Title

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Journal

Trends in Microbiology, 26(9)

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Publication Date

2018-09-01

DOI

10.1016/j.tim.2018.04.001

Peer reviewed

HHS Public Access

Author manuscript

Trends Microbiol. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Trends Microbiol. 2018 September ; 26(9): 769–780. doi:10.1016/j.tim.2018.04.001.

Emerging Role of Retromer in Modulating Pathogen Growth

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Abstract

Intracellular pathogens have developed elegant mechanisms to modulate host endosomal trafficking. The highly conserved retromer pathway has emerged as an important target of viruses and intravacuolar bacteria. Some pathogens require retromer function to survive. For others, retromer activity restricts intracellular growth; these pathogens must disrupt retromer function to survive. In this review, we discuss recent paradigm changes to the current model for retromer assembly and cargo selection. We highlight how the study of pathogen effectors has contributed to these fundamental insights, with a special focus on the biology and structure of two recently described bacterial effectors, *Chlamydia trachomatis* IncE and *Legionella pneumophila* RidL. These two pathogens employ distinct strategies to target retromer components and overcome restriction of intracellular growth imposed by retromer.

Keywords

Retromer; Sorting Nexin; IncE; RidL; *Chlamydia trachomatis*; *Legionella pneumophila*

Challenges faced by vacuolar pathogens

Intracellular pathogens have evolved versatile strategies for successful survival inside the host cell [1, 2]. Some bacteria, such as *Listeria* and *Shigella*, escape from a membrane-bound compartment into the cytosol. Others, including *Chlamydia*, *Legionella*, *Coxiella*, and *Salmonella*, replicate in a **parasitophorous vacuole** (see Glossary) within the host cytosol. This membrane-enclosed compartment physically separates the pathogen from the cytoplasm and serves as a protective barrier from cytosolic sensors of the host innate immune response. However, living “in a bubble” requires specific survival strategies, including the ability to modulate host pathways from within this membranous compartment for nutrient acquisition and mechanisms to avoid fusion with lysosomes and autophagosomes. To overcome these challenges and to create a protected niche, Gram-negative **intravacuolar pathogens** often employ specialized protein export systems,

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including the **type III secretion system (T3SS)** or the **type IV secretion system (T4SS)**, through which they directly translocate up to hundreds of **effectors** from the bacteria across the vacuolar membrane into the host [2]. Many of these effectors reprogram host cell trafficking pathways, allowing intravacuolar pathogens to survive within the hostile intracellular environment [2].

Modulating **retromer**-dependent trafficking is emerging as a common survival strategy for intracellular pathogens [3]. Retromer is a highly conserved multiprotein complex that is involved in recycling of cargo from endosomes to various cellular compartments [4–6]. Some pathogens depend upon retromer for intracellular survival and growth [3]. For others, including the important human vacuolar bacterial pathogens *Chlamydia trachomatis* and *Legionella pneumophila*, retromer restricts bacterial growth [7–9]. Here we explore current advances in our understanding of retromer assembly and cargo selection, review reported interactions between intracellular pathogens and retromer, and describe new work that reveals how secreted effectors from *C. trachomatis* and *L. pneumophila* interfere with retromer function to overcome pathogen restriction. These studies provide new insights into retromer biology, which have important implications for understanding a broad array of human diseases, including cancer, diabetes, and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [4, 10, 11].

Retromer components

Retromer is a multi-subunit protein complex, conserved from *Saccharomyces cerevisiae* to humans, that mediates recycling or **retrograde trafficking** of protein "cargo" from endosomes to either the *trans*-Golgi network (TGN) or to the plasma membrane [4–6] (Figure 1, Key Figure). These protein cargos are varied and range from transmembrane receptors and transporters to adhesion molecules. Retromer is composed of two distinct sub-complexes of tightly assembled subunits: (i) the heterotrimeric vacuolar protein sorting (VPS) complex, which engages and concentrates cargo to be transported and (ii) one or more sorting nexins (SNXs) that bind to endosomal membranes and organize the formation of tubulovesicular carriers that transport cargo to their final destination. In yeast, the VPS and SNX subcomplexes form a relatively stable heteropentameric complex [12, 13] while in mammalian cells, the association of the VPS complex with the SNX complex is more transient [14, 15]. In some literature, "retromer" refers specifically to only the VPS complex. In other published works and in this review, "retromer" refers to the VPS and SNX sub-complexes.

