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Review

Coats, Tethers, Rabs, and SNAREs Work Together to Mediate the Intracellular Destination of a Transport Vesicle

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Tethering factors have been shown to interact with Rabs and SNAREs and, more recently, with coat proteins. Coat proteins are required for cargo selection and membrane deformation to bud a transport vesicle from a donor compartment. It was once thought that a vesicle must uncoat before it recognizes its target membrane. However, recent findings have revealed a role for the coat in directing a vesicle to its correct intracellular destination. In this review we will discuss the literature that links coat proteins to vesicle targeting events.

Introduction

The transfer of material between organelles is mediated by carrier vesicles that continually bud from one membrane and fuse with another. In the past few decades, considerable progress has been made in identifying the molecular machinery that maintains and regulates membrane traffic (for a review see Bonifacino and Glick, 2004; Mellman and Warren, 2000). Each vesicle transport reaction can be divided into four essential steps that include vesicle budding, transport, tethering, and fusion (Bonifacino and Glick, 2004). These steps are tightly regulated to ensure that vesicles generated from a donor compartment are delivered to their correct acceptor compartment. Although much has been learned about these processes, exactly how a carrier vesicle finds and fuses with its target organelle remains an unanswered question.

In this review we will discuss how tethering factors may work together with coat proteins to mediate the intracellular targeting of vesicles. Coat proteins, which are required for vesicle budding and cargo selection, have recently been shown to play an important role in tethering a vesicle to its correct target membrane (Cai et al., 2007).

Four Essential Steps in Vesicle Transport

Vesicle budding is mediated by protein coats (Bonifacino and Lippincott-Schwartz, 2003; Kirchhausen, 2000; McMahon and Mills, 2004). Protein coats are dynamic structures that cycle on and off membranes. They are recruited from the cytosol onto donor membranes by small GTPases of the Arf1/Sar1 family that regulate their assembly (Springer et al., 1999). Coats deform flat membranes into round buds, which leads to the release of coated vesicles (Figure 1). Coat proteins also participate in cargo selection through the recognition of sorting signals present in the cytoplasmic domain of transmembrane cargo proteins. Clathrin was the first coat to be identified (Pearse, 1975). Clathrin-coated vesicles are mainly derived from the plasma membrane or the *trans*-Golgi network (TGN) and are transported to endosomes (Owen et al., 2004). Subsequent studies identified two nonclathrin coats, COPI (coat protein complex I) and COPII (coat protein complex II), that mediate vesicle transport in the early secretory pathway. COPI primarily acts from the Golgi to the endoplasmic reticulum (ER) and between Golgi cisternae, while COPII mediates traffic from the ER to the Golgi (Barlowe et al., 1994; Letourneur et al., 1994; Waters et al., 1991). In the last decade, several other nonclathrin coats have been identified (Godi et al., 2004; Seaman et al., 1998; Wang et al., 2006).

After budding, vesicles are transported to their final destination by diffusion or by motor-mediated transport along a cytoskeletal track (microtubules or actin, see Figure 1). The molecular motors kinesin, dynein, and myosin have all been implicated in this process (Hammer and Wu, 2002; Matanis et al., 2002; Short et al., 2002). The third step in vesicle-mediated membrane traffic is tethering (Figure 1). Tethering is a term used to describe the initial interaction between a vesicle and its target membrane. It precedes the pairing of transmembrane SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) on apposing membranes, an event that leads to membrane fusion (Söllner et al., 1993). Proteins and protein complexes called tethers or tethering factors have been identified in nearly all membrane-trafficking events (Sztul and Lupashin, 2006; Whyte and Munro, 2002). Together with Rabs, small GTPases of the Ras superfamily, tethers play a critical role in determining the specificity of vesicle targeting.

The last step in vesicle-mediated transport is the fusion of the vesicle with its target membrane (Figure 1). Fusion is thought to occur by the pairing of SNAREs, a family of membrane proteins that are related to three different neuronal proteins: synaptobrevin, syntaxin, and SNAP-25. A SNARE on a transport vesicle (v-SNARE) pairs with its cognate SNARE-binding partner (t-SNARE) on the

