Received: 7 March 2018 | Revised: 11 April 2018 | Accepted: 13 April 2018 | Uncorrected manuscript published: 18 April 2018

DOI: 10.1111/tra.12574

WILEY Traffic

REVIEW

Endosomal receptor trafficking: Retromer and beyond

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Funding information
Center for Scientific Review, Grant/Award Number: R01-DK073639, R01-DK107733; National Natural Science Foundation of China, Grant/Award Number: 31671477; Iowa State University; Roy J. Carver Charitable Trust

The tubular endolysosomal network is a quality control system that ensures the proper delivery of internalized receptors to specific subcellular destinations in order to maintain cellular homeostasis. Although retromer was originally described in yeast as a regulator of endosome-to-Golgi receptor recycling, mammalian retromer has emerged as a central player in endosome-to-plasma membrane recycling of a variety of receptors. Over the past decade, information regarding the mechanism by which retromer facilitates receptor trafficking has emerged, as has the identification of numerous retromer-associated molecules including the WASH complex, sorting nexins (SNXs) and TBC1d5. Moreover, the recent demonstration that several SNXs can directly interact with retromer cargo to facilitate endosome-to-Golgi retrieval has provided new insight into how these receptors are trafficked in cells. The mechanism by which SNX17 cargoes are recycled out of the endosomal system was demonstrated to involve a retromer-like complex termed the retriever, which is recruited to WASH positive endosomes through an interaction with the COMMD/CCDC22/CCDC93 (CCC) complex. Lastly, the mechanisms by which bacterial and viral pathogens highjack this complex sorting machinery in order to escape the endolysosomal system or remain hidden within the cells are beginning to emerge. In this review, we will highlight recent studies that have begun to unravel the intricacies by which the retromer and associated molecules contribute to receptor trafficking and how deregulation at this sorting domain can contribute to disease or facilitate pathogen infection.

KEYWORDS

endosome, receptor trafficking, retriever, retromer, sorting nexin, WASH

Jing Wang and Alina Fedoseienko contributed equally to this study.

1 | INTRODUCTION

Maintaining cellular homeostasis is necessary for every aspect of cellular life including cell growth, cell death, various signal transduction pathways and immune response[1–3]. Receptor endocytosis and subsequent sorting in endosomes are major pathways to preserve cellular homeostasis. Integral membrane proteins and their associated macromolecules are internalized via endocytosis. They can be further delivered to lysosomes for degradation, or targeted to the trans-Golgi network (TGN) or the plasma membrane for reuse (Figure 1). These processes, known as endosomal protein sorting, are essential for a wide array of physiological functions, including nutrient uptake, developmental and neural signaling, as such, genetic defects in these processes have been linked with pathologies such as neurological disorders and diabetes[1,4,5].

One of the best-characterized protein complexes regulating endosomal sorting is the evolutionarily conserved retromer complex, which was first identified in the yeast *Saccharomyces cerevisiae* 2 decades ago[6]. Retromer is a coat complex that assembles on endosomes, and mediates the transport of receptors that traverse the endosomal compartment on their way to an ultimate physiologic destination such as the plasma membrane or the TGN, and are thus referred to as "cargo" proteins. The core of retromer is the VPS35-VPS26-VPS29 heterotrimer, which is conserved from yeast to human (Table 1). Since the core complex is able to recognize certain cargo, it was termed the cargo-selective complex (CSC). Retromer functions together with a large number of accessory proteins to package cargo into tubular or vesicular structures for transport to the TGN or plasma membrane.

Although the role of retromer in endosomal sorting is well established, recent work has identified additional retromer-dependent and retromer-independent trafficking pathways, and revealed their functions in development and human disease (Figure 1). Several novel regulators, including TBC1d5, TBC1d23, the WASH actin regulatory complex, and the recently described COMMD/CCDC22/CCDC93 (CCC) and retriever complexes have been identified and characterized (Table 1). Accumulating evidence suggests that sorting nexin (SNX) proteins, in addition to binding to membranes play a critical role in cargo selection. In this review, we focus on these newly identified endosomal sorting machineries, and summarize the current understanding of their roles in endosomal trafficking and retrograde vesicular transport. We also discuss how viral and bacterial pathogens exploit endosomal trafficking pathways to promote their replication during infection. We do not intend to discuss every aspect of endosomal sorting due to space limitations, and interested readers are referred to excellent reviews published elsewhere[1,4,5,7].

2 | TBC1D5 REGULATION OF RECEPTOR TRAFFICKING

Study of classic coat proteins including the clathrin/adaptor protein, COPI and COPII (coat protein complex I or II), has revealed that small GTPases are essential for the formation of coated vesicles[8–11]. Similarly, the endosomal-localized Rab7a GTPase is required for the membrane recruitment of the retromer CSC, and this role is conserved in yeast, plants and mammals[12–19]. TBC1d5 is a member of the