The VPS Complex

The VPS complex is composed of VPS26, VPS29, and VPS35 [16]. Until recently, the VPS complex was thought to be the major if not sole determinant for selection and concentration of protein cargo that is recycled through retromer-dependent trafficking [17, 18]. The central protein, VPS35, has an elongated α-helical solenoid structure and functions as a platform on which VPS26 and VPS29 bind independently at opposite ends [16, 19]. VPS26 interacts with the N-terminus of VPS35 [20, 21] and contributes both directly and indirectly to cargo recognition and membrane docking in combination with different SNXs [18]. VPS29

interacts with the C-terminus of VPS35 and functions as a scaffold for association with accessory proteins that regulate retromer activity [18]. VPS29 has a metallophosphoesterase fold with two conserved surface patches on opposite sides [19, 22]. One of these patches interacts with VPS35 [19] whereas the opposite conserved patch binds to the cysteine rich zinc-binding motifs of the adaptor protein **VPS29-ankyrin-repeat protein (VARP)** [23] or with **Tre-2/Bub2/Cdc16 domain family member 5 (TBC1D5)**, a **GTPase activating protein (GAP)** that converts **Rab**7-GTP to Rab7-GDP [24, 25]. The interaction of VARP with retromer is important in endosome to plasma membrane recycling of a subset of transmembrane proteins [23, 26–29], while TBC1D5 regulates interaction of the VPS complex with the membrane [24, 25].

The VPS complex does not have intrinsic membrane binding capacity; rather, its recruitment to the endosome membrane requires activation of Rab7 GTPase by precise orchestration of Rab7-specific GAPs (TBC1D5) and **guanine exchange factors (GEFs)** [25, 30] as well as by cooperative interaction with SNX proteins [18]. The association of TBC1D5 with VPS29 promotes rapid GTP hydrolysis of Rab7, triggering the disassembly and release of the VPS complex from membranes [18]. In yeast, there is evidence that the SNX-BAR subcomplex displaces Rab7 from the VPS trimer during formation of endosomal tubules [31], however whether this happens in mammalian cells is unclear [32].

The Sorting Nexins and the Scission Complex

The defining characteristic of the 33 human SNX family members is the presence of a **Phox-homology (PX)** domain that binds to specific **phosphoinositides** enriched on endosomal membranes [11, 33]. Many SNX family members encode additional domains that further determine their interaction with the various proteins and lipids that are associated with distinct recycling routes [6, 11]. For example, SNX27 encodes a **post-synaptic density/disc large tumor suppressor/zona occludens (PDZ domain)** that recognizes a PDZ binding motif found in diverse receptors (such as the beta-2 adrenergic receptor), facilitating endosome to plasma membrane cargo recycling[4, 34–36]. In contrast, endosome to TGN trafficking utilizes either SNX3 or a heterodimer composed of SNX1 or SNX2 in combination with either SNX5 or SNX6 [37]. This latter subset of SNXs encode a Bin, Amphiphysin, and Rvs (BAR) domain, which senses and induces membrane curvature [11]. These so-called SNX-BARs promote endosomal membrane tubulation. The actin-associated Wiskott-Aldrich syndrome protein and SCAR homologue (WASH) complex binds to the VPS complex, which mediates actin polymerization, tubule scission, and formation of cargo-rich vesicles [38–42]. SNX5 and SNX6 bind the **dynein-dynactin minus-end directed motor complex** and mediate microtubule-based transport of the cargo-containing vesicle to the TGN [43, 44].

Cargo recognition by retromer components

Our understanding of how retromer recognizes a wide variety of protein cargo continues to evolve [4–6, 17, 18, 45–49]. Initial studies suggested that the VPS complex was the sole determinant of cargo selection through its recognition of a shared hydrophobic motif (ΦX(L/M);where Φ is an aromatic amino acid) that is required for retromer-dependent sorting found

in many cargoes, such as the cation-independent **mannose-6-phosphate receptor (CI-MPR)** and the divalent metal transporter 1 (DMT1-II) [50, 51]. However, several recent studies suggest that cargo recognition may be more complex and may involve recognition by the SNX proteins in addition to or independently of the VPS complex [16, 19, 36, 52–57]. For example, the crystal structure of SNX3:VPS26:VPS35 in complex with an extended sequence containing the DMT1-II ΦX(L/M) motif reveals that this recycling signal binds at the SNX3:VPS26 interface and that its recognition involves a conformational change in VPS26 upon SNX3 binding [16]. As SNX3 can also interact with phosphatidylinositol 3-phosphate (via its PX domain), these cooperative interactions ensure a mechanism whereby signal recognition is coupled to membrane recruitment [16]. For SNX27, cargo recognition is mediated by its carboxy-terminal 4.1/ezrin/radixin/moesin (FERM)-like domain [58, 59], which engages NPxY sorting signals, and by its PDZ domain, which binds to proteins that contain PDZ-binding motifs[4, 34–36, 54, 60]. The PDZ domain of SNX27 also binds VPS26 [54, 60]. This interaction increases the affinity of the PDZ domain for cargo, indicating cooperative interactions among VPS and SNX components to select a wide variety of endosome-plasma membrane recycled cargo [54, 60].

