Principles of membrane tethering and fusion in endosome and lysosome biogenesis

Daniel Kümmel and Christian Ungermann

Endosomes and lysosomes receive cargo via vesicular carriers that arrive along multiple trafficking routes. On both organelles, tethering proteins have been identified that interact specifically with Rab5 on endosomes and Rab7 on late endosomes/lysosomes and that facilitate the SNARE-driven membrane fusion. Even though the structure and stoichiometry of the involved proteins and protein complexes differ strongly, they may operate by similar principles. Within this review, we will provide insights into their common functions and discuss the open questions in the field.

Introduction

The biogenesis of organelles of the endolysosomal system is tightly linked to multiple trafficking routes from the Golgi and plasma membrane that are responsible for the delivery of proteins and lipids to the lysosome. During endocytosis, plasma membrane proteins such as amino acid transporters, receptor tyrosine kinases or the low-density lipoprotein (LDL) receptor, are sequestered into vesicles that fuse with the early endosome (EE). If the cargo is separated from the receptor at the EE, the receptor is sorted back the plasma membrane via the recycling endosome. The early endosome then matures into the late endosome (LE), which eventually fuses with the lysosome. Additional trafficking routes deliver lysosomal hydrolases and membrane proteins from the Golgi to the EE and LE, thus providing further cargo load to the endolysosomal system [1]. During the transition from the EE to the LE, ESCRT complexes [2] sort membrane proteins into intraluminal vesicles, which are eventually degraded, whereas sorting nexins and retromer retrieve transport receptors back to the Golgi [3]. These remodeling processes occur before the fusion of LEs with the lysosome. It is thus expected that the machinery responsible for membrane fusion of the endolysosomal system has to be coordinated with processes that occur during endosomal maturation. Within this short review, we will focus in particular on the principles of tethering and fusion at endosomes and lysosomes.

Fusion of intracellular membranes is mediated by a conserved machinery that consists of two functional modules. On the one hand, SNARE proteins are the engines that drive fusion [4,5]. Their assembly generates the force to overcome the energy barrier of lipid bilayer merging and is coupled to disassembly by the adaptor protein αSNAP [6] and the ATPase NSF [7], which ultimately provides the energy for fusion. Efficient SNARE-driven fusion additionally requires SM (Sec1 and Munc18) family proteins, which are thought to act as chaperones and stimulate SNARE fusion [8]. On the other hand, each fusion process requires activated, GTP-bound Rab GTPases, which act as markers of membrane identity and represent landmarks for the recruitment of effectors [9,10]. The Rab machinery can thus include diverse accessory factors that will improve the efficiency and fidelity of fusion.

Tethering factors are Rab effectors that are thought to primarily act by bridging opposing membranes to establish the initial contact between incoming donor vesicles and their acceptor organelle before the engagement of SNAREs [11,12]. Members of this diverse group of proteins are — among others — dimeric coiled-coil proteins and multi-subunit tethering complexes (MTCs). The largest family of MTCs are the Complexes Associated with Tethering Containing Helical Rod (CATCHR), which are characterized by the helical rod-fold of their subunits and have been reviewed elsewhere [13]. These diverse group of MTCs have interaction partners on both the donor and the acceptor compartment in the particular trafficking step they regulate [14]. This is consistent with a role of CATCHRs in tethering, but it should be noted that this activity has not been fully reconstituted biochemically and the molecular mechanism of their function remains elusive.

Whereas CATCHR complexes have been identified on most organelles of the secretory pathway, no homologous complex is found in the endolysosomal system. Here, coiled-coil tethers and Class C MTCs are required for the fusion at EEs, LEs, and lysosomes/vacuoles. Importantly,
tethering function as part of the Rab machinery and the cooperation with the SNARE machinery has been reconstituted in vitro using purified components [15**,16**,17**]. These studies — taken together with the structural characterization of the proteins involved — can serve as a starting point to achieve a better general understanding of the fundamental function of tethering in membrane fusion.

