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Role of vesicle tethering factors in the ER-Golgi membrane traffic

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Abstract

Tethers are a diverse group of loosely related proteins and protein complexes grouped into 3 families based on structural and functional similarities. A well-accepted role for tethering factors is the initial attachment of transport carriers to acceptor membranes prior to fusion. However, accumulating evidence indicates that tethers are more than static bridges. Tethers have been shown to interact with components of the fusion machinery and with components involved in vesicle formation. Tethers belonging to the 3 families act at the same stage of traffic, suggesting that they mediate distinct events during vesicle tethering. Thus, multiple tether-facilitated events are required to provide selectivity to vesicle fusion. In this review, we highlight findings that support this model.

Keywords

Tethering; Rabs; SNAREs; coats; ARFs; ARF guanine nucleotide exchange factors (GEFs); GBF1; membrane traffic; coil-coil tethers; tethering complexes

1. INTRODUCTION

Tethering factors are a diverse group of peripherally associated membrane proteins and protein complexes that bridge newly formed transport vesicles (as well as other types of intracellular transport carriers) with acceptor membranes to ensure correct docking and fusion. Historically, tethers were assigned to two general classes: multi-subunit complexes and coiled-coil proteins. More recent phylogenetic and structural evidence suggest the existence of three distinct functional classes: 1) oligomeric complexes that bind to SNAREs and typically act as Rab effectors (the DCGE group that includes Dsl1 complex, Conserved Oligomeric Golgi (COG) complex, Golgi-associated retrograde protein (GARP) complex, and Exocyst), 2) oligomeric complexes that function as guanine nucleotide exchange factors (GEFs) for Rab proteins (Transport Protein Particle TRAPP I and TRAPP II complexes and HOPS (HOPS is both, a GEF and a Rab effector)); and 3) coiled-coil tethers. Tethers with confirmed roles in membrane trafficking are listed in Table 1.

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Distinct tethers localize to different compartments of the secretory and endocytic pathways and have either been shown to or are proposed to function therein. While some tethers appear selective for a single step of traffic, others seem more general and localize to multiple compartments. It is possible that the same tether may function at distinct stages of traffic by binding to distinct Rabs and/or SNAREs. A single step of traffic may utilize a tether from each of the three functional classes: ER-Golgi traffic appears to require the activity of Dsl1 and COG (both are members of the DCGE class), TRAPP I (a GEF tether) and p115 (a coiled-coil tether). This suggests that different tethers might perform distinct functions in the overall transport process. In this review, we discuss a model of vesicular tethers working together with SNAREs, Rabs, coats and GEFs to ensure correct docking of vesicular membranes prior to vesicle fusion. We focus exclusively on tethers involved in ER-Golgi traffic.

1.1. DCGE TETHERS INVOLVED IN ER-GOLGI TRAFFIC: DSL1 AND COG

The DCGE sub-family of tethers (Dsl1, COG, GARP and exocyst complexes [1]) has been initially grouped together based on the finding that subunits of the Dsl1p, COG, GARP and exocyst complexes share limited sequence homology [1,2] that suggests convergent evolution [2]. Emerging structural information shows significant similarities between folding of key subunits within Dsl1, COG, GARP and the exocyst [3,4]. Partial structures of two COG subunits and four exocyst subunits have been reported [3,5-10]; these structures contain one (Cog2p), two (Sec15, Exo84p), three (Sec6p), or four (Exo70p) helix bundle domains. In each of the exocyst structures, the tandem helical domains form an extended, rod-like array [11]. Crystal structure of two subunits of Dsl1 complex, Tip20p and Dsl1p, resemble known subunits of the exocyst complex [4]. Recently, the X-ray structure of Cog4 establishes an unambiguous link between the COG, exocyst and Dsl1p complexes, providing strong evidence that they are derived from a common evolutionary precursor [3]. The structural similarities suggest a mechanistic similarity in the function of the DCGE tethers. Structural studies of the TRAPP1 multisubunit tethering complex [12], demonstrate that it is structurally unrelated to the DCGE family.

All members of the DCGE family have been shown to interact with SNAREs (Table 1). However, their interactions with other components of the trafficking machinery vary: interactions with known vesicular coats have been observed for Dsl1, COG and GARP, but not the exocyst. Interactions with Rabs have been observed and in some cases shown to be essential for COG, GARP and the exocyst, but not for Dsl1 [13-15]. Whether this reflects lack of knowledge or real differences in mechanisms of action of these tethers remains to be defined.

Dsl1 complex—The yeast Dsl1 trimeric complex is composed of essential subunits, Dsl1p, Tip20p and Dsl3/Sec39 (Table 1). According to the most recent model, the Dsl1p subunit lies at the center of a ternary Sec39p-Dsl1p-Tip20p complex [4]. The Dsl1 complex localizes to the ER [16]. It is stably associated with ER membranes, and this association is independent of Rab GTPases. The ER localization may be mediated, at least in part, by two independent interactions: between Tip20p and the ER SNARE protein Sec20p [16-18] and between Sec39p and the ER SNARE protein Use1 [4,16].

Dsl1 is essential for recycling traffic into the ER. The strongest evidence comes from studies of the *dsl1-22* mutation that causes defects in Golgi-to-ER retrieval of ER-resident SNARE proteins, BiP/Kar2p and integral membrane proteins harboring a C-terminal KKXX retrieval motif. Cells with defects in Dsl1 complex function show massive accumulation of COPI-coated vesicles [19]. Together, the data suggest that Dsl1 is required for fusion of recycling COPI vesicles with the ER. This function of Dsl1 is further supported by the findings that Dsl1p binds the δ -COP [20] and α -COP [21] subunits of the COPI vesicle coat *in vitro*. The δ -COP belongs to the F subcomplex of COPI, while α -COP is part of the B subcomplex [22]. Both

are involved in binding cargo during vesicle formation. Interestingly, Dsl1p binds to COPI subunits at sites identical to those involved in interactions between COPI subunits that stabilize the COPI coat [19,23]. Thus, it is possible that Dsl1 acts to tether COPI vesicles and to destabilize the COPI coat to expose proximal SNAREs and allow close apposition of membranes before fusion.

Like all DCGE tethers, the yeast Dsl1 complex interacts with SNAREs, the three ER Q-SNAREs, Ufe1, Sec20 and Use1p [16]. Dsl1 appears to promote SNARE complex stability [16], suggesting that it also may promote SNARE interactions during fusion. However, direct evidence of Dsl1 catalyzing SNARE complex formation is unavailable. There is also no evidence that Dsl1 complex directly interacts with Rab GTPases.

