Membrane tethers at a glance

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Abstract

Cargo delivery from one compartment to the next relies on the fusion of vesicles with different cellular organelles in a process that requires the concerted action of tethering factors. Although all tethers bridge vesicle membranes to mediate fusion, they form very diverse groups as they differ in composition, in their overall architecture and size, as well as their protein interactome. Yet, their conserved function relies on a common design. Recent data on Class C Vps complexes indicates that tethers play a significant role in membrane fusion beyond vesicle capturing. Furthermore, these studies provide additional mechanistic insights into membrane fusion events and reveal that tethers should be considered as key players of the fusion machinery. Moreover, the discovery of the novel tether FERARI has changed our understanding of cargo transport in the endosomal system by mediating 'kiss-and-run' vesicle-target membrane interactions. In this Cell science at a glance and in the accompanied poster, we compare the structure of the coiled-coil and the multisubunit CATCHR and Class C Vps tether families on the basis of their functional analogy. We discuss the mechanism of membrane fusion and summarize how tethers capture vesicles, mediate membrane fusion at different cellular compartments and regulate cargo traffic.

Introduction

A generally accepted view of cellular trafficking is that cargo-enriched vesicles bud off from precursor membranes, move through the cytoplasm on microtubule tracks and fuse with their target membrane in a highly regulated manner. Membrane fusion relies on three key components: small GTPases of the Rab family, tethering proteins/complexes and soluble NSF-attachment protein receptors (SNARE) proteins (D'Agostino et al., 2017; Ohya et al., 2009; Pieren et al., 2010). To date, approximately 60 Rab proteins have been identified in mammals, which in their active, GTP-loaded state are bound to specific membranes. Together the Rab proteins organize the transport, the tethering to target organelles and the fusion of vesicles. Accordingly, Rab proteins provide identity to trafficking organelles: Rab5 marks endocytic vesicles and early endosomes, Rab7 late endosomes and endolysosomes, and Rab4, Rab10, Rab11 define for instance different recycling pathways to the plasma membrane (Homma et al., 2021). To regulate incoming cargo, target membranes recruit specific tethers, which are able to recognize Rab proteins on vesicles and selectively capture them for fusion (Spang, 2016).

As a second selection system for fusion specificity, target membranes also contain specific Q-SNARE complexes, which are able to engage with R-SNAREs found on the vesicles (Jahn and Scheller, 2006). Mechanistically, the zippering of the SNARE motifs into a four-helix bundle brings the membranes in close proximity to displace the hydration layer from the surface of the membranes and initiate

fusion. This process is chaperoned by Sec1/Munc18 (SM) proteins. However, SNAREs on the opposing membranes can only engage when they are in very close proximity (Jahn and Scheller, 2006). Therefore, tethers have to bring the vesicles close to the target membrane to induce SNARE assembly (Spang, 2016). In this article and the accompanying poster, we compare how different families of tethers are able to capture vesicles and bring them into close proximity to the acceptor membrane. Furthermore, we also describe the mechanism of membrane fusion and the essential role of tethers in this process. Finally, we discuss how the discovery of novel tethers shaped our understanding of endosomal cargo recycling.

Vesicle tethering by different tether families

Tethers are characterized by their ability to bridge membranes and facilitate fusion. On the basis of structural similarities, tethers have been classified into two groups: the coiled-coil tethers and the multisubunit tethering complexes (MTCs) (Brunet and Sacher, 2014).

Coiled-coil tethers are well-represented by the golgin protein family anchored to the Golgi membrane (Gillingham, 2018). They can extend over 100 nm into the cytoplasm and build a mesh-like matrix for the structural and functional integrity of the Golgi apparatus. Although golgins share low sequence homology, they all have coiled-coil motifs, in which alpha helices are curled up around each other like “the strands of a rope” (Gillingham, 2018). Furthermore, most of golgins bind small GTPases of the Rab, Arf and Arl families with their carboxy-teminus determining their specific location within the Golgi (Witkos and Lowe, 2016).

One of the best-characterized golgins is the mammalian GMAP-210, which binds Arf1 on the cis-Golgi membrane with its carboxy-terminal GRAB domain (Witkos and Lowe, 2016). To capture vesicles, GMAP-210 has an N-terminal amphipathic lipid packing sensor (ALPS) motif, which is able to sense the high degree of membrane curvature of vesicles (Drin et al., 2007) (see poster). Although GMAP-210 is the only known golgin containing an ALPS motif, vesicles can also bind the splayed N-terminus of another golgin, the dimeric GCC185 *in vitro* suggesting that these tethers share a common design in order to capture vesicles (Cheung et al., 2015).

