Vesicle tethering complexes in membrane traffic

James R. C. Whyte and Sean Munro*

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

*Author for correspondence (e-mail sean@mrc-lmb.cam.ac.uk)

Journal of Cell Science 115, 2627-2637 (2002) © The Company of Biologists Ltd

Summary

Despite the recent progress in the field of membrane traffic, the question of how the specificity of membrane fusion is achieved has yet to be resolved. It has become apparent that the SNARE proteins, although central to the process of fusion, are often not the first point of contact between a vesicle and its target. Instead, a poorly understood tethering process physically links the two before fusion occurs. Many factors that have an apparent role in tethering have been identified. Among these are several large protein complexes. Until recently, these seemed unrelated, which was a surprise since proteins involved in membrane traffic often form families, members of which function in each transport step. Recent work has shown that three of the complexes are in fact related. We refer to these as the 'quatrefoil' tethering complexes, since they appear to share a fourfold nature. Here we describe the quatrefoil complexes and other, unrelated, tethering complexes, and discuss ideas about their function. We propose that vesicle tethering may have separate kinetic and thermodynamic elements and that it may be usefully divided into events upstream and downstream of the function of Rab GTPases. Moreover, the diversity of tethering complexes in the cell suggests that not all tethering events occur through the same mechanisms.

Key words: Vesicle tethering, Membrane traffic, Exocyst, Sec34/35 complex, COG complex, GARP complex, TRAPP

Introduction

Fusion of intracellular membranes is mediated in many, if not all, cases by SNARE proteins (Chen and Scheller, 2001). The final stage of fusion involves the formation of a bundle of four parallel core SNARE domains, one contributed by the vesicle and three contributed by the target membrane (Fig. 1). Such a trans SNARE complex bridges the two membranes, and its formation is thought to overcome the energy barrier preventing two membranes from fusing. Only particular combinations of four core SNAREs are able to promote fusion in vitro (McNew et al., 2000; Parlati et al., 2000). A simple model is therefore that the SNARE complement of a vesicle and a potential target membrane is necessary and sufficient to determine their compatibility for fusion.

Several aspects of this model have recently been questioned. In particular, it is unclear whether further factors provide specificity, help in SNARE assembly or even assist in the fusion event itself. The rate of trans complex formation is too slow in vitro to account for the rate of membrane fusion observed in vivo, which suggests that accelerating factors are involved (Fasshauer et al., 2002). There is also evidence that in some cases fusion events are regulated downstream of SNARE complex assembly, although the generality of this is unclear (Muller et al., 2002). Most debate, however, has focused on whether interactions between v- and t-SNAREs can account for the specificity of membrane transport events (Pelham, 2001). Biochemical and genetic studies have identified several proteins that appear to play a role in membrane transport steps after vesicle formation. These factors could contribute to the fidelity of vesicle fusion and function in a process that has become known as tethering (Fig. 1). This is the formation of physical links, often over considerable distances, between two membranes that are due to fuse, before trans SNARE complex formation (for reviews, see Guo et al., 2000; Lowe, 2000; Waters and Hughson, 2000). Tethering might represent the earliest stage at which specificity is conferred on a fusion reaction. Both yeast and mammalian systems have been used in the discovery of tethering factors, and, although our understanding of the process is still limited, the emerging picture is of a series of perhaps inter-related steps that determine the specificity of membrane fusion.

What is the evidence that SNAREs do not provide all the specificity in vivo? The ubiquitous distribution of SNAREs on some membranes is not sufficient to account for the fusion of vesicles to localised regions of those membranes. For example, the yeast plasma membrane SNAREs Sso1p and Sso2p are distributed over the entire plasma membrane, and yet vesicles fuse with only certain parts of the membrane during periods of polarised growth (Brennwald et al., 1994). Cleavage of squid synaptic SNAREs with toxins prevents SNARE complex formation but results in the association of more, not fewer, vesicles with the membrane (Hunt et al., 1994). Similarly, the percentage of tethered neuronal vesicles is significantly higher in flies lacking syntaxin or synaptobrevin than in wild-type flies (Broadie et al., 1995). These results are consistent with SNAREs being involved in membrane fusion but dispensable for a prior tethering event that initially attaches the vesicle to its target without causing it to fuse. Vesicle tethering has been observed in an in vitro reconstituted system, which demonstrated that ER-derived vesicles attach to the Golgi apparatus, losing their ability to diffuse freely, in a reaction that is independent of functional SNARE proteins (Cao et al., 1998). A growing number of factors proposed to be involved in tethering have been identified. In many cases, the mode of action, interactions and indeed identities of these factors remain obscure. Although it is still far from clear how tethering

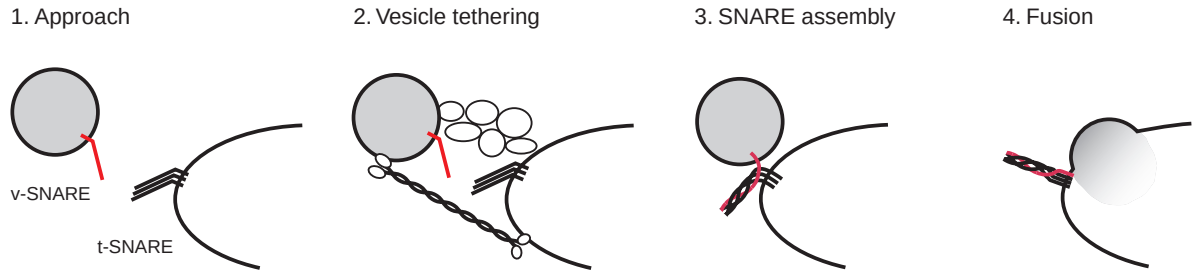

Fig. 1. Steps in the delivery of vesicles to the correct organelle. (1) An intracellular transport vesicle approaches its destination organelle either by diffusion or motor-mediated directed transport. (2) The vesicle is then proposed to be tethered to the organelle by protein complexes and long coiled-coil proteins. (3) A v-SNARE protein on the vesicle then engages a t-SNARE on the target, forming a four-helical bundle whose assembly drives the two bilayers into close proximity, (4) thereby causing membrane fusion. Both vesicle tethering and SNARE assembly have been referred to by others as 'docking', so to avoid confusion we use only the former terms here.

occurs at a molecular level, connections between the variety of seemingly disparate tethering factors are beginning to become apparent and may prove useful in elucidating their roles.

Protein complexes and coiled-coil proteins as potential tethering factors

Two broad classes of molecules are proposed to have a role in tethering: a group of long, coiled-coil proteins and several large, multisubunit complexes. The former have the potential to form homodimeric coiled coils with lengths up to several times the diameter of a vesicle. In yeast, one example is Uso1p, whose importance in tethering is clear since it is an essential protein and the only cytosolic factor required for the tethering of ER-derived vesicles to washed acceptor membranes in the assay mentioned above (Barlowe, 1997). The formation of long coiled coils by Uso1p (Yamakawa et al., 1996) and its mammalian homologue p115 has been observed by electron microscopy (Sapperstein et al., 1995). Likewise, X-ray crystallography has shown that a part of the endosomal tethering protein EEA1 forms long coiled coils (Dumas et al., 2001), and this is generally supposed to hold for the other mammalian examples, the coiled-coil proteins of the Golgi (often called golgins), and their yeast counterparts. These structures have led to the idea that the large coiled-coil proteins are anchored at one end to a membrane, which allows them to 'search' the surrounding space for a passing vesicle, which is then bound by the other end. Although this is an attractive model, there is currently a lack of direct evidence for it.

Although the coiled-coil proteins are known in many cases to bind to a target membrane and/or vesicle, their receptors on the membranes are generally not known. One exception is p115, which tethers COPI vesicles to the Golgi. Its receptors are two other coiled-coil proteins: giantin on the vesicles and GM130 on the Golgi (Sonnichsen et al., 1998). However, the simultaneous binding of p115 to both these molecules has been called into question by the finding that they share, and compete for, a common binding site on p115 (Linstedt et al., 2000). Nevertheless, the in vivo significance of the interaction between p115 and GM130 is demonstrated by the accumulation of transport vesicles and reduction of secretory transport when this interaction is inhibited (Seemann et al., 2000). Giantin is membrane anchored, and GM130 interacts with Golgi membranes through another protein, GRASP65 (Barr et al., 1998). Even in the case of p115, stripped of its complications, the idea that a long, coiled-coil tether links the vesicle and the target has yet to be confirmed. p115 and Uso1p may not even be typical of other large, coiled-coil proteins, since they are unique in having a large globular domain at one end.

