

# Retromer: A Master Conductor of Endosome Sorting

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The endosomal network comprises an interconnected network of membranous compartments whose primary function is to receive, dissociate, and sort cargo that originates from the plasma membrane and the biosynthetic pathway. A major challenge in cell biology is to achieve a thorough molecular description of how this network operates, and in so doing, how defects contribute to the etiology and pathology of human disease. We discuss the increasing body of evidence that implicates an ancient evolutionary conserved complex, termed “retromer,” as a master conductor in the complex orchestration of multiple cargo-sorting events within the endosomal network.

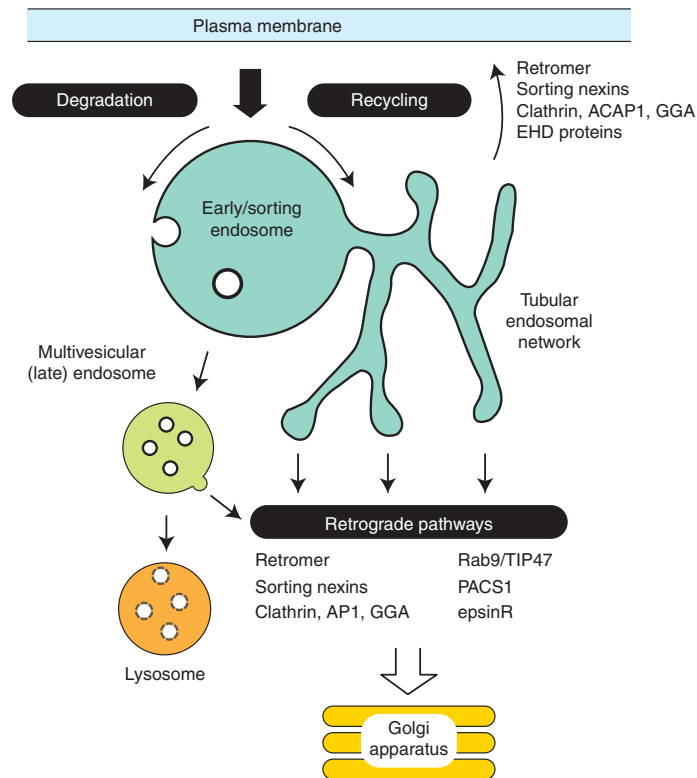
On entering the endosomal network, integral membrane protein cargoes have two fates: either they are sorted from the limiting endosomal membrane into intraluminal vesicles for delivery into the lysosomal degradative pathway (Fig. 1), or they are exported from the endosome via transport carriers that bud from the endosome membrane into the cytoplasm. The endosome export pathways ultimately deliver cargo to the *trans*-Golgi network (TGN) via retrograde pathways, or to the plasma membrane via recycling pathways (Huotari and Helenius 2011). The principal cargoes of the retrograde pathways are sorting receptors, SNAREs, and other molecules whose functions depend on continual retrieval from the endosomal system back to the biosynthetic pathway (Bonifacino and Rojas 2006; Johannes and Popoff 2008;

Burd 2011). Plasma membrane recycling cargoes are especially diverse in structure, as they include numerous nutrient transporters, mitogenic signaling receptors, and cell adhesion receptors (Maxfield and McGraw 2004; Hsu et al. 2012). Although much has been learned regarding signals and sorting mechanisms that confer sorting into the degradative pathway, these aspects of endosome export pathways are poorly understood. There are therefore significant gaps in our understanding of how the endosomal system is integrated with cell physiology and the development of multicellular organisms, and how deficiencies in endosomal sorting contribute to human disease. Here, rather than give a broad overview of the field (we refer the interested reader to many excellent recent reviews: Grant and Donaldson 2009; Henne et al. 2011;

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**Figure 1.** Overview of endosomal sorting pathways. Cargo in the endosome is sorted either into the degradative pathway, leading to its eventual delivery to the lysosome, or into an export pathway that returns it to another organelle for reuse. Recycling export pathways deliver cargo to the plasma membrane, and retrograde export pathways return cargo to the *trans*-Golgi network. Sorting devices that act in each of these export pathways are listed. Transport carriers of the export pathways bud from the tubular endosomal network, whereas sorting into the lysosomal degradation pathway results in packaging of cargo into vesicles that bud into the lumen of multivesicular endosomes.

