

The retromer complex – endosomal protein recycling and beyond

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Summary

The retromer complex is a vital element of the endosomal protein sorting machinery that is conserved across all eukaryotes. Retromer is most closely associated with the endosome-to-Golgi retrieval pathway and is necessary to maintain an active pool of hydrolase receptors in the trans-Golgi network. Recent progress in studies of retromer have identified new retromer-interacting proteins, including the WASH complex and cargo such as the Wntless/MIG-14 protein, which now extends the role of retromer beyond the endosome-to-Golgi pathway and has revealed that retromer is required for aspects of endosome-to-plasma membrane sorting and regulation of signalling events. The interactions between the retromer complex and other macromolecular protein complexes now show how endosomal protein sorting is coordinated with actin assembly and movement along microtubules, and place retromer squarely at the centre of a complex set of protein machinery that governs endosomal protein sorting. Dysregulation of retromer-mediated endosomal protein sorting leads to various pathologies, including neurodegenerative diseases such as Alzheimer disease and spastic paraplegia and the mechanisms underlying these pathologies are starting to be understood. In this Commentary, I will highlight recent advances in the understanding of retromer-mediated endosomal protein sorting and discuss how retromer contributes to a diverse set of physiological processes.

Key words: Retromer, Endosome, Recycling, Sorting, WASH complex

Introduction

The retromer complex is a key component of the endosomal protein sorting machinery and operates by recognising specific membrane proteins – cargo – that are concentrated into discrete regions of the endosomal membrane where tubules form that transport cargo proteins to the appropriate destination. Prominent retromer cargoes include: the cation independent mannose 6-phosphate receptor (CIMPR) (Seaman, 2004; Arighi et al., 2004), the iron transporter DMT1-II/Slc11a2 (Tabuchi et al., 2010), the Wnt transport protein Wntless/MIG-14 (Eaton, 2008), Crumbs (Crb) (Pocha et al., 2011), an apical protein required for cell polarity, and SorL1 (sortilin-related receptor, also known as SorLA), a protein that binds amyloid precursor protein (APP) (Nielsen et al., 2007; Fjorback et al., 2012). By mediating the localisation of many membrane proteins, the activity of the retromer complex has been linked to processes such as lysosome biogenesis, aspects of metazoan development and pathologies such as Alzheimer disease (AD).

There has recently been significant progress in determining how retromer recognises and then partitions its cargo for incorporation into a nascent tubule, thereby mediating efficient retrieval of endosomal proteins. In this Commentary, I will summarise recent findings from studies of retromer-mediated protein sorting and discuss how the advances in understanding the cell biology of retromer have generated new insights into the mechanisms of endosomal protein sorting.

The identification of retromer

The retromer complex was first identified in the yeast *Saccharomyces cerevisiae* and was shown to mediate endosome-

to-Golgi retrieval of the carboxy peptidase Y (CPY) receptor Vps10p. The complex, as characterised in yeast, comprises five proteins that are all encoded by vacuole protein sorting (*VPS*) genes (Seaman et al., 1997; Seaman et al., 1998). The yeast heteropentameric retromer complex can be biochemically and phenotypically dissected into two subcomplexes: a trimer of Vps35p, Vps29p and Vps26p, which mediates cargo selection, and a dimer of Vps5p with Vps17p. The Vps5 and Vps17 proteins are members of the sorting nexin (Snx) family and are believed to mediate tubule or vesicle formation through their C-terminal Bin-Amphiphysin-Rvs (BAR) domains and an intrinsic self-assembly activity (Nothwehr and Hindes, 1997; Horazdovsky et al., 1997; Kurten et al., 2001; Peter et al., 2004). Vps5p, Vps17p and related proteins are frequently described as Snx-BAR proteins to distinguish them from other members of the Snx family (reviewed in Attar and Cullen, 2010).

In the 14 years since retromer was identified, much has been learned about how retromer functions in endosomal protein sorting. Retromer subunits are highly conserved, and in higher eukaryotes, such as mammalian cells, the cargo selective trimer is essentially identical to the yeast complex. In addition, similar to in yeast, the Snx-BAR component is a dimer, whereby a Vps5-like protein associates with an orthologue of Vps17p. For example, in mammalian cells the homologues of Vps5p, namely Snx1 or Snx2 dimerise with a Vps17p orthologue, specifically either Snx5 or Snx6 (Wassmer et al., 2007).

There are, however, important differences between the prototypical retromer in yeast and retromer in higher eukaryotes. A key difference is that, in mammalian cells, retromer is not a

stable heteropentamer but a much more transient association of the cargo-selective trimer (Vps35, Vps26 and Vps29) and the Snx components (Snx1 or Snx2 with Snx5 or Snx6) (Swarbrick et al., 2011). From studies in various model systems including mammalian cells, there are now a growing number of reports of cargo proteins and processes that utilise only one of the retromer subcomplexes without requiring the function of the other (Nisar et al., 2010; Prosser et al., 2010). This difference means that when discussing retromer in mammalian cells or other higher eukaryotes, it is important to specify which functional unit of retromer is being referred to, the cargo-selective trimer or the membrane-bending Snx-BAR dimer. It is also apparent that many of the retromer accessory proteins identified from studies in higher eukaryotes are not conserved in yeast; the implications of this observation are discussed below.