Apart from their involvement in the Golgi and in endosomal fusion (Nielsen et al., 2000), long, coiled-coil proteins are not associated with other transport steps. We therefore focus here on the second class of tethering factor, multisubunit complexes, since there has been recent and rapid progress in their characterisation. The overall molecular function of the complexes is still unknown, but biochemical studies have revealed their subunit compositions and interactions with other membrane-trafficking components. The complexes can thus now be grouped and distinguished on the basis of sequence similarity. The resulting classification suggests that the functions of some complexes differ and that tethering is a complex process that encompasses several steps both upstream and downstream of a stable, physical attachment of a vesicle to a target membrane.

Multisubunit tethering complexes

Seven large, conserved complexes have been proposed to have roles in vesicle tethering at distinct trafficking steps (Fig. 2). In most cases these complexes were initially identified and characterised in yeast. Frequently, the same complex has been identified by more than one laboratory and characterised in more than one organism, which has led to a confusing variety of names. It is to be hoped that a standard nomenclature for each complex will soon be adopted, as has recently been agreed for the COG complex (Table 1); we shall use a single name for each complex here. The seven complexes can be divided into two groups: those that have a common domain at the N-terminus of at least some, if not all, of their subunits (Whyte and Munro, 2001), and those that do not (Fig. 3). The family of complexes related to each other by virtue of their shared domain, which we shall call the 'quatrefoil' complexes, consists of the exocyst (termed the Sec6/8 complex in mammalian studies), the conserved oligomeric Golgi (COG) complex [previously known as the Sec34/35 complex, and in mammals also as the Golgi transport complex (GTC) or ldlCp complex] and the Golgi-associated retrograde protein (GARP) complex (which is also known in yeast as the Vps52/53/54 [VFT]). The other group consists of complexes that seem to

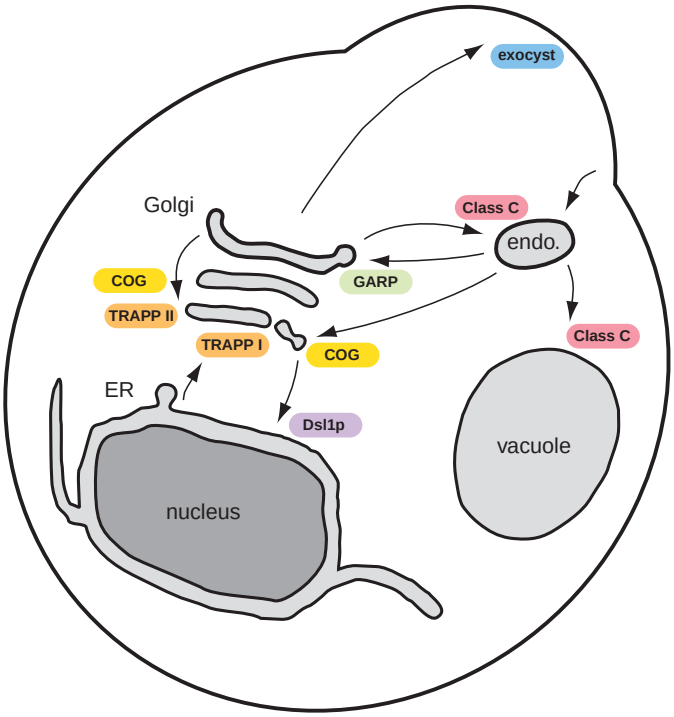

Fig. 2. Putative tethering complexes in the yeast secretory pathway. Protein complexes that have been found to have a role in particular vesicular transport steps are indicated next to those steps. The role of early and late endosomes in yeast is contentious, and so for simplicity this compartment has been shown as a single organelle.

bear no relation to each other or to the quatrefoil complexes and comprises TRAPP, the Class C Vps complex (also known as the Pep3p/Pep5p complex or the homotypic fusion and vacuole protein sorting [HOPS] complex) and the Dsl1p complex.

A family of quatrefoil tethering complexes

The exocyst, COG complex and GARP complex not only share an N-terminal domain in their subunits but all contain a multiple of four subunits. Moreover, the eight components of the COG complex can be divided into two distinct sets of four. Although this use of multiples of four subunits might be coincidental, we tentatively propose that these complexes be collectively termed "quatrefoil" tethering complexes. More speculatively, their quatrefoil nature might reflect interaction with a set of fourfold-symmetric components, such as the core SNARE domains that form the trans SNARE complex.

The exocyst

Probably the best-characterised tethering complex is the exocyst. Most of its eight subunits were originally identified as products of genes whose mutation causes yeast to accumulate vesicles destined for plasma membrane (Guo et al., 1999a; TerBush et al., 1996). The complex is localised to sites of polarised exocytosis in yeast, to which one of the subunits, Sec3p, is localised independently of the cytoskeleton and the secretory pathway (Finger et al., 1998). This contrasts with the localisation of the other components to such sites, which requires both actin and a functional secretory pathway. Sec3p might thus act as a landmark for polarised secretion independently of the rest of the exocyst. One of the other components, Sec15p, binds to Sec4p, the Rab GTPase present on secretory vesicles (Guo et al., 1999b). Moreover, it appears to bind preferentially to the activated, GTP-bound form of Sec4p. Sec10p and Sec15p co-immunoprecipitate from the soluble fraction of cytosol, which indicates that they form a subcomplex. These findings lead to a model in which activated Sec4p on a Golgi-derived vesicle binds to the Sec10p-Sec15p subcomplex, which results in its assembly with Sec3p and the remainder of the exocyst on the plasma membrane and thereby tethers the vesicle (Guo et al., 1999b).

The localisation of Sec3p appears to be mediated by its interaction with the GTP-bound form of two Rho family GTPases, namely Rho1p (Guo et al., 2001) and Cdc42p (Zhang et al., 2001), which also have a role in the polarisation of actin. Vesicles travel along actin filaments en route to the sites of polarised secretion (Karpova et al., 2000; Pruyne and Bretscher, 2000); the Rho GTPases might therefore coordinate the cytoskeletal tracks along which vesicles travel with the machinery that tethers them to their target. The synthetic lethality of the combination of *sec3* and mutations in profilin, which regulates actin polymerisation and depolymerisation, illustrates the importance of the actin cytoskeleton in this process (Finger and Novick, 1997; Haarer et al., 1996).

Table 1. Revised nomenclature for the COG complex

Yeast							Mammals	
New name****	Previous names						New name****	Previous name
Cog1p*			Cod3p††		Sec36p§§	Tfi1p¶¶	Cog1*	ldlBp***
Cog2p*	Sec35p§						Cog2*	ldlCp†††
Cog3p	Sec34p§,¶	Gdr20p**					Cog3	hSec34‡‡‡
Cog4p			Cod1p††	Sgf1p‡‡	Sec38p§§	Tfi3p¶¶	Cog4	hCod1††,§§§
Cog5p			Cod4p††				Cog5	GTC-90¶¶¶
Cog6p			Cod2p††		Sec37p§§	Tfi2p¶¶	Cog6	hCod2††, §§§
Cog7p*			Cod5p††				Cog7*	–†
Cog8p			Dor1p††				Cog8	hDor1††,§§§

*Assignment of the same name to yeast and mammalian versions of Cog1, Cog2 and Cog7 is on functional grounds, since sequence similarity is low. †(Ungar et al., 2002); ‡(VanRheenen et al., 1998); §(VanRheenen et al., 1999); ¶(Kim et al., 1999); **(Spelbrink and Nothwehr, 1999); ††(Whyte and Munro, 2001); ‡‡(Kim et al., 2001b); §§(Ram et al., 2002); ¶¶(Suvorova et al., 2002); ***(Chatterton et al., 1999); †††(Podos et al., 1994); ‡‡‡(Suvorova et al., 2001); §§§(Loh and Hong, 2002); ¶¶¶(Walter et al., 1998); ****(Ungar et al., 2002).

