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Sorting nexins provide diversity for retromer-dependent trafficking events

Peter J. Cullen^{1,3} and Hendrik C. Korswagen^{2,3}

¹Henry Wellcome Integrated Signalling Laboratories, School of Biochemistry, Medical Sciences Building, University Walk, University of Bristol, Bristol BS8 1TD, U.K. ²Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands.

Abstract

Sorting nexins are a large family of evolutionarily conserved phosphoinositide-binding proteins that play fundamental roles in orchestrating the process of cargo sorting through the membranous maze that is the endosomal network. One ancient group of sorting nexin-containing complexes is the retromers. Here we discuss how retromer complexes regulate endosomal sorting, and describe how this is generating exciting new insight into the central role played by endosomal sorting in development, and normal tissue homeostasis.

Introduction: the endosomal network

The endosomal network comprises a series of interconnected membranous compartments that begins at the plasma membrane with internalisation of cargo through endocytosis¹. Internalised cargo enters the early endosome, an endomembrane compartment enriched in phosphatidylinositol 3-monophosphate (PtdIns(3)P) and morphologically characterised by vacuolar and tubular sub-domains². Early endosomes initiate cargo sorting, principally into two distinct pathways: a central endo-lysosomal degradative route, one major function of which is to down-regulate signalling receptors; and a collection of distinct retrieval pathways that recycle cargo, such as nutrient sensing receptors, away from the degradative axis (Figure 1A). While a great deal is known about the mechanistic details of cargo sorting into the endo-lysosomal degradative pathway, and the central role played by PtdIns(3)P³, a mechanistic understanding of how cargo is retrieved from lysosomal degradation is only now beginning to emerge^{4, 5}.

As with a number of membrane trafficking events, retrieval is orchestrated through the assembly of coat complexes, which function to recognise and concentrate specific cargo, drive membrane remodelling and elicit scission to form a cargo-enriched carrier⁶. One family of PtdIns(3)P-binding proteins that have emerging roles as coat complexes in multiple endosomal retrieval pathways are the evolutionarily conserved sorting nexins (SNXs)^{2, 7}. In particular, much of our insight into how these proteins regulate retrieval, and how this basic aspect of cell biology impinges on organism development, has stemmed from the study of the retromer complex.

³To whom correspondence should be addressed. pete.cullen@bristol.ac.uk; Phone: +44-117-3312193; FAX: +44-117-3312168. r.korswagen@hubrecht.eu; Phone: +31-30-2121981; FAX: +31-30-2516464. .

Retromer

Retromer's primary role is to select cargo proteins for retrograde endosome-to-Golgi transport (Table 1). In yeast, retromer is classically considered a multi-protein complex formed by association of two sub-complexes: a stable Vps26, Vps29, and Vps35 trimer and a membrane-bound heterodimer of the SNXs, Vps5 and Vps17⁸ (Figure 1B). The Vps26-Vps29-Vps35 trimer is termed the cargo selective adaptor, on account of Vps35 directly binding to sorting motifs present in the cytoplasmic domains of cargo proteins, while the SNX heterodimer is called the membrane deforming sub-complex to acknowledge its ability to induce and/or stabilise the formation of membrane tubules. Interwoven with these events, retromer recruits additional proteins that aid further cargo capture and packaging^{9, 10}, as well as accessory proteins that regulate maturation and scission of the retromer tubules¹¹⁻¹⁵. Retromer therefore shares a number of similarities with archetypal coat complexes such as COPI, COPII and clathrin coats⁶: it is an evolutionarily conserved, multi-protein assembly designed to select cargo, drive membrane remodelling and elicit carrier scission.

Membrane remodelling – the SNX-BAR sub-complex

In metazoans, gene duplication at the vertebrate ancestor has resulted in two Vps5 orthologs SNX1 and SNX2, whereas duplication of Vps17 in the metazoan ancestor has resulted in SNX5, SNX6 and possibly SNX32^{16,17} (Figure 1B). These SNXs are members of the SNX-BAR sub-family as they contain two membrane-binding modules: a membrane curvature sensing BAR domain (Bin/amphiphysin/Rvs), and a phosphoinositide-binding PX domain (phox homology). The PX domain in SNX1 and SNX2 associates with the endosomal phosphoinositides, PtdIns(3)P and phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P₂)¹⁸. For BAR domains the ability to sense membrane curvature arises upon dimerisation, which forms a positively charged crescent-shaped surface that through electrostatic interactions binds to membranes of positive curvature¹⁹. Dimerisation of retromer SNX-BARs follows a simple rule: one Vps5 equivalent dimerises with one Vps17 equivalent²⁰; eukaryotic lineages between Amoebozoa and Excavata, which lack a Vps17 equivalent, appear to rely upon Vps5 homodimerisation¹⁷. In combining the membrane binding affinities of the PX and BAR domains, retromer SNX-BARs utilise an avidity-based system to sense high curvature sub-domains of the PtdIns(3)P/PtdIns(3,5)P₂-enriched endosomal network^{21, 22}.

