Role of tethering factors in secretory membrane traffic

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INTENSE INVESTIGATION over the past few decades has produced a general framework of the organelles and the factors regulating cargo transport from the endoplasmic reticulum (ER) to the plasma membrane (PM). Initial electron microscopic studies and more recent usage of light and live imaging have identified secretory compartments that handle cargo as it travels to the cell surface. All secretory and transmembrane proteins enter the pathway at the ER and are then sequentially transported through the ER-Golgi intermediate compartment (ERGIC), the Golgi complex, and the trans-Golgi network (TGN) before reaching the PM. Transport between the compartments occurs through small vesicles or larger tubulovesicular and tubular structures.

In all cases, cargo is selected from the donor compartment by being sorted into a vesicle or a larger intermediate that then detaches from the donor compartment. The separation of cargo from resident proteins is mediated by the spatially restricted recruitment of coats. All coats studied to date are recruited to membranes by the active GTP-bound forms of the Sar1/ARF family of small GTPases (reviewed in Ref. 14). The recruitment of the coat is coupled to the sorting of the cargo into a transport intermediate. The sorting of cargo for export from the ER is mediated by the coat protein complex II (COPII) and the Sar1 GTPase. The sorting of cargo for exit from the TGN is mediated by clathrin and the ARF and Arl GTPases. An additional COPI coat recruited by ARF mediates protein sorting at the ERGIC and the Golgi. Available evidence indicates that COPI mediates the recycling of cellular components and does not directly participate in cargo sorting.

After budding from the donor compartment, the transport intermediates traverse a certain distance toward the acceptor compartment and then fuse with it. Membrane fusion is mediated by a family of soluble N-ethylmaleimide-sensitive factor attachment protein receptors, or SNAREs (for review, see Ref. 22). Fusion requires the presence of cognate SNAREs in the membrane of the transport intermediate (v-SNARE) and the target (t-SNARE) compartment. The assembly of a fusogenic SNARE complex involves the formation of a long (~12 nm) parallel four-helix bundle, in which one α-helix is contributed by the v-SNARE associated with the vesicle and three α-helices are contributed by t-SNAREs on the target membrane (102,128). This complex brings the apposing membranes into close proximity and facilitates fusion (for review, see Ref. 134). Currently, >30 SNAREs (36 SNAREs in Ref. 41) have been identified, and all show compartment-specific localization (see Refs. 41 and 46 for reviews). This has led to the proposal that SNAREs are the major determinants of fusion specificity between vesicles and target membranes. However, a growing body of evidence suggests that SNAREs alone are unlikely to confer all the selectivity. SNAREs have been shown to form promiscuous complexes in vitro (23, 151), and many SNAREs interact with multiple cognate SNAREs to facilitate multiple steps of traffic in vivo (reviewed in Ref. 8). For example, the SNAREs Bet1 and syntaxin-5 facilitate two steps of traffic, the fusion of COPIII vesicles to generate later transport intermediates, and the fusion of those transport intermediates with the Golgi (112, 132, 149). It appears that in addition to SNAREs, other proteins participate in membrane-membrane pairing. Accumulating evidence points to a family of loosely related proteins, the tethering factors, as facilitators of membrane recognition before fusion. A direct role in membrane tethering has been documented for only a few members of the family. However, high level of similarity in overall structure, in biochemical properties, and in cellular behavior suggests a conserved function. Two general types of tethering factors have
been described: proteins containing extensive coiled-coil domains and large multisubunit complexes.

COILED-COIL TETHERS

Coiled-coil tethers are characterized by long stretches of heptad repeats where every seventh residue has a propensity for forming an α-helix (73). Many (if not all) coiled-coil tethers are dimers (116, 39). Structurally, they appear to be long, extended rod-like molecules (116). A large part of their appeal as possible tethering moieties lies in this structural characteristic. Long fibrous connections have been observed between vesicles and Golgi membranes and between Golgi cisternae (Fig. 1 and Refs. 58, 94). The coiled-coil proteins appear perfect for such proteinaceous bridges. The approximate length of tethers observed in electron micrographs (~50–300 nm) is consistent with the length of several confirmed and putative coiled-coil protein tethers.

Coiled-coil proteins with documented roles in membrane tethering are listed in Table 1. Their molecular features and the data supporting their function in tethering are discussed below. The triumvirate of p115, GM130, and giantin is discussed first because these proteins have largely driven the current models of membrane tethering.

p115 is a parallel homodimer with two globular heads and a long tail composed of four coiled-coil domains (116). The overall structure is reminiscent of myosin II. The four coiled-coil regions within the tail are separated by proline-rich “hinge” regions that may facilitate rotation relative to the polypeptide backbone. The overall length of p115 (~55 nm) is perfect for a tether, and the presence of the hinges has been proposed to facilitate an “accordion-like” collapse of the tether to bring the vesicle and acceptor membranes into proximity (150). p115 localizes to ER exit sites, the ERGIC and the Golgi, suggesting a possible function at these traffic stations (90, 143). p115 homologs are found in all eukaryotes examined, including yeast (see below), attesting to its fundamental role in membrane homeostasis.

In vivo studies document requirement for p115 at an earlier pre-Golgi stage (2). Inactivating p115 by microinjecting anti-p115 antibodies or depleting p115 from cells prevents ER-Golgi traffic of VSV-G at ER exit sites (2, 105, 106). This finding suggests that p115 might be necessary for the fusion of ER-derived COPII vesicles to generate later transport intermediates. This is strongly supported by the presence of p115 on...

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**Table 1. Golgi-localized coiled-coil proteins with confirmed role in membrane traffic**

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular Weight of Human Protein, kDa</th>
<th>Yeast</th>
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<td>80</td>
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<td>CCAAT displacement protein isoform C</td>
<td>TM</td>
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<tr>
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<td></td>
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<td>CEV14, Trip11, Trip230</td>
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CASP, CDP alternatively spliced product; GMAP, Golgi microtubule-associated protein; GRAB, GRIP-like ADP ribosylation factor-binding domain.
COPII vesicles generated in vitro and by direct evidence that p115 facilitates binding of such vesicles to Golgi membranes (1, 4). The putative site of p115 action in membrane traffic is diagrammed in Fig. 2.