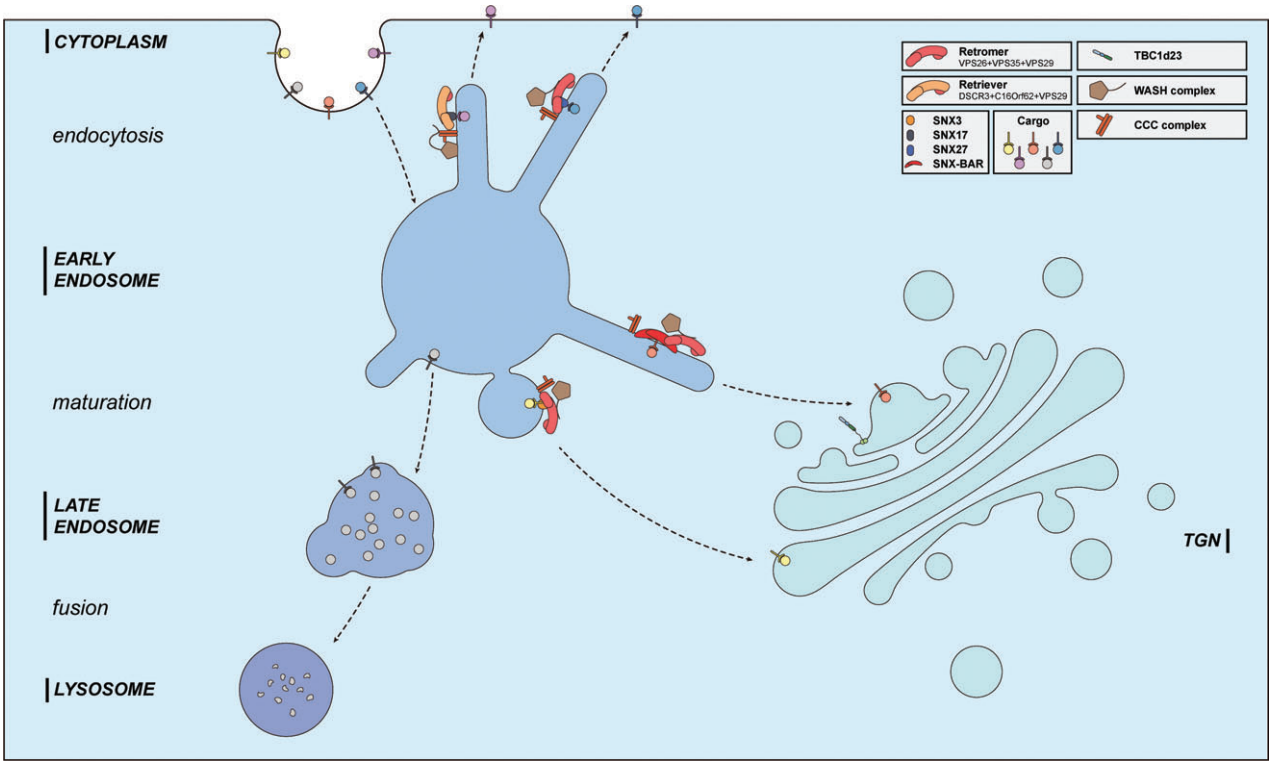

FIGURE 1 Representative trafficking pathways of transmembrane receptors. Transmembrane proteins are internalized into early endosomes via endocytosis. Maturation of early endosomes into late endosomes leads to protein degradation via lysosome. Some proteins are delivered to the plasma membrane or to the TGN with the assistance of retromer, retriever, WASH, CCC and a variety of SNX proteins, thus escaping lysosomal degradation. At the TGN, TBC1d23 and associated proteins are responsible for receiving endosomal vesicles

TABLE 1 Conservation of genes encoding endosomal protein sorting machinery in model organisms

	Homo sapiens	*Mus musculus*	*Danio rerio*	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Arabidopsis thaliana*	*Dictyostelium*	*Saccharomyces cerevisiae*
Retromer	√	√	√	√	√	√	√	√
TBC1d5	√	√	√	√	√	√	√	
SNX-BAR	√	√	√	√	√	√	√	√
SNX3	√	√	√	√	√	√	√	√
SNX27	√	√	√	√	√			
SNX17	√	√	√	√	√			
WASH	√	√	√	√	√[a]		√	
CCC	√	√	√	√	√[b]	√	√	
Retriever	√	√	√	√	√[c]	√	√	
TBC1d23	√	√	√	√	√			

[a] Four homologs of WASH complex components have been reported in *C. elegans* (WASH, Strumpellin, SWIP and CCDC53). A possible FAM21 *C. elegans* homolog (C05G5.2), which is also conserved in other nematode species, could also be identified. However, it remains to be determined whether these proteins associate with each other to form a functional WASH complex and regulate endosomal receptor trafficking in nematodes.

[b] *C. elegans* has CCC subunits: 3 homologs of CCDC93 (C16A11.2, C31E10.6, C31E10.5), no homologs of CCDC22 and one COMMD gene homolog (T28F2.2). It remains to be determined whether these proteins associate with each other and function in endosomal receptor trafficking.

[c] *C. elegans* has a possible retriever subunit, F26G1.1, which according to blast is more similar to VPS35L than to VPS35; interestingly, no VPS26C homolog is apparently present.

Tre2-Bub2-Cdc16 (TBC) family, and was first identified through its association with retromer in yeast-2-hybrid and immunoprecipitation experiments[19,20]. Both human and worm TBC1d5 function as GTPase-activating proteins (GAP) for Rab7a, catalyzing GTP hydrolysis and thus, inactivating Rab7a[21,22]. Over-expression of TBC1d5 was shown to decrease the amount of Rab7a-GTP and reduce the amount of VPS35 on endosomes[19]. Therefore, TBC1d5 was initially proposed to inhibit retromer trafficking; recent evidence, however, suggests that TBC1d5 is more than an inhibitory factor for retromer.

Among all the known endogenous regulators of retromer, TBC1d5 displays the highest affinity toward retromer[21]. TBC1d5 forms a stable complex with retromer, with a dissociation constant of 220 to 450 nM[21,23]. Such an affinity is comparable to that measured between VPS29 and VPS35 (~200 nM), and is at least one order of magnitude higher than the affinity between retromer and its other binding partners, such as SNX3, VARP, Rab7a or the WASH complex subunit FAM21[16,21,23–26]. TBC1d5 harbors a TBC domain on its N-terminus, and a largely disordered C-terminus. The TBC domain mediates the interaction with retromer, through contacting both VPS35 and VPS29[21]. A loop from TBC1d5 binds to a conserved hydrophobic pocket on VPS29, and a second loop may interact with the N-terminus of VPS35. Interestingly, a VPS35 mutant unable to associate with TBC1d5 did not localize properly to endosomes, suggesting that TBC1d5 may play a role in recruiting VPS35 to endosomal membrane[14,21].

The tight association between TBC1d5 and retromer is akin to the Sec23-Sec24 complex in the COPII coat. Similar to TBC1d5, Sec23 is a GAP for the Sar1 GTPase. The interaction between Sec23 and Sar1 is needed for the recruitment of the COPII coat to ER (endoplasmic reticulum) membranes, at least in an in vitro reconstitution system[10,11]. GTP hydrolysis of Sar1, promoted by Sec23, is believed to be essential for the maturation of COPII vesicles and coat disassembly. Past studies have provided mechanistic insights into the formation process of the COPII coat, and recognition of the analogy between the retromer and COPII systems may help to understand how retromer-coated vesicles are formed.

Recently, Steinberg et al have revealed a novel role for both retromer and TBC1d5 in regulating the activation and subcellular localization of Rab7a[27]. They found that Rab7a resides on multiple subcellular organelles in addition to the interface between EEA1+ sorting endosomes and LAMP1/2+ late endosomes/lysosomes. These include the TGN, endoplasmic reticulum and mitochondrial membranes. In the absence of either retromer or TBC1d5, Rab7a-GTP levels substantially increase and Rab7a accumulates over the lysosomal domains, which results in decreased Rab7a mobilization, depletion of inactive Rab7a from other subcellular organelles and defective membrane turnover. Intriguingly, whereas we previously showed that TBC1d5 was critical for the recycling of integrin alpha 5 and CI-MPR, Steinberg et al showed that loss of TBC1d5 did not impact the recycling of the retromer cargoes CI-MPR (cationic mannose 6 phosphate receptor) or GLUT1. The exact reason for the discrepancy remains unclear, but could involve either the way in which the knockout cells were generated or how the trafficking assays were performed.

TBC1d5 also plays a role in autophagy, a process in which cellular components are selectively targeted and degraded[27–30]. Studies by Dikic et al showed that TBC1d5 switches between endosomes and autophagosomes[28,29]. More recently, the Debnath group extended this study and showed that this switch could modulate retromer function under different physiological conditions such that metabolic stress leads to the association TBC1d5 with autophagosomes, promoting the recycling of GLUT1 and glucose uptake[30].

3 | SORTING NEXIN PROTEINS

SNX proteins are characterized by the presence of a particular and highly conserved phox-homology (PX) domain and participate in variety of cellular activities[31,32]. *S. cerevisiae* and human genomes encode 10 and 33 SNXs, respectively[31]. Although PX domains are capable of

contacting PtdIns(3)P, recent studies reveal that certain PX domains can also interact with phosphoinositides other than PtdIns(3)P, and can also interact with a variety of proteins[31]. With endosomes being the major organelle enriched with PtdIns(3)P, the functions of SNXs have been most extensively investigated in this subcellular compartment.

