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Multisubunit Tethering Complexes and Their Role in Membrane Fusion

Review

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Protein trafficking within eukaryotic cells depends on vesicular carriers that fuse with organelles to deliver their lipid and protein content. Cells have developed an elaborate system to capture vesicles at organelles that involves the action of Rab GTPases and tethers. Vesicle fusion then takes place with the help of SNARE proteins. In this review we focus on the role of multisubunit tethering complexes of eukaryotic cells. In particular, we discuss the tethering complexes of the secretory pathway and the endolysosomal system and highlight recent evidence for the role of these complexes in interaction with Rabs, coat recognition and cooperation with SNAREs during the fusion cascade.

Introduction

Eukaryotic cells contain an endomembrane system that consists of morphologically distinct organelles with dedicated functions in protein and lipid glycosylation, protein sorting, and degradation. Transport of lipids and proteins between the organelles, including the transport to and from the plasma membrane, depends on vesicular carriers that are generated at one organelle and consumed at their destination (for review, see [1,2]). Both processes are tightly regulated and balanced to maintain the integrity of both donor and acceptor organelles. The generation of transport vesicles is linked to cargo recruitment and results in membrane deformation by coat proteins, which is then coupled to scission. To be consumed at the correct destination, vesicles need to overcome the distance between the two organelles and must either bring along or acquire the machinery necessary for fusion. In most cells, transport of vesicles occurs along the cytoskeleton. Fusion of transported vesicles at their destination membrane requires the coordinated action of Rab GTPases, tethering complexes and SNAREs (Figure 1A).

Rab GTPases are small GTP-binding proteins with low intrinsic GTP-hydrolysis and GDP-release activities (reviewed in [3]). These proteins serve as molecular switches that exist in two conformations, depending on their nucleotide status (reviewed in [2–4]). In their GDP-bound form, Rabs can be extracted from membranes by GDP-dissociation inhibitors (GDIs), which chaperone the Rab in the cytosol. The recruitment to membranes requires displacement of the GDI, potentially via a GDI-displacement factor (GDF), and subsequent activation by a guanine nucleotide exchange factor (GEF). The GDF and GEF activities may be found in one and the same protein [5]. In their GTP-bound form, Rabs interact with their effectors until they are reverted into their inactive GDP-bound form by a GTPase-activating protein (GAP).

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For their function in fusion, Rabs in the GTP-loaded form activate tethering factors [6]. The cooperation between Rabs and tethering factors is necessary for vesicle capture and is tightly coupled to vesicle fusion [6]. Therefore, membrane-embedded SNARE proteins with a cytosolic coiled-coil domain are required on both the vesicle and the target membrane (Figure 1A). After tethering, SNAREs from opposing membranes bring the two bilayers into close proximity by folding into specific four-helix-bundle complexes, which subsequently leads to fusion and lipid mixing of the bilayers [7].

In general, tethers are thought to bridge membranes by binding to Rab GTPases as well as to SNAREs and thus prepare them for membrane fusion. Tethers may have additional membrane-binding modules to stabilize their interaction with Rabs and SNAREs. This general principle is widely accepted, even though it is surprising that in most cases no direct proof for tethering exists. Two classes of tether have been defined and characterized in eukaryotes: elongated coiled-coil tethers and multisubunit tethering complexes (MTCs; Figure 1B). Within this review, we highlight recent observations on tether structure and function during vesicle targeting and fusion by focusing primarily on MTCs, and discuss the evidence for their activity.

Coiled-Coil Tethers

Coiled-coil tethers are large hydrophilic dimeric proteins comprising two globular heads connected by predicted long coiled-coil domains (for reviews, see [8,9]). Due to their size (up to around 3,000 residues [8]), they can bridge distances of more than 200 nm (Figure 1B). Most coiled-coil tethers have been found in the Golgi apparatus and have been termed ‘golgins’, although some (such as EEA1) are present at endosomes, which, at least in plants, are part of the *trans*-Golgi network (TGN) [10,11]. The function of golgins, and other coiled-coil tethers in general, has been discussed in recent reviews [8,9]. Without going into detail, we will briefly highlight a couple of excellent studies.

First, coiled-coil tethers have been shown to associate with membranes via one of three different modes: they either bind to adaptors, like the small GTPase Arf or Arf-like proteins, via their carboxy-terminal GRIP domains (e.g. golgin-245 and GMAP-210) [12,13]; or they contain a carboxy-terminal transmembrane domain (e.g. giantin and CASP) [14]; or they bind directly to specific lipids (e.g. EEA1) [15]. Coiled-coil tethers, however, also carry binding sites at their amino-terminal regions. GMAP-210, for instance, binds via its carboxy-terminal domain to Arf-GTP on membranes and contains in addition an amino-terminal amphipathic lipid packaging segment (ALPS) motif, which is able to bind to highly curved membranes [16]. Using a truncated GMAP-210 tether, Antonny and colleagues [17] could reconstitute tethering between Arf-GTP-loaded large liposomes and small liposomes. This tether may thus capture small vesicles that travel between Golgi cisternae and then release them after their fusion with the next cisterna [17]. Alternatively, the protein may generate contacts between the cisternae by taking advantage of its abilities to bind both to flat