Besides sensing membrane curvature, certain BAR domains are capable of inducing membrane remodelling leading to membrane tubulation²³. Both SNX1 and SNX2 are predicted to contain amphipathic helices amino-terminal to their BAR domains²⁴. For N-BARs (N-terminal amphipathic helix-BARs), the amphipathic helix forms in the cytosol-lipid interface leading to the formation of surface tension between leaflets of the bilayer¹⁹. The membrane accommodates this tension by generating positive curvature (*i.e.* bends into the cytosol) which is stabilised by the curvature sensing properties of the BAR domain. As the effective membrane-bound concentration of the BAR domain increases²⁵, it is argued that local membrane remodelling is translated into a global re-sculpturing by self-assembly of the BAR domain-containing protein into a higher-ordered helical array^{23, 26, 27}. A model therefore emerges in which retromer SNX-BARs switch between curvature-sensing and curvature-inducing modes and in doing so drive the formation of endosomal tubules²⁸. That retromer SNX-BARs can induce vesicle-to-tubule transitions *in vitro* and *in vivo* is entirely consistent with this model^{21, 29}, as is data that tubules generated in an *in vitro* reconstitution have a diameter comparable with that observed for SNX-BAR-retromer tubules from 3D electron tomography (30-55 nm versus 20-50 nm respectively)^{21, 30}. It should be stressed however, that it remains to be determined if the physiological concentration of retromer SNX-BARs corresponds to the specific concentration range required for switching between curvature-sensing and curvature-inducing modes.

Cargo selection – the VPS26-VPS29-VPS35 sub-complex

The VPS26-VPS29-VPS35 sub-complex is a highly conserved protein assembly that originated before the last eukaryotic common ancestor¹⁷. The core component is VPS35, which forms an extended horseshoe-shaped α -helical solenoid onto which VPS26 and VPS29 independently associate at either distal end^{31, 32}. Interaction with the cytoplasmic domains of cargo proteins is of low affinity and, for the majority of cargoes no strong consensus-binding motif has been described³³⁻⁴³ (Table 1). The VPS26-VPS29-VPS35 adaptor itself lacks any recognisable membrane binding motifs, being associated with endosomes through interaction with the GTP-loaded form of Rab7^{14, 44, 45} (see below for discussion of an additional mechanism for membrane targeting of VPS26-VPS29-VPS35). Binding to Rab7 presumably restricts the diffusion of the VPS26-VPS29-VPS35 adaptor allowing the on-rate of cargo binding to increase favouring a more stable cargo-bound nucleation complex, while the GTP-dependency incorporates timing into the nucleation event. Moreover, as the VPS26-VPS29-VPS35 adaptor also interacts with the Rab GAP TBC1D5¹⁴, fine-tuning of the Rab7 GTPase cycle may regulate the assembly and turnover of the nucleation complex. Indeed, a similar role for Arf1-GTP and Sar1-GTP, and their respective GAP proteins ARFGAP1 and Sec23, in controlling the dynamics of COPI and COPII coats is a well-established concept⁶.

For SNX-BAR-retromer little is known about the regulation of cargo capture: does it rely on chance encounters governed by random diffusion and low-affinity interactions (*i.e.* a stochastic event) or does it incorporate a ‘pre-nucleation’ priming of cargo that may enhance avidity and generate co-operativity in cargo recognition⁴⁷? Indeed, it has been proposed that clathrin and a variety of clathrin adaptors and binding proteins, *e.g.* Hrs, epsinR and RME-8, function to cluster retromer cargo on the endosomal surface prior to SNX-BAR-retromer-mediated sorting (see ⁴⁷ for an extensive review of the interface between clathrin and SNX-BAR-retromer). Finally, it is important to consider how non-SNX-BAR-retromer cargoes are excluded from packaging into the forming tubular profiles. Here, the interwoven aspects of cargo capture/package and membrane re-modelling may combine to exclude unwanted proteins: efficient packing of captured cargo limiting the membrane environment available for non-specific integral membrane proteins, while the geometrical shape of the tubular profile has the effect of reducing lateral diffusion into and out of the forming carrier.