Equally strong evidence for p115 function in membrane tethers comes from studies on its yeast homolog, Uso1p (yUSOu means transport in Japanese). Uso1p was identified as an ER-Golgi transport factor because a temperature-sensitive mutant, uso1-1, blocks the traffic of the main yeast secretory protein invertase to the Golgi (87). Subsequent in vitro studies showed that Uso1p is required for tethering COPII vesicles to Golgi membranes (9). The tethering was proposed to be SNARE independent because normal levels of tethering were observed when SNARE function was inhibited by adding inhibitory anti-SNARE antibodies or by using inactive SNARE mutants (16). However, in both cases, SNARE proteins were present and could theoretically participate in Uso1p-mediated events, despite their inability to catalyze fusion (see below). p115 has been shown to bind the Golgi proteins GM130 and giantin (59, 63, 88).

GM130 is an extended rod-like protein with 6 coiled-coil domains (89). GM130 has an NH2-terminal basic domain of 75 amino acids, which binds to the COOH-terminal acidic patch of p115 (88). GM130 homologs are found in lower eukaryotes, such as Caenorhabditis elegans and Drosophila melanogaster. There is no yeast homolog of GM130, although it has been proposed that the extended tail of Uso1p might be functionally analogous to GM130. GM130 localizes to the cis-Golgi cisternae (89), suggesting that it functions therein. GM130 is tightly bound to Golgi membranes by interacting with the Golgi reassembly stacking protein of 65 kDa (GRASP65) (10).

The requirement for GM130 in traffic has been documented in vivo. The addition of anti-GM130 antibodies to semi-intact cells arrests VSV-G traffic at the Golgi (4). A direct role in tethering is suggested by in vitro studies showing that GM130 is required for COPII vesicle targeting/fusion with the cis-Golgi (83).

Giantin is an extended rod-like type II Golgi membrane protein, with most of its mass projecting into the cytoplasm (62). A COOH-terminal sequence (residues 3059 –3161) adjacent to the transmembrane domain is required for giantin localization to the Golgi (80). Giantin is found only in mammalian species, suggesting a more specialized cellular function acquired during evolution. Giantin localizes to the medial-Golgi, predominantly to cisternal rims of the medial-Golgi (62). The requirement for giantin in secretory traffic has been documented in vivo. The addition of anti-giantin antibodies to semi-intact cells arrests VSV-G traffic at the Golgi (4). Furthermore, a short NH2-terminal fragment of Giantin shown to bind p115 in vitro and in vivo blocks cell-free Golgi reassembly (59).

Giantin is found in in vitro-generated COPII vesicles and has been postulated to tether such vesicles during their recycling from distal to proximal Golgi cisterna. This is supported by the finding that pretreatment of COPII vesicles with anti-giantin antibodies inhibits the docking of these vesicles to Golgi membranes (126).

BRIDGE MODEL OF TETHER FUNCTION

p115 binds GM130 and giantin, and these interactions underlie current models of membrane tethering. A bipartite tether involving p115 and GM130 has been proposed to mediate the tethering of COPII vesicles to cis-Golgi membranes. Since p115 resides on COPII vesicles and GM130 marks the cis-Golgi, their interaction could link COPII vesicles to Golgi elements and facilitate their fusion with the target membrane.
Despite its appeal, recent findings have raised concerns about this model. First, the Krieger laboratory has isolated temperature-sensitive mutant Chinese hamster ovary cells that are defective in the trafficking of the low-density lipoprotein receptor at the nonpermissive temperature of 39.5°C (IdLG cells) (53). Characterization of IdLG cells shows that they lack detectable GM130. When grown at the normal temperature of 37°C, such GM130-compromised cells support traffic of VSV-G to the PM and secrete normally. In addition, the ultrastructure of the Golgi apparatus examined by immunofluorescence and immunoelectron microscopy appears normal. These findings suggest that at normal temperature, removal of GM130 does not compromise traffic. However, GM130 function becomes essential at higher temperatures because incubation at 39.5°C for 12 h causes disassembly of the Golgi into dispersed vesicles. It is possible that GM130 (or a GM130-dependent protein) plays a role in maintaining Golgi structure at higher temperatures (139). Second, the Linstedt laboratory used short interfering RNA (siRNA), coupled with microinjection of plasmid DNA, to reduce the level of endogenous p115 and at the same time express p115 unable to bind GM130 in the same cell (105). The mutant p115 does not bind GM130 and is unlikely to participate in p115-GM130 tethering. Despite this, the cells transport VSV-G to the surface and have normal Golgi structure. Third, the depletion of the Drosophila homolog of GM130 does not perturb the morphology of the secretory compartments or inhibit secretory traffic (56). Together, these findings are inconsistent with an absolute requirement for p115-GM130 tethering in membrane traffic and suggest that the p115-GM130 interaction may have other cellular functions.

In addition to the p115-GM130 tethering of COPII vesicles, a tripartite tether involving p115, GM130, and giantin has been proposed to mediate the tethering of recycling COPI vesicles. The model postulates that giantin in COPI vesicles binds p115, which then binds GM130 on the acceptor cis-Golgi membrane to create a molecular “bridge” (126). This model is indirectly supported by the finding that pretreatment of COPI vesicles with anti-giantin antibodies inhibits both the binding of p115 and the docking of these vesicles to Golgi membranes. Furthermore, a peptide analogous to the NH₂-terminal p115-binding domain of GM130 (and shown to bind p115 in vitro and in vivo and thus likely to prevent p115-GM130 interaction) inhibits docking of COPI vesicles to Golgi membranes when microinjected into cells (126). Further support for the GM130-p115-giantin tether in COPI vesicle tethering comes from experiments in which a GM130 mutant lacking the NH₂-terminal p115-binding domain is overexpressed in cells. Such mutant is unable to form the p115 bridge. Electron microscopic analysis of transfected cells shows more COPI-sized vesicles in the Golgi region, suggesting that COPI vesicles are unable to tether and fuse (59).