In addition to the PX domain, SNXs often possess other domains, including BAR (Bin/Amphiphysin/Rvs), FERM (protein 4.1/ezrin/radixin/moesin) and SH3 domains[31]. The presence of these additional domains allows SNX proteins to be divided into 5 subfamilies: SNX-PX, SNX-BAR, SNX-FERM, SNX-PXA-RGS-PXC and SNX-MIT[31]. Several SNXs from 3 different subfamilies have been the focus of recent studies: SNX3 of the SNX-PX subfamily, SNX1/2/5/6 of the SNX-BAR subfamily and SNX27 and SNX17 of the SNX-FERM subfamily (Figure 2A). These SNXs associate with retromer or the recently identified retriever complex, and mediate distinct endosomal trafficking pathways. Remarkably, recent studies have revealed that these SNXs play a central role in cargo recognition, in addition to binding phosphatidylinositides (PtdIns) (Figure 2B).

SNX3 (SNX-PX subfamily) contains only one structural domain, the PX domain, which binds to PtdIns(3)P (Figure 2). SNX3 is required for endosome-to-TGN retrieval of Wntless in human, worm and fly[33,34]. In *S. cerevisiae*, Grd19p/SNX3 is also needed for cargo trafficking from endosomes to the TGN[35]. Burd et al found that retromer is recruited to the endosomal membrane through both Rab7a and SNX3, and in vitro the recruitment depends on the presence of a transmembrane cargo, DMT1, on the liposome[17]. More recently, a crystal structure of the quaternary VPS35-VPS26-SNX3-DMT1 tail complex revealed that SNX3 binds at the interface of VPS35 and VPS26[36]. Upon binding to SNX3, VPS26 undergoes a conformational change in its cargo-binding motif, which allows recognition of the DMT1 tail by both VPS26 and SNX3. Thus, the SNX3/retromer complex integrates 2 different activities: membrane binding and cargo recognition.

Unlike SNX3, the SNX-BAR proteins possess an additional BAR domain, which can sense membrane curvature and induce membrane tubulation[31,32]. The SNX-BAR proteins can target to endosomes through coincidence detection of membrane curvature and PtdIns. Members of the SNX-BAR subfamily, SNX1 or SNX2, form a heterodimer with SNX5 or SNX6 in cells (Figure 2B). Intriguingly, although the PX domains of SNX1 and SNX2 bind to PtdIns(3)P, the PX domain of SNX6 interacts with PtdIns(4)P[37]. Such an interaction may facilitate the dissociation of SNX-BAR-coated vesicles from motors at the TGN[37]. Interestingly, whereas a wealth of earlier studies[38–40]

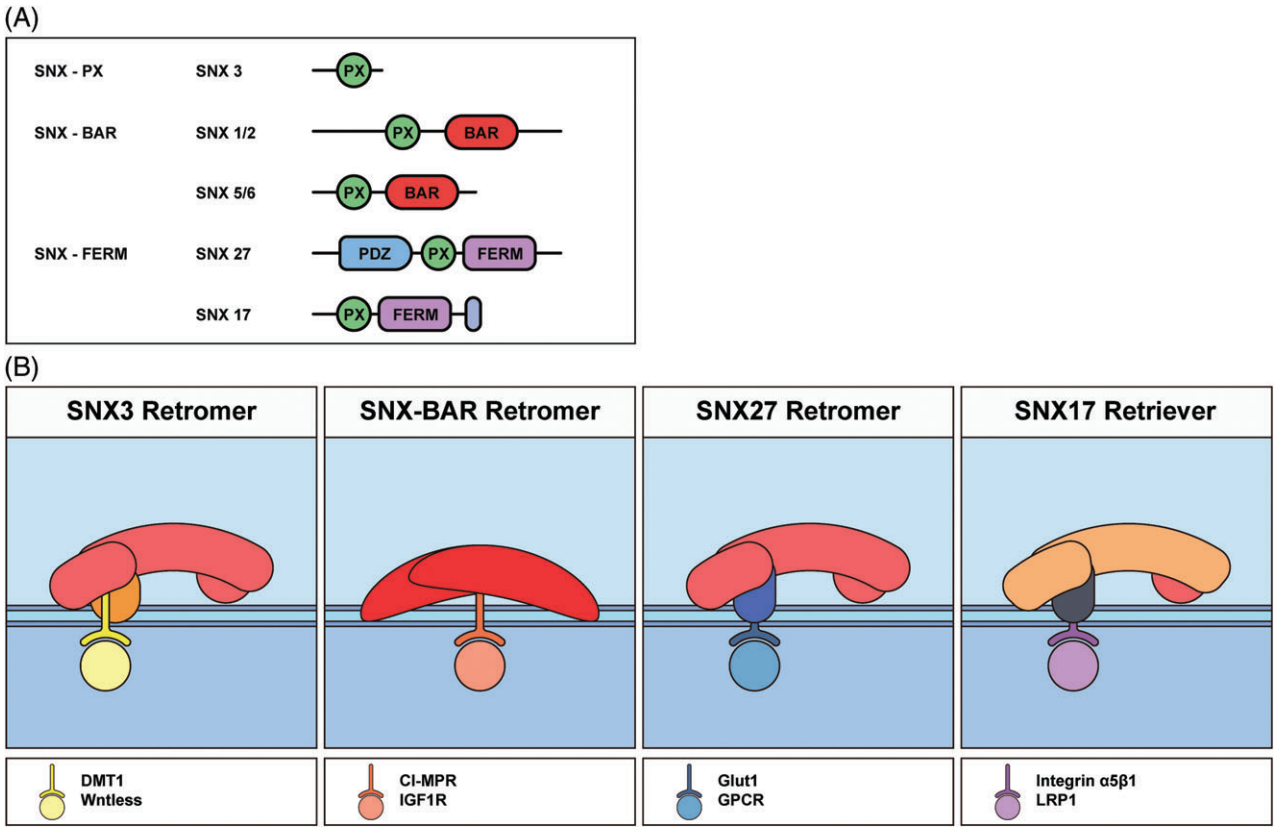

FIGURE 2 Domain organization and functional modes of select SNXs. A, Domain structures of retromer and retriever-associated SNXs. All SNXs share a conserved PX domain. SNX-BAR and SNX-FERM subfamilies possess a BAR or FERM domain, respectively, C-terminal to the PX domain. Within the SNX-FERM subfamily, SNX27 features a unique N-terminal PDZ domain whereas SNX17 has a unique C-terminal tail. B, Distinct cargo recognition modes of SNXs. Representative cargo proteins are listed under the cartoon. SNX3 interacts with both VPS35 and VPS26 subunits of retromer, and cargo recognition is achieved through cooperative action of SNX3 and VPS26. SNX-BAR may directly bind to cargo such as CI-MPR and IGF1R. The PDZ domain of SNX27 recognizes a subset of cargo proteins independent of retromer; however, interaction with VPS26 enhances the affinity of SNX27 for cargo. SNX17 may bridge cargo with retriever through simultaneous interaction with cargo and VPS26C

suggested that the retromer CSC associates with CI-MPR for its endosome-to-TGN transport, 2 recent studies indicate that SNX5 and SNX6 interact with the cytoplasmic tails of CI-MPR (and IGF1R), independent of retromer (Figure 2B)[41,42]. Even more surprisingly, these studies also showed that deletion of SNX1/2/5/6, but not VPS35, leads to a pronounced defect in CI-MPR trafficking[41,42]. The exact reasons for these discrepancies remain unclear, but given that both SNX3 and retromer bind to DMT1, SNX-BAR and retromer may recognize the CI-MPR tail through an analogous mechanism[36]. Future studies will be necessary to address how CI-MPR and IGF1R are recognized and transported.