Additional cargo-specific adaptors

The repertoire of retromer cargoes is expanding with the identification of cargo-specific adaptors that mediate recycling by piggy-backing onto the SNX-BAR-retromer pathway. In yeast, recycling of the Fet3p-Ftr1p reductive iron transporter requires recognition of the cytoplasmic domain of Ftr1p by the sorting nexin Grd19/Snx3p⁹. Grd19/Snx3p physically associates with the SNX-BAR-retromer thereby allowing recycling of the Fet3p-Ftr1p back to the plasma membrane via the Golgi apparatus⁹ (Figure 3B). Moreover, two novel WD-40 domain proteins, Ere1 and Ere2 (Endosomal Recycling proteins) also appear to function as adaptors for SNX-BAR-retromer-mediated sorting: Ere1 interacts with the arginine transporter Can1 regulating its recycling via the SNX-BAR-retromer pathway⁴⁸. In mammalian cells SNX27, a PDZ domain-containing SNX, interacts with the carboxy-terminal PDZ ligand of the β 2-adrenergic receptor (β 2AR), and by doing so acts as an adaptor for recycling of this receptor through SNX-BAR-retromer decorated tubules¹⁰. Interestingly, SNX27 associates with the SNX-BAR-retromer through binding to the Wiskott-Aldrich syndrome protein and SCAR homologue (WASH) complex¹⁰ and, furthermore, mediates recycling of β 2AR through a direct Rab4-dependent endosome-to-plasma membrane pathway rather than via the TGN^{10, 49}. SNX27 also associates with, and regulates the endosomal sorting of other PDZ ligand-containing transmembrane spanning

proteins: 5-hydroxytryptamine type 4 receptor (5-HT₄R)⁵⁰; the G protein-gated inward rectifying potassium (GIRK, or Kir3) channels^{51, 52}, and the NMDA receptor subunit NR2C⁵³. Whether recycling is mediated through the SNX-BAR-retromer pathway, and if this is via the TGN or directly back to the plasma membrane, remains to be established. SNX27 also contains a FERM-like domain and hence may function as an adaptor for NPxY motif-containing cargoes. Indeed, the FERM-like domain of a closely related protein SNX17 (this lacks a PDZ domain) has been shown to regulate the endosomal recycling of a number of NPxY-containing proteins⁵⁴⁻⁵⁶. Whether SNX17 also functions as an adaptor for the SNX-BAR-retromer pathway remains to be determined. Based on these examples, and the precedent set by other coat complexes, it would appear that a multitude of cargo-specific adaptors, many of which may be SNXs, have evolved to utilize the core machinery of the SNX-BAR-retromer pathway to allow for recycling of specific cargo.

Accessory proteins for SNX-BAR-retromer

In addition to cargo-specific adaptors the area of concentrated VPS26-VPS29-VPS35 and SNX-BAR binding sites act to recruit a number of accessory proteins that stabilise further tubule growth and ultimately drive the scission of retromer tubules. Among these is EHD1, a member of the carboxy-terminal Eps15 homology domain (EHD) family¹¹. These proteins have similarities to dynamin GTPases⁵⁷, being capable of assembling into oligomeric structures, inducing liposome tubulation *in vitro*, and hydrolysing ATP (not GTP)⁵⁸. Binding of EHD1 to SNX-BAR-retromer is sub-stoichiometric and is required to stabilise tubule formation possibly by assisting further sculpturing of the maturing tubule¹¹. In addition, given the similarity to dynamin, it is tempting to speculate that ATP hydrolysis may induce conformation changes that lead to scission of SNX-BAR-retromer tubules⁵⁷. EHD proteins also associate with proteins containing the tripeptide NPF motif and so EHD1 may recruit additional proteins required for further cargo capture and/or processing of the SNX-BAR-retromer tubules⁵⁸.