Recruitment of retromer to the membrane

Recruitment of the retromer subcomplexes to the endosomal membrane precedes or occurs concomitantly with the selection of cargo proteins – a process mediated by the Vps35–Vps29–Vps26 trimer. Simultaneously, the Snx-BAR dimer assembles to promote formation of endosomal membrane tubules into which cargo proteins are sorted. Following tubule formation, the retromer proteins then dissociate from the membrane so that further rounds of cargo sorting can occur. Recruitment of the retromer subcomplexes to the endosome is therefore a key element of the regulation of retromer function.

Recruitment of the Snx dimer

The recruitment of the Snx-BAR dimer is mediated through binding to phosphatidylinositol (PtdIns) 3-phosphate [PtdIns(3)P] by the Phox homology (PX) domain, which is present in each Snx protein (Cozier et al., 2002). Production of PtdIns(3)P in both yeast and mammalian cells occurs as a result of the activity of the phosphoinositide 3-kinase (PI3K) Vps34 (Stack et al., 1993). Vps34 is itself regulated by the Vps30 protein in yeast or its metazoan homologue Beclin1 (Burda et al., 2002; Ruck et al., 2011). Studies in mammalian cells have shown that RNA interference (RNAi)-mediated loss of the cargo-selective complex does not prevent the recruitment of the Snx-BAR dimer, demonstrating that the Snx-BAR dimer associates with the membrane independently of the cargo-selective complex (Seaman, 2004; Arighi et al., 2004).

Recruitment of the cargo-selective complex

How the cargo-selective complex is recruited to the membrane is an important question because its recruitment is necessary for cargo selection. Unlike the Snx-BAR dimer, none of the proteins that comprise the cargo-selective complex contain a known lipid-binding domain. It has now been shown that the small GTPase Rab7a is required for recruitment of the cargo-selective trimer (Rojas et al., 2008; Seaman et al., 2009). The association between the cargo-selective complex and Rab7a is conserved through evolution and was first reported in studies from enteric amoeba (Nakada-Tsukui et al., 2005).

Recent evidence has linked the activity of the ceroid-lipofuscinosis neuronal 5 (CLN5) protein to the regulation of the recruitment of the cargo-selective complex through Rab7a (Mamo et al., 2012). CLN5 is mutated in Batten disease, a lysosomal storage disorder, and has been shown to bind to the sortilin protein, a known cargo for retromer (Seaman, 2004;

Seaman, 2007; Canuel et al., 2008). Loss of CLN5 expression results in reduced levels of active Rab7a and compromised retromer recruitment, and leads to degradation of sortilin and CIMPR (Mamo et al., 2012). Unlike Rab7a, CLN5 does not appear to be conserved across all eukaryotes and, therefore, simpler organisms (e.g. yeast) appear able to dispense with CLN5 function for the recruitment of the retromer cargo-selective complex. The molecular details of how Rab7a interacts with the cargo-selective complex remain to be determined. Additionally, as Rab7a is also present on lysosomes, whereas retromer is not, it is logical that Rab7a cannot be the only determinant of retromer–membrane association.

Along with Rab7a, Snx3 has been implicated in mediating the recruitment of the cargo-selective retromer complex (Harterink et al., 2011). Unlike the Snx-BAR proteins, such as Snx1 and Snx2, Snx3 has no BAR domain but contains a PX domain with a high affinity for PtdIns(3)P (Yu and Lemmon, 2001). The role of Snx3 in mediating the recruitment of the cargo-selective complex appears to be similar to that of Rab7a. Silencing of either Snx3 or Rab7a, by using small interfering RNA (siRNA), results in the displacement of the cargo-selective complex from the membrane. Recent studies have now shown that Snx3 associates exclusively with Vps35, and that both Snx3 and Rab7a are required for proper recruitment of the cargo-selective complex, as loss of Snx3 in a background in which either Rab7a, or even the constitutively active Rab7a Q67L mutant is overexpressed, results in displacement of the cargo-selective complex (Vardarajan et al., 2012).

The requirement for both Rab7a and Snx3 to promote recruitment of the cargo-selective complex, therefore, places the site of action of retromer at a discrete region of the maturing endocytic system where both Snx3 and Rab7a are present (Box 1).