Huotari and Helenius 2011; Johannes and Wunder 2011; Hsu et al. 2012), we discuss the role of an evolutionary conserved complex, termed “retromer,” that plays a key role in exporting cargo from the endosomal system and is increasingly implicated in many other facets of endosome function.

### CARGO EXPORT FROM THE ENDOSOME

Early studies of endocytosis and lysosomal turnover indicated that the turnover rates for cell-surface receptors and their ligands vary substantially, with that of transmembrane receptors being relatively long-lived, and ligands short-lived. This led to the discovery of plasma mem-

brane recycling pathways, whereby internalized receptor–ligand complexes dissociate in the acidic lumen of the endosome and the receptor then exits the endosome with the bulk flow of membrane lipids that are returned to the plasma membrane (Maxfield and McGraw 2004; Hsu et al. 2012). For the plasma membrane recycling of the transferrin receptor (TfnR), one of the most intensively investigated recycling cargoes, initial ideas evoked a sorting signal-independent model in which iterative formation of an extensive network of endosomal tubules ensured that bulk membrane flow occurred through exit from the endocytic network (Mayor et al. 1993). Central to this proposal was the geometric consideration that the relatively large

ratio of membrane surface area-to-luminal volume of the tubular endosomal network (TEN), when compared with the vacuolar domain of the endosome, effectively serves to segregate integral membrane proteins from luminal content by default (Maxfield and McGraw 2004). It is now clear, however, that the majority of endocytic recycling is mediated in a signal-dependent manner through the engagement of specific sorting signals by a variety of sorting devices (Hsu et al. 2012). Signal-mediated sorting ensures robust endosome function and allows for detailed regulation in response to changes in the cellular environment.

Endosomal cargo export pathways originate from multiple, functionally distinct gateways along the entire endosome maturation pathway. Plasma membrane recycling is mediated by clathrin-containing coat complexes, including clathrin-ACAP1 (that recognizes LE, RE, KR, and PLSLL sequences) and clathrin-GGA3 (Prorich motifs), that recognize cell adhesion receptors and nutrient transporters (Dai et al. 2004; Li et al. 2007; Parachoniak et al. 2011). Similarly, other, less well described sorting factors such as sorting nexin-17 (SNX17) (NPxY, NxxY, and NxxF sequences), EHD proteins (NPF motif), and sorting nexin-27 (SNX27) (PDZ ligands) also contribute to plasma membrane recycling (Burden et al. 2004; Joubert et al. 2004; Braun et al. 2005; van Kerkhof et al. 2005). Endosome-to-TGN retrograde sorting is similarly complex, with contributions by clathrin in conjunction with AP1, PACS1, and epsinR (Meyer et al. 2000; Crump et al. 2001; Saint-Pol et al. 2004; Johannes and Popoff 2008; Shiba et al. 2010; Johannes and Wunder 2011). In addition, export of the mannose-6-phosphate receptor from the late endosome appears to be regulated by the TIP47/Rab9 assembly (Lombardi et al. 1993; Díaz and Pfeffer 1998; Carroll et al. 2001; also see Bulankina et al. 2009).

### THE RETROMER COMPLEX

Pioneering studies of protein sorting in the yeast endolysosomal system led to the identification of an endosomal coat protein complex named

“retromer” that was found to be required for retrieval of a TGN sorting receptor (Vps10) from the endosome to the TGN (Seaman et al. 1998). The initial biochemical characterization of yeast retromer showed that it is composed of five proteins that can be dissociated into two subcomplexes (Horazdovsky et al. 1997; Seaman et al. 1997, 1998). One subcomplex contains a heterodimer of the Vps5 and Vps17 sorting nexins (SNX), which possess Bin/Amphiphysin/Rvs (BAR) domains (SNX-BAR proteins) that can induce and/or sense the formation of membrane tubules (Carlton et al. 2004; van Weering et al. 2012a). The other retromer subunits constitute a trimeric complex of Vps26, Vps29, and Vps35 that does not have intrinsic membrane-binding activity and relies on association with the Vps5-Vps17 for endosome recruitment (Seaman et al. 1998; Haft et al. 2000; Liu et al. 2012), or, independent of Vps5-Vps17, on association with the Rab7 ortholog, Ypt7, for recruitment to the vacuole membrane (Liu et al. 2012). The VPS26:VPS29:VPS35 heterotrimer has been shown to recognize cargo proteins and is therefore termed the cargo-selective complex (abbreviated “CSC”) (Seaman et al. 1998; Nothwehr et al. 2000; Norwood et al. 2011; Fjorback et al. 2012).