Newly reported studies demonstrate that transport of some retromer cargo can occur by a SNX-BAR-dependent but VPS-independent mechanism [56, 57]. Quantitative proteomics was utilized to define the SNX-BAR interactome, revealing distinct sets of known retromer cargoes that interact with SNX1, SNX2, SNX5, and/or SNX6 [56, 57]. Surprisingly, the CI-MPR, which transports newly synthesized lyososomal hydrolases from the TGN to late endosomes for ultimate delivery to lysosomes [61], was found to selectively associate with SNX5 and SNX6 through its conserved ΦX(L/M) motif, but not with SNX1 and SNX2 [56, 57]. This finding was unexpected, as several prior studies had suggested that CI-MPR associates with retromer via the VPS complex: yeast two hybrid studies demonstrated that the CI-MPR was able to bind directly to VPS35 [62], and depletion of VPS complex components was shown to disrupt CI-MPR recycling, resulting in a failure to deliver lysosomal hydrolases to the lysosome [62, 63]. In these more recent studies, however, RNAi-mediated protein depletion and CRISPR-mediated gene deletion studies show that loss of the VPS complex has no effect on the distribution of the CI-MPR between endosomes and the TGN [56, 57]. In contrast, loss of SNX1/2/5/6 causes the CI-MPR to accumulate, i.e. to be “stuck” in the endosome, indicating that SNX-BARs can directly mediate transport of subsets of cargo without the VPS complex. Altogether, these findings suggest that cargo selection and transport is a complex process [17, 45].

Retromer components are targeted by intracellular bacteria and viruses

The retromer complex is emerging as a commonly recognized target of intracellular pathogens, including both viruses and bacteria [3] (Table 1). Many of these microbes require retromer for successful intracellular replication and survival. Indeed, depletion of specific retromer components inhibit specific steps in the intracellular life cycle of vaccinia virus [64], hepatitis C virus (HCV) [65], human papilloma virus (HPV) [66], *Coxiella burnetii* [67], and *Salmonella enterica* serovar Typhimurium [66, 68]. The role of retromer in intracellular survival is most easily explained for pathogens that require an acidic intracellular environment for vacuole maturation, such as *C. burnetti* [67] and *S.*

Typhimurium [68, 69]. For some viral pathogens, such as HPV16 or vaccinia virus, retromer may help transport viral particles to the TGN or to recycling endosomes [64, 70].

Although much remains to be learned about how pathogens regulate retromer, studies of the interactions between viral effectors and specific retromer component have revealed an enormous diversity of strategies (Table 1). Some viral effectors recruit retromer components to viral replication sites to promote infection. For example, the NS5A protein from HCV interacts with VPS35 and may be important for recruiting retromer and its cargo, CI-MPR, to replication sites [65], potentially as a mechanism to deliver host factors that promote viral growth, such as Tip47 [71–73], which interacts with CI-MPR [74, 75]. Other effector proteins may mimic retromer cargo to "hitch a ride" from endosomes to the TGN either to escape lysosomal degradation or to gain to access to the nucleus. Thus, influenza virus M2 [76] and human immunodeficiency virus (HIV) envelope (Env) protein bind the VPS complex to traffic from endosomes to the TGN [77]. Interestingly, depletion of the VPS complex leads to enhanced surface levels of Env, suggesting that retromer-mediated recycling of Env may finely tune the release of virions from the cell surface. HPV16 L2 major capsid protein encodes multiple elements, including a ΦX(L/M) motif, an NPxY motif, and a non-canonical PDZ binding motif, that mediate interactions of L2 with the VPS complex, SNX17 and SNX27, respectively, to cooperatively direct retrograde trafficking of viral DNA [70, 78–80]. Some viral effectors regulate retromer activity during infection. Herpesvirus saimiri tyrosine kinase-interacting protein (Tip) interacts with VPS35, leading to redistribution of VPS35 from early endosomes to lysosomes and inhibition of retromer function [81]. While Tip is not required for viral replication, the ability of Tip to inhibit retromer activity may contribute to Tip-dependent transformation of T cells [82]. HPV E6 is an oncoprotein that interacts with the PDZ domain of SNX27 and modulates retromer-dependent trafficking of the glucose transporter GLUT1, which leads to high levels of glucose uptake and potentially contributes to HPV malignancy [83]. Vaccinia virus K7 protein interacts with VPS26 and VPS35 [84]. While K7 is not essential for vaccinia virus replication, it has been speculated that the K7-retromer interaction could play a role in viral transport, viral uncoating, or cargo transport [85].