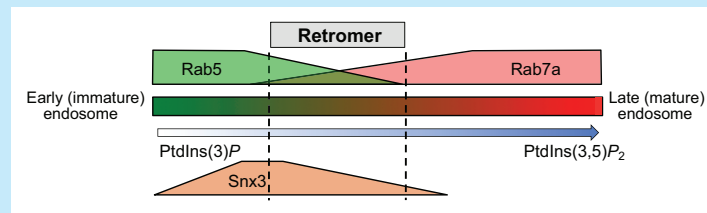
Fine tuning the recruitment of the cargo-selective complex

A number of proteins have recently been identified as associating or directly interacting with the cargo-selective retromer complex. One of these is TBC1D5 – a member of the Tre2-Bub2-Cdc16 (TBC) family of Rab GTPase-activating proteins (GAPs) (Seaman et al., 2009). TBC1D5 binds directly to Vps29 (Harbour et al., 2010). Overexpression of TBC1D5 results in displacement of the cargo-selective complex from the membrane similar to RNAi knockdown of Rab7a or expression of a constitutively inactive Rab7a mutant. Mutation of conserved arginine and glutamine residues in the TBC domain that are required for catalytic activity abolishes the ability of TBC1D5 to displace the cargo-selective complex, which is consistent with the hypothesis that TBC1D5 regulates the membrane association of retromer through its action on a Rab protein – Rab7a being the most obvious and likely candidate (Harbour et al., 2010). Indeed studies in *C. elegans* have shown that RBG-3, the worm homologue of TBC1D5, is a potent GAP for Rab7 (Mukhopadhyay et al., 2007).

Are there additional factors that contribute to the recruitment of the cargo-selective complex? It has been reported that Snx1 and Snx2 together regulate recruitment of the cargo-selective complex and RNAi knockdown of both Snx1 and Snx2 appears to displace the cargo-selective complex from the membrane (Rojas et al., 2007). However, although an interaction between Snx1 and the cargo-selective complex protein Vps29 is detectable, it is very weak and unlikely to have a key role, such as the recruitment of the cargo-selective complex (Swarbrick et al., 2011). Furthermore, cells derived from transgenic *Snx1*^{-/-}, *Snx2*^{-/-} mice display normal localisation of the cargo-selective

Box 1. Snx3 and Rab7a mediate the recruitment of the the cargo-selective retromer complex and thereby define the region of the endocytic pathway where retromer operates

The endocytic pathway, represented by the green–red bar, is a continuum of maturing endosomes that acidify as part of the maturation process. Rab5 present on early endosomes promotes PtdIns(3)*P* synthesis required for Snx3 membrane association. Early endosomes are relatively rich in PtdIns(3)*P* which is converted to PtdIns(3,5)*P*₂ during maturation. Rab conversion in which Rab7a supplants Rab5 is a key event in endosome maturation and is associated with production of membrane tubules (van Weering et al., 2012). The respective levels of endosomal Rab5, Snx3, and Rab7a vary during endosome maturation and are represented by the sloping polygons. The requirement for both Snx3 and Rab7a to mediate the recruitment of the cargo-selective retromer complex therefore defines the region where retromer operates. The diagram is adapted with permission from Michael E. Harbour (Michael E. Harbour, Novel interactions of the cargo-recognition subcomplex of retromer, Ph.D Thesis. University of Cambridge, 2012).



complex (M.N.J. Seaman, unpublished observation). An alternative explanation is that RNAi-mediated knockdown of Snx1 and Snx2 depletes endosomes of cargo proteins that might contribute to the stable membrane association of the cargo-selective retromer complex following initial recruitment through interactions with Rab7a and Snx3.

Retromer interacts with and recruits the WASH complex

In addition to TBC1D5, the cargo-selective retromer complex also interacts with the macromolecular WASH complex (Harbour et al., 2010). The WASH complex was identified by affinity purification of one of its components, the actin-nucleation-promoting factor Wiskott-Aldrich syndrome homologue 1 (Wash1). The other components of the WASH complex are Fam21, strumpellin, CCDC53 and KIAA1033 (Derivery et al., 2009) [KIAA1033 is also known as SWIP, for strumpellin and WASH interacting protein (Jia et al., 2010)].

The interaction between the cargo-selective complex and the WASH complex is necessary for the endosomal localisation of the WASH complex (Harbour et al., 2010). Vps35 mediates the association of the cargo-selective complex with the WASH complex and is able to interact with both Wash1 and Fam21. It is the Vps35–Fam21 interaction, however, that is key to the recruitment of the WASH complex to endosomal membranes. Fam21 binds to Vps35 through its extended unstructured ‘tail’ domain, which is over 1000 amino acids in length and contains multiple binding sites for Vps35. The Fam21 tail also binds the actin-capping proteins CAPZa and CAPZb, and the FKBP15/WAFL protein (Harbour et al., 2012; Jia et al., 2012).