For a number of coat complexes, further membrane re-modelling, including the efficiency of membrane scission, are intricately linked with the underlying actin and microtubule cytoskeleton^{59, 60}. This is also the case with the SNX-BAR-retromer where the Vps17 orthologs SNX5 and SNX6 associate with the p150^{glued} component of dynactin and hence couple to the minus-end directed microtubule motor dynein^{15, 61}. Interestingly, uncoupling to the dynein motor leads to extended SNX-BAR-retromer tubules through an apparent decrease in the efficiency of tubule scission¹⁵. More recently, the association of VPS26-VPS29-VPS35 to the WASH complex has established a link with actin polymerisation^{12, 13}. WASH is a member of the WASP family that regulates the actin nucleating properties of the Arp2/3 complex⁵⁹. WASH is found associated with a regulatory complex composed of FAM21, SWIP, strumpellin and CCDC53^{12, 62}, and interacts with VPS26-VPS29-VPS35 sub-complex through binding of VPS35 to FAM21, with additional interactions between VPS35 and SNX1/SNX2 to WASH and FAM21 further stabilising the association^{12, 13}. Knock down of WASH leads to the formation of elongated SNX-BAR-retromer tubules that align along microtubules, presumably due to association with the dynactin-dynein motor complex¹². Again this phenotype appears to arise from a decrease in the efficiency of tubule scission^{12, 62}. Importantly, the WASH complex associates with the scission factor dynamin-II, and CAPZ, a capping protein for the barbed ends of actin filaments which, by promoting branching leads to the generation of longitudinal force^{12, 62, 63}. The SNX-BAR-retromer therefore assembles two opposing force-generating systems: a motor-dependent pulling force on the tubule and a pushing force on the endosomal vacuole generated by a localised burst of actin polymerisation^{15, 62}. These forces appear to combine to increase membrane tension thereby enhancing the efficiency of membrane scission elicited by either line tension

formed by lipid phase separation or by constriction/twisting mediated by dynamin-II and possibly EHD1⁶⁴.

Once the coated tubular carrier has been generated, for fusion with the recipient compartment the carrier must undergo uncoating. For COPII this requires the GAP activity of Sec23, with an analogous function being achieved by recruitment of ARFGAP1 during biogenesis of COPI transport carriers⁶⁵. Binding of VPS26-VPS29-VPS35 to TBC1D5 may therefore perform a similar function in the SNX-BAR-retromer pathway¹⁴. In addition, one cannot exclude a role for phosphoinositide turnover, and the need for ATP hydrolysis to destabilise the high avidity nature of the coat complex and reprime components for further rounds of coat assembly.

Pathway progression

The preceding discussion has focused on individual processes that are classic elements of coat complexes (Figure 2). How are these separate events co-ordinated to achieve pathway progression? By their very nature coat complexes are characterised by low affinity interactions and the SNX-BAR-retromer is no exception^{66, 67}. In a pathway defined by low affinity interactions, many individual steps are dependent on the integration of the affinities from a series of interactions to generate the required avidity for progression from one step to another⁶⁸. This builds into the pathway dynamic instability, as progression is dependent upon the required number of nucleating cargo-bound VPS26-VPS29-VPS35 adaptors, and the correct level of membrane re-modelling SNX-BAR sub-complexes and other accessory proteins. In turn this leads to proof reading, where pathway progression can be checked at various steps and aborted if the necessary affinities (*i.e.* interactions) are not in place⁶⁹. While we currently lack a great deal of basic information, for example quantified affinities and a true detailed network view of protein:protein and protein:lipid interactions required for SNX-BAR-retromer function, it would be very surprising if this pathway did not conform to an avidity-based system for pathway progression that includes a series of defined checkpoints.

SNX-BAR-retromer does not however conform to all aspects of a classic coat complex. For example, unlike conventional coats SNX-BAR-retromer does not form an electron dense layer on membranes²¹. Moreover, for a number of coats the subunits that stabilise membrane deformation, such as clathrin and Sec13/Sec31, do not themselves interact directly with the membrane. For example, clathrin coats are formed by sequential layering, with first the adaptors binding the membrane and forming the cargo-bound nucleation complex, followed by the binding and assembly of clathrin⁷⁰. This ensures co-ordination of membrane deformation with cargo sorting, and avoids membrane remodelling in the absence of cargo. In contrast, retromer SNX-BARs have their own mode of membrane association entirely independent of the VPS26-VPS29-VPS35 adaptor¹⁷. How does the SNX-BAR-retromer avoid the formation of unwanted endosomal tubules devoid of enriched cargo? One potential answer lies with the low affinity of the interaction between the SNX-BAR sub-complex and the VPS26-VPS29-VPS35 adaptor⁶⁷. If the SNX-BAR dimer preferentially associates with the cargo-bound VPS26-VPS29-VPS35 adaptor, then the elevation in the effective concentration of the membrane re-modelling complex would be co-ordinated with cargo selection, consistent with the low affinity interactions required for pathway progression. Indeed, SNX-BAR-retromer tubulation occurs with the greatest frequency at the Rab5-to-Rab7 early-to-late endosomal switch^{30, 45}. What advantages arise from having a membrane re-modelling coat component directly binding to membranes? One possibility is an increased level of regulation which may stem from the mutually exclusive binding to PtdIns(3)P versus PtdIns(3,5)P₂. Hence, PtdIns(3)P-to-PtdIns(3,5)P₂ switching may constitute an additional point of regulation for the SNX-BAR-retromer pathway^{71, 72}.