However, the bridging model for GM130-p115-giantin tethering of COPI vesicles also has grown less seductive. First, all of the concerns raised above for the p115-GM130 tether are equally applicable to the bridging model. For example, the finding that p115 mutant lacking the GM130 and the giantin-binding domain supports traffic is not consistent with a requirement for bridge formation (105, 106). In addition, the bridge model requires that p115 binds to both GM130 and giantin at the same time. Surprisingly, the GM130 and the giantin binding sites in p115 map to the same COOH-terminal acidic domain, and the proteins compete for p115 (63). Finally, giantin appears to be present only in mammalian cells, arguing against a conserved role in membrane tethering. Together, the results suggest that the interactions of p115 with GM130 and giantin may be sequential, rather than simultaneous, and that they may facilitate events other than vesicle tethering.

**OTHER COILED-COIL PROTEINS INVOLVED IN MEMBRANE TRAFFIC**

In addition to p115, GM130, and giantin, other Golgi-localized coiled-coil proteins have been implicated in membrane traffic. Although all regulate a key aspect of membrane homeostasis, the exact mechanisms of their action may vary.

CCAAT-displacement protein (CDP) alternatively spliced product (CASP) was originally identified as an alternatively spliced product of the **CUTL1** gene that codes for the nuclear CDP (60, 91, 93). CASP lacks the DNA-binding motifs of CDP and localizes to the Golgi (30). Like giantin and golgin-84, CASP is a type II transmembrane protein with a large coiled-coil cytoplasmic domain. CASP appears to be a dimer linked by disulfide bonds within the ER lumen.

A possible role of CASP in traffic was inferred from studies of its yeast homolog. CASP in yeast (**COY1**, **COY1** is not essential but shows genetic interactions with **Gos1p** and Sec22p, two SNAREs implicated in ER-Golgi traffic (30). Gos1p has also been implicated in intra-Golgi traffic. Subsequent studies in mammalian cells showed that microinjection of soluble CASP inhibits retrograde traffic within the Golgi (77). The exact molecular events regulated by CASP during traffic remain to be defined.

GCP60 (also known as golgin-60) was identified as an interactor of giantin (125). GCP60 is an extended coiled-coil protein localized to the Golgi ribbon and to adjacent vesicles. The giantin-binding domain is essential for GCP60 localization, and GCP60 without this domain does not target to the Golgi. GCP60 is likely to participate in ER-Golgi traffic or in the maintenance of Golgi structure because its overexpression inhibits ER to Golgi traffic and disrupts Golgi architecture. The exact function of GCP60 and its role in tethering remain to be elucidated.

Golgil microtubule-associated protein of 210 kDa (**GMAP210**) is a human **cis**-Golgi protein (45). **GMAP210** is involved in membrane traffic because overexpression of **GMAP210** blocks anterograde transport of both a soluble form of alkaline phosphatase and the transmembrane hemagglutinin of influenza virus at a pre-**cis**-Golgi stage (101). Overexpression of **GMAP210** also inhibits the retrograde transport of Shiga toxin B-subunit at a stage between the Golgi and the ER. Whether the effect on retrograde traffic is a consequence of disrupted anterograde traffic, or vice versa is currently unknown. In cells expressing high levels of **GMAP210**, the Golgi is disrupted into ~10–20 clusters composed of hundreds of small 50-nm vesicles. The data are consistent with a possible tethering function for **GMAP210** because increased levels of **GMAP210** may lead to massive accumulation of tethered vesicles and prevent their fusion.

A function in membrane events is also suggested by the yeast homologue of **GMAP210**, **RUD3**. Like **GMAP210**, **RUD3**...
Rud3p contains extensive coiled-coil regions and localizes to the yeast cis-Golgi compartment containing Och1p (136). Rud3p-depleted cells secrete underglycosylated invertase, suggesting a disturbance in Golgi function (51, 136). The exact molecular mechanism of Rud3p action is unknown. Interestingly, RUD3 shows genetic interactions with USO1 because it was identified in a genetic screen for multicopy suppressors of a mutation in USO1 (137). Whether Rud3p and Usolp interact physically and what the functional significance of such interaction may be remains to be explored.

Golgin-45 is a coiled-coil Golgi protein shown to interact with GRASP55 via a COOH-terminal sequence (119). Golgin-45 appears to be important in membrane traffic because overexpression of golgin-45 causes disruption of the Golgi, with Golgi components localizing to punctate structures dispersed throughout the cell. Equally telling, depletion of golgin-45 by siRNA causes Golgi disruption and arrests VSV-G traffic. Interestingly, golgin-45 depletion causes a BFA-like phenotype, with Golgi enzymes relocating to the ER, Golgi matrix proteins localizing to punctate structures dispersed throughout the cell, and the cargo protein VSV-G arresting at the ER. The data are compatible with a role for golgin-45 in COP II vesicle tethering. However, the exact function of golgin-45 in traffic remains to be defined.

Golgin-84 is a coiled-coil integral membrane protein with a single transmembrane domain close to its COOH terminus (13). The transmembrane region of Golgin-84 is similar to that of giantin and CASP. Cross-linking indicates that golgin-84 forms dimers. Golgin-84 localizes to the cis-Golgi network and is enriched on tubules emanating from the lateral edges of, and often connecting, Golgi stacks (117). A tethering/stacking function for golgin-84 is suggested by the finding that overexpression or depletion of golgin-84 results in fragmentation of the Golgi ribbon (21). The exact mechanism of golgin-84-mediated tethering is unknown but is likely to involve intra-Golgi recycling (77).