The role of the WASH complex in endosomal protein sorting

The role of the WASH complex in regulating endosomal protein sorting is beginning to be understood. Through its ability to promote formation of branched actin networks on endosomes, the WASH complex might work to create actin-stabilised microdomains that function to constrain retromer cargo proteins in discrete endosomal regions, thereby concentrating cargo at sites of tubule formation (Puthenveedu et al., 2010). Alternatively, the

actin-stabilised microdomains could function as signalling platforms, where localised signalling events are regulated, or restrict the movement of certain lipids in the plane of the endosomal membrane, thereby stabilising or preserving the integrity of a lipid microdomain. Additionally, the Wash1 protein has been reported to interact with microtubules (Gomez and Billadeau, 2009) and might, therefore, facilitate endosomal protein sorting through microtubule-based mechanisms, although presently it is not clear how this could be achieved.

The WASH complex is widely conserved and, in *Dictyostelium*, is required for retrieval of the vacuole (V)-ATPase from a degradative organelle. Failure of WASH-complex-mediated V-ATPase retrieval results in an accumulation of phagolysosomes that are unable to excrete undigested material (Carnell et al., 2011). In mammalian cells, the WASH complex has been shown to be important for the trafficking of specific proteins including the β 2-adrenergic receptor, a membrane-spanning G-protein-coupled receptor (GPCR) that is endocytosed upon ligand binding, and, following the action of the WASH complex, recycled back to the cell surface through tubules (Temkin et al., 2011). In this instance, the recycling of the β 2-adrenergic receptor requires Snx27, a sorting nexin that is distinct from the Snx-BAR proteins and might function as an adaptor that links the β 2-adrenergic receptor to the retromer machinery. Additionally, the function of the WASH complex is required for the recycling of the α 5 β 1 integrin, and inhibition of α 5 β 1 integrin recycling reduces invasive cell spreading (Zech et al., 2011).

The localisation of the WASH complex to endosomes requires the cargo-selective retromer complex (Harbour et al., 2010). This requirement therefore extends the functions of retromer beyond endosome-to-Golgi retrieval and implicates it in the endosome-to-plasma membrane pathway for recycling specific cargo such as the β 2-adrenergic receptor (Fig. 1).

In addition to mediating branched actin filament formation, the WASH complex also regulates scission of endosomal tubules through the association with dynamin-2 (Derivery et al., 2009). The strumpellin subunit of the WASH complex is mutated in hereditary spastic paraplegia (HSP), a peripheral neuropathy, and several other genes known to be mutated in HSP encode proteins that function at endosomes to regulate protein localisation (reviewed in Blackstone et al., 2011). Pronounced tubulation of