In mammalian cells, a Dsl1-like complex composed of ZW10, RINT-1 and NAG has been identified [24,25]. Depletion of ZW10 or RINT-1 results in central, disconnected cluster of Golgi elements and inhibition of Golgi protein recycling to the ER. No vesicle accumulation was observed at the light microscopy level. Mammalian Dsl1 complex interacts with ER-localized t-SNAREs Syntaxin-18, BNIP1 and p31 [24,25]. It is not known whether mammalian Dsl1/ZW10 complex directly interacts with Rab GTPases, but both, knock-down of Golgi-localized Rab6, or the expression of the dominant-negative GDP-restricted Rab6 suppressed Golgi disruption induced by ZW10 knockdown [26].

COG complex—COG is an octameric complex composed of Cog1-8 subunits and is found in all organisms from yeast to mammalian cells (Table 1) [27]. Based on genetic and biochemical studies [1,28-30] and images of quick-freeze/deep-etch/rotary-shadow purified COG complexes [27], the eight subunits of the COG complex are grouped into two sub-complexes: Cog 1–4 (Lobe A) and Cog 5–8 (Lobe B) that are assembled in a bi-lobed structure. Protein-protein interaction studies suggest that each lobe contains a heterotrimeric COG sub-complex (COG2–4 and COG5–7). The two lobes of the complex are bridged by the COG1/COG8 heterodimer [28-30]. Secondary structure algorithms for COG subunits suggest that COG subunits are predominantly α -helical.

Light microscopy revealed that COG complex is preferentially associated with *cis*- and *medial*-Golgi membranes [1,31,32]. Electron microscopy in mammalian cells showed Cog1 localized to the tips and rims of the Golgi cisternae as well as on vesicles [33]. Thus, COG is positioned to mediate tethering of vesicles trafficking to the Golgi and within the Golgi. The exact mechanism that restricts COG localization to Golgi cisternae is currently unknown.

The COG complex interacts genetically and physically with subunits of the COPI coat. Yeast Cog2 interacts with γ -COPI in yeast two hybrid assay [13], while antibodies to human Cog3p co-precipitate β -COPI [34]. These findings led to hypothesis that COG acts as a tether that connects COPI vesicles with *cis*-Golgi and perhaps *medial*-Golgi membranes during retrograde traffic [13]. This model is consistent with findings that *cog2* and *cog3* temperature-sensitive yeast mutants accumulate multiple vesicles at the non-permissive temperature [35]. Additional support for this function of COG comes from mammalian cells where siRNA-mediated depletion of Cog3p leads to the accumulation of vesicles carrying Golgi glycosylation enzymes, the Golgi SNAREs GS15 and GS28 and the *cis*-Golgi glycoprotein GPP130 [34]. Importantly, COG-depleted cells do not support the retrograde trafficking of Shiga and SubAB toxins [34,36], even though they are competent for anterograde trafficking of VSVG protein to the PM.

Further support for COG being involved in retrograde membrane trafficking of Golgi resident proteins is provided by data showing that mutations in COG subunits lead in humans to congenital disorders of glycosylation (CDG Type II) [3,37-45]. Mutations in COG subunits

severely alter the localization and function of Golgi glycosylation machinery [1,13,37,39, 46-48], resulting in defects in the processing of N-linked glycan chains (for review, see [49]). Such alterations in glycosylation cause type II CDGs that lead to a wide variety of neurological and developmental abnormalities (for review, see [49]).

Genetic and physical interactions have been detected between the COG complex and Golgi SNARE proteins [13,32,34,50-53]. Yeast COG complex interacts *in vitro* with the SNARE domain of the Golgi t-SNARE Sed5p and preferentially binds to the quaternary Sed5p-containing SNARE complexes. The mammalian hCog4p subunit directly binds to Syntaxin-5a, the mammalian homologue of Sed5p[32]. The same subunit is simultaneously interacting with Syntaxin-5 partner Sly1p that belongs to the Sec1/Munc18 (SM) protein family [54]. Defects in the function of mammalian COG complex lead to a significant decrease in Golgi SNARE mobility, an accumulation of uncomplexed Syntaxin-5, and a decrease in the steady-state level of intra-Golgi SNARE complexes. Together, the data suggest that the COG complex enhances formation and/or stability of intra-Golgi SNARE complexes [32]. Thus, COG complex appears to have dual functions of mediating vesicle recognition via interactions with COPI coat and directly facilitating SNARE-catalyzed fusion events. These two activities are likely to reside in different subunits of the COG complex: Cog2p binds to COPI coat, while Cog4p interacts with SNARE and SM proteins.

COG complex is an effector of multiple Golgi-localized Rab proteins. Purified yeast COG complex preferentially binds to Ypt1-GTP, and to a lesser extent to Ypt6-GTP [13]. Mammalian Cog6p binds to GTP-restricted Rab1, Rab6 and Rab41, while Cog4p preferentially interacts with Rab30-GTP [55].

Recently, a novel direct interaction between Cog2p and the coiled-coil tether p115 was uncovered and is described below [56]. This interaction was shown to be essential for Golgi ribbon structure.

1.2. GEF TETHERS INVOLVED IN ER-GOLGI TRAFFIC: TRAPPI AND TRAPP II

TRAPPI complex in yeast contains seven subunits (Table 1) [53,57-59]. Additional three subunits (trs130, trs120 and trs65; Table 1) are present in the TRAPP II complex [60]. The yeast 300 kDa TRAPPI complex has been shown to have a dumbbell shape by single-particle electron microscopy [12]. One of the lobes contains the trs20-trs31-bet3 heterotrimer, while the other lobe contains the bet3-trs33-bet5 heterotrimer. The two lobes of TRAPPI are bridged by the trs23 subunit ([12].

TRAPP complexes are different from other tethers, most of which are Rab effectors, because they uniquely act as Rab activators. In yeast, TRAPP complexes have been shown to act as guanine nucleotide exchange factors for the Rab GTPases Ypt1p and Ypt31/32p [61,62]. Initially, TRAPPI and TRAPP II have been shown to specifically act as GEFs for Ypt1p[60, 61]. The GEF activity can be reconstituted *in vitro* with only five of the seven TRAPPI subunits [12,63]. Interestingly, all these subunits are also present in TRAPP II. Subsequent studies have suggested that the addition of the TRAPP II-specific subunits (Trs120p/Trs130p) changes the substrate specificity of the GEF and allows it to activate Ypt31p/Ypt32p[64]. Further work will be needed to unequivocally define whether TRAPP II is a GEF for Ypt1p alone, Ypt31p/Ypt32p alone, or both. Mammalian TRAPP II specifically activates Rab1 [65].