Recent studies have revealed the exciting finding that retromer can function as a host **restriction factor** that limits the intracellular replication or survival of some intravacuolar pathogens [8, 9]. In the following sections, we discuss new advances in our understanding of how *C. trachomatis* and *L. pneumophila* effectors interfere with retromer function and how these studies reveal new insights into retromer biology.

Chlamydia hijacks SNX5/6 to counteract host restriction by retromer

C. trachomatis is an obligate intracellular pathogen that is a leading cause of bacterial sexually transmitted and ocular infections and an important cause of non-congenital infertility and blindness, respectively [86]. Understanding the pathogenesis of *C. trachomatis* infections is critical to the development of a successful vaccine, which remains elusive [87]. All *Chlamydia* species share a common intracellular developmental cycle in which they alternate between an infectious, spore-like elementary body (EB), and a non-infectious, metabolically active reticulate body (RB) [88, 89]. EBs are internalized by receptor-

mediated endocytosis into a membrane-bound compartment—the inclusion—that avoids fusion with lysosomes and components of autophagosomes. Within the inclusion, the EB differentiates into an RB and bacterial cell division commences. The inclusion interacts with multiple host organelles to acquire nutrients while evading host immune responses. After replicating over a 24–72-hour time period, RBs re-differentiate into EBs, which are released to infect neighboring cells.

As part of their intracellular survival strategy, *Chlamydia* encodes a T3SS through which they secrete effectors into the host cell. These secreted effectors include a class of proteins unique to Chlamydiales, the Inclusion membrane proteins (Incs), which are translocated from the bacteria through the T3SS and inserted post-translationally into the inclusion membrane [90]. These proteins encode two closely spaced membrane-spanning domains, ~30 amino acids each, separated by a short linker [91]. Once inserted into the inclusion membrane, their N- and C-terminal domains extend into the host cytoplasm, where they are ideally poised to interact with host molecules in order to modulate interactions at the host-pathogen interface [92].

The function of many Incs has been elusive, in large part due to the limited genetic tractability of *Chlamydia* [93]. To circumvent these limitations, an unbiased **affinity purification-mass spectrometry (AP-MS)**-based screen was performed in human cells transiently expressing epitope-tagged *C. trachomatis* Incs to identify putative host binding targets [8]. The resultant Inc-human protein-protein interactome predicted a robust interaction between IncE and the SNX-BAR proteins; notably, VPS complex components did not co-affinity purify with the IncE-SNX-BAR complex [8]. Consistent with this finding, a quantitative proteomics study of inclusions isolated from infected cells revealed that SNX-BAR proteins were enriched in the inclusion proteome [94]. In vitro studies demonstrate that the IncE C-terminal domain is necessary and sufficient to bind directly to the PX domains of SNX5/SNX6 and that this region of IncE does not bind to the corresponding PX domains of SNX1/2 [8, 95, 96]. Depletion of SNX5 alone or in combination with SNX6 enhances the production of infectious progeny but does not diminish inclusion size or quantity [8, 94]. Together, these results suggest that retromer functions as a host restriction factor to limit later steps of *Chlamydia* infection. Whether retromer restricts RB replication or differentiation of RBs toEB s remains to be determined.

Recently, three groups independently solved the structure of IncE bound to the PX domain of SNX5 [7, 95, 96]. The crystal structures are similar and demonstrate that the C-terminus of IncE binds directly to a highly conserved hydrophobic groove in the PX domain of SNX5 at a site that is distinct from the SNX5 phosphoinositide-binding site. This binding site is present in SNX6 but is absent in the closely related SNX1/2 proteins, explaining the exquisite specificity for IncE binding to SNX5/6. The measured submicromolar binding affinity of SNX5-IncE [96] may explain why the SNXs appear to be recruited away from the endosomes and the TGN to the inclusion membrane. Mutation of key conserved residues in the hydrophobic groove of SNX5 diminishes binding to IncE both in vitro [95, 96] and in vivo [7, 95, 96] and abrogates recruitment of SNX5 to the inclusion during *C. trachomatis* infection [7, 96]. Together, these results demonstrate the importance of the hydrophobic groove in the IncE:SNX5/6 interaction.

IncE binding residues in SNX5/6 are highly conserved from worms to humans [7, 95, 96], suggesting that this hydrophobic surface might normally mediate a native interaction with a host macromolecule in the absence of infection. A comparative quantitative AP-MS approach identified several host proteins whose binding to SNX5 is dependent on the same residues to which IncE binds, including the CI-MPR [7]. The finding that CI-MPR co-immunoprecipitates with SNX5 is consistent with the subsequently reported SNX-BAR interactome, which, as described above, also identified CI-MPR as a binding partner for both SNX5 and SNX6 (but not SNX1 and SNX2) [56, 57]. Further studies demonstrate that *C. trachomatis* infection disrupts the CI-MPR:SNX5 interaction [7]. Moreover, addition of an IncE C-terminal peptide competes with binding of CI-MPR to SNX5, indicating that IncE and CI-MPR compete for the same binding surface on SNX5 [7]. Finally, transient production of IncE disrupts CI-MPR trafficking from the endosome to the TGN [8, 95]. These findings are consistent with the emerging notion that SNX-BAR proteins mediate recognition and transport of some cargo, such as the CI-MPR, independently of the VPS complex.