Lastly, 2 members of the SNX-FERM subfamily, SNX27 and SNX17, have been shown to be critical for recycling of numerous cell surface proteins (Figure 2). In addition to the PX domain, both SNX27 and SNX17 contain a FERM domain, which recognizes NPxY/NxxY motifs found in a variety of proteins including growth factor receptors, solute carriers and integrins[43]. Although SNX27 contains a unique PDZ domain at its N-terminus, SNX17 does not harbor this domain, but instead possesses a unique C-terminal polypeptide sequence. Intriguingly, it is these 2 unique regions that determine the cargo selectivity of these 2 SNXs. The PDZ domain of SNX27 binds directly to VPS26 and cargo proteins with PDZ-binding motifs including glucose transporter GLUT1 and the β2-adrenergic receptor (β2AR)[44–48]. Thus, SNX27 mediates cargo recycling through a retromer-dependent manner. In contrast, SNX17 utilizes its unique C-terminal tail to interact with DSCR3 (VPS26C) of the retriever complex (see below), whereas its FERM domain binds to NPxY/NxxY motif-containing cargo proteins such as β1 integrin[49]. As a result, SNX17 mediates the recycling of α5β1 integrin in a retromer-independent, retriever-dependent manner. Collectively, through association with retromer or retriever, SNXs provide cargo specificity and help to establish diverse endosomal trafficking pathways.

4 | WASH COMPLEX

The WASP family promotes actin nucleation via the Arp2/3 complex, to facilitate cell migration/invasion, cell-cell adhesion, endocytosis/phagocytosis, cytokinesis and intracellular membrane transport[50,51]. WASH1 shares the conserved VCA (verprolin connecting and acidic) domain (also known as the WCA domain) with other members of the WASP family, including WASP/N-WASP and WAVE[50–52]. In cells, WASH1 tightly associates with 4 other proteins, including FAM21 (WASHC2), Strumpellin (WASHC5), Strumpellin and WASH1-interacting protein (SWIP or WASHC4) and CCDC53 (WASHC3) (Figure 3A)[53–55]. The WASH complex is conserved in many eukaryotic taxa, including some unicellular organisms, but is not found in the yeast *S. cerevisiae* (Table 1). Although some controversy exists, in general, depletion of individual subunits impacts the stability of the other subunits. For example, mouse embryonic fibroblasts lacking WASH show a dramatic effect on CCDC53 stability and incorporation with the remaining complex members, whose levels are also diminished[56]. FAM21 is comprised of a head domain (~220 amino acids), which is necessary to interact with other members of the WASH complex, and an extended C-terminal tail containing 21 repeats of a novel acidic motif (L-F-$[D/E]_{3\text{-}10}$-L-F), termed the LFa motif[25,55]. The FAM21 tail has been suggested to function as an endosomal signaling hub recruiting numerous proteins, including the actin-capping protein CapZ, ANKRD50, FKBP15, TBC1d23, RME-8 and the CCC complex (Figure 3A)[25,55,57–63].

WASH1 predominantly localizes to endosomes, but can be also found associated with other organelles and even within the nucleus[64]. In the cytosol, WASH complex-mediated actin polymerization has been observed to function in several distinct endosomal recycling pathways: (1) endosome-to-Golgi retrieval of CI-MPR,[54] which also requires retromer, SNX3 and SNX-BAR proteins; (2) endosome-to-cell surface recycling of the transferrin receptor (TfnR), the β2-adrenoceptor (β2AR), the glucose transporter GLUT1, the copper transporter ATP7A and α5β1 integrin[53,56,60,65–67]. Interestingly, whereas recycling of TfnR, β2AR, ATP7A and GLUT1 requires retromer activity, recycling of α5β1 is retriever-dependent (see below). (3) WASH complex is also involved in epidermal growth factor receptor (EGFR) delivery to lysosomes[68]. It should be noted that currently no evidence supports that retromer or retriever is directly required for the delivery of cargo to the degradation pathway. SNX6 appears to be needed for EGFR degradation, which may or may not be related to retromer activity[69]. (4) Finally, the WASH complex can also associate with BLOC-1 (biogenesis of lysosomal organelles complex-1), but the significance of this interaction remains to be determined[70,71].

How does the WASH complex function to promote trafficking in such diverse pathways? Most of our understanding is derived from the better-characterized retromer-WASH pathway. In conjunction with SNX proteins, retromer mediates both endosome-to-TGN retrieval, and endosome-to-plasma membrane recycling. The WASH complex directly associates with retromer through an interaction between the LFa motif of FAM21 and VPS35[25,57]. Knockdown of VPS35 in cells decreases the amount of endosomal-localized WASH complex; however, a significant amount of the WASH complex is still present on endosomes in VPS35-KO cells, suggesting that the WASH complex can localize to endosomes in both a retromer-dependent and -independent manner[25,49,57]. Current data suggests a model by which WASH-mediated actin polymerization promotes retromer trafficking: (1) endosome-bound proteins are recognized by specific combinations of retromer and SNXs; (2) retromer recruits the WASH complex through a direct interaction; (3) the WASH complex is activated through ubiquitination or other yet to be characterized mechanisms, promoting actin polymerization via Arp2/3 complex recruitment and activation. A combination of the action of BAR domains, motor proteins and actin polymerization leads to the formation of tubular structures; (4) subsequently, actin polymerization, together with the activity of the dynein-dynactin complex, kinesin microtubular motor and/or Dynamin II, promotes the fission of tubular structures; (5) ultimately, tubular vesicles carrying various cargo proteins are delivered to their final destinations (Figure 3B). In addition to functioning in retromer-dependent receptor trafficking, the WASH complex may function in retromer-independent pathways through similar mechanisms (Figure 3C). For instance, the SNX17-retriever complex recognizes a different subset of endosomal cargo (see below). These proteins associate with the WASH complex, which may help to form