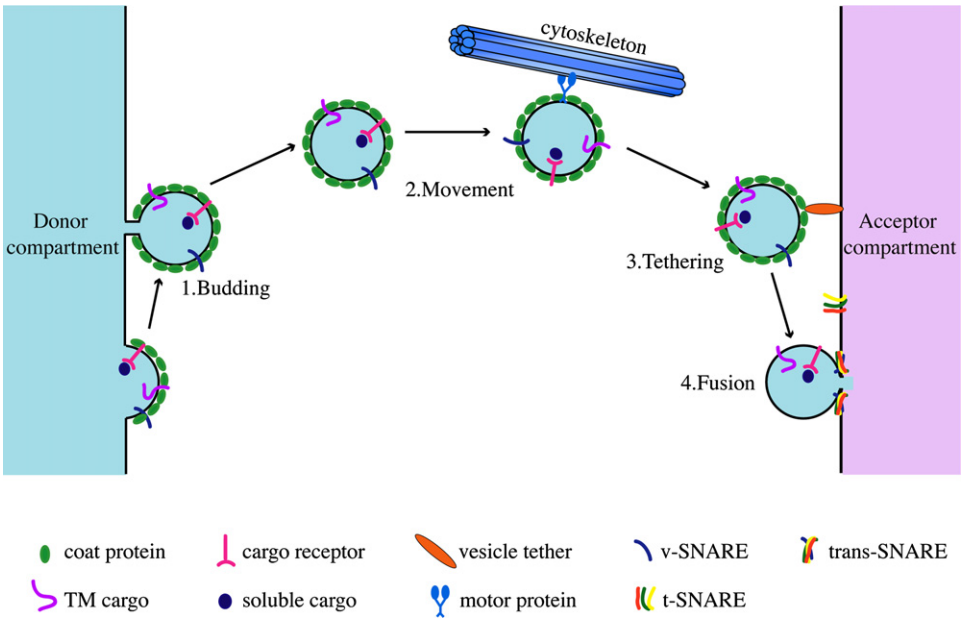

Figure 1. The Four Essential Steps in Vesicle Transport

(1) Budding: coat proteins are recruited onto the donor membrane to induce the formation of a vesicle. Cargo and SNAREs are incorporated into the budding vesicle by binding to coat subunits. (2) Movement: the vesicle moves toward the acceptor compartment by diffusion or with the aid of a cytoskeletal track. (3) Tethering: tethering factors work in conjunction with Rab GTPases to tether the vesicle to their acceptor membrane. (4) Fusion: the vesicle-associated SNARE and the SNARE on the acceptor membrane assemble into a four-helix bundle (trans-SNARE complex), which drives membrane fusion and the delivery of cargo.

appropriate target membrane (Rothman, 1994; Söllner et al., 1993). The energy derived from the assembly of the SNARE complex has been postulated to drive the fusion of two lipid bilayers (Hanson et al., 1997; Lin and Scheller, 1997). However, factors acting downstream from the SNAREs may also contribute to membrane fusion (Muller et al., 2002; Ungermann et al., 1998). While the specific role of the SNAREs in membrane fusion remains to be determined, there is little doubt they play a key role in this process. For a recent review on SNAREs and their role in membrane fusion, see Jahn and Scheller (2006).

The pairing of SNAREs was once postulated to drive the specificity of vesicle targeting (Söllner et al., 1993). However, several lines of evidence indicate this is not the case. First, since SNAREs are recycled after each fusion event, the same SNARE will be present on both anterograde- and retrograde-directed vesicles. Thus, the mere presence of a SNARE cannot be the sole determinant of the direction in which a vesicle is traveling. Furthermore, the interactions of SNAREs are promiscuous (Tsui and Banfield, 2000; von Mollard et al., 1997), and the disruption of SNARE complex formation does not block vesicle tethering (Broadie et al., 1995; Hunt et al., 1994). These findings indicate that SNAREs do not mediate the first point of contact between a vesicle and its target. Instead, tethers, which act upstream of the SNAREs, appear to perform this function.

The Structure of Multisubunit Tethers

Although tethers are required for the initial interaction of a carrier vesicle with its target membrane, not all putative tethers have been shown to bind to vesicles. Almost all tethering factors fall into two broad categories: long putative coiled-coil proteins and multisubunit complexes. This review will focus on the role of multisubunit complexes in vesicle tethering. The interaction of coats with tethers is best documented with this class of tethers.

All large multisubunit complexes implicated in tethering were first found in the yeast *Saccharomyces cerevisiae* (for review see Whyte and Munro, 2002). To date eight conserved complexes (COG, CORVET, Dsl1, exocyst, GARP/VFT, HOPS/Class C VPS, TRAPPI, and TRAPPII) acting in exocytic and endocytic trafficking events have been identified (see Figure 2). Five of these complexes are required for secretion in vivo: TRAPPI (ER-Golgi), Dsl1 (Golgi-ER), TRAPPII (intra-Golgi/endosome-late Golgi), COG (endosome-early Golgi), and the exocyst (Golgi-plasma membrane; endosome-plasma membrane) (Andag et al., 2001; Cai et al., 2005; Munson and Novick, 2006; Reilly et al., 2001; TerBush et al., 1996; Whyte and Munro, 2001). Three of these complexes are required for vacuolar protein sorting in vivo: CORVET (late Golgi-endosome), HOPS (endosome-vacuole) and GARP/VFT (endosome-late Golgi) (Conibear et al., 2003; Peplowska et al., 2007; Peterson and Emr, 2001). The HOPS complex is also required for vacuole-vacuole fusion (Price et al., 2000). The exocyst is needed for polarized growth, and it tethers a variety of vesicles to exocytic sites (Munson and Novick, 2006). Thus, some tethers act in only one trafficking event, while others may participate in more than one.

Structurally the best characterized tethering complexes are the ~300 kDa TRAPPI complex (Sacher et al., 2001)