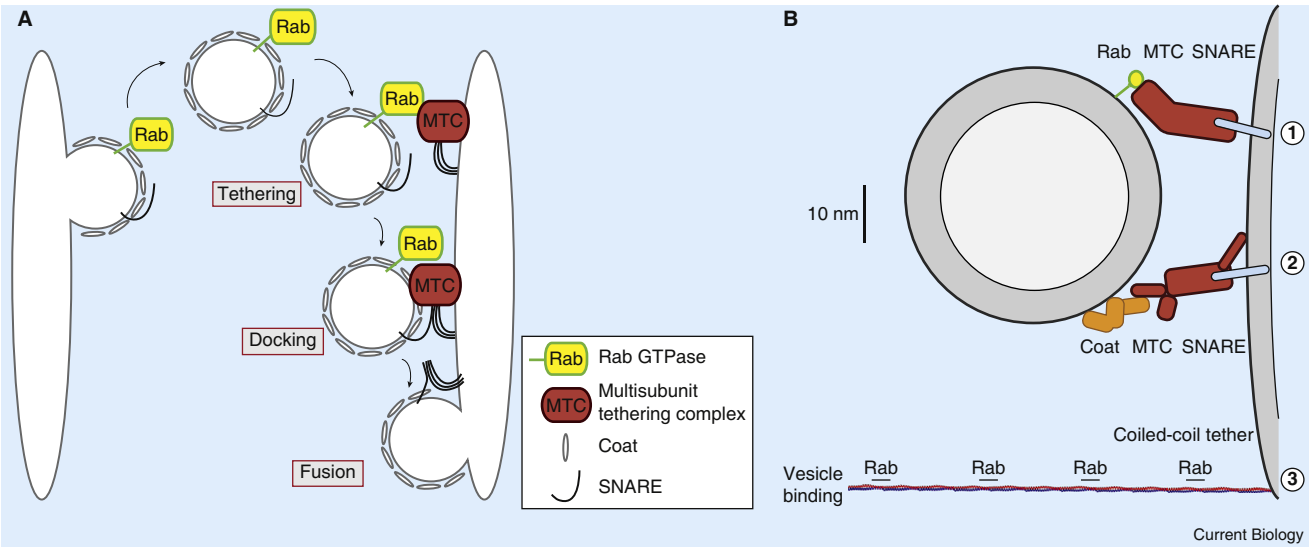

Figure 1. Role of tethers in vesicle recognition and fusion.

(A) Targeting and fusion of a transport vesicle. The consecutive steps of tethering, docking and SNARE-mediated fusion are indicated. (B) Three modes of tethering at organelle membranes. All structures are drawn to scale. The indicated vesicle is 70 nm in diameter. MTCs (red) are scaled according to the published Dsl1p complex structure (pdb:3k8p), the coat is sized according to available AP-2 structures (pdb:2jkt, 2iv8), the Rab corresponds to Ypt7 (pdb:1ky2), and the SNARE complex was sized according to pdb:3ipd. Due to the hypervariable domain of the Rab GTPase, it may reach out >10 nm from the surface of the vesicle. The depicted coiled-coil tether contains 1,000 residues. Three modes of interaction are shown: MTC-mediated bridging either (1) via Rab-GTP (yellow) and SNAREs (blue) or (2) via coat recognition and SNAREs. Coiled-coil tethers (3) have membrane-binding sites at each end, and multiple Rab-interaction domains along their entire length [18,19].

membranes (via the GTPase Arf1) and to highly curved membranes at the edges of the Golgi cisternae (via its ALPS motif). Coiled-coil tethers could thus be responsible for the stacking of the Golgi and might protrude from the Golgi in a tentacle-like manner.

Second, the coiled-coil sequence is not just required for bridging distances but also contains multiple binding sites for Rab GTPases [18,19] (Figure 1B). Golgins may thus provide a Rab-binding matrix that could facilitate local diffusion of Rab-loaded vesicles through the Golgi stacks [19].

Multisubunit Tethering Complexes

The second group of tethers that act throughout the endomembrane system and at the plasma membrane are the MTCs. These at first glance comprise a much more divergent family of proteins than the coiled-coil tethers as they consist of three to ten subunits that differ in size between 50 and 140 kDa per subunit (Table 1 and Figure 2). This suggests a combination of several functions within one protein complex. Given that they contain multiple subunits, elongated MTCs may span distances of up to 30 nm, which is sufficient to capture vesicles, but much shorter than the >200 nm distances that coiled-coil tethers can span (Figure 1B). In general, MTCs seem to couple the recognition of membranes via Rab GTPases with the subsequent SNARE-mediated membrane fusion (Figure 1B; mode 1). Binding of the Rab in its GTP-bound form occurs in most cases via one so-called effector subunit. Moreover, tethering complexes cooperate with SNARE proteins and may even 'proofread' their assembly, as observed in the case of the vacuolar HOPS complex (discussed below). MTCs are therefore multipurpose protein complexes.

Initially, MTCs were considered to form a divergent family of proteins with multiple evolutionary origins, but some similarities between subunits soon came to light [20]. The composition of MTCs seems to differ between species, which led to the suggestion that some subunits may perform specialized functions in some organisms [21]. Recent structural observations and sequence comparisons instead suggest a more coherent picture ([22–25], and reviewed in [26]), which allows MTCs to be divided into two general groups: those required for membrane fusion with organelles of the secretory pathway (i.e. Dsl1p, COG, GARP and the exocyst — recently renamed 'CATCHR' for complexes associated with tethering containing helical rods); and those of the endolysosomal pathway (CORVET and HOPS). In addition, eukaryotic cells have the TRAPP complex, a multisubunit GEF that does not fit into either of the two categories, but rather combines coat recognition with tethering (Figure 2 and Table 1), and will be discussed separately. In the following sections, we briefly highlight the composition and function of each complex, before turning to their general function in trafficking pathways.