**Tethering and fusion of early endosomes**

Fusion of early endosomes in mammalian cells is regulated by Rab5 — and driven by the R-SNARE VAMP4 and the Q-SNARES Syntaxin13, Vti1A and Syntaxin6 [18,19]. In reconstitution experiments with synthetic endosomes it could be shown that in addition to activated Rab5 and disassembled SNAREs, phosphoinositol-3-phosphate (PI-3-P) and two Rab effectors are needed for efficient fusion: EEA1 (early endosome antigen 1) and the Rabenosyn-5–Vps45 complex [17**]. The proteins involved in EE tethering combine membrane, Rab and SM protein binding in one functional unit. We will briefly highlight some structural features of each component.

EEA1 is a long coiled-coil protein with protein and lipid interacting domains at its termini. An N-terminal C2H2 Zn-finger domain binds GTP-Rab5 with high affinity (Figure 1a) [20]. The C-terminus contains a FYVE domain that binds PI-3-P with moderate affinity [21] and an adjacent Rab5 binding motif, however, Rab5 binding at this region is not required for endosomal localization of EEA1 [22]. The N-terminal and C-terminal domains of EEA1 are separated by >1000 residues that are predicted to form a continuous coiled-coil structure of ~160–180 nm and to mediate dimerization of EEA1 [23]. The homodimeric quaternary structure explains the robust membrane recruitment of EEA1 as it allows the membrane interactions of two FYVE domains to multiply in affinity (Figure 1b). By containing two endosomal binding sites at the ends of a rigid rod, EEA1 has been suggested to tether early endosomes in preparation of their fusion [23].

Rabenosyn-5 also contains a C2H2 Zn-finger domain that interacts with Rab5 [20] and a FYVE domain that binds PI-3-P, both located in the N-terminus of the protein [24]. At the C-terminus, a helical domain represents a further binding site for Rab5 [25] (Figure 1c). In contrast to EEA1, Rabenosyn-5 is a monomeric protein, but the endosome interaction sites at the opposing ends of the protein would also allow Rabenosyn-5 to act as a tether. Importantly, Rabenosyn-5 forms a complex with Vps45 [24], the SM protein required for early endosome fusion, and thereby links the Rab and SNARE machinery [26].

A third essential effector of Rab5 is the coiled-coil protein Rabaptin-5 [27,28] (Figure 1d). It forms a complex with Rab5, the GEF for Rab5, and thus promotes the activation of Rab5 [27]. Although Rabex-5 alone has some affinity for endosomal membranes and nucleotide exchange activity towards Rab5, the Rabaptin-5/Rabex-5 complex is much more potent [29]. Rabaptin-5 binds to an autoinhibitory helix of Rabex-5 and thus activates Rabex-5 [30]. The result is the amplification of a primary Rab5 activation event through a positive feedback loop.

Interestingly, even though each of the coiled-coil tethering factors has binding sites for Rab5, only the combination of Rabenosyn-5, EEA1 and Rabaptin-5 was
sufficient to reconstitute membrane fusion in vitro [17**]. This suggests that successful fusion requires an organization that integrates Rab-driven membrane tethering with SM protein and SNARE function. Along those lines, the more simple yeast machinery contains no EEA1 or Rabaptin-5, but Vac1 (corresponding to Rabenosyn-5) which binds Rab, PI-3-P and recruits an SM protein [31,32*,33*] and thus fulfills the same core requirements.