GMAP-210 also has Rab2-binding sites along its sequence, which have been shown to function downstream of ALPS-mediated vesicle tethering. It was proposed that vesicles could hop along the Rab binding sites (Sato et al., 2015), which would bring the vesicle into closer proximity to the target membrane. Alternatively, since all golgins contain Rab-binding sites and form a meshwork around the Golgi, vesicles could travel through the tether network by association and dissociation with multiple Rab-binding sites (tentacular meshwork model) (Sinka et al., 2008)(see poster). Yet, other models for moving the vesicles closer to the target membrane were proposed based on the

structural properties of golgins, as they contain highly flexible regions between predicted coiled-coil domains and thus sites at which golgins could bend to bring the captured vesicles closer to the membrane (rigid hinge model) (Cheung et al., 2015). Moreover, it was also proposed that golgins can undergo a flexible collapse onto the Golgi surface as GCC185 is shorter and more flexible than initially thought (Cheung and Pfeffer, 2016; Cheung et al., 2015). Although coiled-coil tethers have been found mainly at the Golgi, the endosomal system can also use coiled-coil tethers such as EEA1, which is recruited to phosphatidylinositol 3-phosphate (PtIns3P)-containing early endosomes. Binding of EEA1 to Rab5 vesicles results in an entropic collapse in the structure of EEA1 that pulls the tethered vesicle towards the target membrane for fusion (Murray et al., 2016)(see poster). As an extension of the collapse model, the proposed conformational changes may also allow golgins to hand over the vesicles to shorter tethers, such as the golgin-84 and the TATA element modulatory factor (TMF), that can interact with the multisubunit conserved oligomeric Golgi (COG) complex (Gillingham, 2018; Miller et al., 2013; Sohda et al., 2010). Nevertheless, it is still unclear for many coiled-coil tethers which model correctly describes the mechanism for vesicle tethering. It is also conceivable that different coiled-coil tethers employ different ways to capture vesicles and bring them closer to the target membrane. Furthermore, even though golgins also interact with monomeric SNARE proteins as it has been shown for p115, GCC185 and GM130, in contrast to MTCs, the function of this interaction remains elusive (Cheung and Pfeffer, 2016, Wang *et al.*, 2015).

Similar to coiled-coil tethers, MTCs also connect membranes by binding to Rabs. However, in contrast to long coiled-coil tethers, MTCs can only bridge a shorter distance (probably around 50 nm) and evolved to fulfil more complex functions during membrane fusion (Shvarev et al., 2022) (see poster). They can be divided further into two classes: the CATCHR complexes (Complexes Associated with Tethering Containing Helical Rods) that mainly act on the secretory pathway and the endolysosomal Class C Vps complexes, HOPS (Homotypic fusion and vacuole Protein Sorting), CORVET (class C core Vacuole/Endosome Tethering), CHEVI (class C Homologs in Endosome-Vesicle Interaction) and FERARI (Factors for Endosome Recycling And Rab Interactions) (Lürick et al., 2018; Nickerson et al., 2009).

Although CATCHR complexes differ in the number of their subunits and the corresponding subunits share low sequence similarities, CATCHR complexes are grouped together based on their structural resemblance. Notable examples of the CATCHR complexes include Dsl1(NRZ complex in metazoan) localized to the ER and essential for Golgi-to-ER trafficking, the conserved oligomeric Golgi (COG) complex that mediates retrograde transport through the Golgi, the Golgi-associated retrograde protein (GARP) that acts as a Golgi-associated tether regulating endosome to TGN recycling, the endosome associated retrograde protein (EARP) found on endosomes, and finally the exocyst

complex that tethers vesicles to the plasma membrane for secretion (Lürick et al., 2018) (see poster and Table1). All CATCHR complexes are conserved from yeast to mammals and consist of evolutionarily related □-helical proteins and form long flexible legs as determined by negative-stain electron microscopy (EM) (Chou et al., 2016; Yu and Hughson, 2010). Furthermore, current low-resolution EM studies show that the subunit organization of the GARP complex is very similar to that of COG1-4 subcomplex. Since EARP only differs from GARP in one subunit, it likely adapts the same subunit organization (Chou et al., 2016) (see poster).