Tethers Required along the Secretory Pathway and the Golgi

The Dsl1p, COG, GARP and exocyst complexes were initially identified in separate studies, which focused on characterising their roles in defined trafficking steps within the secretory pathway (Dsl1, COG, exocyst) and retrograde transport to the Golgi (GARP) (Figure 2). Recent structural studies highlighted, however, that all four complexes might have common α-helical compact folds, suggesting a similar function despite divergent evolution [27]. Indeed, the structures of the GARP subunits Vps53 and Vps54 [28,29] look similar to previously described structures of subunits of related complexes like Dsl1, Cog4 and the exocyst's Sec6 [23,24,30].

The Dsl1p complex is located at the endoplasmic reticulum (ER) membrane and serves as an acceptor of

Table 1. Properties of tethering complexes.

Tethering complex	Localization	Functions between	Subunits	Rab or Rho GTPase	Interacting SNAREs	Interacting coat/ AP complex	Lipid interaction
Dsl1p	ER	Golgi → ER	Dsl1 *Dsl3 (Sec39)* *Tip20*	Ypt1 (?)	Use1, Sec20	COPI	
COG	Golgi	Endosome → cis Golgi	Cog1 *Cog2 (Sec35)* *Cog3 (Sec34)* Cog4 Cog5 Cog6 Cog7 Cog8	Ypt1	Sec22 Gos1 Sed5 Ykt6	COPI	
GARP	TGN	Endosome → TGN	*Vps51/Ang2* Vps52 *Vps53* *Vps54*	Ypt6	Tlg1		
Exocyst	Plasma membrane	Vesicle → plasma membrane	Sec3 Sec5 *Sec6* Sec8 Sec10 **Sec15** Exo70 Exo84	Sec4 RalA Rho1 Rho3	Sso1,2 Snc1,2 Sec9		Sec3, Exo70
CORVET	Endosome	TGN → early endosomes	**Vps8** Vps3 Vps11 Vps16 Vps18 *Vps33*	Vps21 (Rab5)			
HOPS	Vacuole	MVB → vacuole, and vacuole → vacuole	**Vps41** Vps39 Vps11 Vps16 Vps18 *Vps33*	Ypt7 (Rab7)	Vam3 Vam7 Vti1 Nyv1	δ-subunit of AP-3	
TRAPPI	Golgi	ER → Golgi	Bet3 (2x) Bet5 Trs20 Trs23 Trp31 Trs33	Ypt1		COPII	
TRAPPII	Golgi	Endosome → Golgi	TRAPPI + Trs65 Trs120 Trs130 Tca17	Ypt1, Ypt31/ 32		COPI	
TRAPPIII	Phagophore	Golgi→ lysosome	TRAPPI + Trs85	Ypt1			

Subunits that bind SNAREs are indicated in italics and Rab effectors are shown in bold. For TRAPP, the subunits required for GEF activity are underlined. Blank boxes for interactions of tethers with SNAREs, coats or lipids indicate that no data have been reported, to our knowledge.

Golgi-derived coat protein complex I (COPI). It consists of three subunits — Dsl1, Dsl3/Sec39, and Tip20 — which form a stable complex with three Q-SNAREs of the ER — Use1, Ufe1 and Sec20. SNAREs may thus serve as membrane anchors for the Dsl1p complex at the ER membrane [24,31,32]. The Dsl1p complex recognizes COPI-coated vesicles and tethers them to the ER [26,33], thereby combining tethering with SNARE assembly [24]. The interaction of Dsl1p with the yeast Rab GTPase Ypt1 (the homologue of mammalian Rab1) is dispensable for membrane binding, but might be required for function [34] because Ypt1 interacts with Ufe1.

The COG complex functions as a MTC within the Golgi [35]. This complex interacts with Ypt1/Rab1 and consists of eight subunits that comprise two lobes consisting of COG2–COG3–COG4 and COG5–COG6–COG7, linked by a heterodimer of COG1 and COG8 [36,37]. Moreover, the COG complex binds directly to the Sec1/Munc18-homologous (SM) protein Sly1 [38] and to the SNARE Sed5 (yeast homologue of mammalian syntaxin 5) [39]. Mutations in the COG complex are associated with glycosylation defects and subsequent diseases [23].

The GARP tethering complex cooperates with the Rab GTPase Ypt6/Rab6 in tethering endosome-derived vesicles to the TGN [40,41] and consists of four subunits — Vps51, Vps52, Vps53, and Vps54 [42–47]. Vps51 specifically binds to the yeast SNARE Tlg1 [48] and to syntaxin 6 in mammalian cells [47]. In addition, the entire mammalian GARP complex directly interacts with the SNAREs syntaxin 6, syntaxin 16 and VAMP4 [45]. Thus, the GARP complex, like the COG