Unexpected surprises: retromer-mediated sorting of Wntless

An interesting aspect of defining the mechanistic details of the SNX-BAR-retromer has been the insight obtained into the interface between endosomal retrieval and developmental biology (Table 1). This is particularly evident with the regulation of Wnt signalling. The seven-transmembrane-domain protein Wntless^{41-43,73-77}, which binds to Wnts (lipid-modified glycoproteins with roles in development, adult tissue homeostasis and disease⁷⁸), is a cargo for the VPS26-VPS29-VPS35 adaptor (Table 1). Wntless is required for the transport of Wnts from the Golgi apparatus to the cell surface, where their release generates short and long-range morphogenic gradients⁷⁹⁻⁸¹. Continuous Wnt secretion is needed to maintain these gradients and is achieved through Wntless undergoing endocytosis prior to VPS26-VPS29-VPS35-mediated retrieval back to the TGN^{73-77,82,83}. Disruption of VPS26-VPS29-VPS35 results in inefficient retrieval of Wntless and missorting into the lysosomal degradation pathway⁷³⁻⁷⁷. The resultant loss of Wntless manifests as a strong defect in Wnt secretion, failure of gradient formation and a loss of signalling to Wnt receiving cells⁷³⁻⁷⁷.

Unexpectedly, in *C. elegans* *snx-1* (orthologue of mammalian SNX1/SNX2) and *snx-6* (orthologue of mammalian SNX5/SNX6/SNX32) are not required for VPS26-VPS29-VPS35-dependent retrieval of Wntless: identical data has also been obtained in *Drosophila*¹⁶. Importantly, *C. elegans* express a functional SNX-BAR-retromer as sorting of the CED-1 phagocytic receptor is dependent on the SNX-BAR-retromer complex^{16, 37}. Intriguingly, a separate *C. elegans* SNX, encoded by the *snx-3* gene, is required for several Wnt specific processes¹⁶. SNX3 lacks a carboxy-terminal BAR domain and only contains one recognised domain, the SNX PX domain². In a predicted *snx-3* null the range and penetrance of Wnt-dependent phenotypes are similar to those observed in VPS26-VPS29-VPS35 mutants¹⁶. Moreover, the formation of Wnt morphogenic gradients in both *C. elegans* (EGL-20) and *Drosophila* (Wg) are strongly reduced in mutant *snx-3* strains¹⁶. As with the loss of the VPS26-VPS29-VPS35 adaptors, the Wnt phenotypes observed upon loss of *snx-3* arise from increased lysosomal-mediated degradation of Wntless and decreased secretion of Wnt morphogens¹⁶. Indeed, the Wnt signalling phenotype of the *C. elegans* *snx-3* mutant are fully rescued by overexpression of Wntless¹⁶. Further establishing the evolutionarily conserved function of SNX3 in the regulation of Wnt secretion, knock down of SNX3 expression in mammalian cells also leads to a decrease in steady-state levels of Wntless¹⁶.

The SNX3-retromer complex

SNX3 is an established PtdIns(3)P-binding protein⁸⁴. As the VPS26-VPS29-VPS35-dependent sorting of Wntless is dependent on this phosphoinositide, SNX3 is an attractive candidate for linking PtdIns(3)P to the VPS26-VPS29-VPS35-dependent sorting of Wntless. Indeed, the VPS26-VPS29-VPS35 sub-complex is found associated with immunoprecipitates of SNX3, and moreover, in *in vitro* assays recombinant SNX3 directly binds to the recombinant VPS26-VPS29-VPS35 adaptor¹⁶. These data therefore establish that besides the SNX1/SNX2 and SNX5/SNX6/SNX32-containing SNX-BAR-retromer (Figure 1B), there is a second evolutionarily conserved retromer complex formed by the direct association of the VPS26-VPS29-VPS35-adaptor with the non-BAR domain-containing SNX3¹⁶ (Figure 1C). It is the latter, SNX3-retromer, that regulates endosome-to-TGN retrieval of Wntless. In addition, as SNX3 is important in targeting the VPS26-VPS29-VPS35 sub-complex to an early endosomal compartment¹⁶, at least two independent mechanisms have evolved for the endosomal association of the VPS26-VPS29-VPS35 adaptor: binding to Rab7-GTP for association to endosomes undergoing early-to-late endosomal transition^{14, 45}, and association to SNX3 for targeting to early endosomes¹⁶.