The hexamer Class C Vps complex HOPS also shows elongated extensions (Chou et al., 2016; Shvarev et al., 2022). CORVET is expected to share the same architecture as HOPS since the core subunits are shared. In contrast, the Vps21(Rab5 in metazoan)-binding subunits Vps8 and Vps3 in CORVET are replaced by the Rab7-like Ypt7-binding subunits Vps41 and Vps39 in yeast HOPS (Peplowska et al., 2007; van der Kant et al., 2015). Therefore, CORVET is found on endocytic vesicles and early endosomes (Peplowska et al., 2007; Perini et al., 2014; Richardson et al., 2004; van der Kant et al., 2015) and HOPS on late endosomes, mediating the homotypic fusion of late endosomes and the fusion of mammalian lysosomes or yeast vacuoles with late endosomes, autophagosomes and AP-3 vesicles (Balderhaar and Ungermann, 2013; Pols et al., 2013; Spang, 2016) (see poster). Both tethers are conserved from yeast to humans (Spang, 2016). Although, in mammals, HOPS is recruited onto Rab7-positive late endosome by the Rab7 effector RILP (van der Kant et al., 2015), and the two Rab-interacting subunits also binds Arl8 (via Vps41) (Khatter et al., 2015) and Rab2 (via Vps39) (Kajiho et al., 2016) in addition to Rab7 (Marwaha et al., 2017; Zhang et al., 2021). Recently, in metazoans, FERARI was described on sorting endosomes and on tubular endosomal networks (TENs) and CHEVI on recycling endosomes (Banushi et al., 2016; Cullinane et al., 2010; Solinger and Spang, 2022; Solinger et al., 2020; Solinger et al., 2022). Both complexes share some conserved structural features with HOPS and CORVET. In metazoans, two HOPS/CORVET subunit has an additional homologue, namely the Vps16 homologue VIPAS39 and the Vps33A homologue Vps33B. VIPAS39 in complex with Vps33B forms CHEVI while with another eukaryotic SM protein Vps45 is part of FERARI. While up to date, only two CHEVI subunits have been identified, the six subunits of FERARI has been discovered (Solinger and Spang, 2022; Solinger et al., 2020; Solinger et al., 2022; Spang, 2016; van der Beek et al., 2019).

In addition to vesicle capturing and tethering, MTCs associate with SNAREs and with SM proteins, which gives them additional importance in membrane fusion beyond vesicle tethering. Importantly, all CATCHR complexes interact through specific subunits with SNAREs such as the N-terminal regions of the GARP subunits and COG4 in the COG complex (Chou et al., 2016; Lürick et al., 2018). However, while CATCHR complexes associate only transiently with SM proteins, Class C Vps complexes have

evolved to contain SM protein as member of the complex (Spang, 2016) (see Table 1). Whether this built-in SM protein contributes to a more specific localization or higher stability on membranes remains unclear.

The chaperone activity of tethers in SNARE assembly

Our current understanding of membrane fusion is based on studies using reconstituted proteoliposomes or yeast vacuoles as a model system (Baker et al., 2015; D'Agostino et al., 2017; D'Agostino et al., 2018; Izawa et al., 2012; Ohya et al., 2009; Pieren et al., 2010). During membrane fusion, the space between the two phospholipid-bilayers needs to be dehydrated. This is an energy demanding process since this space is surrounded by the hydrophilic headgroups of the phospholipids. Therefore, vesicle fusion events require a catalyst, a role fulfilled by SNAREs. SNARE complexes assemble during membrane fusion from three Q SNARE proteins, a Qa, Qb and a Qc, located on the acceptor membrane and from an R-SNARE on the membrane of the vesicle (see poster). The monomers are categorized according to whether they contribute with a glutamine (Q) or an arginine (R) residue to the only hydrophilic central layer of the SNARE bundle interface (Jahn and Scheller, 2006). During fusion, the SNARE motifs of the anchored SNAREs zipper into parallel four-helix bundles leading to the formation of the fusion stalk. It is estimated that at least three SNARE complexes are necessary around the fusion pore for successful vesicle fusion (Jahn and Scheller, 2006).