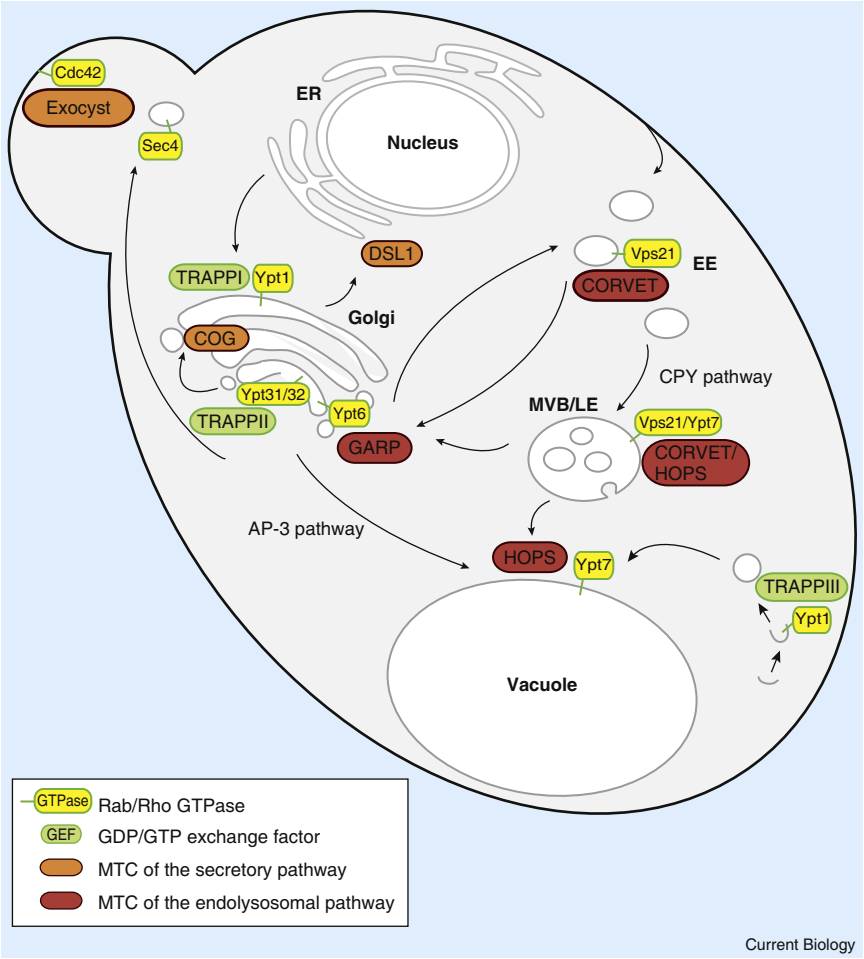

Figure 2. Overview of the intracellular trafficking pathways and the involved tethering complex with their corresponding GTPases.

MTCs are indicated in orange (secretory pathway) and red (endolysosomal system). Rabs are indicated in yellow (using the yeast nomenclature). For details, see text. ER, endoplasmic reticulum; EE, early endosome; MVB/LE, multivesicular body/late endosome; CPY, carboxypeptidase Y, a cargo of the biosynthetic pathway to the vacuole; AP-3, adaptor complex 3.

complex, combines Rab and SNARE binding in one protein complex.

Tethering of exocytic vesicles at the plasma membrane occurs via the octameric exocyst complex, consisting of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84. The exocyst binds to the Rab GTPase Sec4 on secretory vesicles via its subunit Sec15, and to the Rho GTPases Rho1, Rho3 and Cdc42 at the plasma membrane via Sec3. Even though it was initially purified as a single complex [49], several lines of evidence suggest that the exocyst assembles at the sites of exocytosis by binding to Rab and Rho GTPases (reviewed in [22]). Similar to the COG complex, the exocyst interacts with an SM protein, in this case Sec1 [50], and has the ability to bind to SNAREs [51], indicating that it also couples tethering and SNARE assembly.

Tethers between Endosomes and Lysosomes

The 700 kDa CORVET and HOPS MTCs operate sequentially between endosomes and lysosomes [25] (Figure 2 and Table 1). Given that these complexes have four shared subunits (the class C proteins Vps11, Vps16, Vps18 and Vps33) and two additional homologous subunits, the structure of the two hexamers is probably very similar [52]. Moreover, all subunits except the Sec1 homologue Vps33 have a similar domain arrangement, which likely consists of an amino-terminal β-propeller domain and a carboxy-terminal α-solenoid domain [25]. Their structure thus resembles subunits of the nuclear pore complex; structural parallels between coat proteins and nuclear pore proteins have been previously discussed [53]. It is also noteworthy that the shared subunits Vps11 and Vps18 each contain carboxy-terminal RING zinc finger domains, which are important for CORVET and HOPS function [54]. The E3 ubiquitin ligase activity of mammalian hVps18 is also dependent on the presence of this domain [55].

The CORVET complex is most likely required for the fusion of Golgi-derived vesicles with the endosome [56,57]. In addition to the previously mentioned four class C subunits, this complex contains two specific subunits, Vps3 and Vps8 [58]. The CORVET complex binds the Rab GTPase Vps21/Rab5 via Vps8, which can also trigger tethering in a CORVET-independent manner [59]. Vps8 is the third subunit of the CORVET complex that contains a carboxy-terminal RING domain. Vps3, the other CORVET-specific subunit, also binds to Vps21 [58], though its precise role within the complex remains to be determined. It is likely that the CORVET complex interacts with endosomal SNAREs via Vps33 and may thus couple Vps21-GTP binding to SNARE assembly.

The HOPS complex is required for several fusion events at the late endosome and the vacuole, including the fusion of autophagosomes, multivesicular bodies (MVBs), and Golgi-derived adaptor protein 3 (AP-3) vesicles, and the homotypic fusion of vacuoles [60,61]. The two HOPS-specific subunits, Vps41 and Vps39, interact with Ypt7/Rab7 [62,63]. Vps41 binds to Ypt7-GTP as its effector subunit, both from within the HOPS complex as well as on its own [52], whereas Vps39 can interact with Ypt7 independently of its nucleotide state [52,62], but is not the GEF of Ypt7 [64]. Functional studies on yeast vacuole fusion indicate that the HOPS complex is able to promote tethering by binding to Ypt7-GTP and to the vacuolar SNAREs Vam3, Vam7 and Vti1 [65,66].