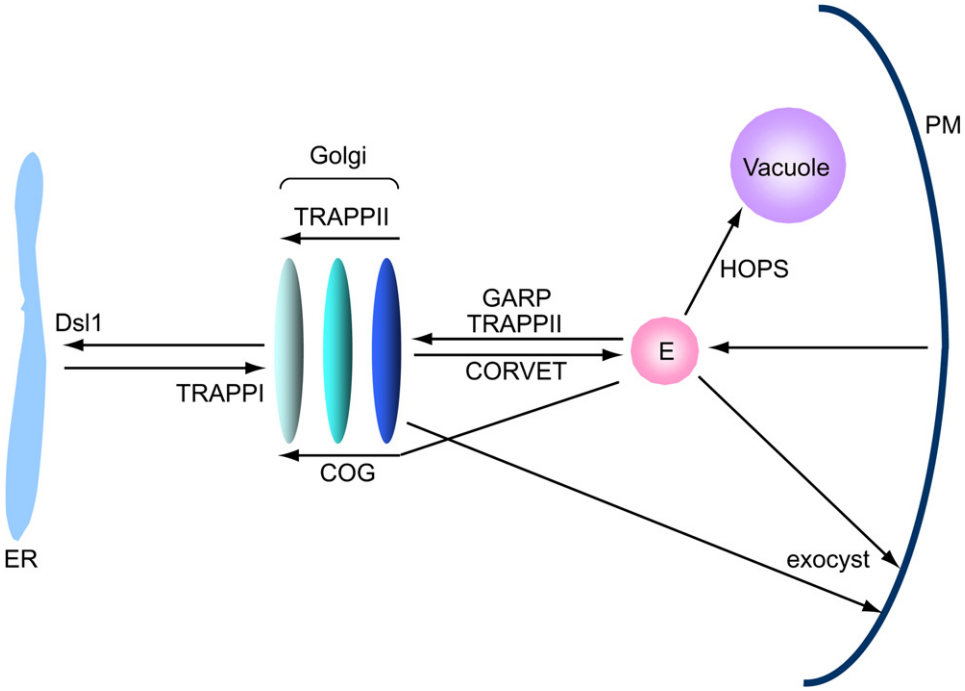

Figure 2. Large Tethering Complexes that Act in the Secretory and Endocytic Pathways
Protein complexes that play a role in vesicle tethering are indicated where they act. TRAPPI acts in ER-to-Golgi traffic. TRAPPII has been implicated in transport events that take place within the Golgi, and from the endosome to the late Golgi. COG has been proposed to tether vesicles from the endosome to the *cis*-Golgi, while the GARP/VFT complex has been implicated in traffic from the endosome to the *trans*-Golgi. The CORVET complex may be required for tethering events between the trans-Golgi and endosome, while HOPS functions between the endosome and vacuole. HOPS also has been implicated in homotypic vacuole fusion. The exocyst complex binds to post-Golgi and recycling vesicles at the plasma membrane.

and the exocyst (TerBush et al., 1996). Yeast TRAPPI has recently been visualized by single-particle electron microscopy (Kim et al., 2006). It is a flattened dumbbell (~65 × 65 × 180 Å) with two lobes. High-resolution structures for subcomplexes of vertebrate TRAPPI were modeled into the EM reconstruction. The trs20-trs31-bet3 heterotrimer was fit into one lobe, while the bet3-trs33-bet5 heterotrimer was fit into the other lobe (Figure 3A). The two lobes of TRAPPI are linked together by the trs23 subunit (Kim et al., 2006). The significance of the presence of bet3 in each lobe of TRAPPI will be addressed below when we discuss the interactions of tethers with coat proteins. TRAPPII contains all the subunits found in TRAPPI, plus three additional proteins (trs130, trs120, and trs65) (Sacher et al., 2001).

The exocyst is a hetero-octameric ~750 kDa complex with subunits that range in size from 50 to 150 kDa (Guo et al., 1999; TerBush et al., 1996). Quick-freeze/deep-etch/rotary-shadow electron micrographs demonstrated that the mammalian exocyst consists of 4–6 "arms." Each arm, which is 35–70 Å wide and 100–300 Å long, emanates from a central point (Hsu et al., 1998). In samples treated with glutaraldehyde, the exocyst looks like a tree. The arms coalesce to a thicker long trunk (~300 Å) with two flexibly disposed long branches (~100 Å). The crystal structure of the Exo70 subunit (see Figure 3B) revealed it to be a long rod (~160 Å) composed of contiguous α-helical bundles (Dong et al., 2005; Hamburger et al., 2006). The C termini of three other exocyst subunits (*Drosophila* Sec15, yeast Exo84, and Sec6; shown in Figure 3B) were shown to be shorter rods composed of contiguous α-helical bundles (Dong et al., 2005; Sivaram et al., 2006; Wu et al., 2005). These structures, together with secondary structure predictions, were the basis for proposing that all exocyst subunits are elongated with related folds (Dong et al., 2005). Two or more of these rods may be aligned in forming the "trunk" and the "branches" of the exocyst.

Based on regions of weak sequence similarity to exocyst subunits, it was proposed that two other tethering complexes, the hetero-octameric COG complex and the heterotetrameric GARP/VFT complex, are structurally and functionally similar to the exocyst (Whyte and Munro, 2001). Secondary structure algorithms for COG subunits suggest that, like exocyst subunits, COG subunits are α-helical. However, quick-freeze/deep-etch/rotary-shadow electron micrographs of glutaraldehyde-fixed mammalian COG subunits showed a bilobed assembly that differs from the exocyst (Ungar et al., 2002). Protein-protein interaction studies suggest that each lobe contains a heterotrimeric COG subcomplex. The COG1/COG8 heterodimer bridges the two lobes of the complex (Fotso et al., 2005; Ungar et al., 2005). Thus, the architecture of the COG complex superficially resembles TRAPPI rather than the exocyst. TRAPPI and COG are similar in that they appear to mediate a single tethering event, while the exocyst