Golgin-97 is a coiled-coil protein associated with the trans-Golgi network (TGN) (20). Golgin-97 appears to be involved in membrane traffic from the endosome to the TGN. This is suggested by experiments from the Hong laboratory that showed a requirement for functional golgin-97 in traffic of Shiga toxin B fragment (internalized from the PM) from endosomes to the TGN (71). Shiga toxin B fragment is arrested in traffic in semi-intact cells supplemented with anti-golgin-97 antibodies or depleted of golgin-97 and in intact cells micro-injected with antibodies or depleted of golgin-97 by siRNA (71). Staging experiments show that golgin-97 is required at a stage means, either as interactors with known tethering molecules or as proteins with conserved coiled-coil structure and localization to either the ERGIC or the Golgi. They are listed in Table 2. A possible role of these proteins in membrane traffic is suggested by their structure and localization, but it remains to be experimentally explored.

### Multisubunit Tethering Complexes

In addition to coiled-coil proteins, multisubunit complexes have been shown to regulate distinct stages of membrane traffic. Like coiled-coil tethers, all show interactions with SNAREs and small GTPases, suggesting a conserved mechanism of action (see below).

Conserved oligomeric Golgi (COG) complex consists of eight subunits (Table 3) (15, 53, 66, 129, 130, 135, 144). A

Table 2. Golgi-localized coiled-coil proteins that may play a role in membrane tethering

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecular Weight of Human Protein, kDa</th>
<th>Yeast</th>
<th>Mammals</th>
<th>Specialized Domains</th>
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<tbody>
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Golgin-245 is indirectly involved in facilitating the motility of Golgi elements toward the microtubule organizing center (MTOC). Golgin-245 does not appear to be critical for anterograde traffic, because VSV-G protein moves from the ER through the dispersed Golgi ministacks to the PM with kinetics analogous to those in control cells (154). The molecular mechanisms of golgin-245 action remain to be investigated.

Golgi reassembly stacking proteins of 55 and 65 kDa (GRASP55 and GRASP65), respectively, are highly homologous coiled-coil proteins shown to participate in stacking of Golgi cisterna in an in vitro assay that reconstitutes Golgi reassembly after mitosis (107). GRASP65 is acylated and is stably associated with cis-Golgi membranes (11). GRASP65 has been implicated in the stacking of cis- and medial-cisterna because of the antibodies to GRASP65 and a truncated GRASP65 block cisternal stacking (11). GRASP65 and GM130 are tightly bound in detergent extracts of Golgi membranes, suggesting that GRASP65 may be the membrane “receptor” for GM130. The GRASP65-GM130 complex can bind p115, suggesting that in addition to cisternal stacking, GRASP65 participates in tethering COP II vesicles (containing p115) to cis-Golgi (10).

GRASP55 is myristoylated and palmitoylated (57) and localizes to the medial-Golgi (122). Similarly to GRASP65, recombinant GRASP55 and anti-GRASP55 antibodies block the stacking of Golgi cisterna (122). Together, the results suggest that GRASP55 and GRASP65 may function in sequential (cis to medial) stages of Golgi stacking. The exact mechanism of their action is unknown.

Additional coiled-coil proteins have been identified by indirect means, either as interactors with known tethering molecules or as proteins with conserved coiled-coil structure and localization to either the ERGIC or the Golgi. They are listed in Table 2. A possible role of these proteins in membrane traffic is suggested by their structure and localization, but it remains to be experimentally explored.
possible involvement in membrane trafficking was initially suggested by studies in yeast showing that COG-interacting genes encode proteins known to function in trafficking (129, 136, 137). Specifically, the COG complex interacts physically and genetically with Ypt1p, intra-Golgi SNAREs, and the COPI coat. These findings led to hypothesis that the COG complex acts as a tether that connects COPI vesicles with cis-Golgi membranes during retrograde traffic (129). This model is consistent with findings that cog2 and cog3 temperature-sensitive yeast mutants accumulate vesicles at the non-permissive temperature (148).

A function in intra-Golgi tethering is even more strongly suggested by findings from the Lupashin laboratory. siRNA-mediated depletion of Cog3p in HeLa cells (Cog3p depletion is accompanied by reduction in Cog1, Cog2, and Cog4) leads to the accumulation of vesicles carrying the Golgi SNAREs GS15 and GS28 and the cis-Golgi glycoprotein GPP130 (156). A prolonged depletion of the COG complex results in extensive fragmentation of the Golgi ribbon. However, the fragmented Golgi membranes maintain their juxtanuclear localization and cisternal organization and are competent for anterograde trafficking of VSVG protein to the PM. In contrast, Cog3 depletion inhibits the retrograde trafficking of the Shiga toxin. Together, the data suggest a function for COG complex in intra-Golgi recycling of COPI vesicles. This is further supported by the finding that the mammalian COG complex physically interacts with COPI (156).

The COG complex seems to be a jack-of-many trades: in addition to its intra-Golgi function, the yeast COG has been proposed to be involved in cargo sorting during exit from the ER (81), to function as a vesicle tether in anterograde ER-to-Golgi traffic (136, 137, 148), and to be required for proper localization of yeast enzymes in the TGN (127). Whether all these functions involve tethering or whether distinct subunits of the octomer may have additional functions remains to be explored.

Ultrastructural examination of purified COG complexes shows dumbbell-like structure of two globular subcomplexes connected by a short extension (135, 144) (Fig. 3). This may reflect a function of the subcomplexes to “mark” either the vesicular or the target membrane. The pairing of the subcomplexes to form the holocomplex would ensure correct membrane tethering.

