggest that this is unlikely. For example, treatment of neurons with tetanus or botulinum toxins, which specifically proteolyze SNAREs, does not result in depletion of docked synaptic vesicles from the presynaptic plasma membrane [10]. Similarly, deletion of synaptic v-SNAREs or t-SNAREs in *Drosophila* does not prevent synaptic vesicles from associating with the presynaptic plasma membrane [11]. Furthermore, although t-SNAREs are dispersed on the plasma membranes of axons [12] and yeast [13], vesicle docking occurs only at defined sites: the active zone in neurons and the bud tip in yeast.

Table 2

Definitions of terms used in this review.

Terms	Definitions
Accessory factors	Components that are important for one (or a few) trafficking steps but are not members of a family used in all steps. Tethering factors fall into this category.
Core components	The components that have family members common to all trafficking steps, namely Rabs, SNAREs, and t-SNARE associated proteins of the Sec1p family.
SNARE-docked	Membrane interaction mediated, at least in part, by *trans*-SNARE pairing.
Tethering	Adherence of membranes preceding, and independent of, *trans*-SNARE pairing.

These results imply that other factors are responsible for, or at least contribute to, targeting specificity.

Tethering in the early secretory pathway

One of the most intensively studied membrane-trafficking steps is endoplasmic reticulum (ER) to Golgi traffic in the yeast *Saccharomyces cerevisiae*. The 'core components', involved in the consumption of ER-derived vesicles at the Golgi are related to those of many other steps: the v-SNAREs Bet1p, Bos1p, and Sec22p; the t-SNARE Sed5p; the t-SNARE-associated protein Sly1p; and the Rab GTPase Ypt1p. In addition to these core components, several 'accessory factors', which do not seem to have close evolutionary relatives employed at other steps within the cell, are also required. These accessory proteins are thought to function in membrane tethering.

Two of the accessory factors for ER to Golgi transport in yeast are Uso1p [14], a homodimeric molecule with two heads and an extraordinary long coiled-coil tail [15], and Sec35p [16], a novel 32 kDa protein present in both cytosolic and membrane-associated pools [17••]. Genetic studies have shown that both Uso1p and Sec35p act upstream of the core components (Rab/SNAREs/t-SNARE associated protein) because *USO1* or *SEC35* deletions can be suppressed by overexpression of the ER to Golgi v-SNAREs, *YPT1*, or a dominant allele of *SLY1* [17••,18]. *In vitro* experiments with a system that can resolve the stable interaction of ER-derived vesicles with the Golgi from their subsequent fusion have shown that both Uso1p [19] and Sec35p [17••] are required for vesicle tethering. Importantly, this Uso1p- and Sec35p-dependent tethering step precedes, and is independent of, the essential function of the SNAREs and Sly1p [20••]. These findings suggest that tethering might occur without *trans*-SNARE complex assembly, although this has not been formally tested.

Vesicle tethering *in vitro* is inhibited by guanine nucleotide dissociation inhibitor (GDI) [20••], a protein that extracts membrane-bound Rab proteins. Interestingly, GDI treatment was shown to remove both Ypt1p and Uso1p, suggesting that Ypt1p regulates Uso1p binding to membranes [20••]. This finding — in combination with genetic studies that place Ypt1p function downstream of, or parallel to, the function of Uso1p and Sec35p — suggests that Ypt1p may be a link between the upstream events of vesicle tethering and downstream SNARE-mediated functions [18,20••].

Another component that may function in vesicle tethering is an ~800 kDa protein complex with ten subunits, termed TRAPP [21••]. TRAPP was identified by virtue of the interactions of a gene encoding one of its subunits, *BET3*, with the v-SNARE gene *BET1*. *In vitro* studies have shown that TRAPP is not required for vesicle formation, rather it plays a role in vesicle consumption. As the genetic interactions of two TRAPP genes, *BET3* and *BET5*, with other genes involved in ER to Golgi traffic are very similar to those of *USO1* and *SEC35*, it seems likely that TRAPP functions in the same process, that is tethering. Indeed, the TRAPP complex resides and functions on the *cis*-Golgi, which is consistent with this function.

Tethering in the Golgi

The Golgi apparatus is the hub of a large amount of membrane traffic in the cell and is generally surrounded by a large number of vesicles [22]. Presently there is debate as to whether these vesicles move cargo in the forward direction, as would be suggested by the vesicular transport model, or whether they transport Golgi enzymes in the retrograde direction as suggested by the cisternal maturation model of Golgi transport [23]. Regardless of directionality, both models suggest that the vesicles are derived from one level of the Golgi stack and fuse with another.