There are several potential explanations for why *Chlamydia* may have evolved a strategy to disrupt CI-MPR trafficking. The CI-MPR delivers newly synthesized hydrolases to the endosome, where they are subsequently trafficked to lysosomes [97]. IncE displacement of CI-MPR from retromer and the resulting disruption in the recycling of the CI-MPR from endosomes to the TGN may diminish lysosome function. While *Chlamydia* has been reported to avoid fusion with lysosomes, these organelles are found in close proximity with the inclusion, possibly to provide a source of amino acids [98–101]. Thus, even a subtle perturbation of lysosome function may allow *Chlamydia* to evade lysosomal killing activity while still allowing this organism to access vital nutrients. Alternatively or in addition, IncE may alter recycling of **Niemann-Pick Disease Type C2 Protein (NPC2)**, another cargo of CI-MPR, resulting in cholesterol accumulation in the late endosomes and lysosomes [102]. As late endosomes provide a source of cholesterol for *Chlamydia* [103–105], inhibition of retromer activity could increase the availability of this cholesterol reservoir to *Chlamydia*. Finally, because retromer has been reported to regulate **autophagy** [106–108], it is possible that IncE-mediated disruption of retromer impacts early autophagic processes, such as the maturation of autophagosome, to reduce host-mediated killing.

Legionella RidL binds to Vps29

Legionella spp are environmental facultative intracellular Gram-negative pathogens whose primary reservoir is aquatic single-celled protozoa [109]. Upon inhalation of *L. pneumophila* by humans, this opportunistic human pathogen replicates inside lung macrophages, causing life-threatening pneumonia (Legionnaires' disease) in immunocompromised hosts or in those with pre-existing pulmonary disease. A milder form of disease, Pontiac fever, causes a flu-like illness in normal hosts [86]. *Legionella* spp utilize a conserved mechanism to proliferate in phagocytic cells, be they amoeba or alveolar macrophages [110–112]. Upon entry, *L. pneumophila* replicates within a membrane-bound compartment, the *Legionella*-containing vacuole (LCV) [110]. The LCV intercepts and associates with the early secretory pathway and merges with the endoplasmic reticulum. *L. pneumonphila* utilizes the Icm/Dot T4SS to translocate several hundred effectors into the host cell, many of which are involved

in the remodeling of the LCV by modulating host proteins and lipids. Mutants in the T4SS apparatus are deficient for intracellular growth, indicating that these secreted effectors are required for successful intracellular growth and survival. Despite the importance of T4SS effectors in *L. pneumophila* infection, the function of many of these effectors remains to be understood, as they have complex, multiple, and often redundant functions, perhaps reflecting their multi-host life style [113, 114].

Several lines of evidence suggest that the *L. pneumophila* LCV associates with retromer [3]. Proteomic studies of intact LCVs purified from *Dictyostelium discoideum*, confocal microscopy and imaging flow cytometry demonstrate the presence of retromer components VPS29 and SNX1/2, as well as Rab7 and TBC1D5 [9, 115]. VPS26, VPS29, VPS35, and Rab7 localize to the LCV in *L. pneumophila*-infected cells [9], and depletion of VPS26, VPS29, or the CI-MPR increases intracellular replication ~ two-fold in both amoeba and lung macrophages [9].

The *Legionella* T4SS effector RidL has been implicated in modulating retromer function during *L. pneumophila* infection [9, 116–118]. RidL binds directly to VPS29 in the absence of other *Legionella* proteins. Ectopically expressed RidL impairs retrograde trafficking of Shiga toxin subunit B [9], a process known to be dependent on the VPS complex [119] and SNX1 [120]. RidL is secreted from *Legionella* during infection and localizes to the LCV [9]. A *L. pneumophila ridL* mutant exhibits a 2-fold decrease in intracellular replication in amoeba and in cultured macrophage cell lines, suggesting that RidL promotes intracellular growth [9]. Interestingly, retromer components still localize to the LCV in the absence of RidL but not in a T4SS mutant [9]. Together, these observations suggest that during *Legionella* infection, retromer is recruited to the LCV by an unknown T4SS effector and can restrict *Legionella* growth. However, *Legionella* circumvents this host defense mechanism by secreting RidL. Given the localization of RidL on the LCV and the location of the LCV within the retrograde pathway, it has recently been proposed that the LCV could be an acceptor compartment in the retrograde trafficking pathway where RidL blocks the interaction of the pathogen vacuole with incoming transport vesicles [121]. It is possible that RidL also acts outside of the LCV and inhibits the formation of transport vesicles at endosomes exit sites [121]. Further studies are needed to determine the site of action of RidL and the position of the LCV in the retrograde pathway.