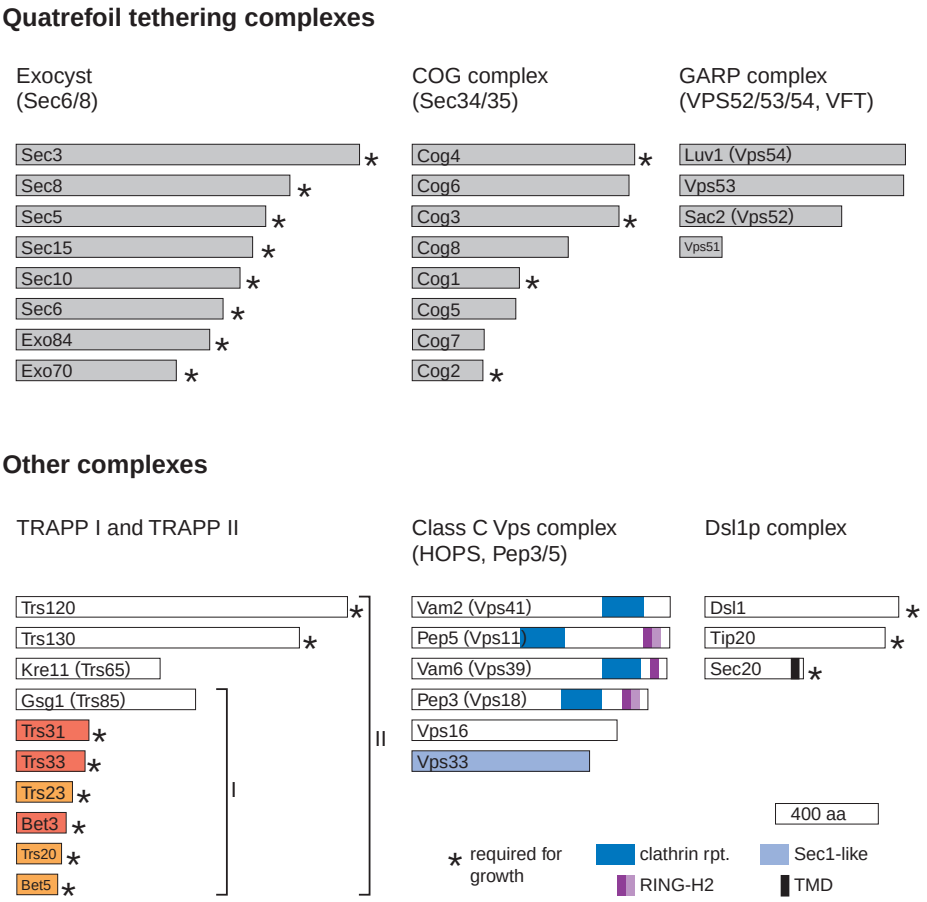

Fig. 3. Composition of proposed tethering complexes. For each complex the known components in the yeast *S. cerevisiae* are shown, arranged by size, and identifiable domains indicated. In each case the standard gene name in the *Saccharomyces* Genome Database is given first, followed by alternative names that have also been used in recent publications. Vps51p is encoded by the open reading frame *YKR020w* (Elizabeth Conibear, personal communication). The two sets of related subunits of the TRAPP complexes are indicated by different colours. Homologues of most of these proteins exist in higher eukaryotes, but in some cases have extra domains. Thus in mammals Sec5 has an N-terminal TIG domain, Exo84 a PH domain, Vam6 a CNH domain (Caplan et al., 2001) and Vam2 a C-terminal RING-H2 domain (Radisky et al., 1997). Vps54 has an N-terminal zinc-finger-like domain in *Drosophila* and *C. elegans*, but not in mammals. Vam6 in both yeast and higher eukaryotes has a conserved half RING domain (C2HC) at its C-terminus. The 'p' has been removed from the yeast protein names for clarity.

The mammalian counterpart of the exocyst consists of homologues of the eight yeast proteins and is also localised to sites of polarised growth (Brymora et al., 2001; Hsu et al., 1996; Kee et al., 1997). In some non-polarised cell types, the exocyst is associated both with the trans Golgi network (TGN) and with the plasma membrane, and antibody inhibition of each pool affects cargo exit from the TGN and delivery to the plasma membrane, respectively (Yeaman et al., 2001). In polarised epithelial cells, antibodies inhibit basolateral but not apical traffic, and overexpression of human Sec10 results in increased synthesis and delivery of secretory and basolateral, but not apical, plasma membrane proteins (Grindstaff et al., 1998; Lipschutz et al., 2000). The mammalian exocyst has been reported to interact with microtubules (Vega and Hsu, 2001) and septins (Hsu et al., 1998), although the localisation of Sec3p in yeast is not dependent on, or coincident with, the septins (Finger et al., 1998). The mammalian exocyst also interacts with Ca^{2+} signalling proteins (Shin et al., 2000) and, as in yeast, it is probably regulated by small GTPases. Sec5 is an effector of the GTPase RalA, and inhibition of Ral function leads to a decrease in the formation or stability of a mammalian exocyst subcomplex containing Sec6 and Sec10 (Brymora et al., 2001; Moskalenko et al., 2002; Sugihara et al., 2002).

The COG complex

The COG complex has been proposed to act as a tether at the Golgi apparatus, although it is unclear which vesicles are its substrates. Wuestehube et al. identified *sec34* and *sec35* mutants in a screen designed to identify yeast genes involved specifically in early stages of the secretory pathway (Wuestehube et al., 1996). Two groups subsequently showed that Sec34p (now Cog3p; Table 1) and Sec35p (Cog2p) associate as part of a large complex (Kim et al., 1999; VanRheenen et al., 1999). Identification of the other six components of the complex (Whyte and Munro, 2001) showed that the eight components fall into two phenotypic groups. The data are best explained by a model in which two distinct classes of vesicle are tethered by the complex to the early Golgi: vesicles recycling within the Golgi, and vesicles recycling to the Golgi from later, endosomal compartments (Fig. 2). Defects in the former process could lead indirectly to a failure in ER-to-Golgi transport, as observed for *sec34* and *sec35* mutants in vivo (Wuestehube et al., 1996) and in vitro (VanRheenen et al., 1998).

A function for the COG complex in recycling of Golgi components is supported by the identification of *COG3* (as *GRD20*) as a gene required for the proper localisation of a TGN protein (Spelbrink and Nothwehr, 1999). Two reports reiterating the identification of a subset of the subunits did not resolve the issue of anterograde versus retrograde transport (Kim et al., 2001b; Ram et al., 2002), but a third shows interactions of the COG complex that support a recycling role (Suvorova et al., 2002). These interactions are with SNAREs involved in intra-Golgi recycling and with the COPI vesicle coat, which is involved in retrograde transport, but not with a

component of the ER-to-Golgi COPII coat. Mutants show Golgi-associated glycosylation and sorting defects at temperatures permissive for the in vitro ER-to-Golgi tethering assay (Suvorova et al., 2002), and indeed a mutant of one of the subunits, Cog1p, shows no defect when tested in the in vitro assay for ER-to-Golgi transport at restrictive temperature (Ram et al., 2002). The ER-to-Golgi tethering defect may therefore be an indirect effect, but a recent report shows a defect in a different in vitro assay that measures tethering of ER-derived vesicles, whether homotypic or to another membrane (Morsomme and Riezman, 2002). The same report suggests that, in addition to a tethering function, the complex could have a separate and unexpected involvement in a sorting event that occurs in vitro to create two classes of ER-derived vesicle (Morsomme and Riezman, 2002). The exact role of the complex remains contentious, but, by analogy with other tethering complexes, the COG complex is expected to interact with a Rab. The most likely interaction would be as an effector of an early Golgi Rab such as Ypt1p (Short and Barr, 2002), and this is supported by an in vitro interaction of the complex with Ypt1p that occurs preferentially in the presence of GTP (Suvorova et al., 2002).

Identification of the eight yeast components of the complex revealed homology to several, characterised and uncharacterised, mammalian proteins (Whyte and Munro, 2001). In particular, it led to the prediction that the mammalian Sec34p-containing complex (Suvorova et al., 2001) is the same as the GTC (Walter et al., 1998), which was purified from bovine brain cytosol on the basis of its stimulatory activity in an intra-Golgi-transport assay. The GTC in turn was already suspected to be the same as the ldlCp complex, a Golgi-associated complex thought to contain ldlBp and ldlCp, two proteins that complement mutant cultured cell lines that have a range of Golgi defects (Chatterton et al., 1999; Podos et al., 1994). Identification of the eight components of the mammalian COG complex has shown these predictions to be correct (Loh and Hong, 2002; Ungar et al., 2002). Interestingly, some of the components appear to be in a subcomplex, which may represent one lobe of the two-lobed structure seen by electron microscopy of the whole complex (Ungar et al., 2002). This division is consistent with the phenotypic division of the yeast proteins into two halves, but the existence of a subcomplex in yeast has not been investigated.

Perhaps the most interesting finding to come from analysis of the components of the COG complex is that some show extensive, albeit distant, sequence similarity to components of the exocyst and the GARP complex, and many of the subunits of all three complexes appear to have at their N-termini a common domain (Whyte and Munro, 2001). An alignment of these domains is shown in Fig. 4A. The domain is predicted to form two short stretches of potential coiled coil or amphipathic helix (not to be confused with the sequences in the long, coiled-coiled tethering proteins such as Uso1p). For at least some of these components, the sequence similarity is likely to reflect more than simply a shared structure, because similarity searches find other components before any other coiled-coil proteins (Whyte and Munro, 2001). The presence of the common domain is not discernible in all of the components by sequence similarity searches. It remains to be seen whether this is because of its absence in some cases or because of an inability to detect a structural similarity at the sequence level owing to sequence divergence. The latter is at least suggested by the presence of regions of predicted short coiled-coil near the N-termini of all components of the human COG complex and exocyst (Fig. 4B). Similar predictions are seen for the yeast COG complex, exocyst and GARP complex (TerBush et al., 1996) (data not shown). The significance of this putative domain is not yet known; it may be involved in assembly of the complex or have some other function. Nevertheless, its existence reveals a similarity between some of the tethering complexes and suggests that they have similar modes of action.