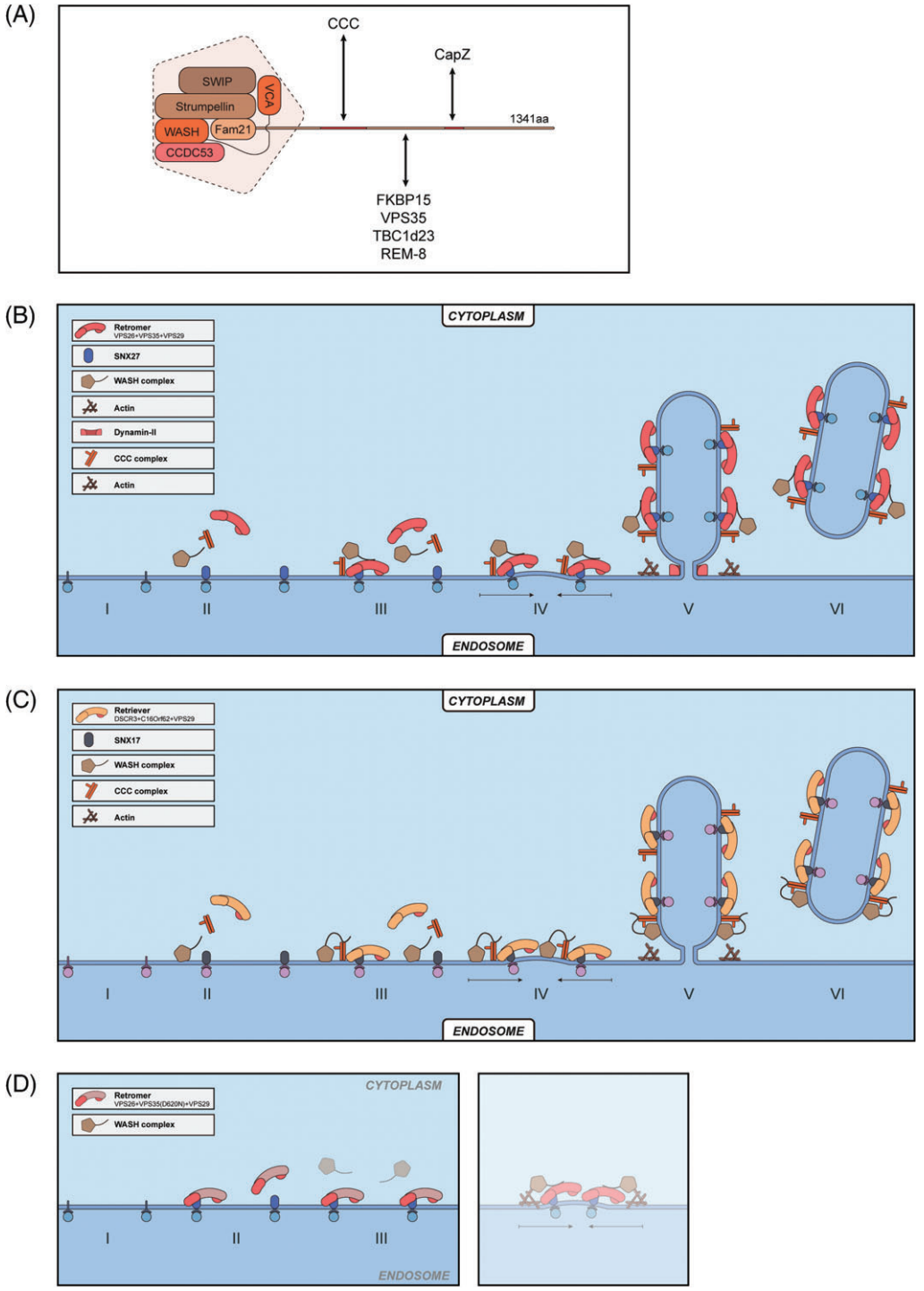

FIGURE 3 Function of the WASH complex in retromer- and retriever-mediated trafficking. A, Organization of the WASH complex and some of its associated proteins. Whereas CCC and CapZ interact with FAM21 fragments containing amino acids 448-631 and 1010-1067 (as indicated), respectively, other proteins either bind to multiple regions of FAM21 or the exact interacting regions are not fully defined. For instance, VPS35 binds to many LFa repeats of FAM21, with highest affinity toward those at the C-terminal end of the tail. B, A model depicting the role of WASH complex in regulating retromer-mediating trafficking. Increasing concentration of cargo (step I) leads to membrane recruitment of the retromer-SNX27 complex (step II), which, in turn, recruits the WASH complex (step III). WASH-mediated actin polymerization could promote the organization of retromer endosomal subdomains (step IV) and formation of retromer tubules (step V). WASH could also function to assist the scission of tubular structures together with other proteins, such as dynamin II (step VI). C, a model depicting the role of WASH complex in regulating retriever-mediating trafficking. SNX17 binds to cargo protein on the membrane, and WASH is recruited to membrane in a retromer-dependent manner (steps I and II). WASH and SNX17 in turn recruit CCC (step III), and retriever complexes (step IV). Similar to its role in retromer trafficking, WASH-mediated actin polymerization could promote the formation of SNX17/cargo/CCC/retriever endosomal subdomains (step V), vesicle budding (step VI), and vesicle scission (step VII). D, A mutation in VPS35 (D620N) diminishes the interaction of the WASH1 complex with the retromer, and impairs retromer-mediated cargo trafficking

discrete endomembrane subdomains and to facilitate vesicular scission through actin polymerization.

The following evidence supports the role of the WASH complex in both the formation of retromer tubules, and subsequent scission. First, in mouse embryonic fibroblasts devoid of WASH1, a collapse of the endolysosomal network to the perinuclear region was observed[56]. There was no F-actin accumulation on the collapsed structures and only re-expression of wild-type WASH1, but not a VCA-deleted mutant, could restore the normal architecture of the endolysosomal system, F-actin accumulation and receptor trafficking[56]. Second, in WASH1-knockdown cells, cargo-laden tubules have been observed suggestive of a defect in actin-dependent tubule scission[54]. Third, recycling β2AR is localized in a subset of tubular endosome microdomains, which do not include degrading receptors and bulk recycling proteins[66]. Recycling of β2AR requires both SNX27 and retromer[44,45]. WASH, but not other WASP family members, including WASP, N-WASP and WAVE, is concentrated on β2AR tubules[66]. Finally, a mutation in VPS35 (D620N), that is associated with early onset Parkinson's Disease (PD) was shown to diminish the interaction of the WASH1 complex with retromer and impact vesicular trafficking from the late endosome as well as impair autophagy[72–77] (Figure 3D). Interestingly, gain-of-function mutations in the FAM21-associated molecule, RME-8, which is involved in coordinating the activity of the WASH complex with the tabulating activities of SNXs[63], were identified in patients with PD and Lewy body pathology[78]. Thus, perturbation of receptor trafficking from the retromer subdomain seems to be a common mechanism contributing to the development of PD.

Due to the importance of WASH-mediated actin polymerization in endosomal trafficking, its activity must be tightly regulated, as is the case for other WASP proteins. Indeed, although recombinant WASH1 is active toward Arp2/3 in vitro, the assembly of WASH1 into the pentameric complex inhibits this activity[55]. Interestingly, the WASH complex has structural similarity to the WAVE complex, which promotes actin polymerization at the leading edge of migrating cells[55,57]. Several distinct mechanisms, including small GTPase Rac, phospholipids and phosphorylation are known to activate the intrinsically inactive WAVE complex[79]. Similarly, mammalian WASH complex can be activated through TRIM27-mediated poly-ubiquitination (K63 linked) on WASH1 at lysine 220[80]. Of note, this lysine residue lies within what would be the meander region of WAVE, and thus, ubiquitination at this residue might activate WASH1 by disrupting an inhibitory fold surrounding the VCA. More recently, USP7, a deubiquitinase that interacts with TRIM27 and the MAGE-L2 cargo adaptor protein, was shown to be required to not only prevent TRIM27 from ubiquitin-mediated degradation, but also regulates WASH1 activity by controlling the level of ubiquitination[81]. Interestingly, the gene-encoding USP7 is mutated in Autism-spectrum disorders, and the same has been noted for MAGE-L2, suggesting that aberrant WASH1 regulation may be involved in the pathology of these neurological diseases[81]. Lastly, it should be pointed out that TRIM27 and MAGE-L2 are not ubiquitously expressed, thus the identification of signaling mechanisms contributing to the activation of WASH in cells devoid of these molecules remains to be determined.