Biochemical characterization of yeast TRAPPs suggests that these complexes are stably anchored to Golgi membranes [59]. TRAPPI co-fractionates with early Golgi compartments and appears to be required for fusion of ER-derived COPII vesicles with the Golgi [60]. The GEF activity is essential for TRAPPI function in membrane traffic, and in cells expressing subunits compromised in GEF activity secretion is inhibited at the non-permissive temperature

[57,59,66]. The TRAPPII subunit Trs120p localizes to the late Golgi, and TRAPPII appears to regulate intra-Golgi traffic and traffic from the early endosome to the late Golgi in vivo [60,67].

In mammalian cells, only one eight-subunit TRAPP complex could be identified according to its size [68]. Human Bet3 is mostly a cytosolic protein that is found both as a monomer and a part of a TRAPP complex [69]. The membrane bound fraction of Bet3 is localized to the transitional ER, and to some extent to endosomes [70]. This is consistent with Bet3 activity at both the ER-Golgi interface and at the late Golgi-endosomal interface. The function of mammalian Bet3 in ER-Golgi traffic was explored in semi-intact cell transport assays [69]. Staging experiments with cytosols depleted of specific components suggest that COPII>Bet3>Rab1> α -SNAP> GS28 SNARE act sequentially to facilitate ER to Golgi traffic of cargo proteins. Interfering with mBet3 function still allowed the budding of COPII vesicles, but blocked formation of pre-Golgi compartments. Thus, Bet3 is required for homotypic fusion of COPII vesicles, which is essential to maintain the structure and integrity of the VTCs and the Golgi network[70].

The key function of TRAPPI in traffic appears to be activation of Ypt1p/Rab1. However, TRAPPI might also have bona-fide tethering function. Interestingly, the Bet3 subunit of TRAPPs interacts with the Sec23 subunit of COPII [63]. COPII vesicles are formed by the initial recruitment of Sec23/Sec24 complex, followed by the recruitment of the Sec13/Sec31 complex. The binding of Bet3 to the Sec23/Sec24 “interior” layer of the coat might suggest that Bet3 (and thus the TRAPPI complex) interacts with COPII vesicles while they are forming. Alternatively, TRAPPI might bind after COPII vesicles partially uncoat. Importantly, a copy of Bet3 is present in each of the two lobes of the TRAPPI complex. Thus, a single TRAPPI complex could simultaneously bind two COPII vesicles and mediate their tethering.

Mammalian TRAPPII is enriched on COPI coated vesicles and buds, but not Golgi cisternae. The depletion of mTrs130 by shRNA leads to increased number of vesicles in the vicinity of the Golgi and the accumulation of cargo in an early Golgi compartment [65]. Mammalian TRAPPII specifically activates Rab1, and its subunits Trs120 and Trs130 co-immunoprecipitates with γ -COP, but not ϵ -COP [65]. Intriguingly, Bet3 does not bind to COP, indicating that only a sub-complex of TRAPPII interacts with the inner layer of COPI coat [65]. The binding to COPI components might mediate the association of TRAPPII with COPI vesicles and would ensure activation of Rab required for the recruitment of other tethers (such as p115 and/or COG) to mediate fusion of COPI vesicles with Golgi cisternae.

Mutation in the human homologue of the Trs20p subunit of both TRAPPs is the cause of the human X-linked disease spondyloepiphyseal dysplasia (SEDL) [71,72]. SEDL causes bone growth defects, resulting in short stature, barrel chest and degenerative joint disease [73]. It is likely that the disease results from defects in the trafficking of type II collagen through the Golgi.

1.3. COILED-COIL TETHERS INVOLVED IN ER-GOLGI TRAFFIC: P115 AND GM130

A family of proteins characterized by extensive coiled-coil domains has been shown to have tethering function. The proteins are (or are predicted to be) highly extended, making them ideal for linking membranes over a relatively large distance [74]. The current model is that coiled-coil proteins mediate long-range tethering (>300 nm) that is subsequently followed by close-range (<12 nm) recognition via the SNAREs. It is unknown how the tethered membranes are brought into close apposition, but tethers may actively participate in this process. Most coiled-coil tethers contain regions of high flexibility “hinges” between the rod-like coiled-coil domains, and it has been suggested that an accordion-like collapse of the tethers might bring the membranes close together. Such collapse or conformational changes in tether structure

could be regulated through binding to Rab GTPases, as all coiled-coil tethers have been shown to bind Rabs.

In addition to acting as membrane bridges, coiled-coil tethers have been shown to interact with SNAREs and might facilitate SNARE complex formation to promote fusion. Coiled-coil tethers also interact with coat components, perhaps to provide an initial level of recognition. By interacting with both, coats and SNAREs, coiled-coil tethers might provide increasingly stringent tethering.

P115—P115 was shown to be a myosin-like homodimer with an N-terminal globular head (~70 kDa), containing two evolutionary-conserved domains (Homology Region, HR1 and HR2), and a smaller (~30 kDa) C-terminal, extended coil-coiled domain containing four heptad repeats (CC1-CC 4) terminated by a short acidic domain [75-77]. The recent crystal structure of the N-terminal domain of p115 shows a novel architecture constructed of 10–12 armadillo-like motifs that are decorated by elongated loops and carry a C-terminal non-canonical repeat [78,79]. The terminal repeat folds into the armadillo superhelical groove and allows homodimeric association [78].

P115 is peripherally associated with multiple compartments of the ER-Golgi interface, including COPII vesicles, Vesicular Tubular Clusters (VTCs), *cis*-Golgi and COPI vesicles. The yeast homologue, Uso1p, is associated with yeast COPII vesicles. Both, p115 and Uso1p are essential for secretory trafficking [75,80-82]. Both proteins have been shown to tether COPII vesicles, either directly to the *cis*-Golgi in the case of Uso1p [83], or to other COPII vesicles and pre-Golgi intermediates in the case of p115 [84]. In addition, p115 is required for cargo traffic from pre-Golgi intermediates to the *cis*-Golgi, as shown by inhibition in traffic at the VTC stage by anti-p115 antibodies [85]. P115 also has been detected on COPI vesicles, suggesting that it participates in traffic between Golgi cisternae [80,86-88]. Precisely how p115 facilitates tethering and fusion of distinct types of vesicles to distinct membranes is unknown, but might involve associations with distinct proteins (such as GM130, giantin and COG) at distinct compartments.