Reconstitution of Golgi protein traffic *in vitro* [24] and subsequent biochemical dissection led to the identification of p115 [25], which is the mammalian homolog of Uso1p [26]. The first evidence implicating p115 in vesicle tethering came from work that showed that p115 could bind distinct membranes together prior to membrane fusion [27]. This p115-dependent tethering might be mediated through a chain of interactions involving at least four proteins: giantin, p115, GM130 (Golgi matrix protein of 130kDa), and GRASP65 (Golgi stacking protein of 65kDa) [28]. Giantin is an integral membrane protein of Golgi-associated coat protein (COP)I vesicles with an approximately 250 nm long coiled-coil cytoplasmic domain [29,30]. It is anchored into the membrane by its carboxyl terminus [29] and interacts with p115 [28], the carboxyl terminus of which in turn interacts with the amino terminus of GM130 [28,31], a coiled-coil protein of the Golgi [32]. Finally, the carboxyl terminus of GM130 binds to GRASP65 [33•], which is thought to be anchored in the Golgi membrane by a myristoyl moiety [34]. Although simultaneous interaction of all these proteins has not been demonstrated, these interactions have the

potential to generate a giantin–p115–GM130–GRASP65 tether that can be traced from the vesicle membrane to the Golgi.

Membrane transport through the Golgi ceases during mitosis [35] and recent results suggest that this regulation is mediated at the level of vesicle tethering. It has been shown that p115 does not bind to mitotic Golgi [36] because the p115 binding site within GM130 is phosphorylated upon entry into mitosis [32]. This phosphorylation is mediated directly by the kinase Cdc2 and involves primarily a single serine within the highly conserved p115-binding site [37••]. This finding provides a nice explanation for why the Golgi apparatus of animal cells disassembles during mitosis: vesicle budding continues but the loss of tethering allows vesicles to diffuse away from, or not re-attach to, the Golgi.

Elegant electron microscopic studies of the Golgi have provided a beautiful complement to these biochemical findings. For example, deep-etch rotary shadowing electron microscopy (EM) [38] and transmission EM [39••] of Golgi have revealed what appear to be proteinaceous links between the Golgi and nearby transport vesicles. It is possible that these tethers are composed of fibers of giantin–p115–GM130–GRASP65, or any of a number of the many coiled-coil proteins that have been identified as Golgi constituents (for example [40•,41–43]). In addition, freeze fracture EM has shown that there is a gradient of density of intra-membranous particles (i.e. integral membrane proteins or protein complexes) across the Golgi stack, and that vesicles have particle densities similar to nearby, not distant, cisternae [39••]. The authors interpret these results to suggest that vesicles are not free to diffuse away from the site of budding. Rather, they are retained by proteinaceous tethers. This mechanism would only allow vesicles to fuse with nearby cisternae and result in an ordered movement of material (be it cargo in the anterograde direction, or enzymes in the retrograde direction) through the stack.

Tethering at the plasma membrane

The final step of secretion — delivery and fusion of Golgi-derived vesicles with the plasma membrane — has been extensively studied in yeast. The core components involved in this trafficking step are: Snc1p and Snc2p (which are functionally redundant); the t-SNAREs Sso1p and Sso2p (again, functionally redundant) and Sec9p; the Rab GTPase Sec4p; and finally Sec1, which is related to Sly1p.

In addition to these core components several accessory factors are required for this step. One of these, the so-called 'exocyst', is a large heptameric protein complex containing Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, and Exo70p [44]. A homologous complex has been identified in mammalian cells [45], where it may be involved be involved in polarized secretion in neurons and epithelial cells [46].

The site of polarized secretion in yeast migrates from the bud tip to the bud neck and back again during the cell cycle, and the exocyst is always localized to these sites [47]. Moreover, one of the exocyst components, Sec3p, localizes at the appropriate secretory site independent of other exocyst polypeptides or components involved in generating cell polarity [48]. Because plasma membrane t-SNAREs in yeast are not localized to the site of secretion, it has been proposed that Sec3p marks the plasma membrane docking site for Golgi-derived vesicles [48].

Recent work [49••] has shown that another exocyst component Sec15p can associate with Golgi to plasma membrane secretory vesicles when overexpressed. Association might be mediated in part through the specific interaction of Sec15p with the GTP-bound form of the Rab Sec4p, which is normally found on these vesicles. Interestingly, Sec15p and another exocyst component Sec10p, can be found in a subcomplex that does not contain other exocyst polypeptides; furthermore, in wild-type cells Sec3p is found exclusively in the heptameric exocyst complex, whereas in the absence of Sec4p function Sec3p is not incorporated into the complex. These results suggest that vesicles might be tethered to the plasma membrane by assembly of the exocyst, with the key player Sec15p (and perhaps Sec10p) localized on the vesicle and the remainder of the complex, including Sec3p, at the docking site on the plasma membrane. This interpretation is supported by analysis of the protein–protein interactions within the exocyst, where a chain of interactions leading from Sec15p to Sec3p have been identified [49••], as well as by genetic studies of the exocyst [50].