Emphasizing the importance of the WASH complex in development and human diseases is the observation that mutations in the complex lead to multiple neurological disorders. A mutation in the gene-encoding SWIP (P1019R) has been observed in patients with non-syndromic autosomal recessive intellectual disability (ID) and results in diminished levels of Strumpellin and other members of the WASH complex[82,83]. In addition, mutations in Strumpellin have been found in patients with autosomal-dominant hereditary spastic paraplegia (HSP), but these mutations do not impact WASH complex assembly or endosomal localization, and thus might either be impacting WASH1 activity directly or could result from other unappreciated cellular functions of Strumpellin[55,84,85]. Lastly, a splice-site mutation in Strumpellin was recently observed in Ritscher-Schinzel/3C syndrome, which among its clinical features includes ID[86]. Although other WASH complex components were not assessed, this splice-site mutation leads to an 8-fold reduction in Strumpellin transcript and an approxximately 60% loss of Strumpellin protein. The mechanism by which WASH deregulation contributes to the pathology of ID syndromes remains to be determined, but likely involves the regulation of vesicle recycling of proteins involved in neuronal maturation, survival or function during brain development.

5 | RETRIEVER COMPLEX

It is of interest that WASH also regulates the recycling of SNX17 cargoes including the integrin α5β1, EGFR, LDLR and Notch ligand Jag1[87–92]. However, how SNX17 couples trafficking of its cargos to WASH-regulated subdomains remained a mystery until very recently[49,93]. Through a series of proteomic and functional studies it was uncovered that SNX17 interacts with C16orf62, DSCR3 and VPS29, among other proteins (Figure 2B). C16orf62 is a distant homolog of VPS35, and has thus been named VPS35L[49]. DSCR3, on the other hand, is predicted to have a fold analogous to VPS26, and has been named VPS26C[49 94]. VPS35L, VPS26C and VPS29 form a stable trimeric complex, and co-elute following size-exclusion chromatography[49]. Due to the similarity with retromer, this new complex has been named "retriever." Knockdown or deletion of VPS35 results in a substantial loss of VPS26A, while VPS35L deletion results in depletion of VPS26C; interestingly, VPS29 is depleted primarily upon loss of VPS35 and not VPS35L[49]. Importantly, despite the similarities between the retromer and retriever complexes, it is not known whether Rab proteins might also participate in the endosomal recruitment of the retriever complex like they do for the retromer complex. However, it is tempting to speculate that mechanisms regulating retromer recruitment might also operate to control retriever recruitment because both retromer and retriever share the VPS29 subunit that interacts with the Rab7a GAP TBC1d5.

Significantly, loss of retriever components (VPS35L and VPS26C) resulted in defective retrieval of SNX17 cargo from the endosome[49,60]. The mechanism by which SNX17 coupled to the retriever was revealed when a highly conserved 4 amino acid sequence in the C-terminus of SNX17 was found to be necessary and sufficient to interact with VPS26C and recruit SNX17 to endosomes where WASH and the CCC complexes reside[49]. Thus, WASH sits at the nexus of 2 major recycling pathways coordinating both SNX17 and SNX27 cargo retrieval (Figure 3).

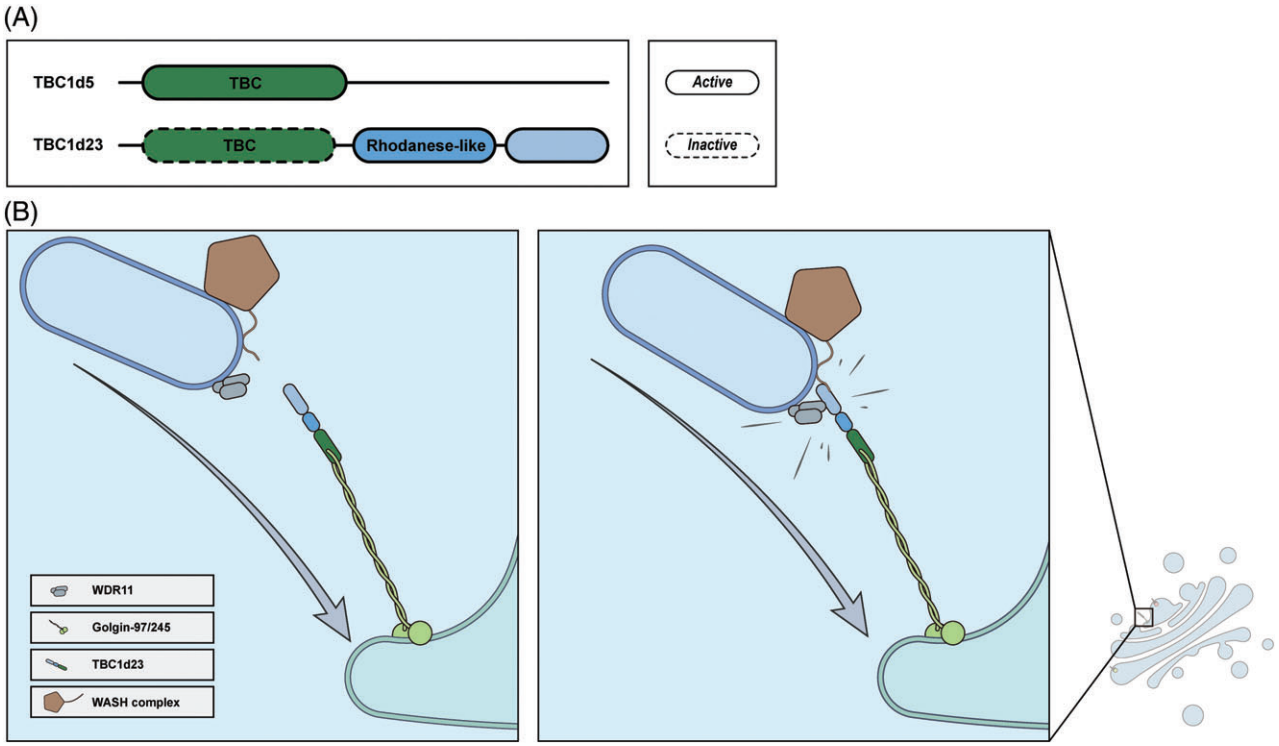

FIGURE 4 Function of TBC1d23 in endosomal sorting. A, Domain organization of TBC1d23 and TBC1d5. Although both proteins possess a TBC domain, TBC1d5, but not TBC1d23, is catalytically active. B, A model depicting the function of TBC1d23 in tethering endosomal vesicles with TGN. Left: The WASH and WDR11 complexes decorate endosome-derived vesicles, and TBC1d23 is localized at the TGN through its interaction with Goglin-97/245. Right: Interaction between TBC1d23 and FAM21 or the WDR11 complex allows tethering and subsequent fusion of endosomal vesicles with the TGN