Tethering via the Class C complexes at endosomes and lysosomes

In addition to the tethering machinery of the early endosome, two large tethering complexes have been characterized in the endolysosomal system in yeast and partially also in metazoans. The CORVET (class C core endosome vacuole tethering) and the HOPS (homotypic vacuole fusion and protein sorting) complexes function at the EE, LE and vacuole in yeast. They will be the main focus of this discussion [34–36]. Both complexes share a common core of four subunits (Vps11, 16, 18, 33) and contain two Rab-specific subunits. CORVET has Vps3 and Vps8, via which it binds the Rab5-like Vps21 protein [37*,38,39*,40–42], whereas the better characterized HOPS complex has Vps41 and Vps39 as specific subunits for the Rab7-like Ypt7 protein [41,43*,44*,45**]. In each complex, five of the six subunits (with the exception of Vps33) have a similar predicted structure consisting of N-terminal β-propeller and C-terminal α-solennoid domains. In some subunits, additional RING domains have been identified [34]. Localization of both complexes requires the interaction with their respective Rab GTPase [45**,46]. Furthermore, in yeast both complexes share the SM protein Vps33 as a sixth subunit and can thus bind SNAREs [47]. Of note, metazoans encode two Vps33 and two Vps16 subunits (a and b), suggesting that the metazoan CORVET and HOPS have further diversified with respect to their SNARE interactions (discussed in [36]).

CORVET likely supports tethering and homotypic fusion of EE via Vps21 [37*,39*], possibly downstream of the Vac1 tether at the yeast EE [46]. On the vacuole, the HOPS complex represents the major regulatory fusion factor that is required for the fusion of Golgi-derived vesicles, endosomes, and autophagosomes with the vacuole as well as homotypic vacuole fusion [43*,44*,48,49]. HOPS is essential for the in vitro fusion of proteoliposomes containing the vacuolar SNAREs, either in the absence or presence of the Rab7-like Ypt7 protein [15**,16**]. The difference is likely due to the inherent affinity of HOPS for phosphoinositides, membranes and SNAREs, which can be bypassed by altering the lipid composition [16**].

Insights into the function of HOPS in membrane fusion were obtained by the analysis of its molecular architecture [45**] (Figure 2a). The electron microscopy (EM) structure revealed that the Rab-specific subunits reside in the large (Vps41) and small (Vps39) portions of a seahorse-like structure, whereas the SNARE-binding Vps33 subunit was found next to Vps41 [45**]. Recently, the structure of Vps33 in complex with a fragment of Vps16 from the fungus Chaetomium thermophilum and humans has been solved [50*,51*] (Figure 2b). It shows a prominent cleft that is found in all SM proteins crystallized so far and supposedly represents a SNARE interaction site. Interestingly, a second SNARE binding site found for some family members, like Munc18, is absent in Vps33. The crystallized C-terminal fragment of Vps16 forms the predicted α-solennoid structure and binds on the outside of Vps33 [50*,51*]. In the context of HOPS Vps33 might be wrapped by the α-solennoid domain of Vps16 and thus anchored to the complex, but leaving the cleft of Vps33 exposed. Such a position would be in agreement with the position of Vps33 in the EM structure. Furthermore, both Rab-specific subunits can interact with activated Ypt7, suggesting that HOPS can bridge two Ypt7-positive membranes. Taking into account that Vps33 is localized proximal to Vps41 in the large head, we speculate that Rab binding and SNARE chaperoning is coordinated during HOPS-dependent fusion. As CORVET likely has a similar architecture, it may function similarly [37*].

Tether function during fusion

The work by the Wickner and Zerial laboratories has identified the core components of the fusion machinery for yeast vacuole fusion and mammalian endosome fusion [16**,17**]. By comparing the findings from two fusion processes in two different model organisms and taking in...
In vivo observations into account, conserved components as well as organizational and functional principles of membrane fusion can be identified (Figure 1). As noted before [52], every intracellular trafficking step requires a set of core factors: one R-SNARE, three Q-SNAREs, an SM protein and a Rab GTPase. Furthermore, NSF/αSNAP are required for the regeneration of SNAREs for multiple rounds of fusion and to recycle them from non-productive cis-SNARE complexes.