The GARP complex

Retrograde traffic from endosomes to the Golgi has not been as extensively characterised as other transport steps, but the GARP complex localises to the TGN and is required for this process (Conboy and Cyert, 2000; Conibear and Stevens, 2000). The GARP complex consists of four proteins (Vps51p, Vps52p, Vps53p and Vps54p) (Conibear and Stevens, 2001; Gavin et al., 2002) (E. Conibear, personal communication), some of which show extensive sequence similarity to other quatrefoil complex components (Whyte and Munro, 2001). Whether it functions as a tethering complex has yet to be established, but strong evidence for this comes from the finding that it is an effector of the Rab Ypt6p and interacts with the SNARE Tlg1p (Siniossoglou and Pelham, 2001). That its components share the domain found in the exocyst and COG complex additionally suggests a tethering function, but confirmation of such a role awaits development of an in vitro assay for this transport step. Genes encoding mammalian homologues of Vps52p, Vps53p and Vps54p are discernible in the databases but have not been characterised.

Non-quatrefoil tethering complexes

Although no enzymatic activity has yet been ascribed to the three similar complexes already described, two of the remaining complexes, although dissimilar in subunit composition, possess guanine-nucleotide-exchange factor (GEF) activity towards Rabs. Yeast contains 11 Rab proteins, whereas humans have at least 60 (Bock et al., 2001). Apart from vesicle tethering, their functions involve interactions of vesicles with the cytoskeleton and possibly vesicle budding (reviewed in Zerial and McBride, 2001). As in the cases of other regulatory GTPases, their function depends on a GDP/GTP cycle. The transition to their active, GTP-bound form is promoted by the corresponding GEF, and they then exert their effects by binding to target molecules (effectors).

The TRAPP complexes

The TRAPP (transport protein particle) complex was originally described as a large protein complex functioning in the later stages of ER-to-Golgi traffic in yeast (Sacher et al., 2000; Sacher et al., 1998). After its initial identification, it transpired that TRAPP represents two distinct complexes. TRAPP I is ~300 kDa in size and contains seven subunits, whereas TRAPP II is ~1000 kDa and contains an additional three subunits (Fig. 3) (Sacher et al., 2001). Subunits of these complexes share some sequence similarity, the six smallest subunits falling into two families of three (Bet3p, Trs33p and Trs31p; and Bet5p,

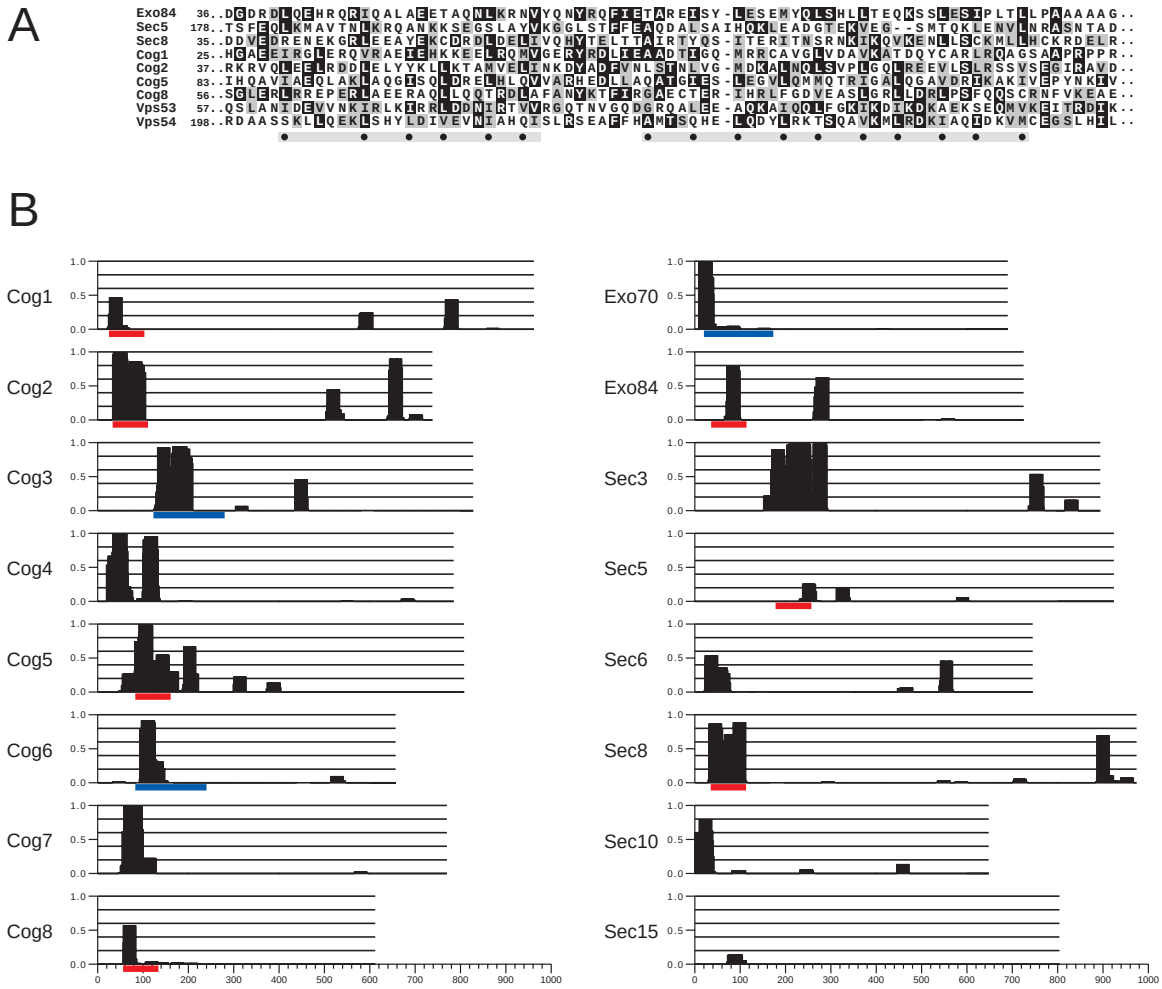

Fig. 4. The presence of a shared domain in components of the COG complex and the exocyst. (A) Sequence alignment of the N-terminal amphipathic helical regions of the indicated components of the human COG complex and exocyst. Residues are shaded if identical (black) or conserved (grey) in at least three proteins. Grey bars show the regions predicted to form coiled-coil (the hydrophobic heptad repeat indicated by black circles). (B) Prediction of the propensity of the subunits of the human COG complex and human exocyst to form coiled coils. The length of the x-axis corresponds to the length of the proteins, with residue numbers indicated at the bottom of the figure. On the y-axis is plotted the probability of a coiled-coil being at each residue of the protein, as determined by the algorithm of Lupas (Lupas, 1996) using the MacStripe program (v2.0b1) with a window length of 28 residues, the MTIDK matrix and weighting of hydrophobic residues. Red bars indicate regions that are aligned in (A). Blue bars indicate longer regions of sequence similarity between Cog3, Cog6 and Exo70 [see alignment in Whyte and Munro (Whyte and Munro, 2001)].

Trs20p and Trs23p) (Sacher et al., 2000). TRAPP I and TRAPP II both cofractionate with an early Golgi marker but not late-Golgi markers and are also present in a cytosolic pool. The Golgi association is stable, in that Bet3p does not relocate to the ER when anterograde ER-to-Golgi traffic is blocked, unlike components that cycle between the ER and Golgi, and the complex remains assembled under these conditions (Barrowman et al., 2000). The Golgi receptor for TRAPP is not known.

TRAPP I, but not TRAPP II, is required for an in vitro ER-to-Golgi transport assay (Sacher et al., 2001). TRAPP I binds to COPII vesicles formed in vitro from permeabilised yeast cells. This binding appears to be independent of other factors, since it occurs even when the COPII vesicles are formed from purified coat components in the absence of cytosol and Golgi. Moreover, if Bet3p is depleted from both vesicles and Golgi, no tethering occurs, which indicates that other factors are not sufficient to tether vesicles in the absence of TRAPP I (Barrowman et al., 2000). TRAPP II, in contrast, might be required for a later transport step. A temperature-sensitive mutant of a TRAPP II-specific subunit accumulates Golgi forms of invertase and CPY, as well as aberrant Golgi structures. Its subunits also show synthetic interactions with mutants of *ARF1* and components of COPI but not COPII (Sacher et al., 2001).