TRAPP — A Multifunctional GEF Complex

As previously mentioned, the TRAPP complex is an exception to the above-described complexes. This complex is found in three forms — named TRAPP I, II, and III — of about 300 kDa (I) and 1,000 kDa (II, III) in size, which would also be consistent with a role for TRAPP in bridging two membranes. All TRAPP complexes have a common core of Bet3 (present

in two copies) [67,68], Bet5, Trs20, Trs23, Trs31 and Trs33 [69]. TRAPPII additionally contains Trs65, Trs120 and Trs130 [70] and a further subunit Tca17/TRAPPC2L that seems to affect TRAPPII stability, assembly or function [71]. The recently identified TRAPPIII complex contains an additional subunit, Trs85 [72]. The different compositions seem to direct the TRAPP complexes to different cellular locations. TRAPPI is required for ER-to-Golgi transport [69,73,74], TRAPPII is required at the TGN [75,76], and TRAPPIII is found at the phagophore and is necessary for autophagy [72].

Despite the differences in subunit composition of the three forms, the TRAPP complex is primarily a multisubunit GEF for the Rab Ypt1 [77], and it has been reported to also act as a GEF for the Rabs Ypt31/32 [74,76]. Structural data have shown that the central subunits — Bet3, Bet5 and Trs23 — interact directly with Ypt1 and together provide GEF activity [78]. TRAPP complexes may therefore activate Ypt1 or Ypt31/32 at different cellular locations, and thus promote tethering.

How does the proposed tethering activity fit with that of the previously discussed MTCs? The most convincing tethering function has been demonstrated for TRAPPI in ER-to-Golgi transport. TRAPPI is able to promote tethering of ER-derived vesicles with the Golgi by binding to Sec23, a subunit of the inner layer of the COPII coat [70,79], and thus facilitates the fusion of COPII-coated vesicles with the Golgi. Therefore, TRAPPI has the ability to recognize coat subunits and couple Rab activation with coat recognition [79]. Functionally, TRAPP-mediated tethering may differ from the MTC-dependent tethering discussed before. TRAPP is not a Rab effector, but a Rab GEF. Due to its shape, the TRAPP complex has been proposed to lie flat on the membrane surface where it activates Ypt1 [68]. Consistent with this idea, TRAPPI contacts a subunit of the inner layer of the COPII vesicle, Sec23 [80], which may require shedding of the outer layer of the COPII coat consisting of Sec13/31. It is possible that TRAPPI is recruited to COPII-coated vesicles via Sec23, activates Ypt1 and thus promotes tethering via the coiled-coil tether Uso1/p115 or the COG complex [81–83]. This would then suggest that the GEF and tethering functions of TRAPPI are coordinated to capture COPII vesicles [79]. Due to their large size and varying composition, it seems likely that TRAPP complexes will have additional functions at different organelles.

Principles of MTC Function

Within the next section, we focus on the MTCs of the exocytic and endocytic pathways, and highlight their common function. We start by discussing their recruitment and assembly at membranes, their function as tethers and conclude with their role in bilayer mixing.

Recruitment of MTCs to Membranes

How are MTCs recruited to membranes? All MTCs are peripheral membrane proteins that seem to associate with membranes via the coordinated interaction with Rab GTPases and SNAREs. As almost all MTCs (except the Dsl1p complex) interact with Rab-GTP via a specific effector subunit, Rab activation and MTC recruitment are probably linked (Figure 1B). MTCs may either associate with membranes via Rab-GTP as preassembled units or they may assemble on membranes as a prerequisite of tethering.

The Golgi-localized COG complex interacts with the Rab GTPase Ypt1 via Cog2 and Cog3 [84], and potentially also interacts with Rab6 [85,86]. The Rab-binding site in the COG complex lies within the Cog2–Cog3 subcomplex [84]. Potentially, the two-lobed structure of COG may allow for binding to multiple Rabs and thus coordinate retrograde trafficking within the Golgi (for review, see [87]). In mammalian cells, Rab33 and Rab6 act sequentially, and the effects of COG depletion can be rescued by depletion of Rab33, suggesting that Rab33 and COG cooperate in the same pathway [86]. Whether COG assembly or dynamic interactions between its two lobes are linked to Rab recognition is currently unknown.

Likewise, GARP binds specifically to the Rab Ypt6 via its subunit Vps52 [41,42]. As GARP has been purified as a heterotetramer from yeast, it remains unclear whether binding to Ypt6 is linked to the assembly of the complex.

For the exocyst, the link between GTPase binding and assembly is an open question, although several studies have shed light on exocyst recruitment to vesicles. The exocyst seems to assemble from two subcomplexes, which are recruited initially onto exocytic vesicles and the recipient plasma membrane (Figure 3, upper panel). Exocytic vesicles initially contain the Rab Ypt31/32, but require the Rab Sec4 for binding to the exocyst. Ypt31/32 is replaced by Sec4, and this step is coupled to the recruitment and assembly of the exocyst complex. Upon transport from the Golgi to the plasma membrane, Ypt32-GTP recruits, with the assistance of phosphoinositide-4-phosphate (PI(4)P), the Sec4 exchange factor Sec2 [88,89]. Following the loss of PI(4)P, presumably induced by a PI 4-phosphatase, Sec2 is activated, generates Sec4-GTP, and recruits Sec15, which then binds the active Sec4 on exocytic vesicles [89,90]. Most of the remaining exocyst subunits also arrive via vesicular transport, presumably by subsequent binding to Sec15 [91]. At the plasma membrane, exocyst subunits bind Rho and Ral GTPases [92,93]. At least two exocyst subunits, Exo70 and Sec3, bind to Rho GTPases (Rho3, Rho1 and Cdc42) and PI(4,5)P_2 [94–99]. It is, however, not yet clear whether the complex acts as one unit or assembles upon vesicle docking to the plasma membrane [22].