6 | CCC COMPLEX

Although a role for COMMD1 in regulating copper homeostasis in dogs and mice had been known for some time, the cellular mechanism was unclear[95]. The explanation for this phenotype emerged when COMMD1 was found to associate with CCDC22 and CCDC93, 2 uncharacterized proteins found to interact with a specific region of the FAM21 C-terminal tail. This interaction is responsible for the recruitment of the CCC complex to endosomes[57,60]. Loss of CCC complex components, as well as loss of the retromer subunit VPS35 or SNX27, lead to impaired endosome to plasma membrane recycling of the copper transporter ATP7A, indicating that CCC is required for recycling of at least certain retromer/SNX27 cargoes[60]. Interestingly, a splice-site mutation in CCDC22 was identified as the causative mutation for X-linked ID in 2 families, a phenotype that resembles defects of WASH function in humans[96–98]. Patients with this mutation showed a substantial loss of CCDC22 protein, which also impacted the levels of CCDC93 and resulted in the loss of COMMD1 from endosomes. Consistent with the idea that the CCC complex is involved in regulating copper transport, patients harboring CCDC22 mutations showed disturbances in copper handling that are similarly seen in animal models of COMMD1 loss.

Another prototypical SNX17 cargo is the LDL receptor (LDLR) and other members of this receptor family[99]. Interestingly, mice lacking COMMD1 in hepatocytes showed high levels of LDL and cholesterol, which was also observed in patients with CCDC22 deficiency and in patients with Strumpellin mutations associated with ID[99]. Significantly, in mice and cells lacking COMMD1, the surface levels of LDLR were diminished, suggestive of a recycling defect. In addition, WASH deficiency also resulted in LDLR trafficking defects resulting in LDLR accumulation in lysosomes. In aggregate, studies of both CCC- and WASH-deficient states suggest that defective LDLR trafficking resulting in reduced cell surface expression likely accounts for the increased cholesterol and LDL levels seen in patients. Consistent with a broader role for the CCC in SNX17-dependent recycling, integrins were also found to be dependent on the CCC complex for proper trafficking[49]. Thus, the studies to date indicate that the CCC complex is recruited to endosomes by the FAM21 subunit of WASH and is required for recycling events mediated by retriever/SNX17 as well as retromer/SNX27. However, the exact mechanism by which it participates in these sorting events remains to be determined.

7 | TBC1D23

In addition to the generation of endosomal vesicles, recent work also provides insights into the mechanisms by which endosomal vesicles are captured by the TGN[61,100]. Interestingly, another member of the TBC family, TBC1d23, plays a critical role in the process. Unlike TBC1d5, TBC1d23 is apparently catalytically inactive due to mutations in key residues important for its GAP activity (Figure 4A). TBC1d23 also harbors a Rhodanese-like domain, which can be found in eukaryotic proteins such as protein phosphatases and ubiquitin

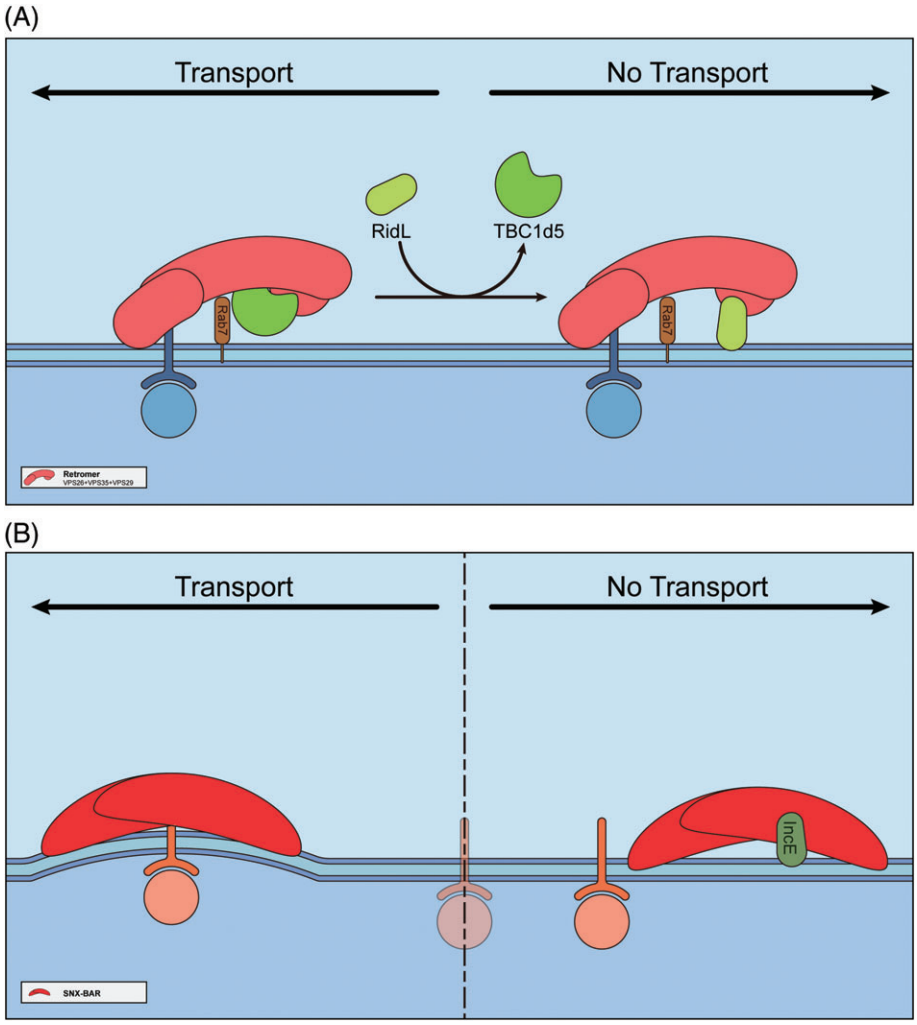

FIGURE 5 Mechanisms of interference of endosomal trafficking by bacterial effector proteins RidL and IncE. A, Model showing how RidL subverts retromer-dependent transport. Left: TBC1d5 is required for retromer-dependent endosomal trafficking. TBC1d5 interacts with both VPS35 and VPS29. Right: During *Legionella pneumophila* infection, RidL replaces TBC1d5, and likely VARP (not shown), to block retromer-mediated trafficking. In contrast with TBC1d5, RidL interacts only with VPS29. B, Model showing how IncE inhibits SNX-BAR-dependent transport. Left: SNX-BAR complex mediates endosome-to-TGN transport of certain proteins, such as CI-MPR. Right: Bacterial effector protein IncE interacts with the PX domain of SNX5/SNX6, and may inhibit their association with cargo proteins

ligases, and a C-terminal domain that bears little similarity with other proteins (Figure 4A).