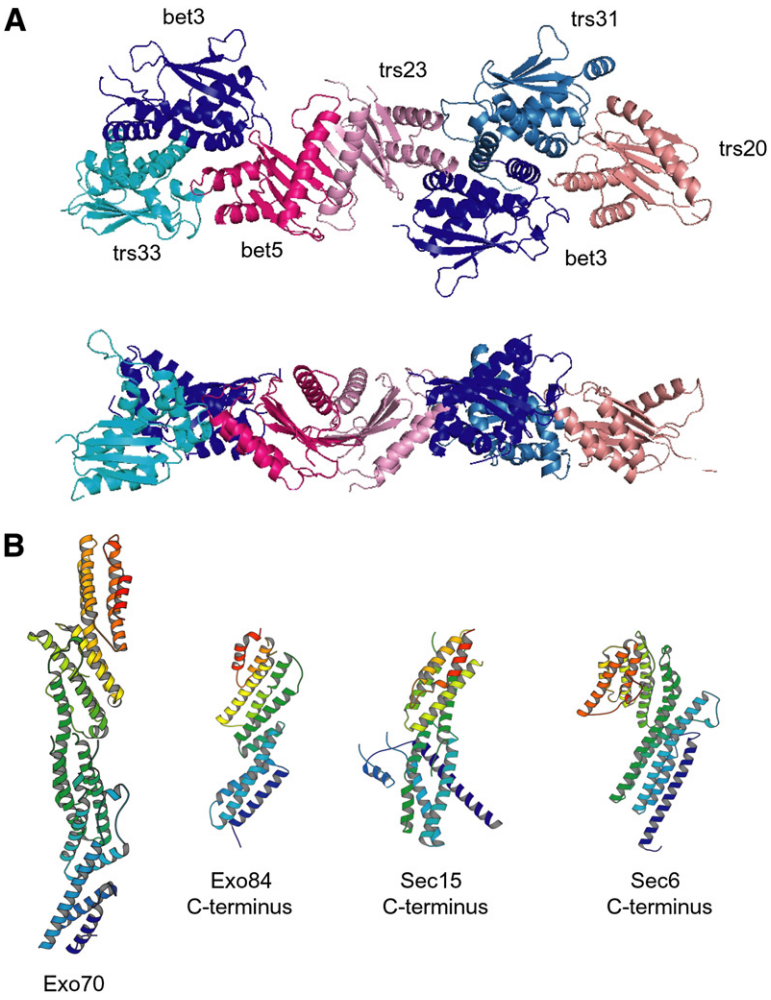

Figure 3. Structures of Tethering Complexes

(A) High-resolution structures of mammalian TRAPP subcomplexes bet3/trs31/trs20 and bet3/trs33/bet5/trs23 have been determined (Kim et al., 2006). bet3, trs31, and trs33 have similar folds, as do trs20, trs23, and bet5. The X-ray structures of the subcomplexes were fit into a low-resolution model of the yeast TRAPPI complex as obtained from single-particle electron microscopy. The subcomplexes are associated as depicted (Kim et al., 2006). The top and bottom views are related by 90° rotation about a horizontal axis.

(B) Structural studies of exocyst subunits reveal they are rods composed of contiguous α-helical bundles. The structures shown are, from left to right, yeast Exo70 and the C-terminal domains of yeast Exo84 (Dong et al., 2005), *Drosophila* Sec15 (Wu et al., 2005), and yeast Sec6 (Sivaram et al., 2006). The fragments are colored from blue at their N termini to red at their C termini.

tethers a variety of vesicles to sites of polarized growth (Cai et al., 2007; Munson and Novick, 2006; Zolov and Lupashin, 2005).

A growing body of evidence implies that tethering is a multistep process that is mediated through interactions with coat proteins, tethers, Rabs, and SNAREs. In the following sections we will review this literature.

Tethers Can Be Rab Effectors and Rab Exchange Factors

Rabs are small GTPases of the Ras superfamily that continuously cycle between the cytosol and membranes. In the cytosol, the GDP-bound form of the Rab is complexed with GDI (guanine nucleotide dissociation inhibitor). Rabs are recruited to membranes with the aid of a GDF (GDI displacement factor) (Dirac-Svejstrup et al., 1997) and are inserted into the membrane via a prenyl group. The membrane-bound Rab is then activated by a specific GEF (guanine nucleotide exchange factor) through the exchange of GDP for GTP (Soldati et al., 1994; Ullrich et al., 1994). Upon activation, the Rab interacts with downstream effectors. Activation of the Rab is terminated when GTP hydrolysis is stimulated by a specific GAP (GTPase-activating protein) (Rybin et al., 1996). To date, 11 Rab proteins have been identified in yeast, and >60 in mammalian cells (Lazar et al., 1997; Pfeffer, 2001).

Do Rabs regulate only one trafficking step, or can the same Rab be required in multiple trafficking events? Initially it was thought that each trafficking event is regulated by a specific Rab. However, now it is clear this is not the case. In fact, genetic studies in yeast imply that some Rabs, like Ypt1p (Rab1 in mammals), act at multiple stages of the exocyctic and endocytic pathway (Bacon et al., 1989; Finger and Novick, 2000). In vivo and in vitro studies have confirmed that Ypt1p (Rab1) is required for ER-to-Golgi traffic, intra-Golgi traffic, and membrane recycling through the early endosome (Bacon et al., 1989; Baker et al., 1990; Davidson and Balch, 1993; Jedd et al., 1995; Lafourcade et al., 2004). For a recent review of Rabs and their role in membrane traffic, see Grosshans et al. (2006).

The best understood function of Rab proteins is probably their role in vesicle tethering. The earliest evidence for the involvement of Rabs in vesicle tethering and fusion was the observation that mutations in the gene that encodes the Rab GTPase *SEC4* lead to the accumulation

of post-Golgi vesicles in yeast (Salminen and Novick, 1987). With the development of in vitro transport assays, other Rabs were shown to participate in vesicle tethering. The yeast homotypic vacuole-fusion assay revealed that Ypt7p is needed at a step that precedes SNARE complex formation (Mayer and Wickner, 1997; Ungermann et al., 1998). Subsequently, the Rab Ypt1p was shown to be required for the tethering of ER-derived COPII vesicles in vitro (Cao et al., 1998).