The COG complex appears to be structurally related to other multisubunit tethering complexes (144). Iterative searches of databases using the NH2-terminal domains of several COG components reveal similarities in the NH2-terminal domains of

Table 3. Multisubunit tethering complexes

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<th>Mammals</th>
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COG, conserved oligomeric Golgi complex; GARP, Golgi-associated retrograde protein complex; TRAPP, transport protein particle. *Asterisks mark additional TRAPP II components.
DSL1 can be suppressed by expression of Sec21p (the Tip20p may function in retrograde traffic to the ER. SNARE Ufe1p. These findings suggest that Dsl1p, Sec20p, and Golgi-to-ER traffic are ER-localized and bind to the ER t-

Fig. 3. Subunit organization of multimeric tethers. The COG complex is composed of two subcomplexes of four subunits each. The Dsl1 complex is a trimer. The exocyst assembles from two subcomplexes: a trimeric complex of Sec15p, Sec10p, and Exo84p on vesicles and a pentameric complex of Exo70p Sec5p, Sec6p, Sec3p, and Sec8p on the PM. The GARP complex is tetrameric. The TRAPP I complex is composed of 7 subunits. Three additional subunits are detected in the TRAPP II complex. The COG, exocyst, and GARP complexes are drawn in similar shapes to stress their structural and sequence similarities. The names of the subunits are those in yeast.

components of the exocyst and the Golgi-associated retrograde protein (GARP) complex. It seems likely that the COG, the exocyst, and the GARP complexes are distantly related assemblies evolved to tether membranes at distinct stages of the secretory pathway. Dsl1 encodes an essential yeast protein localized to the ER (138). Dsp1p coisolates from solubilized membranes with Tip20p and Sec20p (108), forming the Dsl1 complex (Table 3 and Fig. 3). Both Tip20p and Sec20p function in retrograde Golgi-to-ER traffic are ER-localized and bind to the ER t-SNARE Ufe1p. These findings suggest that Dsl1p, Sec20p, and Tip20p may function in retrograde traffic to the ER.

The inviability of strains bearing several mutant alleles of DSL1 can be suppressed by expression of Sec21p (the γ-subunit of the COPI coat complex), suggesting that Dsl1p functions primarily in COPI-mediated retrograde traffic from the Golgi to the ER (138). In support, the dsl1–22 mutation causes defects in Golgi-to-ER retrieval of ER resident SNARE proteins and integral membrane proteins harboring a COOH-terminal KKXX retrieval motif. The dsl1–22 mutation also inhibits the recycling of BiP/Kar2p by the HDEL receptor Erd2p. Furthermore, Dsl1p binds the COPI vesicle coat in vitro (5).

A Dsl1-like complex composed of ZW10, RINT-1, and p31 has been identified in mammalian cells and interacts with syntaxin 18, an ER-localized t-SNARE implicated in membrane trafficking (40). The exocyst complex consists of eight components: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (Table 3 and Fig. 3). All of the subunits are hydrophilic proteins that form a 19.5S complex peripherally associated with the plasma membrane (131). Exocyst subunits are localized to sites of active exocytosis: the bud tip at the beginning of the cell cycle and the mother-daughter cell connection during cytokinesis (24). The Sec3p component of the exocyst is associated with the PM and has been proposed to act as a landmark for incoming vesicles. The incoming vesicles are “marked” by the Sec15p component of the exocyst. The formation of the holoxocyst by linking vesicular and PM exocyst subcomplexes leads to tethering of the vesicles to the PM.

Mammalian homologs of all eight yeast exocyst proteins have been identified (44). Purification of the mammalian exocyst (also called Sec6/8 complex) reveals a combined molecular weight of 743 kDa. The intracellular localization of the exocyst in mammalian cells appears to be influenced by the polarity and the secretory status of the cell. In nonpolarized Madin-Darby canine kidney epithelial cells, the exocyst is mostly cytosolic (33). During Ca2+ -dependent cell-cell adhesion, ~70% of the exocyst is rapidly recruited to sites of cell-cell contact (33).

The exocyst complex appears to function preferentially in delivery to the basolateral PM adjacent to cell-cell junctions. In streptolysin-O-permeabilized Madin-Darby canine kidney cells, anti-Sec8 antibodies inhibit delivery of low-density lipoprotein receptor to the basolateral membrane, but not of p75NTR to the apical membrane (33). The link between the exocyst and cell adhesion machinery is confirmed by the finding that in polarized cells, the exocyst fractionates in a high molecular mass complex with tight-junction proteins and a portion of E-cadherin and coimmunoprecipitates with cell surface-labeled E-cadherin and nectin-2α. Recruitment of Sec6/8 complex to cell-cell contacts can be achieved in fibroblasts when E-cadherin and nectin-2α are coexpressed (152). Similarly, in cultured hippocampal neurons, the exocyst is present in regions of ongoing membrane addition: the tips of growing neurites, filopodia, and growth cones. Similarly to nonneuronal cells, the exocyst appears to be preferentially involved in the PM trafficking of vesicles carrying cargo required for membrane growth, but does not facilitate tethering of synaptic vesicles (85, 86).

Although it is clear that the exocyst plays a central role in exocytosis, little is known about how it is controlled. Given the complexity of the exocyst, one might imagine that it integrates many different inputs. Indeed, recent data (64, 65) identified the exocyst as an effector for five small GTPases, including Sec4, Rho1, Rho3, Cdc42, and, more recently, RapA. It appears that GTPases regulate exocyst assembly by regulating positioning of subunits on the vesicle and on the PM. Specifically, Sec15p associates with secretory vesicles by interacting with the GTP-bound form of the Rab GTPase Sec4p (34). Similarly, Sec3p is recruited to the PM by interacting with the GTP-bound form of Rho1. Rho1 regulates actin dynamics, and the close relationship between actin remodeling and exocytosis is further underscored by the finding that Sec3p also interacts with the GTP-bound form of Cdc42. Other components of the exocyst are also targeted to membranes by GTPases. For example, Sec5p and Exo84 are direct targets for activated Ral.
GTPases (25, 82). The emerging model posits that mammalian exocyst components are present as distinct subcomplexes on vesicles and the plasma membrane and that multiple GTPases regulate the assembly interface of a full octameric exocyst complex through interaction with various components. In this manner, the assembly of the exocyst may integrate various cellular signaling pathways to ensure that exocytosis is tightly controlled (155).

