Retromer revisited: Evolving roles for retromer in endosomal sorting

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The highly conserved retromer complex has been linked to cargo retrieval from endosomes to the trans-Golgi network. In this issue, Kvainickas et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201702137) and Simonetti et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201703015) fundamentally question the current retromer model and demonstrate that in mammalian cells, the individual retromer subcomplexes have functionally diverged to organize multiple distinct sorting pathways.

The highly conserved retromer complex is thought to mediate the retrieval of cargo receptors, including the cation-independent mannose 6-phosphate receptor (CI-MPR), from endosomes to the trans-Golgi network (TGN). In yeast, retromer is formed by two subcomplexes that each contributes distinct functions during cargo recycling. The trimeric vacuolar protein sorting (Vps) core consists of Vps35, Vps29, and Vps26 and directly engages and concentrates cargo molecules in endosomal subdomains. The second subcomplex consists of two members of the sorting nexin (SNX) protein family, Vps5p and Vps17p (Seaman et al., 1998). These SNXs each contain a C-terminal bin-Amphiphysin-Rvs (BAR) domain that allows for self-assembly of the Vps5p/Vps17p dimer into higher-order helical arrays on endosomes, resulting in membrane tubulation and the formation of cargo-containing vesicular or tubular transport carriers. The components of the retromer complex are well conserved in higher eukaryotes, the major difference being the increase in proteins found in the SNX-BAR dimer where either of the Vps5p orthologues, SNX1 and SNX2, dimerizes with one of the Vps17p orthologues, SNX5 and SNX6 (Gallon and Cullen, 2015). However, interactions between the Vps and SNX-BAR subcomplexes are considered much more transient in mammalian cells, and biochemical evidence for their interaction remains elusive. SNX-BAR dimer recruitment to endosomal membranes is not affected by loss of Vps expression, and the commonly accepted functional connection of Vps retromer and the SNX-BAR dimer in mammalian cells is mainly based on their proposed mutual role in CI-MPR transport from endosomes to the TGN (Gallon and Cullen, 2015).

In this issue, the studies presented by Kvainickas et al. and Simonetti et al. fundamentally question the obligatory cooperation of the Vps and SNX-BAR subcomplexes and challenge the ascribed role of Vps retromer in CI-MPR transport. In an impressive set of knockdown, knockout, and rescue studies, the two studies identify the SNX-BAR dimer as the key regulator of CI-MPR retrograde transport in mammalian cells. Loss of SNX dimer function causes the redistribution of CI-MPR from the TGN to endosomes that are positive for the early endosomal marker EEA1, Vps retromer, and the small GTPase Rab7a (Kvainickas et al., 2017; Simonetti et al., 2017), which controls Vps retromer recruitment to endosomes. Thus, in the absence of SNX-BAR dimer function, CI-MPR remains stuck in endosomes even though Vps retromer is efficiently recruited to the organelle. Furthermore, both studies fail to confirm the previously reported role for Vps retromer in CI-MPR recycling (Arighi et al., 2004; Seaman, 2004). Vps retromer loss of function has no effect on CI-MPR distribution between endosomes and the TGN (Kvainickas et al., 2017; Simonetti et al., 2017). Notably, the receptor accumulates in an endosomal subdomain that is spatially distinct from Vps retromer despite the previously reported interaction of the receptor with Vps35 (Arighi et al., 2004). In WT cells, confocal and superresolution microscopy confirmed that the SNX-BAR dimer and CI-MPR strongly overlap in an endosomal subdomain distinct from Vps retromer (Kvainickas et al., 2017; Simonetti et al., 2017), confirming the functional dissociation of Vps retromer and SNX-BAR dimer as an inherent feature of mammalian cells.