P115 interacts with Rab1-GTP and this interaction mediates the recruitment of p115 to membranes [84]. It has been initially proposed that CC1 domain of p115 (residues 650–780) is primarily responsible for Rab1 binding [88], but the recent structural work indicates that the N-terminal HR1 domain of p115 (residues 21–54) is also involved [79]. The identification of the H1 domain as a binding partner for Rab1 raises the possibility that two different Rab1 binding regions may have different functions in the sequential events that direct tethering and t-SNARE assembly.

Because activated Rab1 is required for p115 recruitment to membranes, the GEF activity of TRAPPI and TRAPPII are required on COPII vesicles, VTCs, Golgi membranes and COPI vesicles to promote p115 association with membranes. Thus, a cascade of tethers is required to facilitate traffic. P115 association with membranes is extremely dynamic and p115 undergoes rapid cycles of association and dissociation that is regulated by activated Rab1 and free SNAREs [89]. Membrane association of p115 is probably also influenced by binding to GM130: GM130 binding induces a conformational change in p115 that releases the autoinhibitory folding of p115 C-terminus and allows p115 to interact with Rab1 [90]. *Drosophila* p115 has been shown to bind Rab30 (a possible homologue of mammalian Rab33b) [91], but the functional significance of this interaction is currently unknown.

P115 interacts with a number of ER-Golgi SNAREs (Table 1). P115 appears to preferentially associate with free SNAREs [89,92] and CC1 domain of p115 has been shown to facilitate SNARE-SNARE (Syntaxin5-GS28) pairing in vitro [88]. The interaction of p115 with

unassembled SNAREs might ensure that p115 is only recruited to sites that can participate in *trans*-SNARE complex formation. The function of p115 in facilitating SNARE complex formation might be regulated by Rab1, because binding of Rab1 to p115 causes rearrangements in p115 coiled-coil domains, one of which is essential for SNARE interactions [90]. In support for a fundamental role for p115 in SNARE events, “replacement” experiments in which wild-type p115 was replaced with p115 mutant lacking the SNARE-binding domain show Golgi disruption, suggesting that p115-SNARE interaction is essential [93].

P115 has been shown to bind the coiled-coil proteins GM130 and giantin. The binding site for both proteins lies within the same C-terminal domain of p115, and GM130 and giantin compete for binding to p115 [94]. The functional significance of the interactions between p115 and those two proteins is unclear. Perturbing the p115-GM130 interaction with competing peptides or antibodies inhibits ER to Golgi trafficking and Golgi assembly *in vitro* [95-99]. Similarly, expression of mutant GM130 lacking the p115 binding site, or proteolytic removal of the p115 acidic tail that mediates interaction with GM130 and giantin causes defects in protein trafficking *in vivo* [77,100-102]. However, “replacement” experiments in which wild-type p115 was replaced with p115 mutant lacking the GM130/giantin-binding domain show normal trafficking, suggesting that the p115-GM130/giantin interactions are not essential in that system [93]. A possible explanation for the latter finding is that the p115-GM130/giantin interaction is regulatory and can be bypassed by removal of the C-terminus of p115 [90]. Alternatively, the interaction between p115 and GM130/giantin may increase traffic efficiency as opposed to an absolute requirement in vesicle tethering. Studies described below suggest that GM130 is required for traffic under certain, but not all conditions.

P115 also binds the C-terminal domain (residues 613–669) of Cog2 subunit of the octomeric COG and co-precipitates with other subunits, suggesting that p115 binds the entire COG complex *in vivo* [32,56]. Replacement of wild-type p115 with p115 unable to bind Cog2 in p115-depleted cells results in alterations in Golgi architecture and delayed traffic, indicating that the p115-COG interaction is essential for the maintenance of Golgi structure, presumably by facilitating normal trafficking to the Golgi [56]. The Cog2-binding region on p115 was mapped to residues 200–247, encompassing the Homology Region 2 (HR2) domain of p115. The interaction of p115 and Cog2 is dispensable for the proper targeting of COG complex to the Golgi apparatus [56].

In addition to facilitating tethering and fusion, events that consume vesicles, p115 also may participate in vesicle formation. Usa1p is required for cargo sorting during the formation of COPII vesicles in yeast [103]. Similarly, in mammalian cells depletion of p115 results in the retention of GOS27 SNARE at ER exit sites [56]. A function in cargo selection is not unique to p115, and golgin-160 also has been shown to mediate Golgi sorting of the GLUT4 glucose transporter in adipocytes [104], and in trafficking of the 1-adrenergic receptor and the ROMK channel to the plasma membrane [105,106]. Thus, coiled-coil tethers may participate in cargo sorting during carrier formation. Such function raises the intriguing possibility that tethers may be involved in both, vesicle fusion and vesicle formation.

Additional support for a role in vesicle formation comes from the finding that p115 interacts with the guanine nucleotide exchange factor GBF1 [87]. GBF1 is a guanine nucleotide exchange factor for ARF GTPases and has been shown to be essential for COPI recruitment to membranes [87,107,108]. The p115-GBF1 interaction appears functionally significant because expression of competing peptide *in vivo* leads to Golgi disruption, possibly due to inhibition in traffic [87]. The interaction is not required for the membrane association of either p115 or GBF1. It is more likely that p115-GBF1 binding occurs after both proteins are recruited to membranes and acts to position GBF1 at sites of p115-mediated fusion. Fusion sites receive incoming membranes and need to form recycling vesicles to compensate for membrane

volume. Thus, p115 could act as a central toggle to simultaneously mediate the fusion of incoming vesicles and the formation of recycling vesicles.

Interestingly, p115 interacts with the β -COP component of COPI [109]. The interaction occurs between the conserved WF motif in the appendage domain of β -COP and the E₁₉E₂₁ residues in the Homology Region 1 (HR1) in the head domain of p115. The interaction appears important because studies in which wild-type p115 was replaced with p115 unable to bind β -COP show Golgi disruption. Thus, p115 may participate in COPI-mediated retrograde traffic within the Golgi and from the Golgi to the ER. The interaction may function as a tethering mechanism for linking COPI vesicles to acceptor membranes prior to fusion. However, it can also be proposed that p115 binds free coatamer and positions it adjacent to the site at which GBF1 has just activated ARF. Thus, p115 can be considered a platform that brings into close proximity a GEF to activate ARF (GBF1) and coatamer to promote vesicles formation. At the same time, the p115 platform is positioned at vesicle fusion sites through p115 interactions with Rab1, SNAREs and COG.