Golgi-associated retrograde protein (GARP) complex is composed of Vps51p, Vps52p, Vps53p, and Vps54p (Table 3 and Fig. 3). GARP localizes to late Golgi/TGN (19). Mutations in VPS52, VPS53, or VPS54 do not affect protein traffic through the early part of the Golgi complex, but results in the mislocalization of late Golgi membrane proteins to the vacuole and the secretion of the vacuolar carboxypeptidase Y (19). These defects are consistent with GARP acting in the retrograde trafficking of vesicles from the late endosomal/prevacuolar compartment back to the Golgi/TGN. Defects in VPS52 and VPS54 also inhibit the retrograde traffic of the v-SNARE 

• ROLE OF TETHERING FACTORS

In contrast to TRAPP I, the TRAPP II complex is proposed to mediate intra-Golgi trafficking (112). Mutations in the TRAPP II-specific YPT31 or YPT32, in agreement with the known role of TRAP II as a GEF for the trans-Golgi localized Ypt31/32 (49). Thus each TRAPP complex appears to activate a GTPase acting at a stage of traffic that the TRAPP complex facilitates.

Mammalian TRAPP I homologs have been identified (113) and appear to facilitate the same ER-Golgi stage of secretory traffic. The function of mammalian Bet3 in ER-Golgi traffic was explored in semi-intact cell transport assays (67). 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. The placement of Bet3 (and thereby of TRAPP I complex) upstream of Rab1 is consistent with TRAPP I function as a GEF for Rab1.

RECRUITMENT OF TETHERS TO SPECIFIC MEMBRANES

If tethers provide specificity to membrane fusion, they themselves must be correctly positioned at the cognate membrane. How is this accomplished? Current models suggest that tethers are recruited to membranes by members of the Ras-related GTPases. GTPases proposed/shown to mediate membrane recruitment of a specific tether are listed in Table 4. The participation of Rho, Cdc42, and Rab GTPases appears specific for the exocyst. In contrast, members of the Rab and the ARF/Arl families appear to regulate numerous tethers.

Rab-Mediated Recruitment

Rab GTPases show compartment specificity and are logical candidates for recruiting specific tethers to specific membranes. These GTPases themselves cycle between membrane-bound and cytosolic states in a nucleotide-dependent manner. Membrane-associated, GTP-bound (active) Rabs are able to recruit specific tethering factors to the membrane. p115 binding to membranes is maximal in the presence of the active GTP-bound form of Rab1 (1). The association of Uso1p (yeast p115 homolog) with membranes is also maximal in the presence of active GTP-bound form of Ypt1p (16). However, Rabs are unlikely to act alone to recruit tethers to the membrane. This is suggested by the findings that p115 localizes to membranes in cells expressing a dominant negative inactive Rab1 mutant (3) and that Uso1p can still be recovered with membranes in yeast strains depleted of Ypt1p (7).

It is likely that interactions between tethers and Rabs may relate to functions other than tether recruitment to membranes. This is strongly supported by the finding that the transmembrane golgin-84 and the GRASP65-anchored GM130 bind Rab1, although both proteins are membrane-associated independently of Rab1 (117). It is possible that interactions with Rabs do not recruit tethers, but instead increase the fidelity of tethering by requiring multiple molecular interactions before fusion. Another possibility is that Rabs may regulate the accessibility of tether interactions with SNAREs (see below).

Arl-Mediated Recruitment

ARF-like (Arl) proteins share some but not all characteristics of ARFs. For example, like ARFs, many Arls are myristoylated, but Arls do not mediate ADP-ribosylation of cholera toxin and do not activate phospholipase D (69, 98). The Arl1...
and Arl3 members of the 10-member Arl family have been characterized, and both appear to be involved in trafficking events at the TGN. Active GTP-bound forms of Arls have been shown to facilitate membrane recruitment of proteins containing the GRIP domain (70, 72). The GRIP domain has been identified in golgin-97, golgin-254, GCC88, and GCC185 (54, 72, 84) (see Tables 1 and 2).

GRIP domains lie within the COOH terminus of the respective proteins and are ~45 amino acids long and poorly conserved, except for an invariant tyrosine residue at position 4, followed eight residues later by a phenylalanine or a tyrosine (Fig. 4) (54, 84). GRIP domain proteins have been found in all eukaryotes examined to date, suggesting an essential function. The GRIP domain is required and sufficient for Golgi targeting. Golgi-245 lacking the COOH terminus is not targeted to the Golgi (55), and the GRIP domain of golgin-245 is able to target GFP to the TGN of the parasite *Leishmania mexicana* (79).

Structural studies of the GRIP domain of golgin-245 in complex with Arl1-GTP show that the GRIP domain forms a homodimer that binds two Arl1-GTPs (96, 146). This suggests that the COOH termini of golgins are immobilized at the membrane by Arl1, whereas the majority of the protein either extends from the membrane or aligns on its surface. The bivalent nature of the interactions between the GRIP motif and Arl1 may provide additional control of the location and duration of golgin residency at the membrane, and hence its function.

Studies in yeast have shown that Arl1 is recruited to membranes by the active form of Arl3 (118), suggesting that a cascade of Arls may be required for membrane association of GRIP-containing golgins. This is underscored by the finding that yeast Imh1p (golgin-245 homolog) associates with membranes by a mechanism requiring Arl3p-GTP and Arl1p-GTP (118). A similar mechanism may also operate in mammalian cells because membrane association of human golgin-97 expressed in yeast also requires activated Arl3p and Arl1p (118).