Similarly, Rab exchange on endosomes may accompany the assembly or recruitment of the CORVET and HOPS complexes (Figure 3, lower panel). As mentioned above, both complexes differ in their Rab-interacting subunits. During endocytosis and on endosomes, the exchange factor Rabex5 activates Rab5, which can bind multiple effectors, including the coiled-coil tether EEA1 [100]. The CORVET complex also binds Rab5/Vps21 via its subunit Vps8 [58,59,101,102]. Endosomes (and the related phagosomes) mature by undergoing Rab exchange [103], whereby Rab7 replaces Rab5 in a process that is aided by the Mon1–Ccz1 complex [104,105], which we recently identified as the Ypt7/Rab7 GEF [64]. Due to a change in the Rab composition on endosomes, the CORVET complex is replaced by the HOPS complex, which binds Ypt7/Rab7 via its subunit Vps41 [52,63] (Figure 3, lower panel). Such an exchange may occur by sequential replacement of the CORVET-specific subunits Vps3 and Vps8 for the HOPS-specific Vps41 and Vps39, or by replacing one entire complex for the other [58]. Intriguingly, overexpression of the CORVET subunit Vps3 displaces Vps39 from the HOPS complex [58], suggesting that these tethering complexes may undergo a dynamic turnover. However, it is not currently known either for HOPS or for CORVET whether the complex

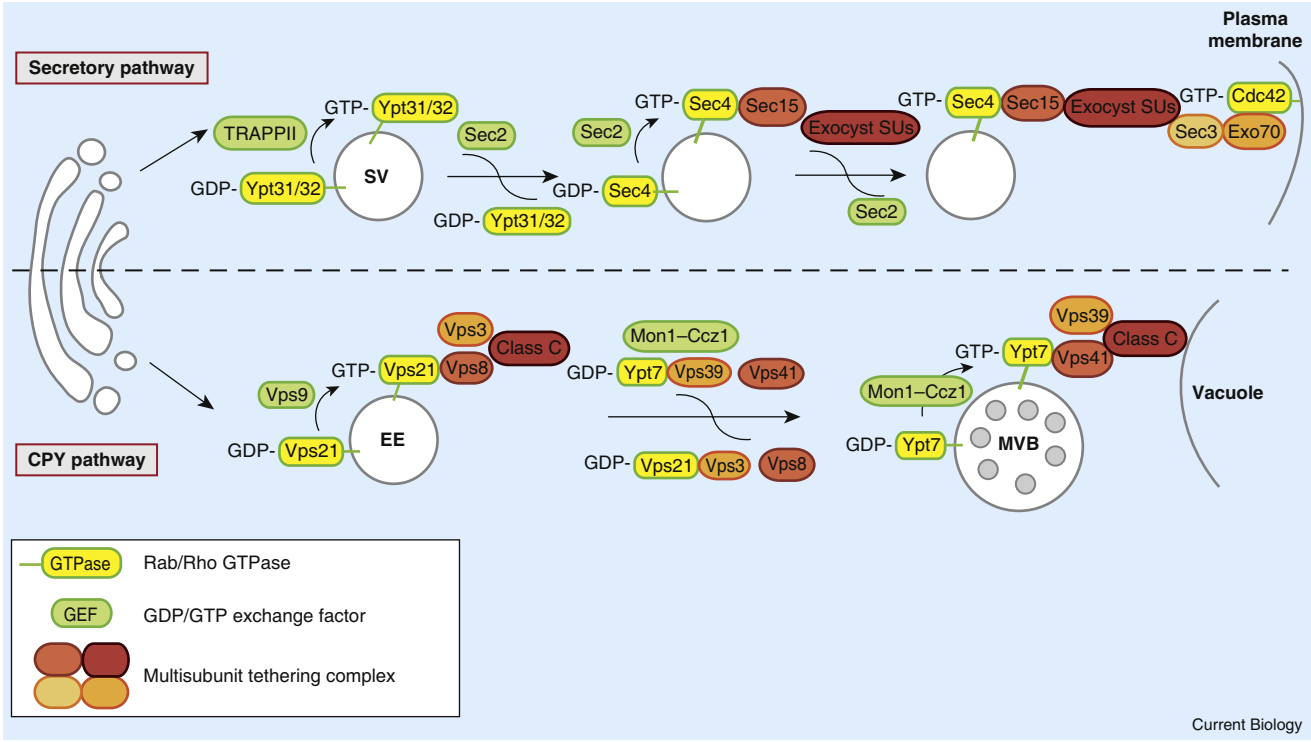

Figure 3. Recruitment of tethering complexes during vesicle maturation.