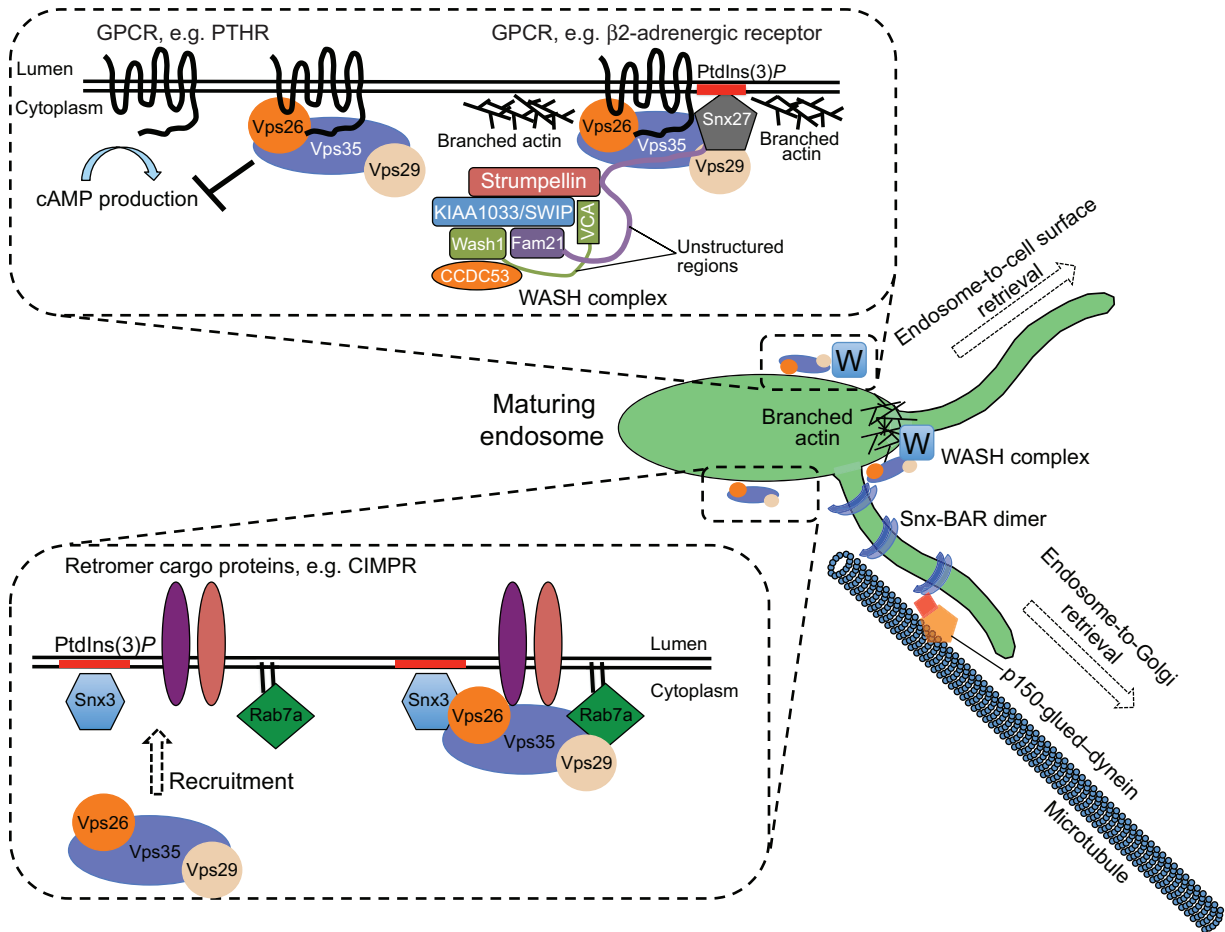


Fig. 1. Schematic depiction of the role of the retromer complex in mediating endosomal protein sorting. The cargo-selective retromer complex operates in the endosome-to-Golgi retrieval pathway to sort cargo, such as CIMPR and sortilin (see Table 1). The interaction of retromer with cargo proteins requires its recruitment to the endosomal membrane, a process that is mediated by Rab7a and Snx3. The sorting nexin (Snx) heterodimer dimer, comprising the Snx-BAR proteins Snx1 or Snx2 and Snx5 or Snx6, mediates tubule formation and links to microtubules through the p150-glued-dynein complex. The cargo-selective retromer subcomplex (Vps35–Vps29–Vps26) recruits the WASH complex, which mediates the production of branched actin networks on the surface of endosomes. The cargo-selective retromer complex together with Snx27 and the WASH complex operate in the endosome-to-cell surface recycling of the GPCR β 2-adrenergic receptor and of other proteins including α 5 β 1 integrin. Additionally, the cargo-selective retromer complex also regulates the signalling activity of another GPCR, the parathyroid hormone receptor (PTHr).

endosomes was observed following RNAi-mediated silencing of strumpellin, possibly hinting that dysregulation of endosomal tubule formation is an underlying cause of HSP (Derivery et al., 2009; Harbour et al., 2010).

A key role for EHD proteins in retromer-mediated endosomal protein sorting

Although loss of WASH complex function results in increased endosomal tubulation, possibly through reduced scission, the tubules that are produced retain a requirement for the Eps15-homology domain 1 (EHD1) protein to stabilise them (Harbour et al., 2010). EHD1 has also been shown to associate with the cargo-selective retromer complex, and loss of function of either EHD1, or its closely related paralogue EHD3, results in an endosome-to-Golgi retrieval defect (Gokool et al., 2007; Naslavsky et al., 2009). Along with EHD1, recent evidence has now shown that the EHD1-interacting protein rabankyrin-5 (Rank-5, also known as ANKFY1) associates with the cargo-selective retromer complex and has a regulatory role in retromer-mediated

endosome-to-Golgi retrieval, although the nature of that role remains to be determined (Zhang et al., 2012).

The precise role of EHD proteins in retromer-mediated endosomal protein sorting also remains to be determined, as does the molecular basis for the interaction between EHD1 and the cargo-selective complex. Studies on the recycling of transferrin receptor and major histocompatibility complex (MHC) class I proteins have shown that EHD1 function is required for the endosome-to-cell surface recycling of these cargo proteins (Rapaport et al., 2006; Caplan et al., 2002). As neither of these proteins is believed to be cargo proteins for retromer, the function of EHD1 might be more generic, for example, it might act as a scaffolding protein that stabilises tubules. Alternatively, it is possible that EHD1 bends membranes or contributes to tubule scission through its ATPase activity (Daumke et al., 2007).

Proteins that associate with the Snx-BAR dimer

Although there remains much to elucidate regarding the control of tubule scission and the factors that contribute to tubule

stability, there is, however, a broad consensus that the Snx-BAR proteins are key to tubule production. The physiological role(s) of the sorting nexin protein family is discussed in detail in recent reviews (Teasdale and Collins, 2012; Cullen and Korswagen, 2012). In retromer-mediated endosomal protein sorting in mammalian cells, Snx1 and Snx2 are functionally interchangeable (Rojas et al., 2007; Schwarz et al., 2002). Both form heterodimers with Snx5 and Snx6, which are equivalent to the Vps5p–Vps17p dimer that constitutes the Snx-BAR retromer component in yeast (Wassmer et al., 2007; Horazdovsky et al., 1997). Snx5 and Snx6 functionally link retromer-mediated endosomal protein sorting to the dynein complex through an interaction with p150-glued (also known as dynactin subunit 1, DCTN1), thereby mechanistically interfacing with the microtubule cytoskeleton (Wassmer et al., 2009; Hong et al., 2009). This might provide a means for nascent Snx-generated tubules to track along microtubules towards the microtubule organising centre where the Golgi is localised.