Importantly, correct SNARE assembly relies on the chaperon activity of SM proteins, which interact with monomeric SNAREs, as well as with intermediate (Qabc; R+Qa; R+Qbc) and complete (R+Qabc) SNARE complexes. For example, the SM protein Vps45 is needed for the Qa-SNARE Tlg2 to form SNARE complexes (Jahn and Scheller, 2006). Likewise, another SM protein Vps33 can intertact with the R-SNARE Nyv1, the Qa-SNARE Vam3 and the Qc-SNARE Vam7, as well as with the complete vacuolar SNARE complex in yeast (Baker et al., 2015; Dulubova et al., 2001; Krämer and Ungermann, 2011; Lobingier and Merz, 2012). Interestingly, Vam3 and Nyv1 bind to Vps33 simultaneously with the overall complex resembling a half-zippered SNARE complex. This intermediate is a fusogenic, partly assembled SNARE complex, which suggests that Vps33 serves as a template for SNARE folding and supports the assembly at rate-limiting concentrations (Baker et al., 2015). Indeed, the built-in SM protein is considered to be an essential part of the membrane-fusion-promoting activity in this family of tethers (Zick and Wickner, 2014); Vps45 is part of the Class C Vps complex FERARI (Solinger et al., 2020), whereas the yeast Vps33 is a HOPS and CORVET complex subunit (Seals et al., 2000; Spang, 2016). In contrast, the CATCHR tether complexes do not contain but interact with SM proteins to facilitate SNARE assembly. Dsl1 and COG are known to interact with the SM protein Sly1

(Laufman et al., 2009; Laufman et al., 2013; VanRheenen et al., 2001). Furthermore, two Dsl subunit Tip20 and Sec39 also directly binds SNAREs , Sec20 (Qb) and Use1 (Qc) respectively, in a way that their SNARE motifs are available for assembly. It is speculated that the SM protein Sly1 would present the Qa-SNARE Ufe1 and would bind the R-SNARE on the vesicle for SNARE-bundle assembly (Travis et al., 2020) (see poster). Moreover, direct interaction of the GARP complex with SNAREs has been shown to regulate SNARE localization and assembly (Pérez-Victoria and Bonifacino, 2009).Taken together, MTCs do not only tether two membranes, they also play an important role in chaperoning SNARE assembly. Interestingly, studies on the yeast HOPS complex indicate additional roles of tethers in membrane fusion as discussed below.

Class C Vps complexes are an essential part of the membrane fusion machinery

Low-resolution negative-stain EM analyses have revealed the overall arrangement of the yeast HOPS complex (Bröcker et al., 2012; Chou et al., 2016). Here, five out of six HOPS subunits (Vps11, Vps16, Vps18, Vps39, and Vps41) share a similar N-terminal β-propeller and a C-terminal α-solenoid domain. The current cryo-EM structure reveals that the HOPS complex adopts a highly extended conformation. Vps11 and Vps18 form the core assembly hub for the four other subunits (Vps39, Vps41, Vps16, and Vps33), each of which have specific functions. Vps41 and Vps39 binds to the Rab7-like Ypt7 by their N-terminal domains. They are positioned at the opposite ends of the complex and show conformational flexibility. The SNARE-binding elements Vps16 and the SM protein Vps33 branch out to the lateral side of the complex as a single unit but is stably connected to the core assembly (Shvarev et al., 2022) (see poster). During membrane fusion, HOPS tethers vesicles through the binding of its Vps39 and Vps41 subunits to Ypt7. The SM protein Vps33 then interacts with SNAREs from the opposing membranes (Baker et al., 2015; Song et al., 2020). Indeed, HOPS was shown to recruit and bind monomer SNAREs (Vam7, Vam3) and catalyze the entry of each Q-SNARE and the R-SNARE into a complex (Sato et al., 2000; Zick and Wickner, 2013). Importantly, in the absence of a Q-SNARE protein, HOPS can mediate the assembly of the rapid fusion intermediates R+Qa or R+QbQc (Song et al., 2020), as well as proofreading trans-SNARE complexes (Starai et al., 2008). Once all SNAREs are recruited, their SNARE motifs zipper up toward their membrane anchor. It is speculated that the backbone of HOPS could act as a “lever arm“ holding on to SNAREs to facilitate zippering (Shvarev et al., 2022). Furthermore, the bulky three-point arrangement of HOPS is expected to cause membrane stress (D’Agostino et al., 2017) and could explain how HOPS catalyzes membrane fusion. Indeed, multiple studies have shown that SNAREs alone lead only to a hemi-fused state of vacuoles, where the outer layer of the lipid-bilayer is fused but the inner layer is separated, preventing content mixing (D’Agostino et al., 2017; D’Agostino et

al., 2018; Pieren et al., 2010). HOPS and its interaction with SNAREs through Vps33 is needed at the hemifusion site to deform the membrane and to increase the fusogenic activity of the SNARE complex, thereby lowering the energy barrier for pore opening and content mixing (D'Agostino et al., 2017; D'Agostino et al., 2018; Pieren et al., 2010; Zick and Wickner, 2014). Zippered SNAREs may then dissociate from HOPS and allow access for α-SNAP and NSF to recycle SNAREs (Shvarev et al., 2022).