The figure shows models that compare Rab exchange on secretory vesicles (SV) and endosomes (EE, MVB), which is accompanied by the recruitment of MTCs. Upper panel: Ypt32-GTP recruits the next GEF Sec2, which in turn promotes Ypt32 displacement. The next Rab Sec4 is then recruited and activated [89], which results in the recruitment of exocyst subunits (SUs e.g. Sec15), and Sec2 displacement. The exocyst may then assemble during tethering [22]. Lower panel: Vps21-GTP recruits CORVET. During maturation, the Vps21 GEF Vps9 is displaced [104], and CORVET may be remodelled into HOPS, while the Rab Ypt7 is activated via the Mon1–Ccz1 GEF complex [64,105]. HOPS then mediates tethering with the vacuole [124]. For details, see text.

assembles on endosomes or remains as one entity throughout endosomal maturation.

Among all tethering complexes, the Dsl1p complex is the only exception with respect to Rab binding because this complex apparently does not require any Rab for its recruitment to membranes. This is probably due to the fact that Dsl1p acts at the ER, which is the starting point of the secretory pathway and is not subject to the constant remodelling that takes place during organelle maturation. As maturation of organelles is tightly linked to Rab exchange cascades, Rab recruitment and tethering function are connected to almost all subsequent organelles.

Recognition of Vesicle Coats by MTCs

Once MTCs are recruited to membranes, how do they recognize vesicles? One possibility is that tethers interact directly with the coat subunits (mode 2 in Figure 1B) [106]. If the coat is indeed one binding site, it then has to be maintained, at least partially, to provide an interaction surface. Indeed, the point at which a coat (such as COPII, COPI or clathrin) is shed is unclear at this point.

Several MTCs have binding sites for vesicle coats. The Dsl1p complex interacts with the COPI coat via a binding site that is also needed to stabilize interactions within the COPI coat [26,33]. Thus, the Dsl1p complex may tether vesicles by recognition of the COPI coat, and assist in uncoating prior to vesicle fusion. Similarly, the COG complex may bind COPI vesicles via the coat, as depletion of COG subunits and COPI results in a similar phenotype [107].

Coat interactions have not been reported for either GARP or the exocyst. Potentially, the exocyst can bridge membranes by assembling from its two subcomplexes, one of which is present on exocytic vesicles, and could thereby initiate fusion [22] (Figure 3, upper panel).

At the yeast vacuole, the HOPS complex is required for several distinct fusion reactions, including fusion between the vacuole and late endosomes or Golgi-derived AP-3-coated vesicles. The Rab effector subunit Vps41 has a dedicated function in the AP-3 pathway by binding to the ear domain of the δ-subunit Apl5 [108,109]. The vacuole casein kinase Yck3, which is transported via the AP-3 pathway to the vacuole [110], phosphorylates Vps41 and controls Vps41 function in the AP-3 pathway [111,112]. As Vps41 function seems to be restricted to the late endosome and vacuole membrane, the interaction of Apl5 with Vps41 might be required to tether AP-3-coated vesicles to vacuoles [113] (mode 2 in Figure 1B). This tethering is controlled by Vps41 phosphorylation [111], and Apl5 indeed partially localizes to the vacuole membrane [113]. Our recent data show that the Vps41 binding site for Apl5 is masked due to membrane binding on the late endosome, and only becomes available upon Yck3-mediated phosphorylation of Vps41 at the vacuole [114]. For the CORVET complex, potential interactions with coats have not been reported.

When a tether binds to the coat and thus captures a vesicle, uncoating should be coupled tightly to SNARE-mediated fusion. To date, all assays addressing the function of tether–coat interactions are based on *in vivo* observations.