Rabs in their GTP-bound form appear to facilitate the recruitment of tethers to specific locations. A well-studied example of a tether that is a Rab effector is the exocyst complex. As mentioned above, the exocyst localizes to sites of polarized growth in yeast and is required to tether post-Golgi and recycling vesicles to the plasma membrane (Munson and Novick, 2006; TerBush et al., 1996). Most exocyst subunits are delivered on post-Golgi vesicles to exocytic sites (Boyd et al., 2004). They are recruited to vesicles via an interaction between the exocyst subunit Sec15p and the GTP-bound form of the Rab Sec4p (Guo et al., 1999). Two other exocyst subunits, Sec3p and Exo70p, localize to the plasma membrane independent of the cytoskeleton and ongoing membrane traffic (Boyd et al., 2004; Finger et al., 1998). The exocyst complex forms at the cell surface when post-Golgi vesicles tether to the plasma membrane (Boyd et al., 2004). In epithelial cells, the exocyst is required for the transport of proteins to the basolateral cell surface (Grindstaff et al., 1998).

The COG and GARP/VFT complexes (Figure 2) are also Rab effectors. COG is a multisubunit tethering complex that mediates membrane traffic at the Golgi apparatus. The yeast COG complex (also called the Sec34p/Sec35p complex) is a Ypt1p effector that has been proposed to tether vesicles from the late Golgi/early endosome to the early Golgi (Suvorova et al., 2002; Whyte and Munro, 2001). GARP/VFT binds to the GTP form of Ypt6p and plays a role in retrograde traffic from endosomes to the trans-Golgi (Conibear et al., 2003; Quenneville et al., 2006; Siniossoglou and Pelham, 2001).

The HOPS complex is the only tethering factor shown to act as an effector of a Rab (Ypt7p) and as a GEF. Homotypic vacuole fusion occurs in ordered steps that includes priming, tethering, docking, and fusion. Proteins that regulate this process assemble into a "vertex ring" around the apposed membranes of tethered vacuoles before fusion occurs (Fratti et al., 2004; Wang et al., 2002, 2003). Ypt7p and HOPS are required for the homotypic fusion of yeast vacuoles and the fusion of transport vesicles to the vacuole (Haas et al., 1995; Mayer and Wickner, 1997; Price et al., 2000; Seals et al., 2000; Wichmann et al., 1992; Wurmser et al., 2000). The HOPS subunit, Vps39p, converts Ypt7p from its GDP-bound form to its GTP-bound form to promote vesicle tethering (Price et al., 2000; Seals et al., 2000; Wurmser et al., 2000).

HOPS shares four subunits, Vps11p, Vps16p, Vps18p, and Vps33p, with the newly identified CORVET complex. CORVET binds to GTP-bound Vps21p, the Rab5 homolog in yeast (Peplowska et al., 2007). Interestingly, human homologs of Vps11p, Vps16p, and Vps18p are also components of a mammalian complex that binds to Rab5-GTP (Rink et al., 2005). These recent findings suggest that the HOPS and CORVET complexes play a role in tethering events that lead to endosome-vacuole (lysosomes in mammals) biogenesis in both yeast and mammalian cells. One of the other subunits of the CORVET complex, Vps3p, has an affinity for Vps21p-GDP (Peplowska et al., 2007). This observation led to the suggestion that Vps3p is an exchange factor for Vps21p. Additional biochemical studies will be needed to test this proposal. Vps9p, which is not a component of the CORVET or HOPS complexes, is an established Vps21p exchange factor (Hama et al., 1999).

All tethers discussed in this review article are Rab effectors, except for the TRAPP complexes. In 2001, the tethers TRAPPI and TRAPPII were identified as exchange factors that specifically activate Ypt1p (Sacher et al., 2001; Wang et al., 2000). TRAPPI only functions in ER-to-Golgi traffic (Sacher et al., 2001), while TRAPPII regulates intra-Golgi traffic and traffic from the early endosome to the late Golgi in vivo (Cai et al., 2005; Sacher et al., 2001). Interestingly, five TRAPP subunits are needed to fully reconstitute Ypt1p exchange activity in vitro (Cai et al., 2007; Kim et al., 2006), and all of these subunits are shared between TRAPPI and TRAPPII. This finding fits well with the observation that both TRAPPI and TRAPPII are Ypt1p exchange factors (Sacher et al., 2001). The activation of Ypt1p by two distinct but related exchange factors may explain how this small GTPase can function in different transport events. However, it has recently been reported that TRAPPII is not a Ypt1p exchange factor, but instead activates Ypt31p/Ypt32p (Morozova et al., 2006). Yeast must contain a Ypt31p/Ypt32p exchange factor distinct from TRAPPII, as previous studies demonstrated that the depletion of TRAPP from yeast lysates results in the loss of Ypt1p, but not Ypt31p/Ypt32p, exchange activity (Wang et al., 2000; Wang and Ferro-Novick, 2002). Additional experiments will be needed to resolve these discrepancies and to determine if TRAPPII acts as a Ypt1p or a Ypt31p/Ypt32p exchange factor in vivo.

Tethers Interact with Coat Proteins to Mediate Vesicle Tethering

Vesicle coats are multilayered complexes (for review see Gurkan et al., 2006). One layer includes adaptor protein (AP) complexes that recognize and select cargo molecules. Another layer includes cage proteins (CPs). CPs assemble into a lattice on the membrane surface to form a scaffold that collects AP-cargo complexes. This links cargo concentration with membrane deformation, which eventually leads to the formation of coated vesicles. As we discuss here, many tethers show biochemical interactions with coat proteins.