Whether SNARE specificity is an encoded feature of SNARE pairing or whether it actually relies on others factors remains an issue of debate. SNAREs can functionally replace each other to a certain extent both *in vitro* (Izawa et al., 2012) and *in vivo* (Brandhorst et al., 2006), while individual SNAREs can operate in more than one fusion step that involve different SNARE partners (von Mollard and Stevens, 1999). Moreover, SNAREs need to be transported through the secretory pathway and/or the endosomal system to the membrane where they mediate fusion. Therefore, early endosomes contain multiple sets of SNAREs. In addition to the putative early endosomal SNAREs, SNAREs involved in exocytosis and late endosomal fusion are also present. However, fusion is mediated by early endosomal SNAREs with little or no role of the exocytic and late endosomal SNARES during homotypic fusion (Brandhorst et al., 2006). In contrast to *in vivo*, *in vitro* reconstituted proteoliposomes with early endosomal SNAREs fuse with exocytic or late endosomal SNAREs, suggesting that SNARE pairing is regulated (Brandhorst et al., 2006). However, it is still unclear how the cell manages to select one set of SNAREs for fusion, while silencing others. Although it was shown that the specificity of SNARE pairing relies on the Qabc SNAREs and this composition is tightly regulated (Izawa et al., 2012), tethers appear to be prime candidates to regulate specificity (Lobingier and Merz, 2012).

FERARI, the tether that mediates kiss-and-run

The endosomal system developed efficient and multifunctional tethers whose role is not restricted to vesicle capturing and tethering but that also have essential chaperone activity in SNARE assembly and specificity, as well as catalytic activity in fusion pore opening. Intriguingly, another new function was observed for the recently-discovered FERARI complex (Solinger et al., 2020). FERARI has been described in *C. elegans* and in mammalian cells as a tether, which localizes to sorting endosomes and the TEN. FERARI can mediate the fusion of Rab5- and Rab-10 positive endosomes through its Rabenosyn5 subunit and of Rab11 containing endosomes through the Rab11FIP5 subunit. Intriguingly, FERARI also contains EHD1, which is a dynamin-like pinchase (Solinger et al., 2020) and was found to

regulate the recycling of cargoes from sorting endosomes and TEN through a kiss-and-run mechanism, whereby cargo carriers fuse with the target membranes for a few seconds while retaining their round shapes and subsequently pinch off when cargo loading has finished (Solinger et al., 2020; Solinger et al., 2022) (see poster). The discovery of FERARI has also led to a new model of how recycling cargoes are returned to the plasma membrane and how cargo flow through the endosomal system is regulated (Solinger and Spang, 2022).

Perspectives

Taken together, the newly acquired knowledge on tethers in recent years, particularly regarding the MTCs, has led to a change in our view of their function from simple vesicle catchers to sophisticated machines that regulate various aspects of membrane traffic.

Yet, many questions remain unanswered. Firstly, the mechanistic description of the FERARI-mediated kiss-and-run is currently lacking and it is still unclear how FERARI contributes to membrane fission. EHD1 could oligomerize around the fusion stalk and with its ATPase activity pinch off the endosome or alternatively, several FERARI complexes could be at the kiss-and-run site providing the critical concentration for EHD1 (Solinger and Spang, 2022). The membrane flattening during this kiss-and-run may either be prevented by EHD1 itself or by the pulling force of the actin cytoskeleton (recruited by the FERARI subunit Ankyrin) at the fusion stalk (Solinger and Spang, 2022).

Furthermore, giving the complexity of trafficking pathways in mammalian cells, additional tethers or a more complex cooperation between tethers may exist. How tethers contribute to the specificity of cargo traffic and whether they interact with adaptors to regulate cargo flow remain open questions.

Acknowledgements

We would like to thank Sung Ryul Choi for critical reading of the manuscript.

Declaration of competing interests

The authors declare no competing interests.