Whereas there is considerable divergence of sorting nexin proteins between species, CSC is an ancient protein assembly that emerged before the last common eukaryotic ancestor and has been strikingly conserved throughout eukaryotic evolution (Koumandou et al. 2011). Hence, the CSC trimer is considered to constitute the core functional component of retromer. Structural and sequence-based analysis predicts that human VPS35 is composed of 17 two-helix repeats that fold into an  $\alpha$ -solenoid that is typical of vesicle coat proteins (Hierro et al. 2007). Mammals express two Vps26 orthologs, VPS26A and VPS26B (Kerr et al. 2005), that possess an arrestin fold and bind to the highly conserved amino-terminal region of VPS35 (Shi et al. 2006; Collins et al. 2008). VPS29 possesses a metallophosphoesterase fold (Collins et al. 2005; Wang et al. 2005) and it binds to the carboxy-terminal portion of VPS35 (Collins et al. 2008; Norwood et al. 2011) where it is

proposed to scaffold the helical solenoid of VPS35. Very little is known regarding the manner by which CSC recognizes cargo. Most retromer cargoes possess at least one simple hydrophobic motif, F/W-L-M/V, required for retromer-dependent sorting (Seaman 2007), and Vps35 has long been considered to provide the sole interface for cargo recognition. However, very recently, VPS26 was shown to bind a FANSHY sorting signal in the cytoplasmic domain of sorLA (Fjorback et al. 2012), and other proteins that associate with CSC, including sorting nexin-3 (SNX3), SNX27, and the retromer SNX-BARs, have been implicated in cargo recognition (Parks et al. 2001; Heydorn et al. 2004; Strohlic et al. 2007; Temkin et al. 2011; Steinberg et al. 2013). It is now appreciated that there exist multiple direct and indirect mechanisms by which CSC recognizes cargo.

#### RETROMER AND THE ENDOSOME MATURATION PATHWAY

By promoting the export of cargo from the endosome, retromer serves to “rescue” proteins from lysosome-mediated turnover and, hence, retromer function is intrinsically coupled to the endosome maturation pathway. Endosomal maturation itself is defined by an increasing number of intraluminal vesicles as the early endosome matures into the late endosome/multivesicular body, an increase in luminal acidification, endosome movement from the cell periphery toward a juxtannuclear localization, and the switching of two key endosome identity cues (Jean and Kiger 2012): the Rab5-positive, phosphatidylinositol-3-monophosphate (PtdIns(3)P)-enriched early endosome switches to become a Rab7-positive, phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P<sub>2</sub>)-enriched late endosome (Huotari and Helenius 2011).

The CSC does not itself bind to endosomes; instead, association to early and maturing late endosomes occurs through a variety of indirect mechanisms. The specific binding of SNX3 to PtdIns(3)P targets the CSC to early endosomes (Harterink et al. 2011; Vardarajan et al. 2012), whereas the ability of VPS35 to bind Rab7-GTP associates the CSC with late endosomes

(Nakada-Tsukui et al. 2005; Rojas et al. 2008; Seaman et al. 2009; Balderhaar et al. 2010; Liu et al. 2012; Zelazny et al. 2013). Rab7 (Ypt7 in yeast) is a principal regulator of late endosome dynamics, and the interaction with VPS35 appears to serve a dual function in recruiting and/or stabilizing CSC on the endosome membrane and in coordinating the timing of cargo export with endosome maturation. In yeast cells, depletion of Ypt7-GTP results in a deficiency in cargo export, even though CSC is assembled with SNX-BARs on the endosome membrane, suggesting that Ypt7 potentiates retromer-mediated cargo export (Liu et al. 2012). In further support for a role in Rab7 regulating retromer activity, in mammalian cells SNX-BAR-coated tubules bud predominantly from endosomes that have recently acquired Rab7 via Rab conversion (van Weering et al. 2012b). In yeast, CSC has been shown to inhibit Ypt7-regulated fusion of late endolysosomal organelles, suggesting that it can also influence endosome maturation indirectly by modulating the amount of Ypt7-GTP (Liu et al. 2012). In mammalian cells, CSC limits Rab7 signaling through the recruitment of TBC1D5, a potential Rab7 GAP (Mukhopadhyay et al. 2007), to the endosome via direct binding to Vps29 (Seaman et al. 2009; Harbour et al. 2010). Interestingly, TBC1D5 also directly associates with the small ubiquitin-like protein LC3, a marker for autophagic membranes (Kabeya et al. 2000) and during starvation-induced autophagy TBC1D5 is relocalized from endosomes to LC3-positive autophagosomes (Popovic et al. 2012). As Rab7-GTP is required for autophagosome maturation, it is tempting to speculate that competitive binding of TBC1D5 to CSC or LC3 might serve to reprogram endosomal sorting to accommodate the ensuing autophagic flux to the lysosome.