The depletion of the mammalian COG3 subunit in HeLa cells results in the accumulation of COPI-coated, COG-complex-dependent (CCD) vesicles carrying the intra-Golgi SNAREs GS15 and GS28. Anterograde trafficking of vesicular stomatitis virus G protein to the plasma membrane was maintained in COG3-depleted cells, while the

retrograde traffic of Shiga toxin was inhibited. Additionally, mammalian COG bound to isolated CCD vesicles, and both mammalian and yeast COG showed specific interactions with COPI subunits (Suvorova et al., 2002; Zolov and Lupashin, 2005). These studies led to the conclusion that the *cis*-Golgi-localized COG complex tethers retrograde COPI vesicles that bud from a *trans*-Golgi/endosomal compartment (Zolov and Lupashin, 2005).

Specific interactions with COPI subunits were also observed with two other putative tethering complexes, TRAPPII and Dsl1. TRAPPII localizes to the late Golgi/early endosome (Cai et al., 2005; Yu et al., 2006). Mutations in genes that encode two different TRAPPII-specific subunits (*TRS130* and *TRS120*) have somewhat different phenotypes. Mutations in *trs130* block intra-Golgi and endocytic traffic, while mutations in *trs120* only block endocytic traffic. TRAPPII also interacts with COPI in yeast lysates, and COPI subunits are mislocalized in *trs120* mutants (Cai et al., 2005). A putative role for TRAPPII is to tether vesicles that recycle through the early endosome and Golgi (Cai et al., 2005).

Dsl1 is a large ER-localized complex implicated in the tethering of retrograde COPI vesicles that direct traffic from the Golgi to the ER (Andag et al., 2001; Reilly et al., 2001). The central acidic domain of the Dsl1p component interacts in vitro with the δ- and α-subunits of the COPI coat. The δ-subunit is a constituent of the F subcomplex of COPI that shares sequence similarities with clathrin adaptor components. The α-COPI subunit belongs to the B subcomplex of the COPI coat, which is thought to function like clathrin (Andag and Schmitt, 2003). Thus, Dsl1p interacts with both the F and B subcomplexes of the COPI vesicle coat.

While several different large tethering complexes have been shown to interact with coat subunits and coat complexes, the function of these interactions has been unclear. Direct evidence that coat proteins play a role in vesicle tethering has come from a recent study that defined the TRAPPI-binding partner on COPII vesicles (Cai et al., 2007). The formation of COPII vesicles is initiated when the small GTPase Sar1p recruits the cargo adaptor complex Sec23p/Sec24p (Yoshihisa et al., 1993). This leads to the capture of transmembrane cargo by Sec24p (Kuehn et al., 1998; Miller et al., 2003). Sec23p/Sec24p then recruits the Sec13p/Sec31p complex, which induces coat polymerization and membrane deformation (Stagg et al., 2006). In yeast, COPII vesicles are believed to tether and fuse directly with the *cis*-Golgi (Baker et al., 1988; Ruohola et al., 1988). In mammalian cells, COPII vesicle tethering is thought to be largely a homotypic event (Xu and Hay, 2004). Homotypic COPII vesicle fusion results in the formation of membrane structures called VTCs (vesicular tubular clusters), which are the compartments where soluble cargo concentrates (Martinez-Menarguez et al., 1999). Anterograde cargo then moves from VTCs to the *cis*-Golgi (Ben-Tekaya et al., 2005; Presley et al., 1997; Scales et al., 1997).

TRAPPI is a stage-specific tether that binds directly to COPII vesicles (Sacher et al., 2001). The most conserved component of the TRAPPI complex is Bet3, as yeast and mammalian Bet3 (mBet3) are 54% identical (Sacher et al., 1998). Immuno-EM studies demonstrated that mBet3 resides on COPII-coated vesicles and COPII budding profiles, and in vitro mBet3 is required for homotypic COPII vesicle tethering (Yu et al., 2006). Additional studies demonstrated that Bet3 mediates COPII vesicle tethering via an interaction with the coat subunit Sec23 in both yeast and mammalian cells (Cai et al., 2007). These findings link vesicle budding to tethering and show that the mechanism of COPII vesicle tethering is conserved between yeast and mammals. The structure of TRAPPI (Kim et al., 2006) is consistent with a homotypic tethering model where a copy of Bet3, in each of the two lobes of the TRAPPI complex, interacts with a different coated vesicle to bring two vesicles together. Although TRAPPI and TRAPPII share seven subunits, only TRAPPI binds to COPII vesicles (Sacher et al. 2001). One or more of the three TRAPPII-specific subunits must mask the Sec23p-binding site on Bet3p. Recently, another putative tethering factor that participates in ER-to-Golgi traffic, Grh1p (yeast homolog of GRASP65), was reported to interact with the Sec23p/Sec24p complex (Behnia et al., 2007).

Although it was long thought that the vesicle coat is removed soon after the carrier vesicle is formed (see review in Bonifacino and Glick, 2004), these new findings imply that vesicles remain coated until the coat is recognized by a specific tether. The observation that TRAPPI binds to a component of a cargo adaptor complex links vesicle tethering to cargo recognition. This finding provides a simple mechanism to explain how the directionality of vesicle transport is maintained. It also suggests that tethering factors may integrate the recognition of a vesicle to the process of vesicle uncoating.