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Journal of Cell Science

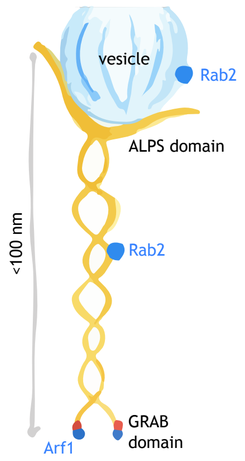
Membrane Tethers at a Glance

Viktória Szentgyörgyi and Anne Spang

Structural comparison of different tether families

1. Coiled-coil tethers

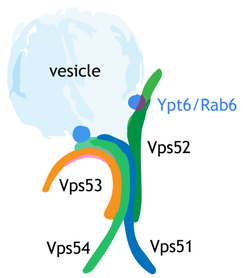
GMAP-210 (Golgin)*



2. Multisubunit tethering complexes (MTCs)

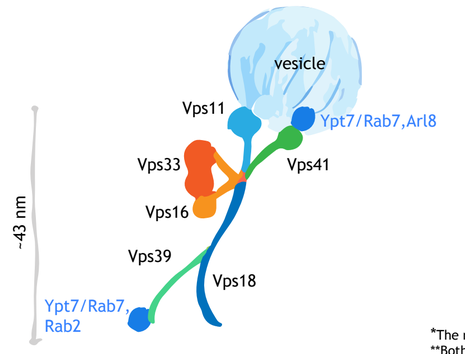
CATCHR complexes

GARP complex**



Class C Vps complexes

HOPS complex**



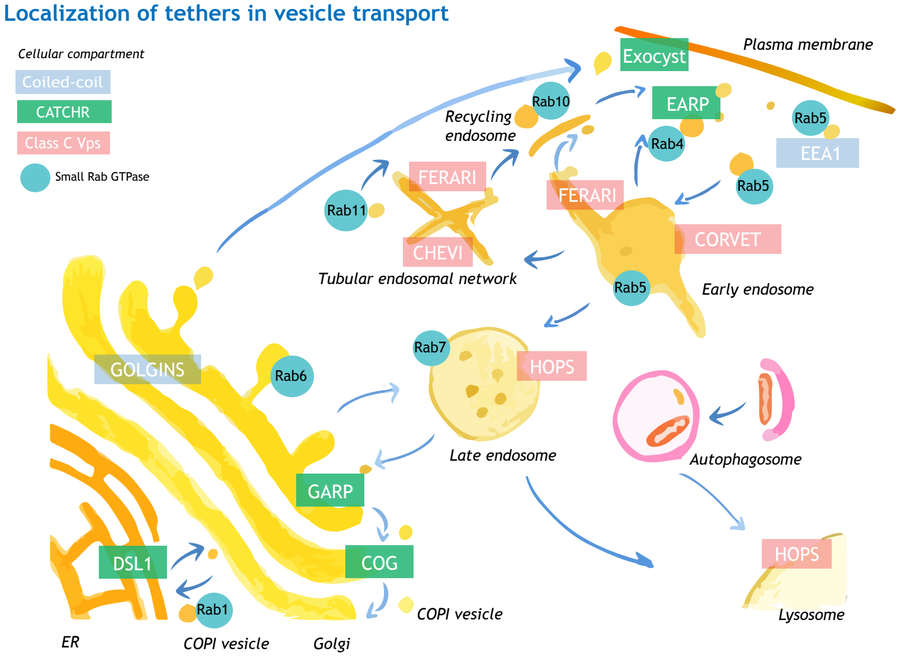
Tethers
Tethers are extended proteins or multisubunit protein complexes that bridge membranes and facilitate their fusion.

Small GTPases
Small GTPases are recruited onto membranes when activated by nucleotide exchange factors (GEFs). They organise the transport of vesicles and endosomes including vesicle biogenesis, transport, tethering and fusion.

Tethers are recruited onto membranes and capture vesicles by binding small GTPases of the Arf, Arl and Rab families.

*The mammalian GMAP-210 has homologs from yeast to vertebrates.
**Both the GARP and the HOPS complex is conserved from yeast to metazoans.

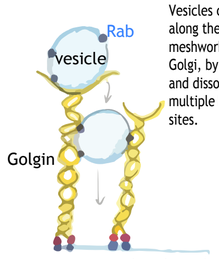
Localization of tethers in vesicle transport



Coiled-coil tethers localize mainly to the Golgi-network, CATCHR complexes are the tethers of the secretory system, Class C Vps complexes are tethers of the endosomal system.