#### THE DIVERSITY OF RETROMER FUNCTION IN CELL FUNCTION AND ORGANISM PHYSIOLOGY

Biochemical and genetic studies have identified a wide array of cargoes and cellular processes that require the CSC including the establishment of cell polarity through trafficking of

Crumbs and Scribble (Pocha et al. 2011; Zhou et al. 2011; Lohia et al. 2012), regulation of the morphology and geometry of epithelial tubes during *Drosophila* development through transport of Serpentine (Dong et al. 2013), formation of Wnt morphogenic gradients through transport of the Wnt sorting receptor Wntless (Belenkaya et al. 2008; Franch-Marro et al. 2008; Pan et al. 2008; Port et al. 2008; Yang et al. 2008), transcytosis of the polymeric immunoglobulin receptor (Vergés et al. 2004), apoptotic cell clearance through sorting of the *Caenorhabditis elegans* phagocytic receptor CED-1 (Chen et al. 2010), cell polarity and organ initiation through trafficking of the *Arabidopsis thaliana* PIN auxin efflux carriers (Jaillais et al. 2007), and mitochondria to peroxisome transport through association with the mitochondrial-anchored protein ligase MAPL (Braschi et al. 2010). Moreover, there is increasing realization that perturbed CSC function is linked to a number of human diseases. CSC deficiency plays a role in nonamyloidogenic versus amyloidogenic processing of amyloid precursor protein (APP) (He et al. 2005; Small et al. 2005; Muhammad et al. 2008; Wen et al. 2011; Choy et al. 2012) and the pathogenesis of Alzheimer's disease (Siegenthaler and Rajendran 2012). In addition, a specific VPS35 mutation, Asp620Asn, has been linked to late-onset autosomal-dominant familial Parkinson's disease (Vilariño-Güell et al. 2011; Zimprich et al. 2011), the loci for human VPS26A has been genetically associated with type 2 diabetes in South Asians (Kooner et al. 2011), and the endosomal transport of various pathogens (*Salmonella*, Herpesvirus saimiri, *Coxiella burnetii*, human papillomavirus, *Legionella pneumophila*) and toxins (Shiga toxin) is also mediated in part by the CSC (Bujny et al. 2007, 2008; Popoff et al. 2007; Kingston et al. 2011; Finsel et al. 2013; Lipovsky et al. 2013; McDonough et al. 2013).

### FUNCTIONALLY DISTINCT RETROMER COMPLEXES

It is now appreciated that retromer CSC functions as a component of multiple distinct sorting devices. The first indication of the diversity

of retromer in multicellular organisms came from a biochemical and genetic study of the role of retromer in regulating the retrograde endosome-to-TGN transport of Wntless, and hence the formation of Wnt morphogenic gradients in *C. elegans* and *Drosophila* (Belenkaya et al. 2008; Franch-Marro et al. 2008; Pan et al. 2008; Port et al. 2008; Yang et al. 2008). Here the functional retromer is composed of the direct binding of the CSC to the early endosome associated non-BAR domain-containing SNX3, an association that is independent of the SNX-BAR retromer subunits (Harterink et al. 2011; Zhang et al. 2011). Genetic loss of SNX3 or CSC in *C. elegans* and *Drosophila*, leads to the mis-sorting of internalized Wntless into the lysosomal degradative pathway (this is also observed in mammalian cell culture upon RNAi-mediated suppression), a reduction in the steady-state level of Wntless, and a defect in Wnt secretion and hence Wnt gradient formation (Harterink et al. 2011). Thus, although the SNX3 retromer and SNX-BAR retromer both mediate endosome-to-TGN transport, they constitute distinct, cargo-specific pathways. Indeed, in *C. elegans*, the retromer-mediated sorting of CED-1 is SNX-BAR-retromer dependent but is independent of the SNX3 retromer (Chen et al. 2010; Harterink et al. 2011; Lu et al. 2011). Furthermore, the morphological profile of each transport carrier is distinct: SNX-BAR retromer residing on nonbranched tubules 170–230 nm in length and 20–50 nm in diameter (Mari et al. 2008), whereas SNX3 retromer is associated with small clathrin-coated vesicular profiles (Harterink et al. 2011). These results indicate that there are distinct forms of retromer that are defined via CSC associations with different sorting nexins and that these retromers act on distinct sets of cargo.