The identification of SNX3 and SNX-BAR-retromers raises a number of interesting questions of which perhaps the most intriguing is: In sharing a common cargo adaptor how do two distinct retromers function to differentially sort endosomal cargo? As discussed above, in mammalian cells SNX3 and the SNX-BAR-retromer SNXs appear spatially separated along the endosomal maturation pathway¹⁶. Although there is some overlap between their distributions, SNX3 is principally associated with early endosomes whereas the SNX-BAR-retromer functions at endosomes undergoing early-to-late endosome transition^{16,84,85}. One can argue therefore that endocytosed Wntless initially enters the SNX3-retromer labelled early endosome, where binding to the VPS26-VPS29-VPS35 adaptor initiates endosome-to-TGN retrieval (Figure 3A). In the absence of SNX3, internalised Wntless follows one of two routes: missorting into intraluminal vesicles and hence lysosomal degradation, or retrieval back to the TGN via the SNX-BAR-retromer. In the absence of SNX3 the steady-state level of Wntless is defined by the relative flux through these pathways. As Wntless levels are greatly reduced upon loss of SNX3, the lysosomal degradative pathway appears to dominate¹⁶. Thus although some internalised Wntless may be retrieved by the SNX-BAR-retromer pathway⁸⁶, in the absence of SNX3 this is insufficient to maintain the necessary level of Wnt secretion required to establish and maintain the morphogenic gradient for normal development.

Clearly another important issue relates to how SNX3-retromer co-ordinates cargo selection with membrane re-modelling in order to form a cargo-enriched carrier. As SNX3 lacks a BAR domain, tubular-based sorting may not be a characteristic of this pathway. Indeed, Wntless appears to exit the SNX3-labeled early endosome via small SNX3-decorated vesicular carriers that are labelled with clathrin^{16,87}. This would be consistent with the proposed association of clathrin to SNX3, although the direct versus indirect nature of this interaction remains unclear^{16,87}. SNX3-retromer and SNX-BAR-retromer therefore sort their respective cargoes through two morphologically distinct carriers.

Returning to issues relating to pathway progression, precise control of the level of PtdIns(3)P is vital for efficient SNX3-retromer mediated retrieval of Wntless in *C. elegans* and *Drosophila*. MTM-6 and MTM-9 are two PtdIns(3)P 3-phosphatases of the myotubularin family that form a complex and regulate Wntless trafficking^{88,89}. Mutation of *mtm-6* leads to a defect in Wntless recycling through an excess of PtdIns(3)P that results in a more pronounced association of SNX3 with endosomes and a reduction of Wntless levels through lysosomal degradation⁸⁸. These data suggest that with elevated levels of PtdIns(3)P the dynamic exchange of SNX3 between cytosolic and membrane-bound forms is perturbed to an extent that adversely affects pathway progression. Indeed, partially knocking down expression of the PtdIns 3-kinase *vps-34* in *mtm-6* mutants, and hence lowering the excess level of PtdIns(3)P, partially restores the Wntless trafficking phenotype (*i.e.* pathway progression)⁸⁸. Alongside emerging evidence that VPS34 may reside in complexes with specific myotubularins^{90,91}, this suggests that tight regulation of PtdIns-to-PtdIns(3)P switching may be an important aspect of SNX3-retromer function.

Finally, in identifying the SNX3-retromer it becomes important to reassess those cargoes whose sorting has been described to be VPS26-VPS29-VPS35-dependent (Table 1), in order to determine whether their retrieval is mediated via SNX3-retromer, SNX-BAR-retromer or a combination of both pathways (*i.e.* like the Grd19p/Snx3p SNX-BAR retromer in yeast (Figure 3B)). In so doing the validity of the spatial segregation model will be tested, as will issues relating to the influence cargo binding has on the SNX complex to which the VPS26-VPS29-VPS35 adaptor associates.