Vacuole tethering in *Saccharomyces cerevisiae*

The inheritance of yeast vacuoles involves fusion of vacuolar fragments derived from the mother cell [51]. This homotypic fusion event has been reconstituted *in vitro* [52] and has been used to identify the factors and to dissect the mechanism of membrane tethering, docking and fusion. The system is particularly powerful because it can be manipulated to allow numerous intermediates along the reaction pathway to be trapped and, in several cases, the intermediates can be 'chased' to complete the reaction. This property allows clear ordering of the biochemical events and requirements.

In this system, the vacuoles have both v-SNAREs (Nyv1p and Vam7p) and a t-SNARE (Vam3p) [53,54] and these must be primed by the ATPase NSF (Sec18p in yeast) in order to allow fusion. NSF-dependent priming involves the disassembly of *cis*-SNARE pairs and the loading onto the t-SNARE of a factor called LMA1 (a heterodimer of thioredoxin and a protease inhibitor) [55,56]. The next step is a tethering event that is dependent on the Rab GTPase Ypt7p [57,58••]. Tethering is not dependent on the SNAREs, however, because anti-SNARE antibodies

do not inhibit this step even though they can inhibit the overall reaction [58••].

Importantly, the tethering event can be reversed by extraction of Ypt7p from the membrane with GDI — but only if *trans*-SNARE pairing has not occurred. This suggests that the GTPase plays is integral role in the tethered state and is not simply required for its formation. Once *trans*-SNARE pairing has taken place, removal of Ypt7p does not result in separation of the membranes. This corresponds to a 'SNARE-docked' state, where the membranes are tightly coupled by *trans*-SNARE pairs. Finally, membrane fusion occurs in a step that appears to be independent of the presence of *trans*-SNARE pairs [58••] and that has been shown to require Ca^{2+}/calmodulin [59].

Endosome tethering

The fusion of endosomes in mammalian cells requires Rab5, and many of the proteins that collaborate with Rab5 in this process have been identified. Rabaptin5–Rabex5 is a protein complex that consists of a Rab5 guanine nucleotide exchange factor (Rabex5 [60]) and a Rab5 effector (Rabaptin5) [61]. The net effect of these two activities is to generate and stabilize the GTP-bound form of Rab5, which then becomes associated with the endosome membrane along with the Rabaptin5–Rabex5 complex [60]. Another important factor is EEA1, a large predominantly coiled-coil endosomal protein [62] with a zinc-binding 'FYVE finger' [63]. EEA1 also binds phosphatidylinositol-3-phosphate [64] and Rab5•GTP [65•].

It has recently been shown that EEA1 is required for homotypic endosome fusion [65•,66•] and that this protein can tether endosomal membranes to one another [67••]. Similar to the ER to Golgi and vacuole systems described above, tethering seems to be independent of *trans*-SNARE pairing because it can occur in the absence of NSF-mediated SNARE priming, which is presumably a prerequisite for *trans*-SNARE pairing [67••]. Another important finding of this work was that although the function of EEA1 is enhanced by Rab5, high levels of EEA1 can tether endosomes in the absence of Rab5 function. This suggests that Rab5 may act upstream of EEA1 in the process of endosome–endosome tethering but does not preclude the possibility that they act together.

Golgi to endosome transport in yeast has been shown to require an EEA1 homolog, termed Vac1p (or Pep7p or Vps19p) [68,69]. Recent studies [70•,71•] have shown that Vac1p can interact with the GTP-bound form of Vps21p, the Rab protein required at that step. Interestingly, Vac1p also interacts with phosphatidylinositol 3-phosphate and Vps45p, which is the endosomal t-SNARE-associated Sec1p-family member. The authors suggest that Vac1p may act as an adaptor protein that integrates multiple inputs to co-ordinate some aspect of vesicle targeting, perhaps tethering.

Common features of tethering systems

One of the common features of membrane tethering systems is their dependence on the large family of structurally and functionally related Rab GTPases. In contrast, different intracellular transport steps utilize entirely unrelated tethering factors. For example, Uso1p, the exocyst, and EEA1 are used in the early secretory pathway at the plasma membrane or at the endosome, respectively. This feature leads one to wonder about the evolutionary basis of vesicle targeting mechanisms. It is reasonable to assume that the families of core components (Rabs, SNAREs, and t-SNARE-associated proteins) arose by gene duplication and divergence, generating related but distinct targeting systems that could be employed to achieve transport between evolving systems of membrane-bound compartments. It would then follow that step-specific tethering factors evolved to enhance or embellish the functionality of an established core system. In this case, one might expect accessory components to enhance an existing vesicle targeting interaction, rather than inserting themselves as intermediaries into an already functional system. Thus, evolutionary considerations might lead one to predict that tethering factors act in concert with Rabs, rather than functioning between Rabs and SNAREs.