How might RidL binding to VPS29 interfere with retromer function? The recently solved crystal structure of the N-terminal region of RidL, either alone or in complex with VPS29 or with the VPS29-VPS35 retromer subcomplex, provides a possible mechanism [116–118]. The structure reveals a beta-hairpin loop protruding from the N-terminus of RidL that inserts into and binds with high affinity to a conserved pocket on VPS29. This region of VPS29 is normally occupied by a structurally similar loop found in the Rab7 GAP TBC1D5 and in the adaptor protein VARP [23, 24, 117]. In vitro, RidL binds with higher affinity to VPS29 compared to TBC1D5 [117, 118] or to VARP [117] and competes with TBC1D5 for binding to retromer in solution [116–118]. Upon ectopic production, a protein comprising this region of RidL co-localizes with retromer at endosomes and displaces TBD1D5 from retromer in a manner dependent upon residues within the beta-hairpin loop [116–118]. Likewise, *L. pneumophila* strains producing RidL exhibit a modest decrease in the amount of TBC1D5

associated with the LCV compared to a strain lacking RidL [116]. Together, these results indicate that RidL is capable of competing with TBC1D5 and/or VARP for binding to VPS29.

Understanding the link between the RidL-mediated displacement of TBC1D5 and/or VARP from VPS29 and the role of retromer in restricting *Legionella* growth is of great interest. If RidL displacement of TBC1D5 interferes with retromer function, then *Legionella* should replicate more efficiently in the absence of TBC1D5, assuming that loss of TBC1D5 would phenocopy the effect of RidL. Surprisingly, *Legionella* replication is impaired in *D. discoideum* deleted for TBC1D5 [116]. One possible explanation for this unexpected result is that binding of RidL to VPS29 not only blocks retromer activity during infection but may also liberate TBC1D5 and/or VARP from retromer to function in non-retromer pathways that promote *Legionella* growth [116]. In the case of TBC1D5, these additional functions could include modulating Rab7 at other membranes [116, 122] and/or regulating autophagy through the interactions of TBC1D5 with ATG9 and LC3 [106, 107]. In addition, TBC1D5 may have promiscuous GAP activity towards other Rab GTPases that promote *Legionella* infection [116]. In this way, displacement of TBC1D5 by RidL from retromer would not be functionally equivalent to loss of TBC1D5.

It will be interesting to determine whether RidL contributes in other ways to *Legionella* infection. Its ability to displace VARP may also contribute to disruption of retromer function. In addition, given the overall large size of RidL and the very small region of RidL that is sufficient to displace TBC1D5/VARP from VPS29, it is possible that other portions of RidL may bind retromer components to inhibit retromer activity or may interfere with non-retromer pathways that could restrict *Legionella* growth.

Concluding Remarks and Future Perspectives

Intracellular vacuolar pathogens must reprogram host trafficking pathways to establish a unique replicative niche, obtain nutrients, and prevent recognition by the host cell defense system [2]. Retromer-mediated trafficking is emerging as a previously unappreciated target of these pathogens. Viral proteins and bacterial effectors may hitch a ride on retromer. In addition, some intravacuolar pathogens may benefit from delivery of cargo transported by retromer to their site of replication. In both of these scenarios, intracellular microbes would require retromer-dependent trafficking for survival. However, other intravacuolar pathogens may subtly disrupt retromer as a mechanism to alter lysosome function and escape degradation. Recent studies reveal that *C. trachomatis* IncE and *L. pneumophila* RidL encode small structural motifs that have the potential to outcompete native retromer-host interactions that rely on highly conserved hydrophobic surfaces. Intriguingly, these pathogen effectors do so by targeting different retromer subcomplexes: *Legionella* targets the VPS complex whereas *Chlamydia* targets the SNX complex. These disparate strategies might have evolved as a consequence of the natures of compartment in which the pathogen resides. Further studies of pathogen-retromer interactions will likely reveal additional insights into the intricate details of retromer assembly and function. In summary, retromer is emerging as a critical host cell innate defense mechanism to restrict the growth and survival of vacuolar pathogens (see Outstanding Questions).

Outstanding Questions

- How does retromer restrict the growth of *Legionella* and *Chlamydia*?
- Does retromer restrict intracellular replication of other pathogens?
- Does disruption of retromer by RidL or IncE alter lysosome function and/or autophagy?
- Does RidL alter TBC1D5 and/or VARP function during *Legionella* infection?
- Are there other functions of RidL that contribute toits role in*Legionella* intracellular replication?
- Does IncE disrupt binding of other cargo to SNX5/6?
- What is the functional significance of direct cargo recognition by SNXs?