Looking ahead: the possibility of other SNX coat complexes

The precedent set by the function of SNXs in SNX-BAR-retromer and SNX3-retromer has placed greater emphasis on the importance of the SNX components in defining separate endosomal retrieval pathways that function through morphologically distinct carriers. With emerging evidence that specific SNXs also function as adaptors for cargo recruitment into the SNX-BAR-retromer pathway (*e.g.* SNX27 and possibly SNX17^{9, 10}, and one should not exclude evidence that retromer SNX-BARs can themselves interact with cargo *e.g.* 92), the central role of this evolutionarily conserved protein family in endosomal sorting appears even more complex than first envisaged⁷. Establishing the credentials of other SNX-BAR and SNX-PX proteins as potential coat components will certainly lead to new insights into the mechanistic basis and complexities of endosomal sorting⁹³⁻⁹⁵. In turn, by enhancing the molecular understanding of this fundamental aspect of cell biology we will undoubtedly obtain greater insight into the exciting link between endosomal sorting, development and human disease.

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B

Yeast SNX-BAR-retromer

Mammalian SNX-BAR-retromer

C

Mammalian SNX3-retromer

Figure 1. Sorting itineraries in the mammalian endosomal network and the distinct SNX-BAR-retromer and SNX3-retromer complexes

(A). Internalised cargo destined for degradation, such as the epidermal growth factor receptor (EGFR), are first sorted into intraluminal vesicles (ILVs) and by a process of endosomal maturation arrive in late endosomes/multivesicular bodies (MVBs)³. Fusion of MVBs with the lysosomal compartment brings about degradation of cargo associated with ILVs⁹⁶. In contrast, retrieval from the degradative pathway of, for example the nutrient sensing Transferrin receptor (TfR), occurs back to the plasma membrane via fast or slow recycling routes, while retrieval of the cation-independent mannose 6-phosphate receptor (CI-MPR) occurs back to the *trans*-Golgi network (TGN). The later route, often referred to

as retrograde transport, comprises a number of distinct pathways, which include those dependent on Rab9- and SNX-BAR-retromer⁴. Enrichment of different phosphoinositides within compartments that make up the endosomal network is shown, including the early endosomal PtdIns(3)P. (B). The distinct cargo-selective and membrane deformation sub-complexes that together form the yeast and mammalian SNX-BAR-retromers. The mammalian genome contains two VPS26's. (C). The evolutionarily conserved mammalian SNX3-retromer complex. Gene duplication has resulted in two orthologs of *C. elegans* *snx-3*, SNX3 and SNX12.

Figure 2. A proposed pathway for SNX-BAR-retromer-mediated sorting

Activation and cargo capture is initiated by association of the VPS26-VPS29-VPS35 sub-complex to early-to-late endosomes through interaction with the GTP-loaded form of Rab7, thus forming the nucleation complex. Not depicted are the possible roles of clathrin and various clathrin-adaptors and binding proteins in cargo clustering prior to formation of the nucleation complex⁴⁷. Incorporation of other cargo into the nucleation complex is achieved through specific cargo adaptors, and the SNX-BAR dimer enters the nucleation complex either by direct binding from the cytosol and/or lateral movement on the early-to-late endosomal surface. This increases the effective concentration of retromer SNX-BARs resulting in their switch to curvature inducing mode and re-model the membrane into tubular profiles. An area of concentrated VPS26-VPS29-VPS35 and SNX-BAR-binding sites is therefore formed, onto which accessory proteins are sequestered, leading to further re-modelling of the maturing tubular profile. Generation of opposing forces through actin polymerisation and motor activity aids carrier scission through either line tension and/or mechano-enzymatic fission possibly catalysed by dynamin-II and/or EHD1. Uncoating of the isolated carrier, possibly requiring ATP hydrolysis and/or Rab7-GTP hydrolysis, occurs either immediately after scission or upon tethering to the recipient compartment. See text for more details.