**ARF-Mediated Recruitment**

Active ARF appears to mediate membrane recruitment of GMAP210. Membrane association is mediated by a GRIP-related Arf-binding (GRAB) domain at the COOH terminus of GMAP210 (31). The GRAB domain is related to the GRIP domain, but the conserved tyrosine residue found at position 4 in all GRIP domains is replaced by a leucine residue (Fig. 4). This leucine is essential for GRAB domain function because mutations lead to loss of membrane targeting of the yeast homolog of GMAP210, Rud3p (31). GMAP210 localizes to the cis-Golgi, whereas all known GRIP-domain proteins associate with the TGN.

**DIRECT MEMBRANE INTERACTION?**

The nature of the TRAPP receptor(s) remains to be determined, but recent structural studies provide insight into the mechanism for TRAPP association with membranes. The crystal structure of mouse Bet3 reveals a dimeric structure with hydrophobic channels (52, 133). The channel entrances are located on a putative membrane-interacting surface that is...
distinctively flat, wide, and decorated with positively charged residues. Both a channel-blocking mutation and a charge-inversion mutation on the flat surface of the yeast Bet3p lead to conditional lethality, incorrect localization, and membrane trafficking defects. These data suggest a molecular mechanism for Golgi targeting and anchoring of Bet3 that involves the charged surface and insertion of Golgi-specific hydrophobic moieties into the channels. The essential Bet3 subunit could then direct other TRAPP components to the Golgi. The stable association of the TRAPPs with Golgi membranes has been proposed to mark these membranes for incoming COPII and COPI vesicles (112).

**MEMBRANE DYNAMICS OF TETHERS**

Photobleaching-based approaches to track the behavior of GFP-tagged tethers indicate that at least some of them undergo cycles of association and dissociation from the membrane. GRASP-GFP cycles between the Golgi membrane and the cytosol with kinetics analogous to those of eCOP-GFP (142). The t1/2 for membrane cycling of eCOP is ~20 s (104), suggesting that GRASP65 cycles with similar kinetics. Because GRASP65 appears to be the membrane receptor for GM130, it is likely that GM130 also rapidly cycles on and off the membrane. Another tether that undergoes rapid (t1/2 < 10 s) cycling is p115. The continuous association/dissociation of tethers may provide rapid cycles of proofreading that ensure correct membrane pairing. Whether other tethers also rapidly cycle is under investigation. Not all tethers undergo cycling: for example, the TRAPP complex (12) and a subset of the exocyst (34) appear to be tightly bound to membranes. How such complexes remain at their spatial coordinates despite continuous membrane flow remains to be explored.

**WHAT DO TETHERS DO?**

**Bridging of Membranes**

The initial model, based largely on the morphological observations of long tethers between vesicles and the Golgi (Fig. 1), regards coiled-coil tethers as structural bridges spanning two membranes. In this model (see Fig. 5, top), a tether on the vesicle recognizes a cognate tether on the target membrane and forms a molecular link that holds the apposing membrane together to facilitate pairing of the v- and t-SNAREs. The tethers may remain bound while the trans-SNARE complex forms and fusion occurs, or they may dissociate before fusion. In both cases, the tethers initiate membrane connection but do not participate in subsequent SNARE events. Tethers show compartment-specific localization, and each tether could provide specificity to the bridging reaction and impose membrane selectivity before SNARE engagement.

The multisubunit complexes can also be fitted into the bridging model, albeit with some modifications. Complexes such as TRAPPs appear stably associated with target membranes (Fig. 5, bottom), and it is likely that they recognize specific determinants on the incoming vesicles. The nature of such vesicular “postal codes” is unknown. Another mechanism of tether formation is presented by the exocyst in which some subunits of the holocomplex associate with the vesicle and

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**Fig. 5. A model for tethering during membrane traffic.** 1) A vesicle approaches the target membrane. The movement can be by diffusion or by motor-mediated process. 2) The vesicle tethers to the target membrane by coiled-coil proteins or through multimeric tethering complexes. Tethering can occur at distances of >200 nm. 3) The cognate v-soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) on the vesicle and t-SNAREs on the target compartment pair to form trans-SNARE complexes. This process is sometimes referred to as “docking.” 4) The assembly of SNARE complexes drives membrane fusion. Transported cargo is incorporated into the membrane of the target compartment or released into its lumen. The tethers dissociate from the membrane or remain stably associated with the target membrane. The details of these events are discussed in the text.
some with the PM; their interaction forms a functional exocyst complex and links the vesicle and target membranes.

The model diagrammed in Fig. 5 shows tethers binding to membrane receptors that are distinct from SNAREs. However, the fact that many tethers show physical interactions with SNAREs (Table 4) may indicate that SNAREs are the membrane receptors for tethers. In light of this possibility, at least two types of tether-SNARE interactions can be envisioned. First, it is probable that a tether on one membrane binds a SNARE on the apposing membrane to form a tether-SNARE link. In this scenario, the tether is still linked to one membrane through an unidentified tether receptor. Alternatively, it is possible that tethers link membranes by simultaneously binding a v- and a t-SNARE.

**SNARE Complex Assembly**

Interactions of tethers with SNAREs may reflect a “bridging” association in which the tether does not influence the activity of the SNARE. Alternatively, or in addition, tether binding may actively promote SNARE complex assembly. Currently, only p115 has been directly shown to facilitate SNARE complex assembly. The first coiled-coil motif of p115 shows similarity to the SNARE motif present in all SNAREs (121). Using the SNARE-related motif from p115, Shorter and colleagues showed that addition of this p115 region stimulates SNARE complex formation in vivo in yeast (121). This result is consistent with a model in which Uso1p inactivates the inhibitory function of Stry1p and allows SNARE complex assembly.

This is supported by the finding that mammalian p115 binds mammalian Stry1 (121).

**Cargo Selection**

A novel role for tethering factors in cargo selection at ER exit sites has been documented. Studies from the Riezman laboratory have shown that GPI-anchored proteins are sorted from other secretory proteins during exit from the ER and that this process requires functional Uso1p. In addition to Uso1p, the sorting also requires functional COG complex because GPI-anchored proteins do not sort away from other secretory proteins in strains with defective Cog2p and Cog3p components.