Initially, targeting and activation of Rabs are needed to mark the site of fusion. This is achieved by membrane-localized GEFs (Rabaptin-5–Rabex-5, Mon1–Ccz1) [27,53,54], which themselves are likely recruited by an upstream Rab as part of Rab cascades and membrane interaction motifs [9,55]. Together with specific lipids, GTP-bound Rab5 will then recruit effectors, which bind multivalent membrane-interacting proteins (EEA1) and SM protein-containing complexes (Rabenosyn-5/Vps45). CORVET and HOPS combine both features in a single tethering complex. The assembled Rab machinery will thereby set the stage for SNARE engagement and fusion, thus contributing to the specificity and efficiency of intracellular transport.

In the light of these observations, how can tethering best be defined? We suggest that the requirement for these factors could be the result of ‘direct’ as well as ‘indirect’ tethering. In this context it is important to note that the initial docking of endosomes has been shown to be independent of SNARE function [56**]. Thus, direct tethering would be equivalent to docking and correspond to the classical definition of tethering, namely the ability of a protein to bridge two membranes [11,12]. EEA1 and

---

Figure 3

Models representing the essential components of the Rab and SNARE machineries involved in (a) homotypic fusion of early endosomes and (b) fusion of vacuoles. Rab GTPases, activated by their cognate GEFs (guanine nucleotide exchange factors) and PI-3-P bind tethers, which bridge opposing membranes and recruit the SM protein, thus representing a link to SNAREs. Whether the Rab and SNARE machineries act simultaneously or consecutively remains unknown.
Rabenosyn-5 would be considered direct tethers, by binding to Rab5 and PI-3-P on one and again Rab5 on the other membrane (Figure 3). HOPS, containing two Ypt7 binding sites, can tether Ypt7-positive liposomes [57**], and CORVET might act similarly during tethering. In both endosome and vacuole fusion, the Rab recruits the respective SM protein via a tether/activity. Although the underlying molecular mechanism is unknown, SM proteins have been described to stimulate SNARE fusion and thus support SNARE assembly [8,58]. This may result in a more stable attachment of opposing membranes and might be considered indirect tethering (Figure 3). The acceleration of fusion by SM proteins, which link the Rab and the SNARE machinery, could also explain in part why tethers are required under physiological conditions.

Whether these principles are also applicable to other intracellular fusion processes will require reconstitution of the Rab and tethering machineries in vitro. However, it is interesting to note that the CATCHR complexes, the most widely distributed family of tethers, could act in a functionally similar way: interactions with lipids and Rab GTPases on opposing membranes have been reported as well as binding to SM proteins.

Acknowledgement

The authors thank Siegfried Engelbrecht-Vandré for critical feedback. Research in the authors’ laboratories is funded by the Deutsche Forschungsgemeinschaft [SFB 944, P11, and UN1115-3 (C.U.)] and KU2537/2-1 (D.K.). C.U. is also supported by the Hans-Müller-Hohenfeld Foundation and State of Lower-Saxony, Hannover, Germany.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest


The authors provided a structural explanation on the effect of Rabaptin-5 on the activation of Rabex-5 as the GEF of Rab5.


This study and Ref. [33] provided the first link of the SM protein Vps45p to early endosome fusion.


see Ref. 32.


The study demonstrates that CORVET can drive tethering and fusion of Rab5p/Vps21p positive membranes.


The authors identified CORVET as a homologous tethering complex of HOPS.


Together with Ref. [44] first demonstration of HOPS as a hexameric complex.


See annotation to Ref. [43].


This electron microscopy-based study revealed HOPS as an elongated particle with two Ypt7-binding sites at opposite ends. It provides a molecular explanation of HOPS function during tethering and fusion.


High resolution structure of the HOPS component Vps33 in complex with a fragment of Vps16, which also provides in vivo evidence for the role of the Vps16-Vps33 interaction.


X-ray structure of the HOPS subunits Vps33 in complex with the C-terminus of Vps16.


This work demonstrates that docking of early endosomes depends on tethers, but not SNARE proteins.


The formal proof that HOPS is able to cluster vacuoles and thus acts as a tether.