Figure 3. Spatial segregation model to account for differential cargo sorting through SNX3-retromer and SNX-BAR-retromer pathways

(A). A model based on spatial segregation to describe differential sorting of Wntless and CI-MPR by SNX3-retromer and SNX-BAR-retromer complexes respectively. Briefly, Wntless bound to Wnt morphogens is secreted to the cell surface where release of Wnt establishes short and long-range morphogenic gradients. Internalised Wntless initially enters the early endosome where it engages the SNX3-retromer for retrieval back to the TGN. In contrast, the transport of CI-MPR bound to newly synthesized hydrolase enzymes exits the TGN and is principally trafficked directly to the endosomal network, entering at a stage of maturation downstream of SNX3-retromer. The acid environment of this compartment leads to

dissociation of the hydrolases, allowing retrieval of the CI-MPR via the tubular SNX-BAR-retromer. It is important to note that this model does not suggest exclusive transport of Wntless and CI-MPR through the SNX3-retromer and SNX-BAR-retromer pathway respectively. It simply reflects that steady-state transport of these cargoes appears to be dependent upon specific retromer complexes. (*B*). As discussed in the text, in yeast recycling of Fet3p-Ftr1p requires recognition of the cytoplasmic domain of Ftr1p by Grd19/Snx3p⁹. Grd19/Snx3p physically associates with the SNX-BAR-retromer thereby allowing recycling of the Fet3p-Ftr1p⁹. The function of SNX3 in Wntless retrieval and Wnt secretion is therefore, fundamentally different to this role of Grd19/Snx3p.

Table 1
Identified retromer cargo proteins

SNX-BAR-retromer and SNX3-retromer regulate a wide variety of cellular functions based primarily, but perhaps not solely, on their ability to regulate retrograde endosome-to-TGN transport. Of great interest is a growing appreciation of how retromer complexes regulate organism development and homeostasis, and equally, how defects in retromer function lead to human disease. Adapted from¹²⁰.

Process	Mechanism
Cargo sorting of intracellular receptors including Vps10, MPRs, DMT1-II, sortilin, SorLA, AtVSR1/AtELP, in various organisms.	Retrieval of the receptors from endosomes-to-TGN. Described tripeptide sorting motifs through which VPS35 associates with cargo being: WLM – CI-MPR; FLV – sortilin; YLL – DMT1-II.
Transcytosis of polymeric immunoglobulin receptor (pIgR) and its ligand, polymeric IgA	Retrieval of internalised pIgR-pIgA, thus allowing for its transport to the opposite plasma membrane.
Maintenance at the plasma membrane of the reductive iron transporter, Fet3-Ftr1, under low iron conditions in <i>S. cerevisiae</i> .	Retrieval of internalised transporter to the TGN mediated in conjunction with the adaptor Grd19/Snx3.
Termination of cAMP by internalized parathyroid hormone receptor (PTHr).	Retrieval of PTHR from endosomes-to-TGN.
Recycling of β 2-adrenoreceptor.	Retrieval of internalised receptor directly to the plasma membrane in conjunction with the adaptor SNX27.
Cell polarity and organ initiation mediated by the phytohormone auxin, and plant growth and senescence in vegetative organs in <i>A. thaliana</i> .	Retrieval and polarised delivery of the auxin efflux carrier, PIN. Transport of proteins to protein storage vacuoles (PSVs).
Secretion of Wnt morphogens and generation of Wnt gradients during development.	SNX3-retromer regulated retrieval of the Wnt receptor/chaperone, Wntless, from endosomes-to-TGN to allow for further rounds of Wnt secretion.
Endocytic trafficking, signaling and tumor suppression in <i>D. melanogaster</i> .	Inhibition of Rac1-dependent actin polymerisation.
Apoptotic cell clearance in <i>C. elegans</i> .	Retrieval of internalized phagocytic receptor, CED-1, back to cell surface.
Maintaining apico-basal polarity and integrity of epithelial tissues in <i>D. melanogaster</i> .	Retrieval of the apical determinant Crumbs from endosomes. Neurodegenerative disease Retrieval of the amyloid precursor protein (APP), its sorting receptor (SorLA) or its processing peptidase (β - and γ -secretase). Link with late on-set Alzheimer's disease. VPS35 mutation, Asp620Asn, associated with late on-set Parkinson disease.
Trafficking of pathogens (<i>Salmonella</i> , Herpesvirus saimiri), and toxins (e.g. <i>Shiga</i> toxin).	Retrieval of membrane and cargo from Salmonella-containing vacuole. Endosomes-to-TGN transport of internalized toxins. VPS35 target of HVS Tip protein, which by inhibiting retromer contributes to efficient T cell transformation.
Type 2 Diabetes	Genome-wide association study identified variants in VPS26A in South Asians.
Mitochondria to peroxisome transport	Association with mitochondrial-anchored protein ligase, MAPL.