Snx1 and Snx2 have been shown to associate with the WASH complex, possibly through binding to the head domain of Fam21 (Gomez and Billadeau, 2009; Harbour et al., 2012). The functional significance of this association has yet to be fully determined, although the initial report of the association of Snx proteins with the WASH complex concluded that the WASH complex is an important regulator of retromer-mediated endosome-to-Golgi retrieval (Gomez and Billadeau, 2009). This conclusion was, however, based on an assessment of CIMPR localisation rather than quantification of the function of the endosome-to-Golgi pathway. Additionally, RNAi silencing of either strumpellin or KIAA1033/SWIP does not inhibit the endosome-to-Golgi retrieval of a CD8–CIMPR reporter protein (Harbour et al., 2010).

Another protein interacting with Snx1 is RME-8, a member of the DNAJ-domain-containing protein family that binds to heat-shock protein chaperones and thereby could regulate macromolecular assembly (Girard et al., 2005; Popoff et al., 2009; Shi et al., 2009). Loss of RME-8 function results in compromised CIMPR retrieval, similar to that observed for knockdown of Snx1 by RNAi (Carlton et al., 2004). Whether RME-8 has role in regulating Snx1 assembly is currently unknown. The interaction partners of the retromer complex are summarised in Fig. 2.

Retromer interactions with other membrane protein sorting machinery

Does retromer interface with other endosomal protein sorting machinery? As yet there is no definitive answer to this question but clues are emerging that suggest that some form of crosstalk between retromer and the endosomal sorting complex required for transport (ESCRT) machinery might occur. First, there are two reports that retromer proteins interact with the ESCRT-0 component Hrs-1. In one study (Popoff et al., 2009), the cargo-selective retromer complex was found to associate with Hrs-1, although other studies have failed to detect this particular interaction (Seaman et al., 2009; Harbour et al., 2010). Snx1 has also been reported to bind to Hrs-1 (Chin et al., 2001), but, again, this interaction has not been confirmed in other studies. More recently, data from an analysis conducted in Trypanosomes has indicated that retromer and the ESCRT machinery might be coordinated through the Rab28 protein because RNAi against Rab28 results in increased turnover of both the trypanosome

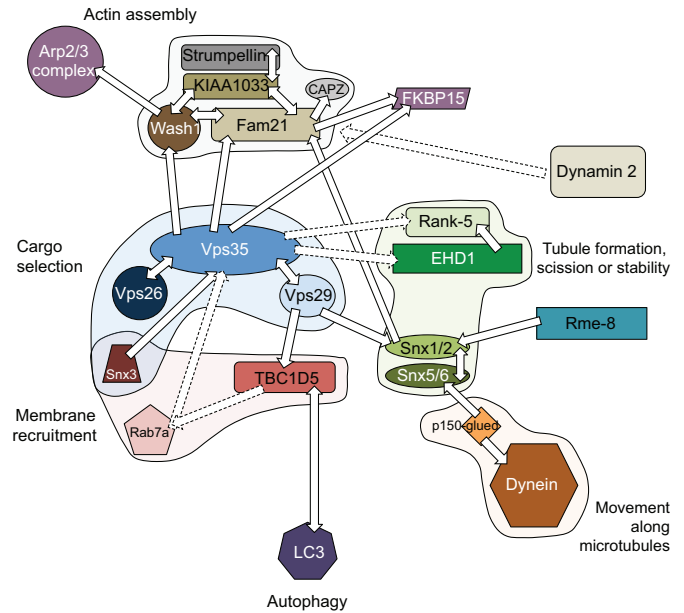


Fig. 2. Schematic diagram of the interactions between retromer and its associated proteins. Here, the many retromer-interacting proteins that have been identified from studies in higher eukaryotes (e.g. mammalian cells) are depicted. Proteins have been grouped according to function, e.g. Snx3, Rab7a and TBC1D5 regulate the membrane association of the cargo-selective retromer complex. Arrows indicate the relationship between the respective proteins, e.g. Vps35 interacts with Fam21 to mediate recruitment of Fam21 and the WASH complex. In some cases, arrows are double-ended as the respective proteins are mutually dependent. Arrows with a dashed outline indicate an interaction that has not yet been experimentally proven, but is likely based on indirect evidence, e.g. native immunoprecipitations.

Vps26 and Vps23/TSG101 (Lumb et al., 2011). Whether the effect of loss of Rab28 on retromer is direct remains to be determined.