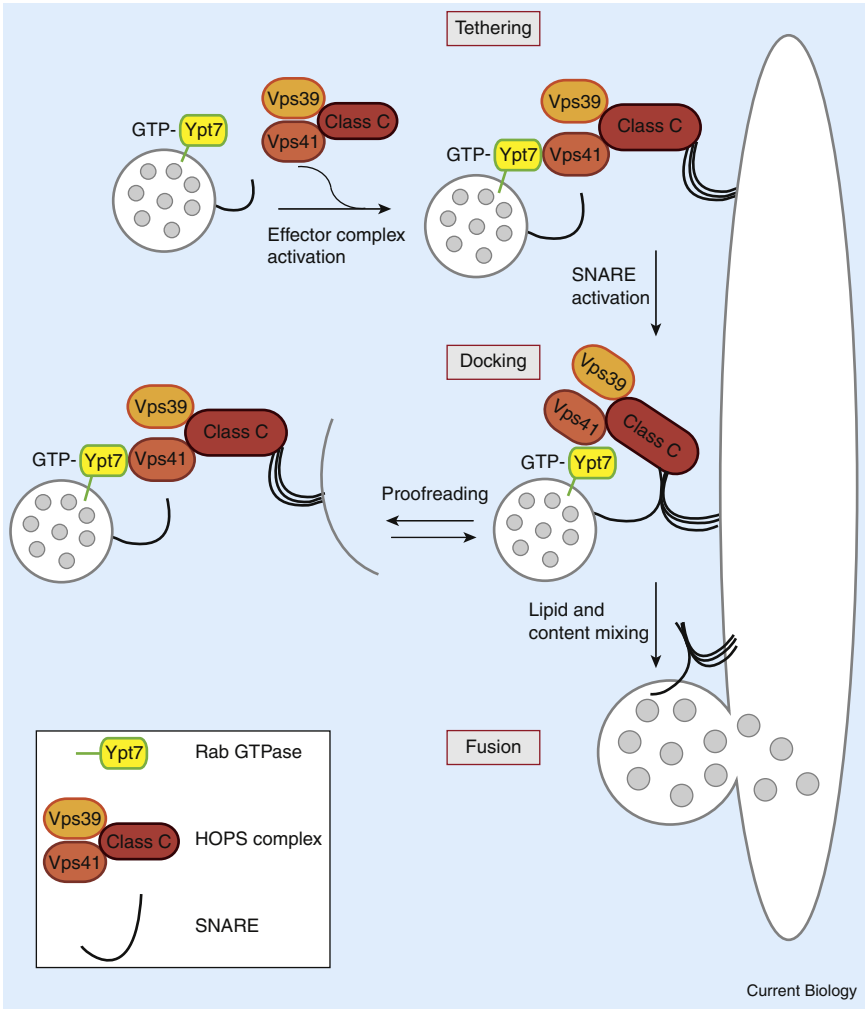

Figure 4. Role of the HOPS tethering complex in vacuole fusion.

HOPS mediates tethering by binding the Rab Ypt7, lipids, and SNAREs [65,124]. HOPS can proofread the correct assembly of *trans*-SNARE complexes. If incorrect, Sec17/18 (homologue of mammalian α-SNAP/NSF) promotes disassembly [126].

Thus, fusion assays will need to be carried out to test the function of tethers in coat recognition prior to fusion.

Coupling of Tethering and SNARE-Mediated Fusion

We consider Rab-GTP binding of MTCs as part of the recruitment reaction; however, MTCs also bind to SNAREs. This binding could be required to stabilize the MTC or one of its subunits on membranes or could be required to couple tethering to SNARE-mediated membrane fusion. Both cases may not be exclusive but part of a concerted scenario of MTC function.

The Dsl1p complex, for instance, binds the amino-terminal domain of the SNAREs Use1 and Sec20 at the ER membrane [24,32], presumably positioning the Dsl1p complex as a kind of 'antenna' on the ER membrane. SNARE binding may be required for membrane association, as Dsl1p does not interact with a Rab. In addition, the complex promotes the assembly of ER SNAREs *in vitro* [24], suggesting that the Dsl1p complex combines tethering with the initiation of membrane fusion.

Similar models have been proposed for the remaining MTCs. COG interacts with the Golgi Q-SNARE Sed5 and stabilizes intra-Golgi SNAREs [39,84]. GARP has several SNARE-binding domains [42,45], and the exocyst recognizes the SNARE Sec9 via its Sec6 subunit [51].

In vitro fusion assays have only been developed for the HOPS complex, which binds SNAREs [115] and strongly stimulates SNARE-mediated liposome fusion [116] (Figure 4). Moreover, the HOPS complex tethers membranes by binding to Ypt7/Rab7 on one membrane and SNAREs on the other [65,66], which may represent a common mode of action for tethering complexes. The HOPS complex promotes fusion [116] but also proofreads the SNARE complex [117] and may thus provide a paradigm for MTC-mediated control of membrane fusion (Figure 4).

One of the subunits of the HOPS (and CORVET) complex that is likely to be involved in SNARE-mediated fusion is the SM protein Vps33 [58,118,119]. SM proteins are dedicated SNARE-binding proteins that control membrane fusion (for review, see [120]). As previously mentioned, both the COG complex and the exocyst bind SM proteins; the COG complex interacts with Sly1 [38], and the exocyst with Sec1 [50]. Whether the cooperation of SM proteins with MTCs is a general feature of tethering complexes is not yet clear. It is, however, striking that only the CORVET and HOPS complexes require Vps33 as an integral subunit.

Summary and Outlook

We envision MTCs as flexible bridges. Except for Dsl1p, they are recruited to membranes by binding to a Rab GTPase in its GTP-bound form and recognize SNAREs and/or coats during tethering (Figure 1B). Some MTCs, such as the exocyst and the HOPS complex, also interact directly with membranes by binding to phospholipids. The binding of MTCs to SNAREs likely promotes SNARE complex formation and subsequent lipid bilayer mixing, a process that may be proofread by MTCs. It is also possible that some tethering complexes actively promote fusion by bending membranes, as shown for C2 calcium-binding domain proteins or synaptotagmins [121–123].

This general scheme is probably too simplistic as not all parameters fit to all MTCs. Some of the discrepancies listed are the result of the fact that we currently have an incomplete picture for many MTCs. For instance, an *in vitro* fusion assay has been established only for the HOPS complex, and this assay may reproduce many, but probably not all, of the *in vivo* functions of the complex. The HOPS complex is also the only MTC for which tethering has been demonstrated *in vitro* using proteoliposomes that carry SNAREs and Ypt7 [65,124]. For the COG complex, the function of multiple

Rab-binding sites is still confusing. Likewise, the interaction of tethers with coats has been described in multiple systems [106] and agrees with the postulated function for the Dsl1p complex, although it is possible that the reported interaction between MTCs and coats also occurs on the same membrane and not just during tethering. If tethering requires the interaction of tethers and coats, then uncoating would need to be linked to fusion. Presently, we are lacking fusion assays that are able to resolve such tethering events. We also do not yet understand whether tethers exist as stable entities or undergo dynamic remodelling, depending on the growth status of the cell, the trafficking pathway or Rab activation status. Due to their large size, we also expect that tethering complexes will be linked to multiple regulatory processes. Similar to SNAREs, which were initially isolated as complexes and are the product of a fusion reaction [125], assembled MTCs may be a product of successful fusion as well. At least for the exocyst complex, this seems to be the case. Given that some general principles of MTC function appear to be conserved, we expect that some of these questions will be answered within the coming years.

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