Immobilised TRAPP I and TRAPP II both act as exchange factors for the early Golgi Rab Ypt1p (Sacher et al., 2001), and there is conflicting evidence about whether either acts as an exchange factor for the late Golgi Rabs Ypt31p and Ypt32p (Jones et al., 2000; Wang et al., 2000). It is also not clear whether or how the Ypt1p exchange activity is regulated, and why Uso1p (a long coiled-coil protein and Ypt1p effector) is required in vitro to tether COPII vesicles to the Golgi (Cao et al., 1998) when TRAPP I alone can bind to COPII vesicles.

Perhaps the combined interactions are required for proper tethering; binding of TRAPP I to vesicles could stimulate its GEF activity, resulting in recruitment of Uso1p by Ypt1p and additional binding of the vesicle by Uso1p.

At least seven of the TRAPP subunits are well conserved in mammals and are present in a large complex (Gavin et al., 2002; Sacher et al., 2000). The mammalian complex(es) has not been extensively characterised but has been shown to localise to the Golgi (Gecz et al., 2000; Sacher et al., 2000). Mutations in the homologue of yeast Trs20p are responsible for the human disease spondyloepiphyseal dysplasia tarda (SEDL) (Gecz et al., 2000). The non-lethality of this X-linked skeletal disorder might be caused by the presence of an additional, autosomal, version of the *SEDL* gene that appears to be a processed pseudogene but is nonetheless expressed.

The Class C Vps complex

The Class C Vps complex was identified through characterisation of the many yeast mutants that show defects in the sorting of proteins to the vacuole. Such vacuolar protein sorting (*vps*) mutants have been classified on the basis of their phenotypes, and Class C mutants are those that lack coherent vacuoles altogether (Raymond et al., 1992). All four of the mutants in this class (Pep3p, Pep5p, Vps16p and Vps33p) are part of a very large (38S) complex that appears to function at two distinct transport steps (Rieder and Emr, 1997). It was first identified as being involved in fusion to the vacuole of both transport vesicles and other vacuoles. At the vacuolar surface, the Class C Vps complex has another two components: Vam2p (Vps41p) and Vam6p (Vps39p). The latter is a GEF for the Rab Ypt7p (Wurmser et al., 2000). The complex is also an effector of Ypt7p (Seals et al., 2000) and also binds to the unpaired vacuolar SNARE Vam3p. This binding is probably through Vps33p, which is a member of the Sec1 (or Munc18) family of SNARE-binding proteins (Jahn, 2000; Sato et al., 2000). This has led to a model in which the Class C complex recruits Vps39p to both the vacuole and incoming vesicles. Vps39p activates Ypt7p, which in turn acts on the complex to promote a tethering interaction. Inhibition of Vam3p is then relieved, which allows trans SNARE complex formation and fusion to proceed.

The components of the complex do not show any clear homology to other known Rab GEFs or to other tethering complexes. However, several of the components have a domain related to the repeating structure of clathrin (Conibear and Stevens, 1998) and also contain RING-H2 domains, which are zinc-binding motifs that mediate a number of protein-protein interactions. In yeast and *Drosophila*, mutations in the RING-H2 domain abrogate function (Rieder and Emr, 1997; Sevrioukov et al., 1999). In many other proteins, RING domains serve to recruit ubiquitin-ligases (Borden and Freemont, 1996; Joazeiro and Weissman, 2000), and given the recent revelation of ubiquitin as a key sorting determinant in the endocytic/vacuolar pathway, they might have a similar function here (Hicke, 2001). Human homologues of Class C Vps subunits have recently been characterised and found to be in a complex localized on late endosomes/lysosomes (Caplan et al., 2001; Kim et al., 2001a; McVey Ward et al., 2001).

The same complex also appears to function in Golgi-to-endosome transport. This is indicated by genetic and physical interactions between Class C mutants and genes encoding proteins known to promote tethering and fusion at the endosome (Peterson et al., 1999; Tall et al., 1999). Furthermore, Class C mutants show allele-specific defects in either Golgi-to-endosome or endosome-to-vacuole transport (Peterson and Emr, 2001). Parallels with the vacuolar function of the Class C complex have yet to be addressed. However, the Class C Vps complex is not the only factor that has been proposed to contribute to tethering in the endosomal system. In mammalian cells the long coiled-coil protein EEA1 appears to act as a tether during homotypic fusion of early endosomes (Christoforidis et al., 1999). There is no clear homologue of EEA1 in yeast, although it was initially proposed that Vac1p (Pep7p) might be related, and both are effectors for Rab5 GTPase (Vps21p in yeast) (Peterson et al., 1999; Tall et al., 1999). However a second Rab5-effector, Rabenosyn-5, appears to be more related to Vac1p, although the latter has a RING domain that is absent from the mammalian protein (Nielsen et al., 2000). The function of Vac1p and Rabenosyn-5 is unknown, but yeast lacking Vac1p show defects in delivery of proteins from the Golgi to the endosome (Weisman and Wickner, 1992). Vac1p interacts genetically and physically with components of the Class C Vps complex, and so it is possible that Vac1p and Rabenosyn-5 are involved in recruiting the Class C Vps complex to endosomal membranes (Peterson and Emr, 2001; Srivastava et al., 2000; Webb et al., 1997). However both Vac1p and Rabenosyn-5 also bind to the Sec1-related protein Vps45p (Burd et al., 1997; Nielsen et al., 2000). Thus it is also possible that there are further proteins stably associated with Vac1p or Rabenosyn-5, which form another entirely distinct tethering complex that acts on endosomal membranes.

Dsl1p complex

So far, there is no clearly described mechanism to tether Golgi-derived vesicles to the ER in this vital recycling pathway. If there is such a mechanism, it may well involve Dsl1p, a peripheral membrane protein of the ER that is required for retrograde traffic and binds to another peripheral membrane protein, Tip20p (Andag et al., 2001; Reilly et al., 2001). Tip20p is part of a complex that contains the SNARE-like membrane protein Sec20p, and together they bind to the ER SNARE Ufe1p (Lewis et al., 1997; Sweet and Pelham, 1993). It therefore seems likely that a complex consisting of at least Dsl1p-Tip20p-Sec20p exists and that it interacts with the SNARE Ufe1p. Mutants of *TIP20* are synthetically lethal in combination with those of several subunits of the Golgi-to-ER (COPI) vesicle coat (Frigerio, 1998), and Dsl1p interacts with a COPI subunit in two-hybrid assays (Reilly et al., 2001). Clarification of the function of the Dsl1p complex awaits further investigation of its biochemical role and protein-protein interactions, but interestingly Dsl1p has a short stretch of predicted coiled coil near its N-terminus, in common with components of quatrefoil tethering complexes.

Mechanistic ideas

An important but unresolved question is how tethering factors promote fusion of the vesicle to the correct organelle. The mechanism may be purely kinetic, with the vesicle being

tethered within the vicinity of its destination and having an increased probability of undergoing SNARE-mediated fusion. Alternatively, the mechanism may be thermodynamic, the tethering factors actively promoting SNARE-mediated fusion in response to vesicle binding. This might be either by release of inhibition of SNAREs or by activation of the SNAREs on the vesicle and target (see below). Of course, both kinetic and thermodynamic mechanisms could operate simultaneously, mediated by the same or different sets of tethering factors. One possibility is that the long, coiled-coil proteins are kinetic tethers that passively hold the vesicle and do not need to transduce signals about vesicle binding along their length, whereas the multi-protein complexes are thermodynamic tethers that actively promote interaction between vesicle and target. Whether there is any cross-talk between the two classes of tethering factor has yet to be explored.

Interactions with SNAREs

Some t-SNAREs possess, in addition to the core SNARE domain, an N-terminal domain that can bind to the core domain and thereby prevent its participation in a trans SNARE complex. An attractive idea is that tethering complexes might relieve this autoinhibition by promoting an open conformation of the N-terminal domain relative to the core domain; recent evidence supports the idea that tethering complexes bind to such N-terminal domains although not necessarily that the complexes thereby relieve autoinhibition.

Deletion of the N-terminal domain of Vam3p results in a significant reduction in homotypic vacuolar fusion in yeast and a significant reduction in the formation of trans SNARE complexes (Laage and Ungermann, 2001). The Class C Vps complex also binds much less efficiently to the truncated Vam3p, which suggests that the reduction in fusion is caused by an inability of the Class C Vps complex to promote trans SNARE complex formation. The GARP complex provides another example of the binding of a putative tethering complex to an N-terminal SNARE domain, that of Tlg1p (Siniossoglou and Pelham, 2001).