Besides its well-established roles in endosome-to-TGN trafficking, it has recently emerged that retromer also plays a central role in endosome-to-plasma membrane recycling (Feinstein et al. 2011; Temkin et al. 2011). In mammalian cells, the endosomal recycling of the  $\beta$ 2-adrenergic receptor ( $\beta$ 2-AR) is dependent on the presence of a carboxy-terminal PDZ ligand that acts as a sorting signal for direct

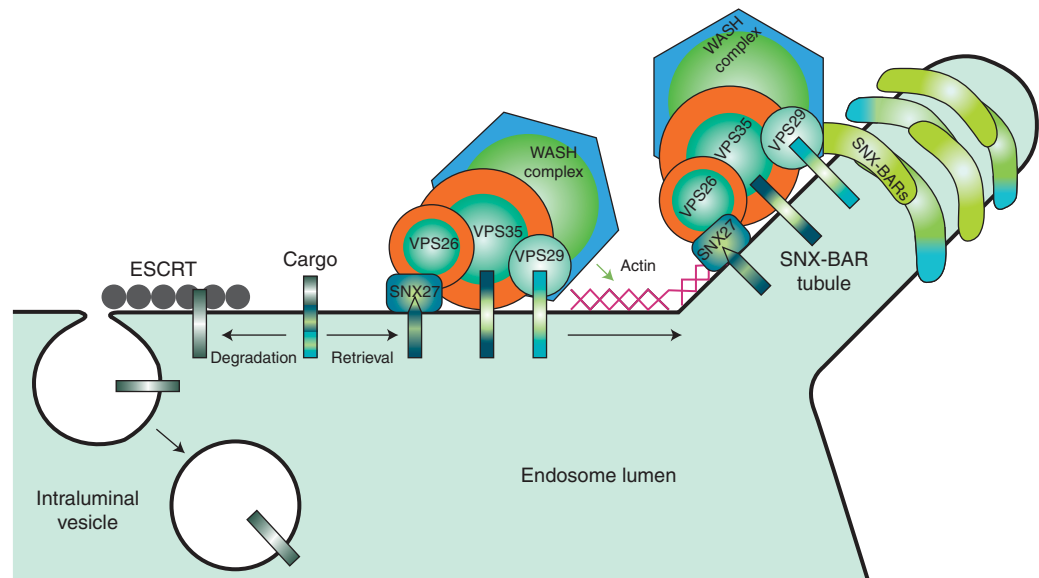
recycling to the plasma membrane (Cao et al. 1999). The PDZ domain of SNX27 binds directly to this signal, and by engaging the CSC mediates  $\beta$ 2-AR recycling through SNX-BAR-retromer decorated tubules (Lauffer et al. 2010; Temkin et al. 2011). Indeed, the CSC constitutes a major endosome recycling hub. In HeLa cells, the endosome-to-plasma membrane recycling of >150 transmembrane spanning proteins would appear to be dependent on the CSC (Steinberg et al. 2013). Of these, >70 cargos require the presence of SNX27 for their recycling, many of which contain PDZ ligands, whereas others contain NPxY/NxxY sorting motifs (Steinberg et al. 2013). Because SNX27 harbors a functional FERM-like domain that can engage NPxY/NxxY-based sorting signals (Ghai et al. 2013), the cargo adaptor function of SNX27 in retromer-mediated recycling appears to embrace both PDZ ligand and NPxY/NxxY sorting motif recognition. Like the situation with the SNX3 retromer, loss of SNX27 or CSC leads to cargo missorting into lysosomal-mediated degradation (Temkin et al. 2011; Steinberg et al. 2013). This reinforces that a primary role of the CSC, and its associated cargo adaptors, is to prevent missorting of selected cargos into the lysosomal degradative pathway.