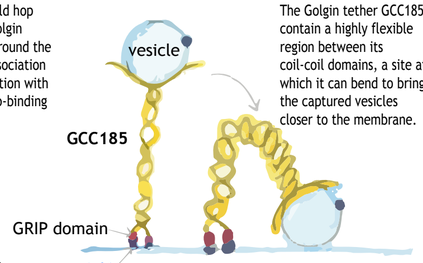
Tethers bring vesicles close to the target membrane

Tentacular meshwork model



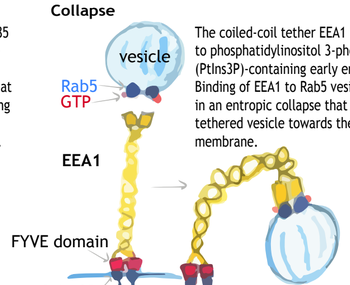
Vesicles could hop along the Golgin meshwork around the Golgi, by association and dissociation with multiple Rab-binding sites.

Bent/rigid hinge model



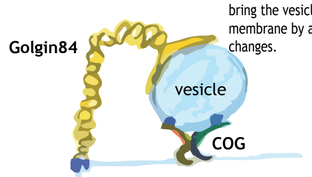
The Golgin tether GCC185 contain a highly flexible region between its coil-coil domains, a site at which it can bend to bring the captured vesicles closer to the membrane.

Collapse



The coiled-coil tether EEA1 is recruited to phosphatidylinositol 3-phosphate (PtIns3P)-containing early endosomes. Binding of EEA1 to Rab5 vesicles results in an entropic collapse that pulls the tethered vesicle towards the target membrane.

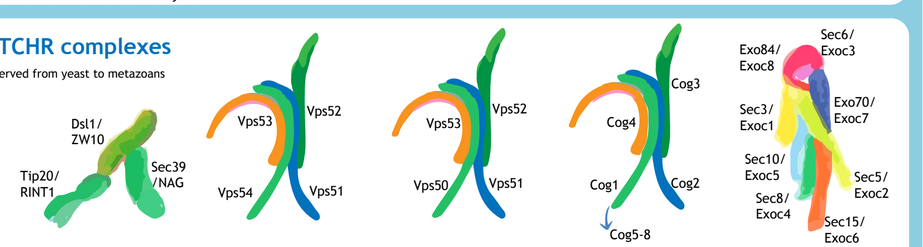
Hand it over to MTCs



Coiled-coil tethers interact with MTCs as it has been shown for Golgin84 and the MTC COG complex. In this model, coiled-coil tethers bent to hand over vesicles to the sorter MTCs which would bring the vesicles close to the target membrane by assumed conformational changes.

CATCHR complexes

Conserved from yeast to metazoans



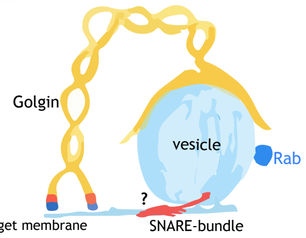
Small GTPase*	Dsl1/NRZ complex	GARP complex	EARP complex	COG complex	Exocyst
	Rab8,Rab18	Rab6	Rab4	Rab1,Rab6	Rab8,Rab11,Cdc42, Rho1/3,RalA/B

*mammalian

MTCs regulate SNARE-bundle assembly

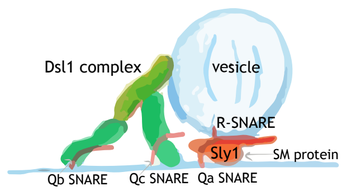
In yeast

Coiled-coil tethers



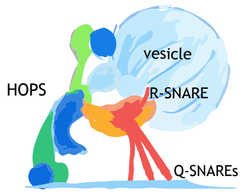
No confirmed regulation of SM and SNARE proteins.

CATCHR complexes



SNARE binding and interaction with SM chaperons.