The Golgi apparatus has a specific set of proteins, called golgins, which are able to capture vesicles from endosomes, endoplasmic reticulum or the Golgi itself [101,102]. Among them, golgin-97 and golgin-245 are responsible for receiving endosome-derived vesicles. Shin et al found that TBC1d23 bridges the interaction between golgin-97, golgin-245 and endosomal vesicles[61]. The TBC domain of TBC1d23 binds to a conserved region of golgin-97 and golgin-245, and the C terminus binds to FAM21 (Figure 4B). Critically, deletion of TBC1d23, or deletion of both golgin-97 and golgin-245 leads to defective endosome-to-Golgi trafficking of CI-MPR and TGN46[61]. Thus, TBC1d23 links WASH-coated endosomal vesicles to the TGN during endosome-to-TGN retrieval.

In addition to golgin-97, golgin-245 and the WASH complex, TBC1d23 also interacts with a trimeric complex consisting of WDR11, FAM91A1 and C7orf75 (Figure 4B) [61,100]. Similar to the WASH complex, the WDR11 complex associates with vesicles derived from endosomes, but not with the TGN[100]. The interaction between the WDR11 complex and TBC1d23 is proposed to promote the tethering of vesicles to the TGN; however, the exact mechanism remains to be elucidated. Intriguingly, homozygous mutation in TBC1d23 has been recently linked with pontocerebellar hypoplasia, a developmental disorder characterized by impaired growth of the pons and cerebellum, furthering emphasizing the importance of vesicular trafficking in neuronal development and function[103,104].

8 | MANIPULATION OF ENDOSOMAL PROTEIN SORTING BY PATHOGENS

Given the importance of endosomal sorting pathways for cellular homeostasis and other cellular functions, these pathways emerge as opportune targets by a variety of viral and bacterial pathogens[105]. Pathogens target key sorting protein machineries, such as retromer, SNX proteins and the WASH complex, to promote cellular entry and replication during infection, and to evade degradation. For instance, the envelope glycoprotein of the HIV type-1 and the tyrosine kinase-interacting (Tip) protein of the herpesvirus saimiri bind directly to retromer during viral infection[106,107]. Human papillomavirus type 16, and the effector IncE of Chlamydia trachomatis, target to SNX27 and SNX5/SNX6, respectively[108–110]. The WASH-FAM21 complex is required by Vaccinia Virus for cellular entry and subsequent intracellular transport[111,112]. Among all cases, 2 bacterial proteins, RidL and IncE, have been most extensively studied. Biochemical, structural and cellular studies have collectively revealed not only important bacteria-host interactions, but have also uncovered important, but previously less appreciated host regulatory mechanisms.

Legionella pneumophila is the causative agent of Legionnaires' disease. During infection, these bacteria reside in a special membrane compartment known as the Legionella-containing vacuole (LCV),[113] which delivers nearly 300 different proteins, known as effectors, to exploit various host functions including vesicle transport. The effector RidL appears to target to retromer and interfere with retromer function, which has been shown to restrict intracellular growth of *L. pneumophila*[114]. RidL is a large protein with over 1100 amino acids, but lacks sequence homology to known proteins. The interaction between RidL and retromer is mediated by the N-terminal 200 amino acids of RidL and the VPS29 subunit of retromer[23,115,116]. RidL contacts VPS29 with a dissociation constant of approximately 200 nM, similar to the affinity between TBC1d5 and VPS35/VPS29[23,116]. Structural and biochemical studies reveal that both RidL and TBC1d5 bind to a highly conserved hydrophobic surface of VPS29, opposite to the VPS35-binding surface[20,23,115,116]. Remarkably, both RidL and TBC1d5, and likely VARP, interact with VPS29 through a conserved P-L/I motif[23,24,116]. Outside of this motif, these 3 proteins bear little sequence similarity. In fact, RidL and TBC1d5 have opposite main chain directions in their aligned regions[116]. Consistent with structural analysis, RidL competes with TBC1d5 and VARP in vitro and in vivo[23,116]. Thus, RidL interferes with retromer trafficking by outcompeting critical endogenous regulators (Figure 5A). In addition to retromer, the N-terminus of RidL also associates with the endosomal lipid PtdIns(3)P, which aids in the recruitment of RidL together with retromer[116]. Despite these studies, functions of the C-terminus of RidL remain unclear[117]. Since many multiple-domain Legionella effectors have related functions in each of their domains, RidL likely regulates endosomal trafficking through other mechanisms, such as post-translational modifications of retromer or related proteins.

Another pathogenic protein that has been extensively characterized is the Chlamydial effector protein IncE[110,118–121]. Chlamydiae infection leads to human blindness, respiratory and genital tract diseases. Chlamydiae replicates within a unique membrane-bound compartment, termed the inclusion. IncE is one of the effector proteins localized on the inclusion membrane, which specifically interacts with SNX5/SNX6 and inhibits retromer- and SNX5/SNX6-mediated endosomal trafficking[110]. Structural studies from 3 different groups revealed that IncE binds to a conserved hydrophobic groove in the PX domain of SNX5[118–120]. Although the exact inhibitory mechanisms exerted by IncE remain to be determined, 2 observations indicate that IncE may function through interfering with the interaction between SNX5 and its cargo, such as IGFR1 and CI-MPR (Figure 5B)[118–120]. First, a SNX5 mutant unable to bind to IncE shows decreased binding to IGFR1 and CI-MPR[120]. Second, overexpression of IncE reduces co-localization of CI-M6PR with VPS35, and inhibits the delivery of CI-MPR to the TGN[118]. Therefore, 2 distinct types of bacteria utilize diverse mechanisms to interfere with endosomal sorting.

9 | CONCLUSIONS

The last several years have seen remarkable progress in the field of endosomal sorting. In addition to the well-established retromer-dependent trafficking pathway, retriever is found to mediate retromer-independent endosomal trafficking. Remarkably, retriever shows a certain degree of homology to retromer, including complex organization, protein sequences and interaction with SNXs. Additionally, several novel protein machineries have been identified and characterized to regulate endosomal sorting including the WASH complex, TBC1d5, TBC1d23, the CCC complex and WDR11. The identification of these complexes has allowed the dissection of the mechanistic details of retromer membrane recruitment, cargo recognition, vesicular transport and vesicle tethering with target membranes. Despite this progress, many key questions remain to be answered in the field including but not limited to: [1] are there other complexes that function similar to retromer and retriever? [2] what is the exact role of the WASH and CCC complexes in retromer and retriever-mediated trafficking? [3] since endosomal proteins can be targeted to both the plasma membrane and the TGN, how are specific trafficking routes selected? [4] why do viral and bacterial pathogens target endosomal trafficking pathways? Undoubtedly, answers to these questions will lead us to a better understanding of endosomal receptor trafficking and their functions in organismal development and human disease.

The Editorial Process File is available in the online version of this article.

ACKNOWLEDGMENTS

We thank members of our laboratories for critical discussions, Mr. Chengxin Weng for help with figures and apologize for our colleagues whose work could not be cited here due to space constraints. This research is supported by Natural Science Foundation of China (NSFC) grants #31671477 (D.J.), NIH grants R01DK073639 (E.B.) and R01DK107733 (D.D.B. and E.B.). D.J. is a "One Thousand Talents" program scholar.

Conflict of interest

The authors declare no financial conflicts of interest.

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How to cite this article: Wang J, Fedoseienko A, Chen B, Burstein E, Jia D, Billadeau DD. Endosomal receptor trafficking: Retromer and beyond. *Traffic*. 2018;1–13. https://doi.org/10.1111/tra.12574