At present, the best evidence that Rabs function upstream of a tethering component is provided by studies of Rab5 and EEA1 [67••]. In this work, Christoforidis *et al.* showed that high levels of EEA1 could drive endosome–endosome fusion *in vitro* even in the presence of GDI, a treatment that should sequester Rab5. The most straightforward interpretation of this result is that Rab5 acts upstream of EEA1. It is also possible, however, that Rab5 acts in concert with EEA1 but, in the absence of Rab5, high levels of EEA1 could drive the endosome fusion reaction. If Rabs function primarily to recruit a tethering factor onto membranes, their action will appear to be required concomitant with the function of the tethering factor. If a tethering factor has another means by which to associate with membranes (such as EEA1 binding to phosphorylated phosphatidylinositol lipids), however, Rab function could be bypassed. We favor the idea that Rabs function to recruit tethering factors onto membranes and to co-ordinate membrane tethering with downstream events such as *trans*-SNARE pairing [20••].

Conclusions

A large body of data now indicates that membranes become tethered together prior to SNARE complex formation and subsequent membrane fusion. Unexpectedly diverse tethering factors have been identified; their only common feature is their tendency to form elongated, coiled-coil structures and/or to assemble into large, multimeric assemblies. All of the tethering events described to date are intimately related to the function of a distinct Rab

GTPase. Thus, it now seems likely that the specificity of membrane targeting is achieved through the combined actions of Rabs, tethering factors, and SNAREs.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Palade G: **Intracellular aspects of the process of protein secretion.** *Science* 1975, **189**:347-358.

2. Simons K, Zerial M: **Rab proteins and the road maps for intracellular transport.** *Neuron* 1993, **11**:789-799.

3. Brennwald P, Novick P: **Interactions of three domains distinguishing the Ras-related GTP- binding proteins Ypt1 and Sec4.** *Nature* 1993, **362**:560-563.

4. Dunn B, Stearns T, Botstein D: **Specificity domains distinguish the Ras-related GTPases Ypt1 and Sec4.** *Nature* 1993, **362**:563-565.

5. Söllner T, Whiteheart SW, Brunner M, Erdjument-Bromage H, Geromanos S, Tempst P, Rothman JE: **SNAP receptors implicated in vesicle targeting and fusion.** *Nature* 1993, **362**:318-324.

6. Fischer von Mollard G, Nothwehr SF, Stevens TH: **The yeast v-SNARE Vti1p mediates two vesicle transport pathways through interactions with the t-SNAREs Sed5p and Pep12p.** *J Cell Biol* 1997, **137**:1511-1524.

7. Holthuis JC, Nichols BJ, Dhruvakumar S, Pelham HR: **Two syntaxin homologues in the TGN/endosomal system of yeast.** *EMBO J* 1998, **17**:113-126.

8. Lupashin VV, Pokrovskaya ID, McNew JA, Waters MG: **Characterization of a novel yeast SNARE protein implicated in Golgi retrograde traffic.** *Mol Biol Cell* 1997, **8**:2659-2676.

9. • Yang B, Gonzalez L Jr, Prekeris R, Steegmaier M, Advani RJ, Scheller RH: **SNARE interactions are not selective. Implications for membrane fusion specificity.** *J Biol Chem* 1999, **274**:5649-5653.

Eleven purified recombinant SNARE proteins were used to generate 21 different SNARE complexes, all of which were quite stable as assessed by their thermal melting profiles. There was no correlation between complex stability and whether the subunits were employed at the same step *in vivo,* suggesting that the thermodynamics of SNARE interactions cannot be the sole determinant of membrane trafficking specificity.

10. Hunt JM, Bommert K, Charlton MP, Kistner A, Habermann E, Augustine GJ, Betz H: **A post-docking role for synaptobrevin in synaptic vesicle fusion.** *Neuron* 1994, **12**:1269-1279.

11. Broadie K, Prokop A, Bellen HJ, O'Kane CJ, Schulze KL, Sweeney ST: **Syntaxin and synaptobrevin function downstream of vesicle docking in *Drosophila.*** *Neuron* 1995, **15**:663-673.

12. Garcia EP, McPherson PS, Chilcote TJ, Takei K, De Camilli P: **rbSec1A and B colocalize with syntaxin 1 and SNAP-25 throughout the axon, but are not in a stable complex with syntaxin.** *J Cell Biol* 1995, **129**:105-120.