The molecular mechanism by which vesicle uncoating is regulated is still unclear. The disassembly of the COPI and COPII coats is thought to occur subsequent to the hydrolysis of GTP by the Arf1/Sar1 family of proteins. In the case of the COPII coat, Sec23p is the GAP for Sar1p, and this GAP activity is further stimulated by the Sec13p/Sec31p complex (Antonny et al., 2001; Yoshihisa et al., 1993). Although GTP hydrolysis is required for COPII vesicle uncoating, the COPII coat remains associated with vesicles for a considerable amount of time after Sar1p is hydrolyzed (Barlowe et al., 1994). The binding of secretory cargo to the coat cargo adaptor complex stabilizes the COPII coat on vesicles after Sar1p is released from membranes (Forster et al., 2006; Sato and Nakano, 2005).

It was initially thought that ArfGAP1 induces COPI vesicle uncoating (Bremser et al., 1999; Goldberg, 1999; Reinhard et al., 2003). However, more recent findings have revealed that ArfGAP1 functions as a basic component of the COPI coat. In vitro studies have demonstrated that the incorporation of cargo into COPI vesicles depends on the hydrolysis of ARF1-GTP (Lanoix et al., 1999; Nickel et al., 1998). When ArfGAP1, ARF1, and coatomer were used to reconstitute vesicle formation, purified COPI coated vesicles were depleted of ARF1 and enriched in ArfGAP1 (Yang et al., 2002).

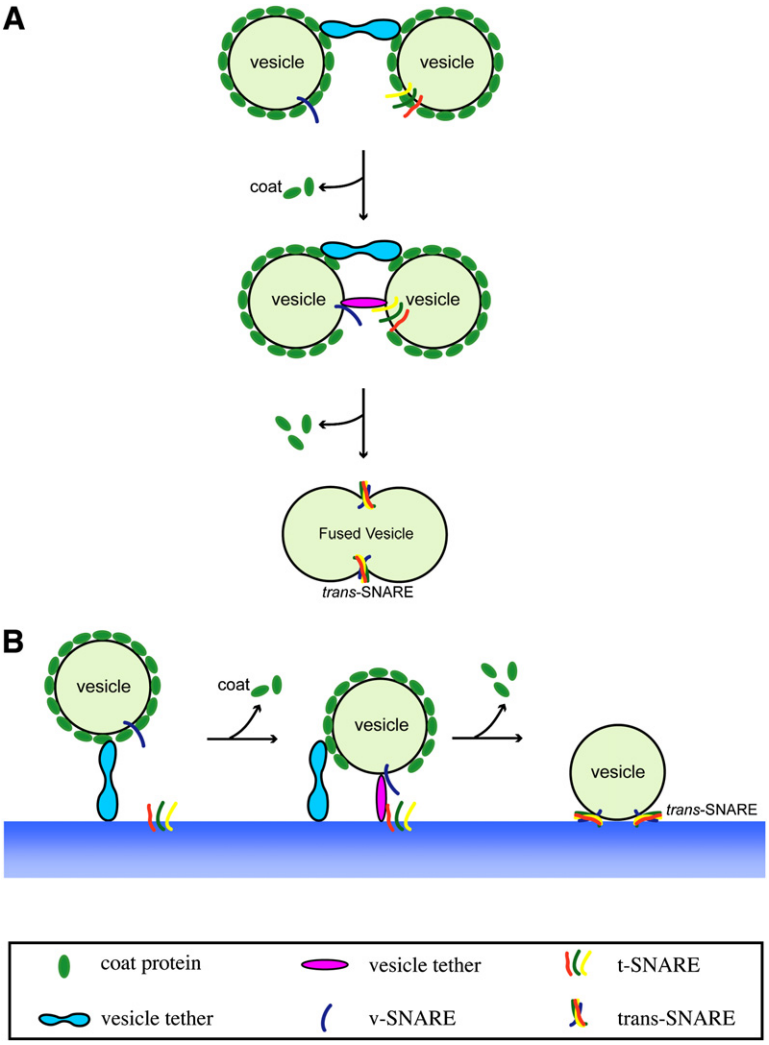

Figure 4. Vesicles Tether via an Interaction with Coat Proteins

(A) In homotypic tethering, the tether (blue vesicle tether) links two coated vesicles together via an interaction with a coat subunit. This interaction is maintained as regions of the vesicle uncoat and the SNAREs are exposed. Other tethers (red vesicle tether) may then promote SNARE pairing and membrane fusion.

(B) In heterotypic tethering, the tether (blue vesicle tether) targets the vesicle to its acceptor compartment through a direct interaction with a coat subunit. As the vesicle uncoats, the SNAREs are exposed. This is then followed by the formation of trans-SNARE pairs (red vesicle tether) and membrane fusion.

Tethers Interact with SNAREs

How do tethering factors coordinate the recognition of a vesicle to the process of membrane fusion? Many tethers physically interact with SNAREs. Tethering factors may bring the vesicle in closer contact with its target compartment after vesicle uncoating (Malsam et al., 2005) to increase the probability of SNARE interactions. Alternatively, tethering factors may actively promote SNARE-mediated membrane fusion by stimulating the formation of trans-SNARE complexes (Shorter et al., 2002).

Studies on the well-characterized coiled-coil tether, p115, support both models. Rab1-GTP recruits p115 onto COPII vesicles as vesicles bud from the ER (Allan et al., 2000). On vesicles, p115 interacts with a select set of COPII vesicle-associated SNAREs (Allan et al., 2000). The recruitment of p115 to membranes is dependent on SNAREs and is required for COPII vesicle tethering and VTC formation (Bentley et al., 2006; Brandon et al., 2006). The most amino-terminal coiled-coil region of p115, which is weakly homologous to a SNARE motif (Weimbs et al., 1997), also stimulates SNARE complex assembly (Shorter et al., 2002). Therefore, p115 may not only tether vesicles to their target membrane via an interaction with SNAREs, it also catalyzes SNARE-mediated membrane fusion. Uso1p, the yeast homolog of p115, is also required for the assembly of the ER-to-Golgi SNARE complex in vivo (Sapperstein et al., 1996). Thus, in addition to participating in fusion, the SNAREs appear to act upstream of fusion to recruit tethering machinery.