Is there a role for clathrin in retromer-mediated endosomal protein sorting? This question remains to be conclusively answered, but has been discussed in some detail in a recent review (McGough and Cullen, 2011). Clathrin-coated buds on endosomes close to sites of retromer localisation have been reported, and proteomic-based studies have identified retromer proteins in semi-pure preparations of clathrin-coated vesicles (Popoff et al., 2007; Popoff et al., 2009; Shi et al., 2009; Borner et al., 2006). In contrast, however, a recent study has concluded that clathrin and retromer are present on distinct transport intermediates (Borner et al., 2012). Additionally, there are currently no reports of an interaction between clathrin and either the cargo-selective retromer complex or the Snx dimer, but clathrin has been reported to associate with Snx3 (Skånland et al., 2009). Recent data has indicated that the CHC22 isoform of the clathrin heavy chain functions in endosomal protein sorting, but presently it is not known whether this is in concert with retromer (Esk et al., 2010). Further studies are required to elucidate whether clathrin has a formal role in retromer-mediated endosomal protein sorting.

Evolutionary conservation of retromer-interacting proteins

The retromer complex is conserved through out the eukaryotic kingdom (Koumandou et al., 2011), but this is not true of all the

retromer-interacting proteins. For example, RME-8 and EHD1 are conserved across many eukaryotic species, but interestingly, both are absent in yeast. The WASH complex and TBC1D5 are also not conserved in yeast, and it appears yeast are able to operate efficient endosome-to-Golgi retrieval with a simplified set of machinery. Yeast might therefore provide the best guide of which proteins are fundamentally required for retromer-mediated endosomal protein sorting, and which function in a regulatory or facilitating role.

How and why yeast employ only minimal machinery for mediating endosome-to-Golgi retrieval is subject to speculation but a plausible explanation centres on evolutionary variation of the *VPS29* gene that results in the gain of additional protein sequences very close to the region of Vps29 that mediates binding to TBC1D5 (Harbour and Seaman, 2011). It is also tempting to speculate that, in yeast, retromer function is restricted to the endosome-to-Golgi pathway and that, as a result, retromer-interacting proteins such as the WASH complex that are required for endosome-to-plasma membrane recycling have been lost.

Cargo and the physiological processes that require retromer

The numerous accessory proteins that interact with either the cargo-selective retromer complex or the Snx-BAR dimer all contribute to the efficiency of retromer-mediated endosomal protein sorting. It is the cargo proteins that depend on retromer for their localisation that ultimately determine the extent to which retromer-mediated endosomal protein sorting governs many diverse and physiological processes (Table 1). Although most studies of cargo sorting in the endocytic pathway indicate that both the cargo-selective trimer and the Snx-BAR dimer are involved in mediating the localisation of the respective membrane protein, there are now examples where only one retromer subcomplex is required. For example, the P2Y1 receptor requires the Snx-BAR dimer, but not the cargo-selective trimer, for its localisation, whereas the opposite might be true for the Wntless/MIG-14 protein in *C. elegans* (Nisar et al., 2010; Harterink et al., 2011).

Wntless/MIG-14 requires retromer for its retrieval

The identification of the requirement for retromer to retrieve the Wntless/MIG-14 protein establishes a role for retromer in

developmental processes involving the Wnt morphogen. Defective Wntless/MIG-14 retrieval results in degradation of the Wntless/MIG-14 protein and leads to reduced Wnt secretion and the abolishment of long-range signalling from Wnt morphogen gradients (Pan et al., 2008; Yang et al., 2008; Belenkaya et al., 2008; Port et al., 2008; Franch-Marro et al., 2008). Genetics-based studies centred on Wnt signalling in *C. elegans* have been successful at identifying other components of the retromer pathway including the myotubularins, which dephosphorylate PtdIns(3)P to regulate PtdIns(3)P-dependent processes, such as the association of Snx proteins with endosomes (Silhankova et al., 2010).

Studies in *C. elegans* have revealed a role for Snx3 in retromer-mediated Wntless/MIG-14 retrieval (Harterink et al., 2011; Zhang et al., 2011). These studies have also reported that the Snx-BAR element of retromer (i.e. Snx1) is not required for Wntless/MIG-14 retrieval and led to suggestions that two functionally distinct retromer complexes operate in endosomal protein sorting in *C. elegans*, a Snx3-containing retromer, and a Snx-BAR-containing retromer complex (Harterink et al., 2011). Presently, however, there is no compelling biochemical evidence for the existence of two distinct retromer complexes, and quantitative localisation analysis of Snx3, Snx1 and the Vps26 subunit of the cargo-selective retromer complex indicate that Snx3 and Snx1 co-localise more extensively than Snx3 and Vps26 – an observation somewhat at odds with the concept of two distinct retromer complexes. Independent studies of Snx1 function in *C. elegans* have also shown a role for Snx1 in mediating Wntless/MIG-14 localisation (Shi et al., 2009). Therefore, currently, there are some apparent discrepancies regarding the requirement for Snx1 in Wntless/MIG-14 trafficking.