13. Brennwald P, Kearns B, Champion K, Keranen S, Bankaitis V, Novick P: **Sec9 is a SNAP-25-like component of a yeast SNARE complex that may be the effector of Sec4 function in exocytosis.** *Cell* 1994, **79**:245-258.

14. Nakajima H, Hirata A, Ogawa Y, Yonehara T, Yoda K, Yamasaki M: **A cytoskeleton-related gene, *uso1*, is required for intracellular protein transport in *Saccharomyces cerevisiae*.** *J Cell Biol* 1991, **113**:245-260.

15. Yamakawa H, Seog D-H, Yoda K, Yamasaki M, Wakabayashi T: **Uso1 protein is a dimer with two globular heads and a long coiled-coil tail.** *J Struct Biol* 1996, **116**:356-365.

16. Wuestehube LJ, Duden R, Eun A, Hamamoto S, Korn P, Ram R, Schekman R: **New mutants of *Saccharomyces cerevisiae* affected in the transport of proteins from the endoplasmic reticulum to the Golgi complex.** *Genetics* 1996, **142**:393-406.

17. •• Van Rheenen SM, Cao X, Lupashin VV, Barlowe C, Waters MG: **Sec35p, a novel peripheral membrane protein, is required for ER to Golgi vesicle docking.** *J Cell Biol* 1998, **141**:1107-1119.

This paper identifies Sec35p as a novel peripheral membrane protein of 32 kDa, deletion of which results in a severe growth defect that can be suppressed by the Rab protein Ypt1p or a gain of function mutant to the t-SNARE-associated protein Sly1p. These genetic data suggest that Sec35p acts upstream of, or in collaboration with, Ypt1p and Sly1p. *In vitro* experiments indicate that Sec35p is required for tethering of ER-derived vesicles to the Golgi.

18. Sapperstein SK, Lupashin VV, Schmitt HD, Waters MG: **Assembly of the ER to Golgi SNARE complex requires Uso1p.** *J Cell Biol* 1996, **132**:755-767.

19. Barlowe C: **Coupled ER to Golgi transport reconstituted with purified cytosolic components.** *J Cell Biol* 1997, **139**:1097-1108.

20. •• Cao X, Ballew N, Barlowe C: **Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins.** *EMBO J* 1998, **17**:2156-2165.

Genetic and biochemical manipulation of an *in vitro* system for ER to Golgi transport in yeast was used to show that vesicle tethering does not require the essential function of the ER to Golgi v-SNAREs Bet1p or Bos1p, the t-SNARE Sed5p, or the t-SNARE-associated protein Sly1p; however, tethering does require Uso1p and the Rab protein Ypt1p, which affects Uso1p-membrane binding. Thus, tethering is clearly separable from SNARE function.

21. •• Sacher M, Jiang Y, Barrowman J, Scarpa A, Burston J, Zhang L, Schieltz D, Yates IJ, Abeliovich H, Ferro-Novick S: **TRAPP, a highly conserved novel complex on the *cis*-Golgi that mediates vesicle docking and fusion.** *EMBO J* 1998, **17**:2494-2503.

A large protein complex that contains ten protein subunits, including Bet3p and Bet5p, was immuno-isolated. The complex, termed TRAPP, is localized to the Golgi complex. Genetic and biochemical studies suggest that it plays a role in the tethering or fusion phase of ER to Golgi traffic. Mammalian homologs of TRAPP components were identified with database searches.

22. Farquhar MG, Palade GE: **The Golgi apparatus: 100 years of progress and controversy.** *Trends Cell Biol* 1998, **8**:2-10.

23. Glick BS, Malhotra V: **The curious status of the Golgi apparatus.** *Cell* 1998, **95**:883-889.

24. Balch WE, Dunphy WG, Braell WA, Rothman JE: **Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of *N*-acetylglucosamine.** *Cell* 1984, **39**:405-416.

25. Waters MG, Clary DO, Rothman JE: **A novel 115-kD peripheral membrane protein is required for intercisternal transport in the Golgi stack.** *J Cell Biol* 1992, **118**:1015-1026.

26. Sapperstein SK, Walter DM, Grosvenor AR, Heuser JE, Waters MG: **p115 is a general vesicular transport factor related to the yeast ER-Golgi transport factor Uso1p.** *Proc Natl Acad Sci USA* 1995, **92**:522-526.

27. Barroso M, Nelson DS, Sztul E: **Transcytosis-associated protein (TAP)/p115 is a general fusion factor required for binding of vesicles to acceptor membranes.** *Proc Natl Acad Sci USA* 1995, **92**:527-531.

28. Sonnichsen B, Lowe M, Levine T, Jamsa E, Dirac-Svejstrup B, Warren G: **A role for giantin in docking COPI vesicles to Golgi membranes.** *J Cell Biol* 1998, **140**:1013-1021.