The N-terminal domain of the yeast plasma membrane t-SNARE Sso1p is essential. However, a constitutively open mutant of Sso1p is viable (Munson and Hughson, 2002). Thus the requirement for growth is not that the N-terminal domain be able to bind to the core domain, although the constitutively open mutant does indeed form complexes with other SNAREs more readily. This implies that the N-terminal domain has an activating function as well as an inhibitory role. The activating function could be provided by the binding of the exocyst or another tethering factor, although there is currently no direct evidence for this.

A variation on this idea is that tethering factors relieve the inhibition of SNAREs by Sec1 family proteins (for details, see Waters and Hughson, 2000), which in some cases hold the SNARE N-terminal domain in a closed conformation. In neuronal exocytosis, for instance, nSec1 binds to the monomeric form of the SNARE syntaxin1A, preventing it from interacting with its t-SNARE partners (Misura et al., 2000; Yang et al., 2000). This sort of mechanism may be employed by the Class C Vps complex, one subunit of which is a Sec1 homologue. As ever, the real situation may be more complicated, since some Sec1 proteins might themselves have an activating role in SNARE assembly (Carr and Novick, 2000; Jahn, 2000).

Conclusion

The concept of tethering has grown around the realisation that the specificity of membrane fusion is conferred by more than just the SNAREs. The definition and ordering of events in tethering will be a crucial advance. In this respect, it is useful to consider where a tethering complex lies in relation to Rab activation. The TRAPP complexes and the Class C Vps complex lie upstream of Rab activation, whereas the quatrefoil complexes lie downstream. This reaffirms the idea that the complexes function differently. There could thus be two sorts of tethering event. In the first, a large complex would respond to a vesicle by activating a Rab, causing Rab effectors such as long, coiled-coil proteins to tether the vesicle. TRAPP would operate in this way, and its interactions with vesicles would reflect the start of a signalling event rather than a stable tether. The second sort of event in tethering would rely on large complexes that are Rab effectors and fulfil some function that promotes SNARE-mediated fusion. This function could operate in parallel with long, coiled-coil effectors. The COG complex, the GARP complex and the exocyst would operate in this manner. Of course, the same tethering event could involve a different large complex both before and after Rab activation – for instance, TRAPP II and the COG complex in Golgi recycling. The same complex could even act both upstream and downstream of Rab activation, as described above for the Class C Vps complex. The notion that tethering could involve different mechanisms in different parts of the pathway would account for the fact that, unlike Rabs and SNAREs, tethering complexes and coiled-coil proteins are not ubiquitous in the pathway and distinct protein complexes act in different places. Whatever their exact functions turn out to be, rapid and exciting progress is expected in this area.

We thank Elizabeth Conibear, Chris Kaiser, Vladimir Lupashin and Gerry Waters for communication of results prior to publication, and Alison Gillingham, Bernhard Sommer and the referees for helpful comments on the manuscript.

References

Andag, U., Neumann, T. and Schmitt, H. D. (2001). The coatomer-interacting protein Dsl1p is required for Golgi-to-endoplasmic reticulum retrieval in yeast. *J. Biol. Chem.* **276**, 39150-39160.

Barlowe, C. (1997). Coupled ER to Golgi transport reconstituted with purified cytosolic proteins. *J. Cell Biol.* **139**, 1097-1108.

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

Barrowman, J., Sacher, M. and Ferro-Novick, S. (2000). TRAPP stably associates with the Golgi and is required for vesicle docking. *EMBO J.* **19**, 862-869.

Bock, J. B., Matern, H. T., Peden, A. A. and Scheller, R. H. (2001). A genomic perspective on membrane compartment organization. *Nature* **409**, 839-841.

Borden, K. L. and Freemont, P. S. (1996). The RING finger domain: a recent example of a sequence-structure family. *Curr. Opin. Struct. Biol.* **6**, 395-401.