At this point, it remains unclear whether the differences with earlier studies that assigned a central role to Vps retromer in CI-MPR transport to the TGN originates from the use of new technical approaches such as CRISPR/Cas9, differences in experimental designs and readouts used to score phenotypes, indirect effects of ectopic protein expression, or cell line–specific differences, or if they are caused by other reasons yet to be understood. However, the functional divergence of the Vps core and SNX-BAR dimer in mammalian cells is well in agreement with studies linking Vps retromer to sorting events that direct cargo to the TGN and the plasma membrane (Gallon and Cullen, 2015). Furthermore, Kvainickas et al. (2017) and Simonetti et al. (2017) use a wide range of thoroughly controlled experiments and approaches to conclusively identify the SNX dimer as an independent cargo-binding molecule responsible for the retrograde recycling of CI-MPR and most likely other cargo, thereby shattering the prevailing dogma of retromer organization and function in higher eukaryotes, including mammalian cells.

The functional divergence of the Vps and SNX-BAR subcomplexes during evolution may, in addition to the apparent loss of their physical interaction, also be reflected in the acquisition of new binding partners such as receptor-mediated endocytosis

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The Rockefeller University Press
J. Cell Biol. Vol. 216 No. 11 3433–3436
https://doi.org/10.1083/jcb.201708111

8 (RME-8), EHD1, and the Wiskott-Aldrich syndrome protein and SCAR homologue (WASH) complex, which are not found in yeast. These interactions may offer a window into the functional diversity and physiological roles adopted by the Vps and SNX-BAR complexes. Vps retromer is recruited to endosomes through interactions with the small GTPase Rab7 and in turn recruits the WASH complex, which generates branched actin networks on endosomes. Interestingly, there is mounting evidence that Vps/WASH functions with different SNXs in cargo transport from endosomes to the plasma membrane. For example, Vps/SNX3 mediate recycling of the WNT receptor wntless to the cell surface. In addition, Vps/WASH/SNX27 control the recycling of β2-adrenergic receptor and a wide variety of other cargo (Steinberg et al., 2013). However, the partial overlap of Vps retromer– and SNX27-dependent recycling cargo suggests that Vps retromer as well as SNX27, which directly binds cargo through its FERM and PDZ domains, also functions in an additional recycling pathway or pathways. In the case of cargo dependency on both, it still remains unclear whether SNX27 functions as alternate cargo adapter in parallel to Vps retromer or if cargo is handed over from the Vps to SNX27 as was originally proposed for the relationship between the Vps and SNX-BAR retromer subcomplexes.

The Phox (PX) domain in SNXs was initially characterized as a phosphoinositol 3-phosphate (PI3P) binding domain responsible for protein recruitment to endosomal membranes. Recent structural research identified a protein binding site on the SNX5 PX domain that is targeted by the *Chlamydia trachomatis* protein Inc.E when mammalian cells are hijacked by this intracellular pathogen (Elwell et al., 2017). Importantly, the same binding site mediates interactions with the cargo receptor CI-MPR (Elwell et al., 2017). Moreover, the interactome of all SNX-BAR members, in particular for SNX5 and SNX6, contains numerous cargo proteins (Kvainickas et al., 2017; Simonetti et al., 2017). It is thus likely that the cargo-binding site is at least conserved in the PX domains of SNX5 and SNX6. Yet, there is only partial overlap in the binding partners identified for each SNX, which may reflect differences in protein expression levels during the experiments. Alternatively, these data may indicate that SNX1/SNX2 and SNX5/SNX6 are not as functionally redundant as previously assumed. It will be interesting to see whether live-cell imaging of different SNXs with their potential cargo reveals new layers of cargo specificity in endosomal sorting and leads to further diversity in the map of endosomal sorting routes.

CI-MPR binding and sorting by the SNX-BAR dimer depends on the presence of a WLM peptide motif in the cytosolic receptor tail (Seaman, 2007; Kvainickas et al., 2017; Simonetti et al., 2017). However, not all candidate cargo molecules contain a WLM motif, and therefore, different binding motifs may explain the cargo selectivity for SNX5 or SNX6 seen in the quantitative proteomics analysis. It also remains to be seen whether the PX domains in other SNX-BAR proteins not previously linked to retromer function also function as cargo adapters, and whether this ability may even apply to all SNXs. Defining cargo binding sites and the peptide motifs they engage will provide important new insights into the molecular determinants that create pathway specificity within the complex network of intracellular transport pathways. Notably, neither study identified components of the Vps trimer as SNX-BAR binding partners (Kvainickas et al., 2017; Simonetti et al., 2017), providing further evidence of the physical and functional segregation of the two transport complexes in mammalian cells.