29. Linstedt AD, Foguet M, Renz M, Seelig HP, Glick BS, Hauri H-P: **A C-terminally-anchored Golgi protein is inserted into the endoplasmic reticulum and then transported to the Golgi apparatus.** *Proc Natl Acad Sci USA* 1995, **92**:5102-5105.

30. Linstedt AD, Hauri HP: **Giantin, a novel conserved Golgi membrane protein containing a cytoplasmic domain of at least 350 kDa.** *Mol Biol Cell* 1993, **4**:679-693.

31. Nelson DS, Alvarez C, Gao YS, Garcia-Mata R, Fialkowski E, Sztul E: **The membrane transport factor TAP/p115 cycles between the Golgi and earlier secretory compartments and contains distinct domains required for its localization and function.** *J Cell Biol* 1998, **143**:319-331.

32. Nakamura N, Lowe M, Levine TP, Rabouille C, Warren G: **The vesicle docking protein p115 binds GM130, a *cis*-Golgi matrix protein, in a mitotically regulated manner.** *Cell* 1997, **89**:445-455.

33. • Barr FA, Nakamura N, Warren G: **Mapping the interaction between GRASP65 and GM130, components of a protein complex involved in the stacking of Golgi cisternae.** *EMBO J* 1998, **17**:3258-3268.

This paper documents a direct interaction and maps the binding sites between GM130 and GRASP65, two components of the giantin–p115–GM130–GRASP65 tether that links COPI vesicles with Golgi membranes. The carboxyl terminus of GM130 interacts with a conserved region of GRASP65 similar to PDZ domains, which are often involved in targeting membrane proteins.

34. Barr FA, Puype M, Vandekerckhove J, Warren G: **GRASP65, a protein involved in the stacking of Golgi cisternae.** *Cell* 1997, **91**:253-262.

35. Warren G, Featherstone C, Griffiths G, Burke B: **Newly synthesized G protein of vesicular stomatitis virus is not transported to the cell surface during mitosis.** *J Cell Biol* 1983, **97**:1623-1628.

36. Levine TP, Rabouille C, Kieckbusch RH, Warren G: **Binding of the vesicle docking protein p115 to Golgi membranes is inhibited under mitotic conditions.** *J Biol Chem* 1996, **271**:17304-17311.

37. •• Lowe M, Rabouille C, Nakamura N, Watson R, Jackman M, Jamsa E, Rahman D, Pappin DJ, Warren G: **Cdc2 kinase directly phosphorylates the *cis*-Golgi matrix protein GM130 and is required for Golgi fragmentation in mitosis.** *Cell* 1998, **94**:783-793.
Direct phosphorylation by Cdc2 kinase of a single serine in the Golgi protein GM130 eliminated its ability to function as a receptor for the membrane tethering protein p115. The resulting disruption of tethering might explain why the Golgi disassembles during mitosis: vesicle budding continues while tethering, and thus fusion, is inhibited.

38. Weidman P, Roth R, Heuser J: **Golgi membrane dynamics imaged by freeze-etch electron microscopy: views of different membrane coatings involved in tubulation versus vesiculation.** *Cell* 1993, **75**:123-133.

39. •• Orci L, Perrelet A, Rothman JE: **Vesicles on strings: morphological evidence for processive transport within the Golgi stack.** *Proc Natl Acad Sci USA* 1998, **95**:2279-2283.
Freeze-fracture electron microscopy was used to show that a gradient of density of intramembranous particles exits across the Golgi stack, with the *cis* side having more particles than the *trans* side. Vesicles have an intramembranous particle density similar to the adjacent cisternae suggesting that they are kept near the parent cisternae. Proteinaceous 'strings' were visualized by thin section EM. The authors suggest that processive transport through the Golgi may occur via tethered vesicles rather than freely diffusible vesicles.

40. • Bascom RA, Srinivasan S, Nussbaum RL: **Identification and characterization of golgin-84, a novel Golgi integral membrane protein with a cytoplasmic coiled-coil domain.** *J Biol Chem* 1999, **274**:2953-2962.
An 84 kDa Golgi type II integral membrane protein was identified through use of a two-hybrid screen with a Golgi phosphatidylinositol 4,5-bisphosphate 5-phosphatase as bait. It has structural and sequence homology to giantin and the authors suggest that, like giantin, it might function in transport vesicle tethering.

41. Erlich R, Gleeson PA, Campbell P, Dietzsch E, Toh B-H: **Molecular characterization of *trans*-Golgi p230.** *J Biol Chem* 1996, **271**:8328-8337.

42. Fritzler MJ, Lung CC, Hamel JC, Griffith KJ, Chan EK: **Molecular characterization of Golgin-245, a novel Golgi complex protein containing a granin signature.** *J Biol Chem* 1995, **270**:31262-31268.