Acknowledgments

This research is supported by grants from the National Institutes of Health (RO1 AI122747, R21 AI105561).

Glossary

Affinity purification-Mass spectroscopy (AP-MS).
Epitope-tagged proteins are expressed in cells, affinity purified from cellular lysates using epitope-specific antibodies, and bound proteins are eluted for identification by mass spectroscopy.

Autophagy
regulated intracellular degradation system that disassembles damaged organelles, misfolded proteins, and invading pathogens. The spherical double layered membranous compartment in which degradation occurs is called an autophagosome. Many host proteins are involved in this process, including ATG9 and LC3.

Cation-Independent Mannose-6-Receptor (CI-MPR)
Binds to Mannose-6-Phosphate modified proteins in the TGN, such as lysosomal hydrolases, and transports them by anterograde trafficking to the endosome. As the endosome matures and acidifies, the hydrolases are released from the CI-MPR and transported via vesicular trafficking to the lysosome. The CI-MPR is concentrated and transported by retromer back to the TGN.

Dynein-dynactin minus-end directed motor complex
Microtubules transport cargo using either plus end-directed motors (from the cell center to the periphery) such as kinesin or minus-end directed motors (from the cell periphery to the cell center) such as dynein-dynactin.

Effector
Pathogen-encoded virulence factors.

GTPase activating protein (GAP)
a protein that enhances the intrinsic GTPase activity of small GTPases such as Rab proteins.

Guanine exchange factor (GEF)
a protein that exchanges GTP for GDP on small GTPases such as Rab proteins.

Intravacuolar pathogen
a pathogen that replicates in its eukaryotic host within a membrane-bound compartment (the parasitophorous vacuole).

Niemann-Pick Disease Type C2 Protein (NPC2)
a protein that regulates transport of cholesterol through the late endosomal/lysosomal system. Mutations in the gene are associated with Niemann-Pick disease, type C2, a fatal heriditary lipid storage disorder.

Parasitophorous vacuole
a specialized membrane-bound compartment within a host cell in which pathogens replicate.

Post-synaptic density/disc large tumor suppressor/zona occludens (PDZ domain)
a structural motif of 80–90 amino acids commonly found in eukaryotic signaling proteins that binds to a short beta strand in the C-terminus of proteins.

Phosphoinositide
a class of phospholipids that are phosphorylated derivatives of phosphoinositol and that are localized on the cytosolic leaflet of membranous organelles. Phosphoinositol is comprised of a glycerol molecule with two fatty acid chains and an inositol headgroup, which can be phosphorylated at the 3, 4, and/or 5 position. Phosphatidyl inositol-3-phosphate is found on endosomes. Phosphatidyl inositol-4-phosphate is typically found on the TGN.

Phox-homology (PX) domains
Protein domains that recognize and bind to distinct phosphoinositides, allowing proteins to bind to specific membranous organelles.

Rab protein
a monomeric G protein that is involved in regulating vesicle trafficking. Rab proteins cycle between an active GTP-bound state and an inactive-GDP bound state.

Retrograde trafficking
transport of proteins from the endosome to the Golgi or ER

Retromer
an evolutionarily conserved multi-protein complex involved in recycling protein cargo from the endosome to either the Trans Golgi Network (TGN) or the plasma membrane. Retromer is composed of 2 associated but distinct sub-complexes: the vacuolar protein sorting (VPS) complex and the Sorting Nexins (SNXs). In this review, retromer refers to the VPS and SNX sub-complexes, though in some literature, retromer refers only to the VPS complex.

Restriction factor

Host factors that were originally defined as anti-viral proteins but now include anti-bacterial proteins that counteract or “restrict” pathogen replication.

Type III and type IV secretion systems (T3SS, T4SS)
specialized protein export systems utilized by some Gram-negative pathogens to translocate proteins directly from the bacteria across host membranes. The T3SS evolved from the flagellar motility apparatus whereas the T4SS is evolutionarily related to the conjugation apparatus.

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Highlights

- Retromer recycles host protein cargo from endosomes to the plasma membrane or the *trans*-Golgi network. It is composed of (i) the trimeric vacuolar protein sorting (VPS) complex that engages and concentrates cargo for transport and (ii) one or more sorting nexins (SNXs), proteins that mediate formation of tubulovesicular carriers. The VPS complex and SNXs participate in cargo selection.
- Intracellular pathogens employ diverse strategies to subvert or inhibit retromer function. Retromer can enhance or restrict intracellular pathogen growth and can be considered part of the innate immune response.
- *Legionella* RidL and *Chlamydia* IncE disrupt retromer-mediated restriction of intravacuolar pathogens and structural studies support the emerging concept that the SNX and VPS subcomplexes are functionally distinct units that can independently recruit cargo and organize multiple sorting pathways.