The interaction of SNX1 with RME-8, a DnaJ domain–containing protein involved in the regulation of clathrin dynamics of endosomes, provides a mechanism for the separation of SNX-BAR–enriched subdomains from endosomal regions specialized in protein degradation. RME-8 locally interferes with the organization of endosomal clathrin and the endosomal sorting complexes required for transport (ESCRT) machinery, thereby preventing cargo sorting toward degradation at sites of recycling tubule formation (Norris et al., 2017). In addition, Simonetti et al. (2017) identified the Rab7 GTPase-activating protein (GAP) Tre-2/Bub2/Cdc16 domain family member 15 (TBC1D15) as a binding partner of SNX5 and SNX6. Because Rab7 activity is required for Vps retromer and ESCRT recruitment to endosomes, the presence of a Rab7 GAP as part of the SNX-BAR protein machinery would allow for a negative feedback loop toward the competing protein machineries and establish physical borders on endosomes for SNX-BAR–mediated tubule formation and cargo recycling (Fig. 1). Once we have a more complete picture of the molecular anatomy of the different endosomal sorting pathways, it will be interesting to see which, if any, small GTPases may regulate SNX dimer-mediated transport and how these pathways may interface with the regulation of other Arf- and Rab-mediated endosomal sorting events.

The overwhelming complexity of endosomal sorting pathways is not surprising given the constant influx of cargo from endocytic and biosynthetic routes and the numerous intracellular destinations of endosomal sorting. For example, the SNX-BAR dimer has been linked to cargo sorting to the plasma membrane, the TGN, and the endoplasmic reticulum, raising the question of how one protein complex faithfully distributes diverse sets of cargo to their correct destinations. One possibility would be the creation of distinct protein machineries formed around a SNX-BAR dimer core that establish pathway specificity for the different cargo molecules (Fig. 1). Based on their findings, Kvainickas et al. (2017) propose an intriguing alternate scenario in which endosomal sorting is a two-step event. The initial sorting step, mediated by the SNX-BAR complex, would divert cargo not destined for degradation into a SNX-BAR subdomain or tubule. From there, additional protein machineries then sort cargo according to their destination.

In this scenario, the initial sorting step could ensure rapid removal of recycling cargo away from the degradation machinery and provide the time and dedicated environment for the localized assembly of secondary sorting machineries that direct cargo to their target sites. The arrival of live-cell superresolution microscopy could provide a means to determine whether, for example, Vps retromer/SNX27–mediated recycling represents one such secondary sorting pathway. The recent discovery of the retriever complex, which functions together with the FERM domain containing cargo adapter SNX17 and in which individual protein components show a striking structural similarity to retromer (McNally et al., 2017), may provide another. Alternatively, Vps retromer, retriever, and SNX-BAR dimers could function in distinct, parallel pathways (Fig. 1). In this case, yet to be characterized adaptations in the protein machineries built around the SNX-BAR dimer would determine the final destination of individual cargo. Clearly, evaluation of the two-step sorting model will require further testing and a more detailed characterization of the different protein machineries involved in endosomal cargo sorting.