GM130—One of the most studied coiled-coil proteins is GM130 [110]. GM130 binds stably to GRASP65 that is anchored to the membrane via an N-terminal myristoyl group [111]. The GRASP65-GM130 complex localization is restricted to the *cis*-Golgi [101,112]. Although GM130 interaction with membranes is stable and GM130 is recovered exclusively with membranes during fractionation, FRAP experiments in live cells indicate that GRASP65-GM130 is extremely dynamic and undergoes rapid cycles of association and dissociation from the membrane [113]. This might be expected of a protein complex that regulates a highly dynamic trafficking process.

The function of GM130 in secretory traffic has been predominantly inferred from studies exploring its interaction with p115 (described above) and is still controversial. Addition of anti-GM130 antibodies to permeabilized cells arrests VSV-G traffic at the Golgi [97]. However, GM130 depletion studies report a delay in secretory trafficking, and this has only been observed in some [114,115] but not in other studies [116-118]. Similarly, depletion of GRASP (and thus preventing GM130 association with membranes) doesn't cause a secretory defect, suggesting either a minor or redundant function in traffic, at least under the analyzed conditions [116,119]. Depletion of GM130 in mammalian cells causes the disruption of the Golgi ribbon, but maintains correct stacking of the resulting Golgi mini-stacks [115,116]. It is possible that GM130 functions to tether lateral Golgi elements in what would be considered a homotypic fusion [116]. The same Golgi fragmentation into mini-stacks is observed in cells depleted of p115 [56,120], suggesting that lateral tethering might involve both GM130 and p115. Alternatively, GM130 might work with p115 to facilitate tethering and fusion of VTCs at the *cis*-Golgi. It can be envisioned that slowing down the rate of incorporation of new membranes at the *cis*-face of the Golgi could eventually result in the shortening of the cisterna and subsequent breaks between the fenestrated areas to generate mini-stacks [101,115]. This model would be consistent with the role of the yeast orthologue of GM130, Bug1p in trafficking at the *cis*-Golgi [121]. Thus, GM130 could have a kinetic rather than an absolute function in trafficking.

This suggestion is supported by studies on the IdIG cell line that lacks GM130. In these cells, Golgi architecture and traffic are normal at low temperatures (32–34°C), but Golgi is highly fragmented and traffic is arrested when the temperature is elevated to 39°C [122]. Thus, GM130 function can be bypassed under normal conditions but is essential under conditions that would require increased membrane flow. GM130 might function to improve efficiency of membrane traffic and is not essential in cultured cells within minimal secretory load, but might be important when trafficking rate has to be optimized.

The *Saccharomyces cerevisiae* GRASP orthologue Grh1p is also stably associated with the membrane via an acetylated amphipathic helix and binds the yeast version of GM130, Bug1p [121]. Deletion of Grh1p or Bug1p does not affect yeast cell viability or growth [123], suggesting a minor or redundant role in trafficking. However, both proteins show genetic and biochemical interactions with ER to Golgi trafficking proteins, supporting a role in ER-Golgi traffic. Grh1 interacts with the Sec23/24 component of the COPII coat [121].

GM130 binds to other proteins implicated in ER-Golgi traffic (Table 1). GM130 binds Rab1, Rab2 and Rab33b [124,125], but the functional significance of these interactions is unclear. GM130 binds syntaxin-5 and appears to directly regulate SNARE complex formation by preventing syntaxin-5 from forming complexes with GOS28 [114]. Binding of p115 to GM130 causes a conformational change in GM130, such that it no longer binds syntaxin-5. Syntaxin-5 is then able to interact with GOS28 and perhaps other SNAREs [114]. GM130 interacts with syntaxin-5 through coiled-coils 4–6, a region distinct from coiled-coil 3 involved in binding Rab1, the N-terminus involved in binding p115, and the C-terminus involved in binding GRASP65 [114]. Thus, GM130 may bind many of its partner proteins simultaneously in a nexus of tether-SNARE, tether-Rab and tether-tether interactions to promote tethering and fusion of vesicles during trafficking.

2. INTERACTIONS OF TETHERS WITH OTHER COMPONENTS OF TRAFFICKING MACHINERY

2.1. Tether interactions with Rabs

Almost all known tethers interact with Rabs (Table 1). So far, 11 Rabs have been identified in yeast, and there are more than 60 mammalian Rabs (Reviewed in [126]). Like all GTPases, Rabs cycle between an inactive GDP-bound state and an active GTP-bound form that interacts with effector proteins. Inactive cytosolic Rabs exist in a complex with a guanine nucleotide dissociation inhibitor (GDI). Rabs are prenylated and this lipid modification mediates their association with membranes. Membrane-bound Rabs are activated through a GDP/GTP exchange. Rabs have slow intrinsic GDP/GTP exchange ability and in cells this reaction is facilitated by Rab guanine nucleotide exchange factors (GEFs).

Three complexes (TRAPPI, TRAPPII and HOPS) have been shown to function as Rab GEFs. All three are first recruited to newly formed Rab-less membranes of vesicles and traffic intermediates. Their GEF activity results in membranes of vesicles and acceptor membranes that are loaded with specific GTP-bound Rab molecules, thus allowing the subsequent recruitment of other oligomeric complexes and coiled-coil tethers. Thus, the activity of GEF tethers must precede the function of other oligomeric and coiled-coil tethers that are Rab effectors. This implies that the positioning of GEF tethers on vesicular membranes is the most upstream event in tethering.

The only known tethering complex not yet found to interact with Rab proteins is the ER-localized Dsl1 complex. A possible explanation is that Dsl1 is the most ancient complex of the DCGE tethers subfamily, and is stably associated with ER membrane [21]. Because ER is not generated through continuous membrane flow, the on-off membrane cycling of this complex is not essential for its function. The other three complexes, COG, GRASP and exocyst and the coiled-coil tethers are associated with highly mobile membranes, and their interaction with membranes is transient and highly regulated.

The role of activated Rab in recruiting oligomeric and coiled-coil tethers to membranes is well documented and binding to Rab1-GTP is required for membrane association of p115 [84]. However, other coiled-coil tethers bind Rab-GTP even though their membrane association or localization doesn't depend on the interaction. The transmembrane proteins golgin-84 and

giantin bind to Rab1 [90,127,128], as does GM130, which is recruited to membranes via GRASP65, independently of Rabs [124,129,130]. Thus, it is likely that binding of tethers to activated Rabs has multiple functions, including specific capture and retention of Rab-containing membranes [91]. While tether-Rab interaction may mediate membrane association, it may also trigger conformational changes that regulate tether function.