Golgin-45 also appears relevant for correct function of ER exit sites. In cells depleted of golgin-45 by siRNA, resident Golgi proteins and anterograde cargo VSV-G protein do not exit the ER (119).

In addition, tethers may directly “traffic” specific cargo proteins through the secretory pathway. For example, GM130 specifically interacts with the human ether-a-go-go-related gene (HERG)-encoded potassium channel (110). Overexpression of GM130 decreases HERG current amplitude in Xenopus oocytes, suggesting that overabundance of GM130 causes intracellular retention of HERG and prevents its delivery to the PM. Another cargo protein, the transmembrane TGF-α, has been shown to associate with GRASP55 (57). Mutations in the COOH-terminal domain of TGF-α decrease or abolish its interaction with GRASP55, strongly impair cell surface expression of TGF-α (57). These observations suggest a role for GRASP55 in escorting transmembrane proteins, including TGF-α, during their transport to the cell surface.

Recently, p115 was shown to bind the NH2-terminal cytoplasmic domain of the type II transmembrane insulin-regulated aminopeptidase (IRAP) (43). IRAP is an abundant cargo protein of Glut4 storage vesicles that traffics to and from the plasma membrane in response to insulin. The amino terminus of p115 binds to IRAP and overexpression of an NH2-terminal p115 construct inhibits insulin-stimulated GLUT4 translocation. These findings suggest that p115 may “traffic” IRAP through its complex membrane itinerary. Whether facilitating traffic of cargo is a general function of tethering factors remains to be defined.

**Coat Events**

Perhaps related to cargo selection, tethering factors have been shown to interact with COPII and COPI coats. TRAPP I has been shown to directly interact with COPII components (112), whereas COG directly binds COPI components (129). It is possible that some tethering factors regulate coat dynamics. This is suggested by the finding that p115 interacts with Golgi-specific brefeldin A-resistant factor 1 (GBF1), a GEF for ARF that regulates COP1 dynamics at the ER-Golgi interface (27). Expression of the p115-binding region of GBF1 in cells leads to Golgi disruption, suggesting that the interaction between p115 and GBF1 is functionally relevant. It is unknown whether other tethers interact with GEFs that regulate coat recruitment.


Cytoskeletal Events

Some coil-coiled proteins appear to link membranes to the cytoskeleton rather than to other membranes. For example, the conserved NH2-terminal domain of the cis-Golgi associated Hook3 protein mediates attachment to microtubules, whereas the more divergent COOH-terminal domain mediates binding to organelles (140). Similarly, the TGN-localized CLIPR-59 is proposed to be involved in membrane-microtubule interactions, and its overexpression strongly perturbs endosome-TGN dynamics (100). The proteins bicaudal-D1 (BICD1) and -D2 (BICD2) are coiled-coil and localize to the TGN. They associate with vesicles and with dynactin, an adaptor for the dynein motor that facilitates (−) end-directed movement on microtubules from cell periphery toward the MTOC (42). BICD1 and BICD2 bind to Rab6 and associate with membranes in a Rab6-dependent manner (78,120). Overexpression of BICD1 enhances the recruitment of dynein-dynactin to Rab6a-containing vesicles. Conversely, overexpression of the carboxy-terminal domain of BICD that interacts with Rab6a but not with cytoplasmic dynein inhibits microtubule minus-end-directed movement of Rab6a-containing vesicles (78). It is likely that BICDs capture and load vesicles on microtubules for targeted delivery to the Golgi. The Drosophila golgin lava lamp associates with dynactin, CLIP-190 and Golgi spectrin, and facilitates the movement of the Golgi along microtubules during Drosophila celluarization (97).

Depletion of golgin-245 or p115 results in Golgi disruption, with Golgi ministacks forming adjacent to ER exit sites. This effect is analogous to that observed in cells treated with nocodazole and suggests that golgin-245 and p115 are directly or indirectly involved in facilitating the motility of Golgi elements toward the MTOC.

Signaling Events

Recently, Preisinger and colleagues have shown that the protein kinase YSK1 binds to GM130 (103). This finding suggests that golgins may also serve as tethers for regulatory proteins.

Golgin-160 has been implicated in Golgi disassembly during apoptosis, suggesting that golgin tethers may receive apoptotic signals and coordinately regulate traffic during cellular death (see Refs. 39, 74, and 75 for reviews).

TETHERS AND DISEASE

Evolutionarily conserved tethers are essential genes, and the low frequency of recovered mutations in tethered proteins probably reflects embryonic lethality. Mutation in the human homolog of the Trs20p subunit of TRAPP is the cause of the human X-linked disease SEDL (28, 29). SEDL causes bone growth defects, resulting in short stature, barrel chest, and degenerative joint disease (76). It is likely that the disease may result from defects in the trafficking of type II collagen through the Golgi.

An association between single-nucleotide polymorphism in the Sec8 component of the exocyst and rheumatoid arthritis has recently been reported, although the underlying cause was not defined (36).

A mutation in the COG7 gene leads to congenital orders of glycosylation and result in death within 10 wk after birth (147). The mutation results in reduced levels of cellular COG7 and defective trafficking of the glycosylation machinery. Because the COG abnormality affects many Golgi functions, systemic organ failure results in death of the affected individuals.

In conclusion, despite remarkable progress, the details of the molecular mechanisms of tether function remain unclear. On the basis of available information, it appears that unlike the Rab and SNARE families, in which distinct members share a common mechanism of action, distinct tethers may use distinct mechanisms to facilitate membrane traffic. Classic approaches of biochemistry and yeast genetics are increasingly coupled with approaches that disrupt tethering in mammalian cells or in complex organisms such as flies and mice to provide insight into tether functions. In addition, identification and characterization of human pathologies linked to defects in tethering provide information on the more specific function of tethers in specific tissues. Together, these explorations are expected to provide further insight into tether role in cellular traffic, tissue development and physiology, and human disease.

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