Another example of a tether interacting with a SNARE is the GARP/VFT complex subunit Vps51p that binds to the N-terminal domain of the SNARE Tlg1p (Conibear et al., 2003; Siniossoglou and Pelham, 2001). GARP/VFT, which localizes to the late Golgi in a Ypt6p-dependent fashion (Siniossoglou and Pelham, 2001), has been implicated in tethering endosomal derived vesicles to the late Golgi via an interaction with Tlg1p (Conibear et al., 2003; Conibear and Stevens, 2000). Since Tlg1p is present on Golgi membranes as well, an alternative model is that Tlg1p is a receptor for GARP/VFT on the Golgi. In either case, it is possible that GARP/VFT interacts with Tlg1p to stimulate SNARE complex formation. Tlg1p has an N-terminal domain that forms a three-helix bundle. Like other syntaxins, it has been suggested that the function of Tlg1p is

autoinhibited through an interaction of its N-terminal domain with its C-terminal SNARE motif (Misura et al., 2000). Vps51p may release this autoinhibition as its N terminus binds to the N-terminal domain of Tlg1p (Conibear et al., 2003; Fridmann-Sirkis et al., 2006; Siniossoglou and Pelham, 2001). However, deletions or point mutations that eliminate the binding of Vps51p to Tlg1p do not affect the recycling of proteins from endosomes to the Golgi in vivo (Fridmann-Sirkis et al., 2006). These findings imply that the binding of Tlg1p to Vps51p is not essential for GARP/VFT-mediated vesicle tethering.

Tethering factors, like the exocyst and HOPS complexes, may promote SNARE-mediated membrane fusion by binding to Sec1/Munc18 family proteins (for review see Jahn and Sudhof, 1999; Waters and Hughson, 2000). Recent in vitro studies showing that Munc18 binds to fully assembled SNARE complexes to promote membrane fusion support this hypothesis (Dulubova et al., 2007; Shen et al., 2007). The exocyst binds to Sec1p (Wiederkehr et al., 2004), which in turn binds to the fully assembled Snc/Sso/Sec9p exocytic SNARE complex (Carr et al., 1999). HOPS is the only multisubunit tethering complex that contains a Sec1 homolog, Vps33p. Vps33p binds to the vacuolar t-SNARE Vam3p (Laage and Ungermann, 2001; Seals et al., 2000; Wang et al., 2001). There are conflicting reports on the significance of the HOPS/Vam3p interaction. In one study (Laage and Ungermann, 2001), deletion of the N-terminal domain of Vam3p was reported to reduce the formation of trans-SNARE complexes and homotypic vacuolar fusion in vitro. In another study (Wang et al., 2001), deletion of the N-terminal domain of Vam3p did not disrupt fusion in vitro or in vivo. Recently, HOPS has been shown to bind to the SNARE Vam7p. This interaction may play a role in initiating SNARE complex assembly (Stroupe et al., 2006).

The COG and Dsl1 complexes are two other tethering complexes that exhibit specific interactions with SNAREs. COG interacts with intra-Golgi SNAREs (Suvorova et al., 2002; Zolov and Lupashin, 2005), and Dsl1 binds to ER-localized SNAREs (Kraynack et al., 2005). It has been suggested that Dsl1 maintains the stability of an ER-localized SNARE complex that contains Use1p, Ufe1p, and Sec20p (Kraynack et al., 2005).

Concluding Remarks and Future Perspectives

Initially, tethers were proposed to act as structural bridges. It was speculated that a tether on the target membrane recognizes and binds to a specific determinant on the incoming vesicle to form a molecular link that holds two apposing membranes together. Since different tethers act in different places, it has been suggested that the localization of tethers is likely to be dependent on their interaction with either certain lipids or with activated forms of small GTPases. These lipids and GTPases are usually present on a subset of internal membranes, providing each compartment with a unique identity that allows it to be recognized by tethering factors (Munro, 2002). Tethers must also recognize vesicular components. Coat proteins are likely candidates, as they bind to cargo and are directly recruited from the cytosol to the compartment where they function.

In the past decade, a growing body of evidence has demonstrated that tethers work in conjunction with Rab GTPases and bind to coat proteins. Based on this observation, we propose the following model for vesicle tethering. First, we speculate that in homotypic tethering events, the tether binds to two different coated membrane compartments (Figure 4A). In heterotypic tethering events, only the vesicle would be coated (Figure 4B). In some cases, tethering may be initiated before the Rab is activated (Cai et al., 2007; Wurmser et al., 2000). As domains on the vesicle begin to uncoat, Rab-GTP may recruit other tethers that promote SNARE pairing (Shorter et al., 2002) (Figure 4). The same tether could act both upstream and downstream of Rab activation, or multiple tethers may participate in these events. For example, in yeast ER-to-Golgi traffic several components have been implicated in tethering COPII vesicles to their acceptor compartment (Behnia et al., 2007; Cai et al., 2007; Sacher et al., 2001). Thus, vesicle tethering appears to be a highly regulated process. Additional studies will be needed to determine how coats, tethers, Rabs, and SNAREs work together to control the fidelity of membrane traffic. The finding that a Rab exchange factor is recruited to vesicles by a coat subunit (Cai et al., 2007) raises the intriguing possibility that Rabs regulate vesicle uncoating.

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