Initial models suggested that Rabs provide specificity to compartment recognition during vesicular traffic. However, a single Rab can localize to multiple compartments of the pathway and function therein, and thus can't be the sole determinant of recognition or specificity. Ypt1p has been shown to function at multiple stages of the secretory pathway including COPII vesicle fusion, intra-Golgi traffic and endosomal trafficking [131-136]. Similarly, mammalian Rab1 localizes to COPII vesicles and is required for their fusion *in vitro* [84,130], localizes to VTCs and is required for their trafficking [85,137,138] and localizes to the early Golgi where it is required for intra-Golgi traffic [139]. Presumably, Ypt1p/Rab1 function at distinct stages of traffic by interacting with distinct effectors, including tethering proteins. Tethering may well be a combinatorial "coincidence detection" - thus binding to the Rab would work together with binding to SNAREs, other tethers and/or additional yet unknown membrane receptors to mediate the destination of a vesicle.

2.2. Tether interactions with SNAREs

Many tethers have been shown to interact with SNAREs (Table 1). This interaction might impart targeting information and ensure that only the correct vesicle containing the appropriate SNARE zip code will tether to and fuse with a given acceptor membrane. However, it is likely that tethers can actively promote *trans*-SNARE pairing *in vitro* and facilitate SNARE complex formation *in vivo*. The coiled-coil tether p115 has been shown to interact with and promote SNARE pin formation through one of its coiled-coil domains that bears limited homology to a typical SNARE motif [88]. Dsl1/ZW10, COG and GARP complexes have been shown to actively interact with step-specific SNARE complexes and are implicated in regulation of their assembly and/or stability [21,24,32,54,140]. GM130 regulates the availability of syntaxin-5 to participate in SNARE complex formation [114].

Recently, an evolutionary conservation in sequence has been detected between the MUN domain found in Munc13 and the Sec6 subunit of the exocyst and the Vps53 subunit of GARP [141]. In addition, MUN domains were also detected in Sec3 and Exo70 subunits of the exocyst, the RINT1 subunit of the Dsl1 complex and the COG4 subunit of the COG complex. The MUN domain has been shown to bind SNAREs and is postulated to have a role in promoting assembly of SNARE complexes [142,143]. Thus, it is likely that the MUN domain within the tethering factors also participate in SNARE complex assembly. It should be noted that the tethering complexes containing MUN domains (Dsl1, COG, GARP and exocyst) are evolutionarily related. In addition to directly binding to and promoting SNARE complex assembly, tethers might also influence fusion by binding to and alleviating inhibitory effects of Sec1/Munc18 family proteins [54,144].

2.3. Tether interactions with coats

Numerous tethers have been shown to interact with coat components (Table 1). The oligomeric Dsl1 and COG complexes bind COPI subunits. These interactions are likely to promote the tethering of COPI vesicles to the ER (for Dsl1) and to Golgi cisterna (for COG). The GEF tethers also bind coats: TRAPP1 binds Sec23/Sec24 components of the COPII coat [63] and promotes homotypic COPII vesicle tethering [70]. TRAPP2 binds the γ -COP subunit of COPI [65] and may tether COPI vesicles to Golgi membranes. Coiled-coil tethers also bind coats. Grh1p (yeast homologue of GRASP65) binds the Sec23p/Sec24p subcomplex of COPII coat [121], and might tether COPII vesicles to Golgi membranes in yeast. P115 has been shown to

bind β -COPI [109] and may tether COPI vesicles at VTCs and Golgi. In vitro studies suggest the existence of at least two distinct classes of Golgi-derived COPI-coated vesicle. One class, enriched in Giantin and p24/p25 proteins was efficiently bound to p115 tether, while the second class, enriched in Mannosidase I and II was preferentially interacting with CASP and golgin-84 tethers [86].

The observed interactions between tethers and coats suggest that the engagement of tethers occurs prior to vesicle uncoating. Thus, a model emerges in which tethers use multiple proof reading mechanisms to recognize incoming vesicles - tethers might first interact with coats to ensure that a vesicle of either the anterograde or retrograde type is coming, followed by tether interactions with the SNARE machinery to provide a more stringent level of recognition.

Dsl1p binds COPI subunits at sites involved in interactions that stabilize the COPI complex. Thus, Dsl1p binding may act to destabilize the COPI coat [23]. Similarly, TRAPP interacts with subunits (Sec23/Sec24) of the internal layer of COPII coat, consistent with the suggestion that it might destabilize the outer (Sec13/Sec31) layer of the coat. Thus, it is possible that tethers may perform two coat-related functions: bind coats as part of the recognition process and trigger or facilitate uncoating of vesicles to ensure unhampered SNARE pairing prior to fusion.

3. ESSENTIAL STEPS IN VESICULAR TRAFFIC

Cargo transport between compartments is mediated by carriers, and their formation and fusion have been extensively studied (reviewed in [145-147]). Cargo is first separated from resident proteins into subdomains of the donor compartment that subsequently bud off the compartment. Coats participate in cargo sorting by binding the cytoplasmic tails of cargo proteins and of cargo receptors and by assembling a lattice that deforms the membrane into a bud. All coats are cytoplasmic components that are recruited onto membrane by small GTPases of the ARF/Sar1 family. Coat recruitment is mediated through the active GTP-bound ARF/Sar1 and the GDP/GTP exchange factors that activate ARFs/Sar1 are critical components of the coating process. The GEFs define the sites of ARF/Sar1 activation, and spatially and temporally initiate budding events.

Newly formed vesicles and larger carriers are transported from the donor compartment to the acceptor compartment either by diffusion or via microtubules and motor-mediated process. Both, dynein and kinesin motors have been implicated in ER-Golgi traffic (reviewed in [148])). It has been assumed that vesicles uncoat during transport and that tethering at the acceptor membranes involves uncoated vesicles. Findings that tethers interact with coat components suggest that vesicles remain partially coated during transit and the coat may participate during tethering to confer specificity. Following tethering and uncoating, the membranes undergo fusion mediated by cognate SNAREs on the incoming vesicle and the acceptor membranes. The particulars of SNARE-mediated fusion have been extensively studied and are reviewed in [149,150]. Following fusion, membrane and components of the trafficking machinery are recycled back to the donor compartment by the same cycle of vesicle budding, transport and fusion.

To maintain a constant size of compartments despite continuous flow of membranes, cells must coordinate fusion and fission of transport intermediates. A key question is the molecular mechanism that ensures integration of anterograde and retrograde traffic. A system must be in place that coordinately regulates the rate of budding and fusion. From an engineering standpoint, an efficient means to generate such a dual proofreader is to use a single component to regulate both, budding and fusion. A dual regulatory mechanism would initiate the formation of a recycling vesicle at the same time that an incoming vesicle was fusing into the compartment. We suggest that tethering factors may function to integrate anterograde and retrograde trafficking between compartments.