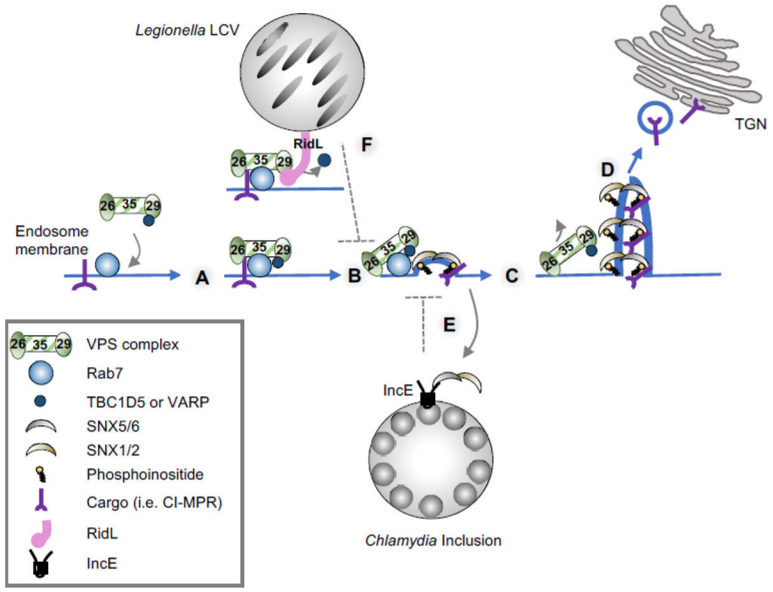

Figure 1. The retromer complex and its interactions with *Legionella* and *Chlamydia*

A. The VPS complex is recruited to the endosomal membrane by Rab7-GTP and by cooperative interactions with SNXs (not pictured here).

B. The SNX-BAR heterodimer associates with membrane phosophoinositides through its PX domains and induces membrane curvature via its BAR domains. SNX5/SNX6 recruit a subset of cargo, including the CI-MPR, into a discrete subdomain for transport to the TGN.

C. TBC1D5, which binds to VPS29, converts Rab7 to its GDP-bound state, leading to the disassociation of the VPS complex from the membrane.

D. Endosomal tubules containing cargo undergo scission. Cargo-rich vesicles are transported to the TGN or to the plasma membrane (not pictured).

E. *Chlamydia* IncE binds with high affinity to the PX domain of SNX5/6, displacing CI-MPR. SNX5/6, along with SNX1/2, are recruited away from retromer to the inclusion. Retromer-dependent recycling of the CI-MPR is disrupted.

F. *Legionella* RidL binds with high affinity to the TBC1D5/VARP binding interface of VPS29, displacing one or both of these proteins. Retromer trafficking is disrupted. The figure is partially adapted from [3, 37]. Dotted lines indicate the proposed mechanism.

Table 1

Summary of pathogen effector-retromer interactions

Effector	Pathogen	Retromer Target	Function	Reference
	VIRUSES			
NS5A	Hepatitis C virus	VPS35	Recruits retromer to deliver cargo and membranes to replications sites	[65, 123]
TIP	Herpes saimiri virus	VPS35	Inhibits retromer activity, may contribute to oncogenic transformation of T cells	[81, 82]
Env	Human immunodeficiency virus 1	VPS26 VPS35	Transports Env to the TGN	[77]
L2	Human papilloma virus	SNX27 VPS complex	Transports viral DNA to TGN	[70, 78, 79]
L2	Human papilloma virus	SNX17	Transports viral DNA to TGN	[80]
E6	Human papilloma virus	SNX27	Alters trafficking of GLUT1 to increase glucose availability	[83]
M2	Influenza A virus	VPS complex	Transports M2 to the TGN to escape lysosome degradation	[76]
K7	Vaccinia virus	VPS35 VPS26	Unknown but may regulate viral transport and/or viral uncoating or inhibit cargo transport	[84]
	BACTERIA			
?	*Salmonella enterica serovar* Typhimurium	SNX1 SNX3	Induces vacuole tubulation and promotes vacuole maturation	[68, 69]
?	*Coxiella burnetti*	VPS29 VPS35 SNX2 SNX3 SNX5 SNX6	Promotes translocation of effector proteins and vacuole maturation	[67]
IncE	*Chlamydia trachomatis*	SNX5 SNX6	Inhibits retromer activity	[7, 8, 95, 96]
RidL	*Legionella pneumophila*	VPS29	Inhibits retromer activity	[9, 116–118]