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

Broadie, K., Prokop, A., Bellen, H. J., O'Kane, C. J., Schulze, K. L. and

Sweeney, S. T. (1995). Syntaxin and synaptobrevin function downstream of vesicle docking in *Drosophila*. *Neuron* **15**, 663-673.
Brymora, A., Valova, V. A., Larsen, M. R., Roufogalis, B. D. and Robinson, P. J. (2001). The brain exocyst complex interacts with RalA in a GTP-dependent manner: Identification of a novel mammalian Sec3 gene and a second Sec15 gene. *J. Biol. Chem.* **13**, 13.
Burd, C. G., Peterson, M., Cowles, C. R. and Emr, S. D. (1997). A novel Sec18p/NSF-dependent complex required for Golgi-to- endosome transport in yeast. *Mol. Biol. Cell* **8**, 1089-1104.
Cao, X. C., Ballew, N. and Barlowe, C. (1998). Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins. *EMBO J.* **17**, 2156-2165.
Caplan, S., Hartnell, L. M., Aguilar, R. C., Naslavsky, N. and Bonifacino, J. S. (2001). Human Vam6p promotes lysosome clustering and fusion in vivo. *J. Cell Biol.* **154**, 109-122.
Carr, C. M. and Novick, P. J. (2000). Membrane fusion. Changing partners. *Nature* **404**, 347-349.
Chatterton, J. E., Hirsch, D., Schwartz, J. J., Bickel, P. E., Rosenberg, R. D., Lodish, H. F. and Krieger, M. (1999). Expression cloning of LDLB, a gene essential for normal Golgi function and assembly of the ldlCp complex. *Proc. Natl. Acad. Sci. USA* **96**, 915-920.
Chen, Y. A. and Scheller, R. H. (2001). SNARE-mediated membrane fusion. *Nat. Rev. Mol. Cell. Biol.* **2**, 98-106.
Christoforidis, S., McBride, H. M., Burgoyne, R. D. and Zerial, M. (1999). The Rab5 effector EEA1 is a core component of endosome docking. *Nature* **397**, 621-625.
Conboy, M. J. and Cyert, M. S. (2000). Luv1p/Rki1p/Tcs3p/Vps54p, a yeast protein that localizes to the late Golgi and early endosome, is required for normal vacuolar morphology. *Mol. Biol. Cell* **11**, 2429-2443.
Conibear, E. and Stevens, T. H. (1998). Multiple sorting pathways between the late Golgi and the vacuole in yeast. *Biochim. Biophys. Acta* **1404**, 211-230.
Conibear, E. and Stevens, T. H. (2000). Vps52p, Vps53p, and Vps54p form a novel multisubunit complex required for protein sorting at the yeast late Golgi. *Mol. Biol. Cell* **11**, 305-323.
Conibear, E. and Stevens, T. H. (2001). The GARP complex is required for retrograde transport from both early and late endosomes to the Golgi. *Yeast* **18**, S135-S135.
Dumas, J. J., Merithew, E., Sudharshan, E., Rajamani, D., Hayes, S., Lawe, D., Corvera, S. and Lambright, D. G. (2001). Multivalent endosome targeting by homodimeric EEA1. *Mol. Cell* **8**, 947-958.
Fasshauer, D., Antonin, W., Subramaniam, V. and Jahn, R. (2002). SNARE assembly and disassembly exhibit a pronounced hysteresis. *Nat. Struct. Biol.* **9**, 144-151.
Finger, F. P. and Novick, P. (1997). Sec3p is involved in secretion and morphogenesis in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **8**, 647-662.
Finger, F. P., Hughes, T. E. and Novick, P. (1998). Sec3p is a spatial landmark for polarized secretion in budding yeast. *Cell* **92**, 559-571.
Frigerio, G. (1998). The *Saccharomyces cerevisiae* early secretion mutant *tip20* is synthetic lethal with mutants in yeast coatomer and the SNARE proteins Sec22p and Ufelp. *Yeast* **14**, 633-646.
Gavin, A. C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J. M., Michon, A. M., Cruciat, C. M. et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**, 141-147.
Gecz, J., Hillman, M. A., Gedeon, A. K., Cox, T. C., Baker, E. and Mulley, J. C. (2000). Gene structure and expression study of the SEDL gene for spondyloepiphyseal dysplasia tarda. *Genomics* **69**, 242-251.
Grindstaff, K. K., Yeaman, C., Anandasabapathy, N., Hsu, S. C., Rodriguez-Boulan, E., Scheller, R. H. and Nelson, W. J. (1998). Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* **93**, 731-740.
Guo, W., Grant, A. and Novick, P. (1999a). Exo84p is an exocyst protein essential for secretion. *J. Biol. Chem.* **274**, 23558-23564.
Guo, W., Roth, D., Walch-Solimena, C. and Novick, P. (1999b). The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *EMBO J.* **18**, 1071-1080.
Guo, W., Sacher, M., Barrowman, J., Ferro-Novick, S. and Novick, P. (2000). Protein complexes in transport vesicle targeting. *Trends Cell Biol.* **10**, 251-255.
Guo, W., Tamanoi, F. and Novick, P. (2001). Spatial regulation of the exocyst complex by Rho1 GTPase. *Nat. Cell Biol.* **3**, 353-360.
Haarer, B. K., Corbett, A., Kweon, Y., Petzold, A. S., Silver, P. and Brown, S. S. (1996). SEC3 mutations are synthetically lethal with profilin mutations and cause defects in diploid-specific bud-site selection. *Genetics* **144**, 495-510.
Hicke, L. (2001). A new ticket for entry into budding vesicles – ubiquitin. *Cell* **106**, 527-530.
Hsu, S. C., Hazuka, C. D., Roth, R., Foletti, D. L., Heuser, J. and Scheller, R. H. (1998). Subunit composition, protein interactions, and structures of the mammalian brain sec6/8 complex and septin filaments. *Neuron* **20**, 1111-1122.
Hsu, S. C., Ting, A. E., Hazuka, C. D., Davanger, S., Kenny, J. W., Kee, Y. and Scheller, R. H. (1996). The mammalian brain rsec6/8 complex. *Neuron* **17**, 1209-1219.
Hunt, J. M., Bommert, K., Charlton, M. P., Kistner, A., Habermann, E., Augustine, G. J. and Betz, H. (1994). A post-docking role for synaptobrevin in synaptic vesicle fusion. *Neuron* **12**, 1269-1279.
Jahn, R. (2000). Sec1/Munc18 proteins: mediators of membrane fusion moving to center stage. *Neuron* **27**, 201-204.
Joazeiro, C. A. and Weissman, A. M. (2000). RING finger proteins: mediators of ubiquitin ligase activity. *Cell* **102**, 549-552.
Jones, S., Newman, C., Liu, F. L. and Segev, N. (2000). The TRAPP complex is a nucleotide exchanger for Ypt1 and Ypt31/32. *Mol. Biol. Cell* **11**, 4403-4411.
Karpova, T. S., Reck-Peterson, S. L., Elkind, N. B., Mooseker, M. S., Novick, P. J. and Cooper, J. A. (2000). Role of actin and Myo2p in polarized secretion and growth of Saccharomyces cerevisiae. *Mol. Biol. Cell* **11**, 1727-1737.
Kee, Y., Yoo, J. S., Hazuka, C. D., Peterson, K. E., Hsu, S. C. and Scheller, R. H. (1997). Subunit structure of the mammalian exocyst complex. *Proc. Natl. Acad. Sci. USA* **94**, 14438-14443.
Kim, D. W., Sacher, M., Scarpa, A., Quinn, A. M. and Ferro-Novick, S. (1999). High-copy suppressor analysis reveals a physical interaction between Sec34p and Sec35p, a protein implicated in vesicle docking. *Mol. Biol. Cell* **10**, 3317-3329.
Kim, B. Y., Kramer, H., Yamamoto, A., Kominami, E., Kohsaka, S. and Akazawa, C. (2001a). Molecular characterization of mammalian homologues of class C Vps proteins that interact with syntaxin-7. *J. Biol. Chem.* **276**, 29393-29402.
Kim, D. W., Massey, T., Sacher, M., Pypaert, M. and Ferro-Novick, S. (2001b). Sgf1p, a new component of the Sec34p/Sec35p complex. *Traffic* **2**, 820-830.
Laage, R. and Ungermann, C. (2001). The N-terminal domain of the t-SNARE Vam3p coordinates priming and docking in yeast vacuole fusion. *Mol. Biol. Cell* **12**, 3375-3385.
Lewis, M. J., Rayner, J. C. and Pelham, H. R. B. (1997). A novel SNARE complex implicated in vesicle fusion with the endoplasmic reticulum. *EMBO J.* **16**, 3017-3024.
Linstedt, A. D., Jesch, S. A., Mehta, A., Lee, T. H., Garcia-Mata, R., Nelson, D. S. and Sztul, E. (2000). Binding relationships of membrane tethering components – The giantin N terminus and the GM130 N terminus compete for binding to the p115 C terminus. *J. Biol. Chem.* **275**, 10196-10201.
Lipschutz, J. H., Guo, W., O'Brien, L. E., Nguyen, Y. H., Novick, P. and Mostov, K. E. (2000). Exocyst is involved in cystogenesis and tubulogenesis and acts by modulating synthesis and delivery of basolateral plasma membrane and secretory proteins. *Mol. Biol. Cell* **11**, 4259-4275.
Loh, E. and Hong, W. (2002). Sec34 is implicated in traffic from the endoplasmic reticulum to the Golgi and exists in a complex with GTC-90 and ldlBp. *J. Biol. Chem.* (in press).
Lowe, M. (2000). Membrane transport: Tethers and TRAPPs. *Curr. Biol.* **10**, R407-R409.
Lupas, A. (1996). Prediction and analysis of coiled-coil structures. *Methods Enzymol* **266**, 513-525.
McNew, J. A., Parlati, F., Fukuda, R., Johnston, R. J., Paz, K., Paumet, F., Sollner, T. H. and Rothman, J. E. (2000). Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. *Nature* **407**, 153-159.
McVey Ward, D., Radisky, D., Scullion, M. A., Tuttle, M. S., Vaughn, M. and Kaplan, J. (2001). hVPS41 is expressed in multiple isoforms and can associate with vesicles through a RING-H2 finger motif. *Exp. Cell Res.* **267**, 126-134.
Misura, K. M., Scheller, R. H. and Weis, W. I. (2000). Three-dimensional structure of the neuronal-Sec1-syntaxin 1a complex. *Nature* **404**, 355-362.
Morsomme, P. and Riezman, H. (2002). The Rab GTPase Ypt1p and tethering factors couple protein sorting at the ER to vesicle targeting to the Golgi apparatus. *Dev. Cell* **2**, 307-317.

Moskalenko, S., Henry, D. O., Rosse, C., Mirey, G., Camonis, J. H. and White, M. A. (2002). The exocyst is a Ral effector complex. *Nat. Cell Biol.* **4**, 66-72.

Muller, O., Bayer, M. J., Peters, C., Andersen, J. S., Mann, M. and Mayer, A. (2002). The Vtc proteins in vacuole fusion: coupling NSF activity to V(0) trans-complex formation. *EMBO J.* **21**, 259-269.

Munson, M. and Hughson, F. M. (2002). Conformational regulation of SNARE assembly and disassembly in vivo. *J. Biol. Chem.* **277**, 9375-9381.

Nielsen, E., Christoforidis, S., Uttenweiler-Joseph, S., Miaczynska, M., Dewitte, F., Wilm, M., Hoflack, B. and Zerial, M. (2000). Rabenosyn-5, a novel Rab5 effector, is complexed with hVPS45 and recruited to endosomes through a FYVE finger domain. *J. Cell Biol.* **151**, 601-612.

Parlati, F., McNew, J. A., Fukuda, R., Miller, R., Sollner, T. H. and Rothman, J. E. (2000). Topological restriction of SNARE-dependent membrane fusion. *Nature* **407**, 194-198.

Pelham, H. R. B. (2001). SNAREs and the specificity of membrane fusion. *Trends Cell Biol.* **11**, 99-101.

Peterson, M. R., Burd, C. G. and Emr, S. D. (1999). Vac1p coordinates Rab and phosphatidylinositol 3-kinase signaling in Vps45p-dependent vesicle docking/fusion at the endosome. *Curr. Biol.* **9**, 159-162.

Peterson, M. R. and Emr, S. D. (2001). The Class C Vps complex functions at multiple stages of the vacuolar transport pathway. *Traffic* **2**, 476-486.

Podos, S. D., Reddy, P., Ashkenas, J. and Krieger, M. (1994). LDLC encodes a brefeldin A-sensitive, peripheral Golgi protein required for normal Golgi function. *J. Cell Biol.* **127**, 679-691.

Pruyne, D. and Bretscher, A. (2000). Polarization of cell growth in yeast. *J. Cell Sci.* **113**, 571-585.

Radisky, D. C., Snyder, W. B., Emr, S. D. and Kaplan, J. (1997). Characterization of *VPS41*, a gene required for vacuolar trafficking and high-affinity iron transport in yeast. *Proc. Natl. Acad. Sci. USA* **94**, 5662-5666.

Ram, R. J., Li, B. and Kaiser, C. (2002). Identification of Sec36p, Sec37p and Sec38p: components of the yeast complex that contains Sec34p and Sec35p. *Mol. Biol. Cell.* **13**, 1484-1500.