4. SPECULATION: TETHERS COUPLE ANTEROGRADE AND RETROGRADE TRAFFIC

We propose that tethers from the three functional groups work together to mediate a coordinated round of anterograde and retrograde traffic. At the ER-Golgi interface, TRAPPI, Dsl1, COG, p115 and GM130 may act in a sequence of events diagramed in Figure 1. We suggest that the cycle is initiated by the interaction between TRAPPI complex and COPII vesicles. This interaction is mediated by direct binding of Bet3 subunit of TRAPPI to the Sec23 subunit of the COPII coat [63]. COPII-bound TRAPPI functions as a GEF and activates (and likely participates in loading) Rab1 GTPase on COPII vesicles. The active form of Rab1-GTP recruits its effector p115 to COPII vesicles (step 1). P115 tethers COPII vesicles to each other and directly promotes formation of trans-SNARE complexes to facilitate homotypic fusion of COPII vesicles to generate VTC. TRAPPI, Rab-GTP and p115 are also present on VTCs, and p115 also facilitates fusion between the newly generated VTCs and additional COPII vesicles (step 2).

At the same time, p115 on VTCs interacts with GBF1 and positions it at sites that have just received an input of membranes and presumably need to form recycling carriers. GBF1 activates ARF, leading to subsequent recruitment of COPI coat and the formation of COPI vesicles for recycling to the ER (step 3). The p115-GBF1 interaction also may underlie the observed exchange of COPII coats for COPI coats at VTCs [151]. COPI vesicles budded from VTCs would be recognized and tethered to the ER membrane through the Dsl1 tethering complex binding to subunits of COPI (step 4). Dsl1 also interacts with SNAREs and might directly facilitate the fusion of COPI vesicles with the ER.

The newly formed VTCs traffic towards the microtubule-organizing center (MTOC) and undergo fusions at the *cis*-Golgi (step 5). Recognition of incoming membranes to pattern on the existing Golgi might be mediated by p115 on VTCs interacting with GM130 and COG on Golgi membranes (dotted lines). The VTCs are predominantly COPI-coated. P115 has been shown to bind β -COP and may mediate tethering of COPI-coated VTCs at the *cis*-Golgi [109]. P115 also facilitates SNARE complex formation, leading to fusion. During VTC transport and fusion, p115 on VTCs membranes continues to interact with GBF1 to facilitate production of recycling COPI vesicles (step 3).

Following VTC fusions at the *cis*-Golgi, the newly formed compartment may receive COPI vesicles from more distal Golgi cisterna (step 6). This event may be mediated through TRAPPI, p115 and COG tethers. TRAPPI is required to provide activated Rab1 to recruit p115 and COG to Golgi membranes. The COPI vesicles may be tethered to Golgi membranes through interactions with COG which binds γ and/or β subunits of COPI coat (dotted lines). Binding of COPI vesicles may also be stabilized through p115 that interacts with β -COP (dotted lines). P115-mediated tethering may involve p115 bound to Golgi membranes through SNARE interactions, or through p115 bound to GM130. Both, p115 and COG directly interact with Syntaxin5 Qa-SNARE, suggesting that both could directly facilitate fusion of COPI vesicles with Golgi membranes.

Throughout the process of tethering and fusion of COPII vesicles and COPI-coated vesicles, p115 may continue to interact with GBF1 and position it at specific sites to promote formation of recycling COPI vesicles (step 7). It is very tempting to suggest that while p115 is facilitating fusion of membranes, it already programs the membranes with GBF1 to nucleate formation of recycling COPI vesicles. Interestingly, COG interacts with p115 and this may ensure that membrane fusion mediated by COG also is coupled to GBF1 and the recycling pathway.

Overall, the available data are consistent with a model in which tethers interact with members of the fusion and the budding machinery to integrate delivery of new membranes into a compartment, while at the same time initiating the removal of membrane from the same compartment. This speculative model will need further experimental evidence to test it.

5. CONCLUDING REMARKS

Models of tethering are constantly evolving. The initial model of tethers as static fibrous bridges between membranes has been expanded to include active participation in the fusion reaction. Tethers are emerging as molecular platforms that facilitate multiple events through interactions with various partner proteins. Tethers are now known to bind coats, other tethers and SNAREs to facilitate membrane recognition and membrane fusion. In addition, tethers have been shown to participate in cargo selection and at least one tether (p115) binds components of the budding machinery (GBF1). Thus, a new model can be suggested in which tethers mediate both, vesicle fusion and vesicle formation. In that model, tethers can be viewed as a mechanism to integrate forward and recycling flow of membranes by engaging the fusion machinery to ensure vesicular input, while simultaneously nucleating the budding machinery for membrane removal. Such coordinated process would ensure a balance in membrane flow through a compartment and maintain compartment size.

Many questions regarding tether function remain. Some of the key issues are: what is the relationship between tethers and coats? do tethers facilitate uncoating? how are tethers regulated to initiate uncoating at a particular stage during transport?

Some tethers are specific to a single compartment while others localize to and function at multiple compartments. How are “one love” tethers recruited to only a single membrane? How are “promiscuous” tethers regulated at different membranes? What additional components regulate their specificity to recognize the correct vesicle? Perhaps tethers that show wide localization perform a less selective role in vesicle recognition and instead function in a general process, such as facilitating SNARE complex formation.

It appears that a single step of traffic may engage numerous mechanistically distinct tethers. What is the order of molecular events at each stage of traffic? Are the functions of distinct tethers in tandem or parallel? Do tethers have multiple functions, depending on what partner they bind at a particular time and site during traffic? Why are different tethers needed at the same step of traffic? Maybe the requirement reflects different populations of transport carriers?

Oligomeric tethers exist in both assembled and partially disassembled forms. It is possible to imagine that the assembly process itself is an important step in vesicle tethering. Is the assembly/disassembly cycle important for the function of these tethers? How the assembly/disassembly cycle is controlled? What is the role of this cycle in the recycling of tethers themselves? How the interaction between different tethers is regulated?

Biochemical, genetic, molecular and structural studies are rapidly expanding our knowledge of the principles that govern membrane traffic. It is likely that many of these questions will be answered soon.

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Abbreviations

siRNA, short (or small) interfering RNA
 PM, plasma membrane
 ER, endoplasmic reticulum
 COPI, vesicle coat protein complex I
 COPII, vesicle coat protein complex II
 VTC, vesicular-tubular cluster
 GEF, guanine nucleotide exchange factor
 TRAPP, transport protein particle
 COG, conserved oligomeric Golgi complex
 GRASP, Golgi re-assembly stacking protein
 TGN, *trans*-Golgi network
 SM, Sec1/Munc18-like proteins
 ARF, ADP-ribosylation factor
 GARP, Golgi-associated retrograde protein complex

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Figure 1.

