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Principles of membrane tethering and fusion in endosome and lysosome biogenesis

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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.

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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

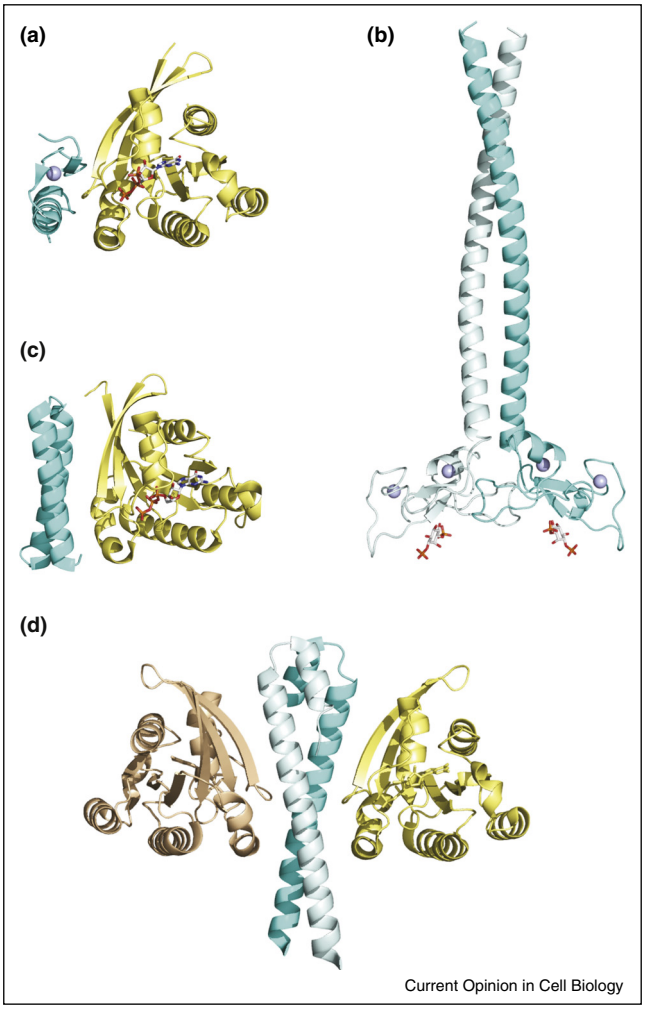
Fusion of early endosomes in mammalian cells is regulated by Rab5 — and driven by the R-SNARE VAMP4 and the Q-SNAREs Syntaxin13, Vti1A and Syntaxin6 [18,19]. In reconstitution experiments with synthetic endosomes it could be shown that in addition to activated Rab5 and disassembled SNAREs, phosphoinositol-3-phospate (PI-3-P) and two Rab effectors are needed for efficient fusion: EEA1 (early endosome antigene 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 C_2H_2 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 in length and mediate dimerization of EEA1 [23]. The homodimeric quarternary 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 C_2H_2 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

Figure 1



Gallery of tethering domains at early endosomes. **(a)** The C2H2 Zn-finger domain of EEA1 (cyan) in complex with Rab5 (yellow) (PDBID: 3MJH). **(b)** Structure of the EEA1 C-terminus, containing the membrane interacting FYVE domain and the adjacent coiled-coil dimerization region (PDBID: 1JOC). **(c)** Effector complex between Rab22 (yellow) and the helical domain from Rabenosyn-5 C-terminus (cyan) (PDBID: 1Z0J). Rab22 has strong homology to Rab5. **(d)** A portion of the Rabaptin-5 coiled-coil dimer (cyan) binds two GTP-bound Rab5 molecules (yellow and brown) (PBDID: 1TU3). Zn^{2+} ions are shown as spheres, GTP and PI-3-P in stick representation.

Rabex-5, 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

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