Interestingly, retromer-mediated recycling of the  $\beta$ 2-AR occurs through a specialized actin-stabilized tubular subdomain (Puthenveedu et al. 2010). Consistent with this, SNX27 lies at the core of a multiprotein complex defined by the binding to retromer, and the independent association with the retromer SNX-BARs and the Wiskott-Aldrich syndrome protein and SCAR homolog (WASH) (Temkin et al. 2011; Steinberg et al. 2013), which by regulating the activity of the Arp2/3 complex mediates the formation of branched actin filaments on the endosomal network (Derivery et al. 2009; Gomez and Billadeau 2009). Given the specific function of each individual component of this multiprotein SNX27-containing complex, one intriguing question is: How does retromer coordinate cargo capture and enrichment with the extensive membrane remodeling that is required to generate a cargo-enriched transport carrier?

## COUPLING RETROMER SORTING TO CARGO EXPORT

Export of cargo from the endosome by SNX-BAR retromer is initiated by the capture of cargo by CSC and other associated adaptors on the vacuolar portion of the endosome thereby preventing their entry into the lysosomal degradative pathway. A key consideration for understanding CSC function is that the functional units of membranes of the endocytic system are specialized microdomains, defined as assemblies of cargo proteins, lipids, and peripherally associated proteins, that partition into discrete networks within the plane of the membrane (Huotari and Helenius 2011). We speculate that CSC defines a subdomain(s) within the endosome membrane that promotes the efficient capture and enrichment of cargo, preventing their entry into the lysosomal degradative pathway and preparing them for subsequent inclusion into the TEN, or for the case of SNX3 retromer, vesicular transport carriers (Fig. 2). Cargo capture takes place on the vacuolar portion of the endosome, which is a region that is essentially flat (at the molecular scale), and subsequent events direct captured cargo into tubules of high relative membrane curvature that are coated with SNX-BAR retromer. Central to the formation of the TEN is the intrinsic ability of a subset of SNX-BAR proteins to self-assemble via interactions involving the BAR domain that can drive and/or stabilize the formation of membrane tubules (Carlton et al. 2004; Wassmer et al. 2007, 2009; van Weering et al. 2012a). In this respect, SNX-BAR proteins constitute an endosomal coat complex (Attar and Cullen 2010; Cullen and Korswagen 2012; Seaman 2012) and a major challenge for the field is to elucidate the mechanism(s) by which CSC orchestrates cargo capture and enrichment with packaging into the formation of these tubular transport carriers.

Although retromer function opposes sorting into the degradative lysosomal pathway, the emerging principles of degradative sorting may be instructive for understanding CSC-dependent packaging of cargo into the TEN. In the degradative pathway, cargo is sorted into vesi-