Table 1

Golgi tethering proteins and protein complexes

Tether	Components / references	Other names	mammals	coats	Small GTPases	SNAREs	others
HOPS	Pep3[152,153] Pep5[154] VAM6[155,156] Vps16[157] Vps33[158] Vps4[155,156]	Vps18, Vps18, VAM18 End1, Vam1, Vps9, Vps11, Vps11 CVT4, VPL18, VPL22, VPS39 SVL6, VAM9, VPT16 CLS14, MET27, PEP14, SLP1, VAM5, VPL25, VPT33	VPS18, hVps18[159] VPS11, hVps11[159] VPS39, hVam6, TLP[160] hVps16[159] VPS33A, VPS33B[159] hVps4[161]		Ypt7[162]	Vam7[163] Vam3[164] Nyv1[165], Vam2[162]	
TRAPP I and TRAPP II	Bet3[66] Bet5[58] Trs85[59] Trs65[59] Trs20[57] Trs23[57] Trs31[59] Trs33[57] Trs120[59] Trs130[59]	GSG1 Kre11 ? ? TRAPPC2, SEDL[166] TRAPPC4, mTrs23[68] TRAPPC5, mTrs31[68] TRAPPC6A, Trs33A, Trs33B[167] TRAPPC9, NIBP[167] TRAPPC10, mTrs130[65]	TRAPPC3, mBet3[68] TRAPPC1, mBet5[68] ? ? TRAPPC2, SEDL[166] TRAPPC4, mTrs23[68] TRAPPC5, mTrs31[68] TRAPPC6A, Trs33A, Trs33B[167] TRAPPC9, NIBP[167] TRAPPC10, mTrs130[65]	Sec23 (TRAPP1)[63], γ -COP[1], (TRAPP1)[65] Rab1[65], Ypt31/32[62]	Ypt1[61,62] Ypt6[52] Rab1[55], Rab6[55], Rab30[55], Rab41[55]		
COG	COG1[1] COG2[50] COG3[51] COG4[1] COG5[1] COG6[1] COG7[1] COG8[1]	Cod3, Sec36[52], Tti1[13] Sec35 Sec34, Grd20[31] Cod1, Sgf1, Sec38[52], Tti3[13] Cod4 Cod2, Sec37[52], Tti2[13] Cod5 Dor1	LdlB[168] LdlC[169] hSec34[170] hCod1[1] GTC-90[171] hCod2[1] hCod5[1] hDor1[1]	γ -COP[13], β -COP[34]	Ypt1[13] Ypt6[52] Rab1[55], Rab6[55], Rab30[55], Rab41[55]	Gos1/ Ykt6/Sed5[13], Sed5[32], GS28, Syntaxin 5[32,34,54]	Slv1[54] Plt1[56], GMI130[32,56]
Dsl1/ZW10	Dsl1[172] Tip20[173] Dsl3[16]	Tip1	ZW10[25] RINT-1[25] NAG[24]	δ -COP[20], α -COP[21]		Sec20/Use1 /Ufe1[174, 175], Syntaxin 18[25,176]	
Exocyst	Sec3[177] Sec5[177] Sec6[178] Sec8[178] Sec10[177] Sec15[178] Exo70[177] Exo84[179]		SEC3L1, EXOC1[180] SEC5L1, EXOC2[179] SEC6L1, EXOC3[181] SEC8L1, EXOC4[181] SEC10L1, EXOC5[182] SEC15L1, EXOC6, SEC15L2[179] EXOC7, SEC70[179] EXOC8, SEC84[179]		Sec4[15], Rho1[183], Rho3[184], Cdc42p[185], RabA[186-190]	Sec9[191]	Sec1[144]
GARP	Vps51[192,193] Vps52[194] Vps53[194] Vps54[194]	API3, VPS67, WHI6 SAC2 CGP1, LUV1, TCS3	ARE1, SAC2, SACM2L, hVPS52[195] FLJ10979, hVps53L[195] HCC8, SLP-8p, VPS54L, hVps54L[196]		Ypt6[14]	Tlg1[192,193], Syntaxin 10[197] Syntaxin6, Syntaxin16, VAMP4[140]	
CASP	[198]	COY1[198]		hSec23[198]			Golgin-84[86]
GCC88	[199,200]				Arl1[199]		

Tether	Components /references	Other names yeast	Interacting proteins related to trafficking					
			mammals	coats	Small GTPases	SNAREs	others	
GCC185	[199]		GCC2, REN53, RANBP2L4		Rab1/ Rab2/ Rab6[201], Arl1[202,203],	Syntaxin16 [204]	CLASP[205]	
GCP60	[206]		ACBD3, GOCAP1, GOLPH1				Giantin[206], Golgin16 [207], p115[96,99], Gcp60[206]	
Giantin	[99,208-210]		GOLGB1, GCP372, Macroglolgin			Rab1[90], Rab6,		p115[96,99], Gcp60[206]
GM130	[95,101,110- 112,125,130,208,211-213]	Bug1[121]	GOLGA2, Golgin-95			Rab1[129,130], Rab2, Rab33b[125]	Syntaxin5[114]	p115[95,96], Giantin, G RASP65[111], COG[32,56], ZFPL1 [214]
GMAP210	[215]	Rud3[51]	TRIP11, TRIP230, CEV14			Arl1[216,217]		
Golgin-45	[124,218]		BLZF1, JEM-1			Rab2[124]		
Golgin-84	[127,128]		GOLGA5			Rab1[127,128]		CASP[86]
Golgin-97	[219]		GOLGA1			Arl1[220] Rab6[208], Rab19, Rab30[91]		
Golgin-160	[211]	GRP1[221]	GOLGA3, MEA-2, GCP170					PIST[222]
Golgin-245	[208,223-230]	Imh1[225]	GOLGA4, p230, tGolgin-1			Arl1[231], Rab2, Rab30[91]		
GRASP55	[124,232-236]		GORASP2, GOLPH6, GRS2			Rab2[124]		Golgin- 45[124], p24[234]
GRASP65	[101,111,112, 225]	Grh1[121]	GORASP1, P65; GOLPH5		Sec23/24[121]			GM130[111], p24[234]
P115	[80- 82,84,87,88,94,95,97,237- 240]	Uso1[82]	VDP, TAP		β -COP[109]	Rab1[84/Y pt1[83], dRab30[91]	GS28, Syntaxin5[88]	Giantin[99], GM130[95], COG2[56], GBF1[87]