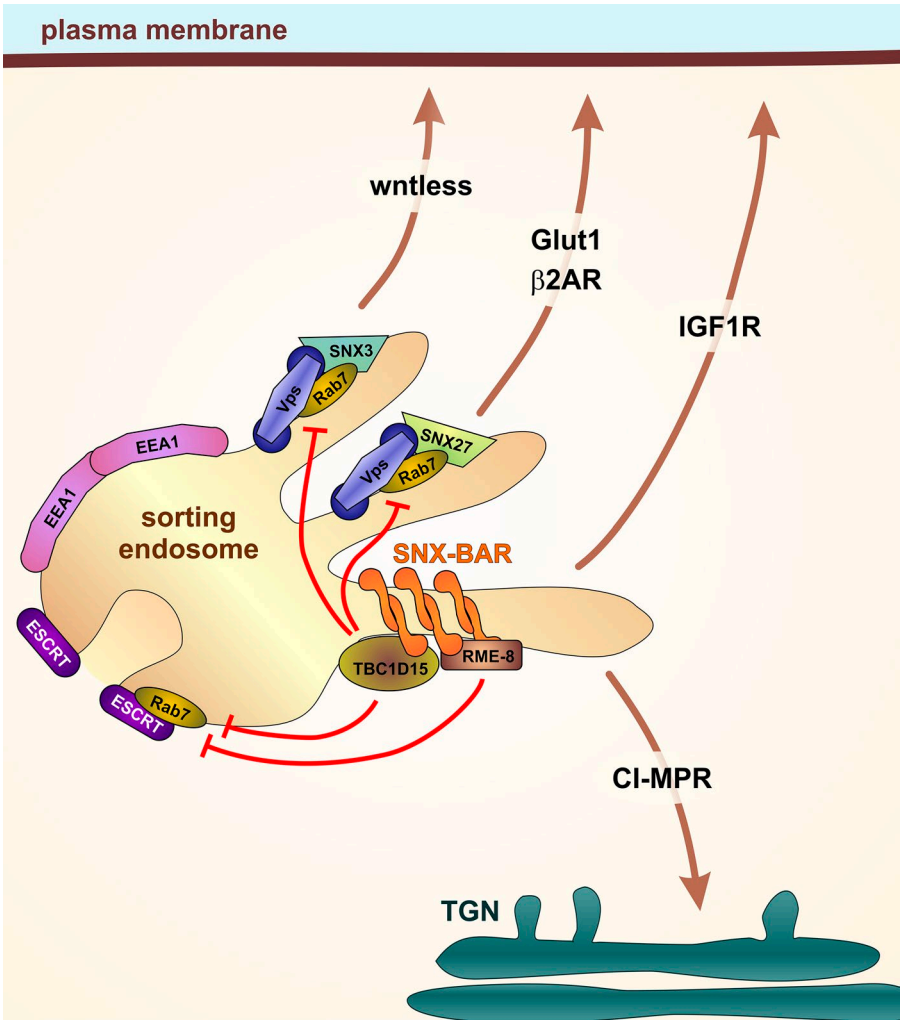

Figure 1. **Vps retromer and SNX-BAR dimer–mediated endosomal sorting pathways in mammalian cells.** Vps retromer and SNX-BAR dimer form distinct subdomains on endosomes away from the ESCRT machinery for protein degradation. Vps retromer engages Rab7 and SNX3 or SNX27 to mediate cargo recycling from endosomes to the plasma membrane. The SNX-BAR dimer sorts select cargo to the plasma membrane and the TGN. Examples of pathway-specific cargo are indicated. Recruitment of RME-8 and TBC1D15 by the SNX-BAR dimer may coordinate negative feedback loops toward Vps retromer and ESCRT function (red arrows) to establish a pathway-specific endosomal subdomain.

The new mechanistic insights into Vps retromer and SNX-BAR dimer function are also important from a medical perspective. Hereditary mutations in Vps35 cause Parkinson's and Alzheimer's disease, and mutations in the WASH complex component strumpellin lead to hereditary spastic paraplegia (Small and Petsko, 2015). Interestingly, these mutations cause cargo accumulation in endosomes and excessive tubulation of the compartment, highlighting a direct link between the efficiency of endosomal sorting events and cellular dysfunction. Therefore, the functional diversity of the Vps retromer and SNX-BAR sorting pathways in mammalian cells has important implications for our understanding of human disease, in particular neurological disorders. A complete understanding of endosome biology and function will improve our ability to manipulate select transport pathways and is likely to open new avenues for the design of therapeutic interventions with high efficacy and limited side effects. Notably, the study by Kvainickas et al. (2017) also revealed that TGN46 sorting from endosomes to the TGN is independent of both Vps retromer and the SNX-BAR dimer, demonstrating the existence of at least one additional retrograde transport pathway in mammalian cells that is yet to be identified. Collectively, the studies by Kvainickas et al. (2017) and Simonetti et al. (2017) demonstrate that there is still a lot to learn about endosomal sorting.

Acknowledgments

The authors declare no competing financial interests.

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