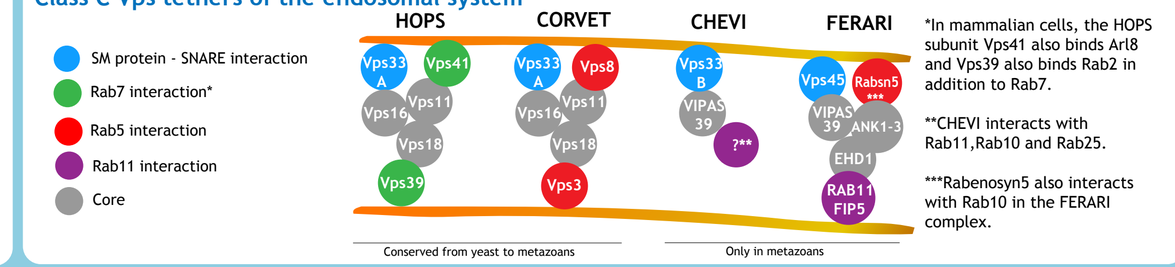
Class C Vps complexes



Direct regulation of SNARE assembly via built-in SM proteins.

SNAREs
Target membranes and vesicles contain a specific set of SNARE proteins. During membrane fusion, their SNARE motifs assemble into a four-helix bundle bringing the membranes in close proximity. This assembly is chaperoned by so called SM proteins.

Class C Vps tethers of the endosomal system



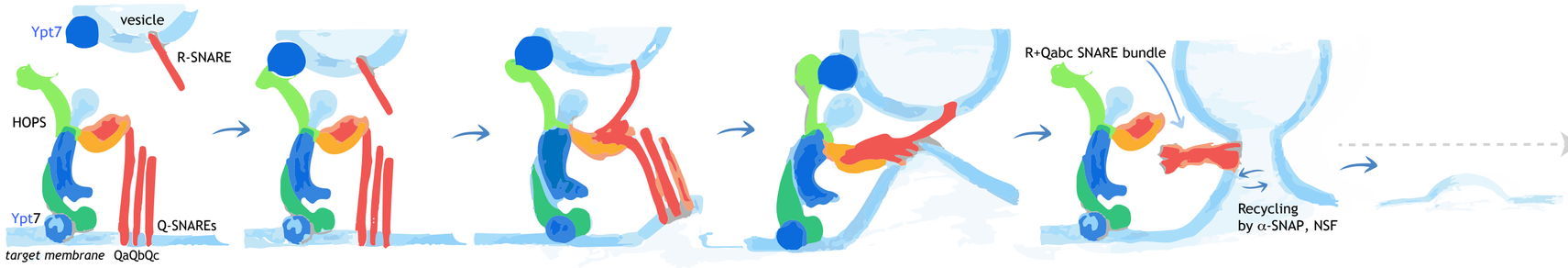
*In mammalian cells, the HOPS subunit Vps41 also binds Arl8 and Vps39 also binds Rab2 in addition to Rab7.

**CHEVI interacts with Rab11,Rab10 and Rab25.

***Rabenosyn5 also interacts with Rab10 in the FERARI complex.

The role of tethers in vesicle fusion

In yeast



Tether recruitment
HOPS is recruited onto the membrane by Ypt7 and engage with SNARE porteins

Rab recognitions and tethering
HOPS recognises Ypt7 on the membrane of the vesicle and tethers the two membranes.

SNARE templating and membrane distortion
HOPS chaperons the assembly of the SNARE proteins and distorts the membrane to lower the energy barrier of membrane fusion.

SNARE zippering
The R and Q SNAREs zipper into a bundle during which membrane fusion occurs.

Pore opening
A fusion stalk is created by the fusion of the vesicle and target membranes via which the content of the two compartment can mix. After fusion, SNARE proteins are recycled by α-SNAP and NSF proteins.

Complete fusion
The vesicle fully flattens and its membranes "dissolves" to the membrane of the target compartment.

Kiss-and-run
In FERARI-mediated membrane fusion, vesicles undergo a kiss-and-run instead of full fusion. EHD1 and actin are proposed mediators of the membrane ***fission***.

Abbreviations

ALPS - Amphipathic Lipid-Packing Sensor motif
CATCHR complexes - Complexes Associated with Tethering Containing Helical Rods
CHEVI - class C Homologs in Endosome-Vesicle Interaction
COG - Conserved Oligomeric Golgi
CORVET - Class C core Vacuole/Endosome Tethering
EARP - Endosome-Associated Retrograde Protein
EEA1 - Early Endosome Antigen 1
FERARI - Factors for Endosome Recycling And Rab Interactions
GARP - Golgi-Associated Retrograde Protein
HOPS - Homotypic fusion and vacuole Protein Sorting
MTCs - Multisubunit Tethering Complexes
SM proteins - Sec1/Munc18 family protein
SNARE - Soluble NSF Attachment protein Receptor

Where it is important to distinguish, the yeast/mammalian homologs are both indicated.