Raymond, C. K., Howaldstevenson, I., Vater, C. A. and Stevens, T. H. (1992). Morphological classification of the yeast vacuolar protein sorting mutants – evidence for a prevacuolar compartment in class-E *vps* mutants. *Mol. Biol. Cell* **3**, 1389-1402.

Reilly, B. A., Kraynack, B. A., VanRheenen, S. M. and Waters, M. G. (2001). Golgi-to-endoplasmic reticulum (ER) retrograde traffic in yeast requires Dsl1p, a component of the ER target site that interacts with a COPI coat subunit. *Mol. Biol. Cell* **12**, 3783-3796.

Rieder, S. E. and Emr, S. D. (1997). A novel RING finger protein complex essential for a late step in protein transport to the yeast vacuole. *Mol. Biol. Cell* **8**, 2307-2327.

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

Sacher, M., Barrowman, J., Schieltz, D., Yates, J. R. and Ferro-Novick, S. (2000). Identification and characterization of five new subunits of TRAPP. *Eur. J. Cell Biol.* **79**, 71-80.

Sacher, M., Barrowman, J., Wang, W., Horecka, J., Zhang, Y., Pypaert, M. and Ferro-Novick, S. (2001). TRAPP I implicated in the specificity of tethering in ER-to-Golgi transport. *Mol. Cell* **7**, 433-442.

Sapperstein, S. K., Walter, D. M., Grosvenor, A. R., Heuser, J. E. and Waters, M. G. (1995). p115 is a general vesicular transport factor related to the yeast endoplasmic reticulum to Golgi transport factor Uso1p. *Proc. Natl. Acad. Sci. USA* **92**, 522-526.

Sato, T. K., Rehling, P., Peterson, M. R. and Emr, S. D. (2000). Class C Vps protein complex regulates vacuolar SNARE pairing and is required for vesicle docking/fusion. *Mol. Cell* **6**, 661-671.

Seals, D. F., Eitzen, G., Margolis, N., Wickner, W. T. and Price, A. (2000). A Ypt/Rab effector complex containing the Sec1 homolog Vps33p is required for homotypic vacuole fusion. *Proc. Natl. Acad. Sci. USA* **97**, 9402-9407.

Seemann, J., Jokitalo, E. J. and Warren, G. (2000). The role of the tethering proteins p115 and GM130 in transport through the Golgi apparatus in vivo. *Mol. Biol. Cell* **11**, 635-645.

Sevrioukov, E. A., He, J. P., Moghrabi, N., Sunio, A. and Kramer, H. (1999). A role for the deep orange and carnation eye color genes in lysosomal delivery in *Drosophila*. *Mol. Cell* **4**, 479-486.

Shin, D. M., Zhao, X. S., Zeng, W., Mozhayeva, M. and Muallem, S. (2000). The mammalian Sec6/8 complex interacts with Ca(2+) signaling complexes and regulates their activity. *J. Cell Biol.* **150**, 1101-1112.

Short, B. and Barr, F. A. (2002). Membrane traffic: exocyst III – makes a family. *Curr. Biol.* **12**, R18-R20.

Siniossoglou, S. and Pelham, H. R. (2001). An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes. *EMBO J.* **20**, 5991-5998.

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

Spelbrink, R. G. and Nothwehr, S. F. (1999). The yeast *GRD20* gene is required for protein sorting in the trans-Golgi network/endosomal system and for polarization of the actin cytoskeleton. *Mol. Biol. Cell* **10**, 4263-4281.

Srivastava, A., Woolford, C. A. and Jones, E. W. (2000). Pep3p/Pep5p complex: a putative docking factor at multiple steps of vesicular transport to the vacuole of Saccharomyces cerevisiae. *Genetics* **156**, 105-122.

Sugihara, K., Asano, S., Tanaka, K., Iwamatsu, A., Okawa, K. and Ohta, Y. (2002). The exocyst complex binds the small GTPase RalA to mediate filopodia formation. *Nat. Cell Biol.* **4**, 73-78.

Suvorova, E. S., Duden, R. and Lupashin, V. V. (2002). The Sec34/Sec35p complex, a Ypt1p effector required for retrograde intra-Golgi trafficking, interacts with Golgi SNAREs and COPI vesicle coat proteins. *J. Cell Biol.* **157**, 631-643.

Suvorova, E. S., Kurten, R. C. and Lupashin, V. V. (2001). Identification of a human orthologue of Sec34p as a component of the cis-Golgi vesicle tethering machinery. *J. Biol. Chem.* **276**, 22810-22818.

Sweet, D. J. and Pelham, H. R. (1993). The *TIP1* gene of *Saccharomyces cerevisiae* encodes an 80 kDa cytoplasmic protein that interacts with the cytoplasmic domain of Sec20p. *EMBO J.* **12**, 2831-2840.

Tall, G. G., Hama, H., DeWald, D. B. and Horazdovsky, B. F. (1999). The phosphatidylinositol 3-phosphate binding protein Vac1p interacts with a Rab GTPase and a Sec1p homologue to facilitate vesicle-mediated vacuolar protein sorting. *Mol. Biol. Cell* **10**, 1873-1889.

TerBush, D. R., Maurice, T., Roth, D. and Novick, P. (1996). The Exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO J.* **15**, 6483-6494.

Ungar, D., Oka, T., Brittle, E. E., Vasile, E., Lupashin, V. V., Chatterton, J. E., Heuser, J. E., Krieger, M. and Waters, M. G. (2002). Characterization of a mammalian Golgi-localized protein complex, COG, that is required for normal Golgi morphology and function. *J. Cell Biol.* **157**, 405-415.

VanRheenen, S. M., Cao, X., Lupashin, V. V., Barlowe, C. and Waters, M. G. (1998). Sec35p, a novel peripheral membrane protein, is required for ER to Golgi vesicle docking. *J. Cell Biol.* **141**, 1107-1119.

VanRheenen, S. M., Cao, X., Sapperstein, S. K., Chiang, E. C., Lupashin, V. V., Barlowe, C. and Waters, M. G. (1999). Sec34p, a protein required for vesicle tethering to the yeast Golgi apparatus, is in a complex with Sec35p. *J. Cell Biol.* **147**, 729-742.

Vega, I. E. and Hsu, S. C. (2001). The exocyst complex associates with microtubules to mediate vesicle targeting and neurite outgrowth. *J. Neurosci.* **21**, 3839-3848.

Walter, D. M., Paul, K. S. and Waters, M. G. (1998). Purification and characterization of a novel 13 S hetero-oligomeric protein complex that stimulates in vitro Golgi transport. *J. Biol. Chem.* **273**, 29565-29576.

Wang, W., Sacher, M. and Ferro-Novick, S. (2000). TRAPP stimulates guanine nucleotide exchange on Ypt1p. *J. Cell Biol.* **151**, 289-295.

Waters, M. G. and Hughson, F. M. (2000). Membrane tethering and fusion in the secretory and endocytic pathways. *Traffic* **1**, 588-597.

Webb, G. C., Zhang, J., Garlow, S. J., Wesp, A., Riezman, H. and Jones, E. W. (1997). Pep7p provides a novel protein that functions in vesicle-mediated transport between the yeast Golgi and endosome. *Mol. Biol. Cell* **8**, 871-895.

Weisman, L. S. and Wickner, W. (1992). Molecular characterization of *VAC1*, a gene required for vacuole inheritance and vacuole protein sorting. *J. Biol. Chem.* **267**, 618-623.

Whyte, J. R. and Munro, S. (2001). The Sec34/35 Golgi transport complex is related to the exocyst, defining a family of complexes involved in multiple steps of membrane traffic. *Dev. Cell* **1**, 527-537.

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

Wurmser, A. E., Sato, T. K. and Emr, S. D. (2000). New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7

GTPase to SNARE-dependent docking and fusion. *J. Cell Biol.* **151**, 551-562.

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

Yang, B., Steegmaier, M., Gonzalez, L. C., Jr and Scheller, R. H. (2000). nSec1 binds a closed conformation of syntaxin1A. *J. Cell Biol.* **148**, 247-252.

Yeaman, C., Grindstaff, K. K., Wright, J. R. and Nelson, W. J. (2001). Sec6/8 complexes on trans-Golgi network and plasma membrane regulate late stages of exocytosis in mammalian cells. *J. Cell Biol.* **155**, 593-604.

Zerial, M. and McBride, H. (2001). Rab proteins as membrane organizers. *Nat. Rev. Mol. Cell. Biol.* **2**, 107-117.

Zhang, X., Bi, E., Novick, P., Du, L., Kozminski, K. G., Lipschutz, J. H. and Guo, W. (2001). Cdc42 interacts with the exocyst and regulates polarized secretion. *J. Biol. Chem.* **276**, 46745-46750.