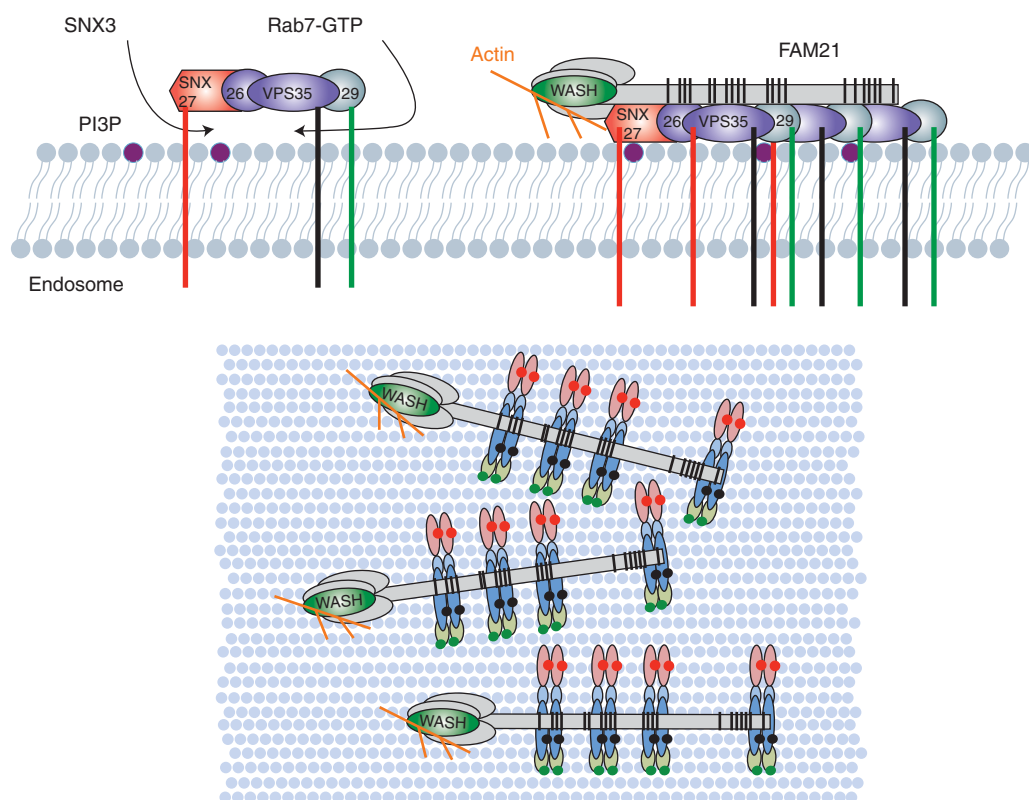


**Figure 2.** SNX-BAR-retromer-mediated cargo exit from lysosomal-mediated degradation. The VPS26:VPS29:VPS35 CSC engage cargo in a signal-dependent manner. Through WASH-mediated actin polymerization a cargo-enriched endosome subdomain is assembled thereby avoiding ESCRT-mediated sorting into forming intraluminal vesicles. Subsequent transport into recycling pathways back to the plasma membrane or retrograde transport to the TGN is mediated through retromer SNX-BAR-mediated tubule formation. Actin polymerization may aid further membrane remodeling and the efficiency of tubule scission to form a cargo-enriched tubular transport carrier.

cles that bud into the lumen of the endosome. Central to our understanding of sorting into intraluminal vesicles is the notion that cargo is first captured in a signal-dependent manner and enriched within a subdomain of the endosomal limiting membrane from where the generation of negative membrane curvature (i.e., bending the endosomal membrane away from the cytosol) occurs concomitantly with the cargo entering intraluminal vesicles (ILVs) (Hurley and Hanson 2010; Henne et al. 2011). Degradative cargo is captured by a cargo-sorting receptor termed “endosomal sorting complex required for transport-0 (ESCRT-0)” and their lateral mobility restricted within an endosomal subdomain defined by ESCRT-0 (Wollert and Hurley 2010) and the formation of a clathrin bilayer coat (Sachse et al. 2002). Subsequently, ESCRT-III promotes the budding of the ILV (Teis et al. 2010; Wollert and Hurley 2010; Henne et al. 2012). Unlike cytoplasmic transport vesicles, ILVs are not surrounded by a pro-

teinaceous coat, implying that once cargo is captured, it enters the forming ILV by default. The insights from endosomal sorting complex required for transport (ESCRT)-dependent sorting point to the key function of establishing a domain of entrapped cargo that serves to direct it into the budding transport carrier. CSC serves a similar function to ESCRT-0 in the signal-dependent capture of cargo that is subsequently transferred into a tubule/vesicle that buds from the endosome into the cytoplasm. In this view, the cargo burden of the transport carrier is defined chiefly by CSC and its associated adaptors (Fig. 3), rather than the specific sorting nexin that functions in sculpting the transport carrier.

How does CSC mediate cargo sorting? One possibility is that it captures and concentrates cargo via a multivalent binding site to CSC proteins. Clear evidence for this is presently lacking, but it is in line with the lack of robust oligomerization of CSC in solution (Norwood



**Figure 3.** A pictorial representation of the proposed CSC retrieval subdomain on the endosomal surface (based on data from Jia et al. 2012). The CSC is associated with the cytosolic face of the early endosome and the maturing late endosome through association with the phosphatidylinositol 3-monophosphate (PI3P)-binding SNX3 and Rab7-GTP, respectively. In addition to cargo selection mediated by the CSC, associated adaptors also engage cargo (the PI3P-binding SNX27 is depicted). Through association of the unstructured tail of FAM21, multiple CSCs, along with their associated cargoes, become enriched into a retrieval subdomain (retrieval zone) in which lateral mobility is restricted as a result of WASH-mediated actin polymerization. In the lower bird's-eye view, two associated CSCs are shown to reflect evidence for dimeric assemblies (Norwood et al. 2011).