43. Misumi Y, Sohda M, Yano A, Fujiwara T, Ikehara Y: **Molecular characterization of GCP170, a 170-kDa protein associated with the cytoplasmic face of the Golgi membrane.** *J Biol Chem* 1997, **72**:23851-23858.

44. TerBush DR, Maurice T, Roth D, Novick P: **The exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*.** *EMBO J* 1996, **15**:6483-6494.

45. Kee Y, Yoo JS, Hazuka CD, Peterson KE, Hsu SC, Scheller RH: **Subunit structure of the mammalian exocyst complex.** *Proc Natl Acad Sci USA* 1997, **94**:14438-14443.

46. Hsu S-C, Hazuka CD, Foletti DL, Scheller RH: **Targeting vesicles to specific sites on the plasma membrane: the role of the Sec6/8 complex.** *Trends Cell Biol* 1999, **9**:150-153.

47. Finger FP, Novick P: **Spatial regulation of exocytosis: lessons from yeast.** *J Cell Biol* 1998, **142**:609-612.

48. Finger FP, Hughes TE, Novick P: **Sec3p is a spatial landmark for polarized secretion in budding yeast.** *Cell* 1998, **92**:559-571.

49. •• Guo W, Roth D, Walch-Solimena C, Novick P: **The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis.** *EMBO J* 1999, **18**:1071-1080.
This work shows that Sec15p can associate with Golgi-to-plasma-membrane vesicles and can physically interact with the GTP-bound form of the Rab GTPase Sec4p, which is normally found on these vesicles. Analyses of the protein–protein interactions within the exocyst indicated that Sec10p and Sec15p can exist in a subcomplex. Finally, it was shown that Sec4p affects integration of Sec3p, which marks the sites of exocytosis on the plasma membrane, into the exocyst complex. These results suggest that vesicles may be tethered to the plasma membrane by Sec4p-regulated assembly of the exocyst, with the key player Sec15p (and perhaps Sec10p) localized on the vesicle and the remainder of the complex, including Sec3p, at the docking site on the plasma membrane.

50. Roth D, Guo W, Novick P: **Dominant negative alleles of SEC10 reveal distinct domains involved in secretion and morphogenesis in yeast.** *Mol Biol Cell* 1998, **9**:1725-1739.

51. Weisman LS, Wickner W: **Intervacuole exchange in the yeast zygote: a new pathway in organelle communication.** *Science* 1988, **241**:589-591.

52. Conradt B, Shaw J, Vida T, Emr S, Wickner W: ***In vitro* reactions of vacuole inheritance in *Saccharomyces cerevisiae*.** *J Cell Biol* 1992, **119**:1469-1479.

53. Nichols BJ, Ungermann C, Pelham HR, Wickner WT, Haas A: **Homotypic vacuolar fusion mediated by t- and v-SNAREs.** *Nature* 1997, **387**:199-202.

54. Ungermann C, Wickner W: **Vam7p, a vacuolar SNAP-25 homolog, is required for SNARE complex integrity and vacuole docking and fusion.** *EMBO J* 1998, **17**:3269-3276.

55. Ungermann C, Nichols BJ, Pelham HR, Wickner W: **A vacuolar v–t-SNARE complex, the predominant form *in vivo* and on isolated vacuoles, is disassembled and activated for docking and fusion.** *J Cell Biol* 1998, **140**:61-69.

56. Xu Z, Sato K, Wickner W: **LMA1 binds to vacuoles at Sec18p (NSF), transfers upon ATP hydrolysis to a t-SNARE (Vam3p) complex, and is released during fusion.** *Cell* 1998, **93**:1125-1134.

57. Mayer A, Wickner W: **Docking of yeast vacuoles is catalyzed by the Ras-like GTPase Ypt7p after symmetric priming by Sec18p (NSF).** *J Cell Biol* 1997, **136**:307-313.

58. •• Ungermann C, Sato K, Wickner W: **Defining the functions of *trans*-SNARE pairs.** *Nature* 1998, **396**:543-548.
This work demonstrated that the homotypic vacuole–vacuole fusion event can be divided into at least four steps: first, separation of *cis*-SNARE pairs so they are competent for later *trans*-interactions (priming), second a tethered state that requires the GTPase Ypt7p for its formation and maintenance but is independent of SNAREs, third a *trans*-SNARE-docked state, and finally the fusion event, which can be uncoupled from SNARE action.

59. Peters C, Mayer A: **Ca^{2+}/calmodulin signals the completion of docking and triggers a late step of vacuole fusion.** *Nature* 1998, **396**:575-580.

60. Horiuchi H, Lippe R, McBride HM, Rubino M, Woodman P, Stenmark H, Rybin V, Wilm M, Ashman K, Mann M, Zerial M: **A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function.** *Cell* 1997, **90**:1149-1159.