et al. 2011). Emerging evidence suggests that actin dynamics on the endosome membrane, regulated by CSC, is a key factor that influences retromer-dependent sorting (Gomez and Billadeau 2009). The WASH complex is formed from the assembly of five proteins: strumpellin, FAM21, SWIP, cdc53, and WASH (Derivery and Gautreau 2010). It is recruited to the endosome membrane through a complex mechanism that in part relies on the binding to the VPS35 CSC subunit (Gomez and Billadeau 2009; Harbour et al. 2010, 2012; Derivery et al. 2012; Jia et al. 2012; Helfer et al. 2013; Park et al. 2013). On the endosome membrane, the WASH

complex activates the Arp2/3 complex, which mediates the nucleation of actin branching to a preexisting actin filament (Rotty et al. 2013). In cultured cells, loss of WASH results in collapse of the endolysosomal system into enlarged endosome-like compartments in which degradative cargo, recycling cargo, retrograde cargo, and lysosomal proteins accumulate (i.e., a mis-sorting phenotype) (Duleh and Welch 2010; Derivery et al. 2012; Gomez et al. 2012; Piotrowski et al. 2013). Interestingly, the carboxy-terminal region of FAM21 possesses 21 copies of a motif distributed over nearly 1000 unstructured amino acids that is recognized by VPS35 (Jia



et al. 2012). The multivalent nature of this interaction (i.e., one FAM21 can associate with multiple copies of CSC) has been suggested to effectively sense the density of CSC and associated cargo adaptors (and hence the density of captured cargo) thereby coordinating cargo capture and enrichment with the formation of a branched actin network that restricts lateral diffusion of the enriched cargo into other sorting pathways (Fig. 3) (Jia et al. 2012). The presence of actin subdomains on the endosome membrane is consistent with such a cargo “retrieval zone” (Sachse et al. 2002; Derivery et al. 2012), akin to the degradative sorting zone formed by ESCRT proteins. Whether these retrieval zones also act to modify the posttranslational status of captured cargo (e.g., ubiquitination and/or phosphorylation), is an interesting open question. Indeed, in higher metazoans VPS35 binds the E3 RING ubiquitin ligase, MAGE-L2-TRIM27, an interaction that localizes this enzyme to endosomes, and through K63-linked ubiquitination of WASH at lysine 220 relieves an autoinhibited WASH conformation leading to activation of endosomal actin polymerization (Hao et al. 2013). Loss of WASH also results in elongated SNX-BAR tubules, raising the possibility that a localized activation of WASH results in a burst of actin polymerization, spatially localized with the retrieval zone, that contributes to the efficiency of fission of sorting tubules coated with filamentous actin (Derivery et al. 2009; Gomez and Billadeau 2009; Puthenveedu et al. 2010). Clearly, understanding the interrelationship between retromer, cargo, and the activity of the WASH complex in regulating the dynamics of actin turnover is sure to generate exciting insight into the formation of sorting subdomains on the endosome membrane.

### CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Although significant research will be required to further define the specific endosomal recycling and retrograde transport pathways, an increasingly important question becomes one of how degradative sorting versus recycling versus retrograde transport are coordinated to achieve a

system capable of actively remodeling sorting itineraries in response to changing metabolic and environmental cues. By addressing this complex question much needed insight will be gained into the wiring between endosomal sorting and the varying physiological needs of cells in tissues and organs, and also a greater understanding of how deficiencies in endosomal sorting, either at the level of individual cargo or in the context of specific coat complexes and their associated protein assemblies, underlie a variety of human diseases. The problem is to identify those elements within the system that constitute the key “conductors” that broadly control endosome sorting. We consider that a growing body of evidence strongly points toward retromer, and in particular the CSC, as a vital evolutionary conserved conductor of sorting within the endosomal network. Exploring the transcriptional and translational control of this heterotrimer and the effects of posttranslation modification on its assembly, endosome association and wider protein:protein interactome are likely to generate significant new insight into the remodeling of endosomal sorting during cell function, organism development, and physiology, and perhaps most importantly, human health and disease.

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