61. Stenmark H, Vitale G, Ullrich O, Zerial M: **Rabaptin-5 is a direct effector of the small GTPase Rab5 in endocytic membrane fusion.** *Cell* 1995, **83**:423-432.

62. Mu FT, Callaghan JM, Steele-Mortimer O, Stenmark H, Parton RG, Campbell PL, McCluskey J, Yeo JP, Tock EP, Toh BH: **EEA1, an early endosome-associated protein. EEA1 is a conserved alpha-helical peripheral membrane protein flanked by cysteine 'fingers' and contains a calmodulin-binding IQ motif.** *J Biol Chem* 1995, **270**:13503-13511.

63. Stenmark H, Aasland R, Toh BH, D'Arrigo A: **Endosomal localization of the autoantigen EEA1 is mediated by a zinc- binding FYVE finger.** *J Biol Chem* 1996, **271**:24048-24054.

64. Patki V, Virbasius J, Lane WS, Toh BH, Shpetner HS, Corvera S: **Identification of an early endosomal protein regulated by phosphatidylinositol 3-kinase.** *Proc Natl Acad Sci USA* 1997, **94**:7326-7330.

65. • Simonsen A, Lippe R, Christoforidis S, Gaullier JM, Brech A, Callaghan J, Toh BH, Murphy C, Zerial M, Stenmark H: **EEA1 links PI(3)K function to Rab5 regulation of endosome fusion.** *Nature* 1998, **394**:494-498.
EEA1, which was shown in other work to tether endosomes together prior to fusion, was shown here to bind directly to Rab5•GTP. There are two Rab5•GTP binding sites within EEA1, one at the amino terminus and one at the carboxyl terminus. *In vivo* and *in vitro* assays were also used to demonstrate that EEA1 is required for endosome fusion.

66. Mills IG, Jones AT, Clague MJ: **Involvement of the endosomal**
• **autoantigen EEA1 in homotypic fusion of early endosomes.** *Curr Biol* 1998, **8**:881-884.
This work shows that EEA1 is required for endosome fusion and complements another study [65•] that shows that EEA1 tethers endosomes together prior to fusion.

67. Christoforidis S, McBride HM, Burgoyne RD, Zerial M: **The Rab5**
•• **effector EEA1 is a core component of endosome docking.** *Nature* 1999, **397**:621-625.
Rab5•GTPγS affinity chromatography was used to identify 22 interacting proteins, including Rabaptin5, Rabex5, and EEA1. These proteins could completely substitute for cytosol in driving an *in vitro* endosome fusion assay, with EEA1 contributing most of the activity. Other eluted proteins, especially Rabaptin5 and Rabex5, stimulated the EEA1 activity. Interestingly, high levels of EEA1 could bypass the requirement for Rab5, suggesting that Rab5 may act downstream of EEA1. Finally, it was shown that EEA1 can tether endosomes together even in the absence of SNARE priming, which presumably precludes formation of *trans*-SNARE pairs.

68. Burd CG, Peterson M, Cowles CR, Emr SD: **A novel Sec18p/NSF-dependent complex required for Golgi-to-endosome transport in yeast.** *Mol Biol Cell* 1997, **8**:1089-1104.

69. Webb GC, Zhang J, Garlow SJ, Wesp A, Riezman H, Jones EW: **Pep7p provides a novel protein that functions in vesicle-mediated transport between the yeast Golgi and endosome.** *Mol Biol Cell* 1997, **8**:871-895.

70. Peterson MR, Burd CG, Emr SD: **Vac1p coordinates Rab and**
• **phosphatidylinositol 3-kinase signaling in Vps45p-dependent vesicle docking/fusion at the endosome.** *Curr Biol* 1999, **9**:159-162.
Two hybrid and co-immunoprecipitation experiments were used to show that Vac1p, which is homologous to EEA1, interacts with the GTP-bound form of Vps21p (the yeast endosomal Rab) and Vps45p (the yeast endosomal Sec1p-family member). The authors suggest that Vac1p, through its ability to bind to phosphatidylinositol 3-phosphate, Vps21p•GTP, and Vps45p, might integrate multiple inputs and thereby facilitate some aspect of vesicle targeting, perhaps vesicle tethering.

71. Tall GG, Hama H, DeWald DB, Horazdovsky BF: **The**
• **phosphatidylinositol 3-phosphate binding protein Vac1p interacts with a Rab GTPase and a Sec1p homologue to facilitate vesicle-mediated vacuolar protein sorting.** *Mol Cell Biol* 1999, **10**:1873-1889.
This work is similar to that of [70•] with further analysis of the role of the Vac1p FYVE domain in Vps21p and Vps45p interactions.