Another genetics-based study reported a role for retromer in the retrieval of the CED-1 protein, which functions as a receptor for apoptotic cells that are cleared through phagocytosis (Chen et al., 2010). After uptake of the receptor and its ligand, the receptor is retrieved from the phagosome to the Golgi for re-secretion to the cell surface in a process that requires both the cargo-selective retromer complex and the Snx-BAR dimer (Chen et al., 2010; Lu et al., 2011). This role is broadly similar to that of the Snx1 and Snx5 proteins in directing membrane retrieval from macropinosomes in mammalian cells (Bryant et al., 2007; Wang et al., 2010).

Table 1. Retromer cargo proteins

Cargo protein	Species identified	Sorting motif	Physiological process	Reference(s)
Vps10p	Yeast	FYVFSN	Hydrolase sorting	(Seaman et al., 1997; Cereghino et al., 1995; Nothwehr et al., 2000)
Ftr1p	Yeast	HLPFTKNLQ ^a	Iron transport	(Strohlic et al., 2007).
Wntless/MIG-14	Flies, Worms	??	Wnt secretion	(Summarised in Eaton, 2008)
CED-1	Worms	??	Apototic cell clearance	(Chen et al., 2010)
CIMPR	Mammals	WLM	Hydrolase sorting	(Seaman, 2004; Arighi et al., 2004; Seaman, 2007)
Sortilin	Mammals	FLV	Hydrolase sorting	(Seaman, 2007; Canuel et al., 2008)
DMT1-II/Slc11a2	Mammals	YLL	Iron transport	(Tabuchi et al., 2010)
pIgA receptor	Mammals	??	Polymeric IgA transport	(Vergés et al., 2004)
Crumbs (Crb)	Mammals, Flies	??	Cell polarity	(Pocha et al., 2011; Zhou et al., 2011)
SorL1/SorLA	Mammals	FTAFANSHY	APP localisation	(Nielsen et al., 2007; Fjorback et al., 2012)
TGN38 and TGN46	Mammals	??	Not known	(Lieu and Gleeson, 2010; Chia et al., 2011)
β2-adrenergic receptor	Mammals	PDZ motif ^b	GPCR	(Temkin et al., 2011)

^aRecognised by Snx3.

^bProbably recognised by Snx27 but not retromer complex proteins.

Retromer mediates iron transporter recycling

Although evidence of a role for Snx3 in retromer-mediated sorting in worms and mammalian cells has been reported recently, the first reports of a role for Snx3 in retromer-mediated retrieval came from an elegant analysis in yeast of the localisation of the Ftr1 protein, a component of the yeast iron-transporter machinery that cycles between endosomes and the Golgi under iron-limiting conditions (Strochlic et al., 2007). Studies in mammalian cells confirmed the requirement for retromer in regulating iron uptake by mediating the localisation of DMT1-II (also known as Slc11a2) (Tabuchi et al., 2010). This study also identified a hydrophobic sequence in the cytoplasmic domain of DMT1-II that is required for the interaction with retromer and that is very similar to motifs found in the cytoplasmic tail of two other retromer cargo proteins, CIMPR and sortilin (Tabuchi et al., 2010; Seaman, 2007; Canuel et al., 2008).

Cell polarity requires retromer

The role of retromer in recycling proteins from endosomes plays a key role in the establishment of epithelial cell polarity through the requirement for retromer to sort Crb, a protein that defines the apical membrane (Pocha et al., 2011; Zhou et al., 2011). Retromer also operates in the transcytosis of the polymeric IgA receptor (pIgAR), which traverses polarised cells from the basolateral to the apical membrane (Vergés et al., 2004). Whether the transport of Crb or the pIgAR to the apical membrane involves an endosome-to-Golgi route (followed by sorting to the apical domain), or a more direct pathway of endosome-to-apical membrane remains to be conclusively determined, and it is possible that both routes are employed.

Cargo recognition

How does retromer recognise cargo? The ability to recognise cargo is integral to the function of retromer but, to date, remains something of a mystery. The Vps35 subunit can interact with the cytoplasmic tail of CIMPR, and genetic studies in yeast strongly support a direct role for Vps35p in cargo binding (Arighi et al., 2004; Nothwehr et al., 2000). The study mentioned above that reported the requirement of retromer to mediate the localisation of DMT1-II also demonstrated that a recombinant cargo-selective trimer directly interacts with a recombinant CIMPR tail or a DMT1-II cytoplasmic tail *in vitro* (Tabuchi et al., 2010). Recent data have revealed that Vps26 can bind directly to retromer cargo proteins, in this case the SorL1/SorLA protein (Fjorback et al., 2012). Here, Vps26 recognises a hydrophobic motif (FTAFANSHY) in the SorL1/SorLA cytoplasmic tail to sort SorL1/SorLA for retrieval. Intriguingly the motif in the SorL1/SorLA cytoplasmic tail is similar to a motif (FYVFSN) present in the yeast Vps10p tail that contributes to the endosome-to-Golgi retrieval of Vps10p (Cereghino et al., 1995), although there is also evidence that additional sequences in Vps10p contribute to interactions with Vps35p (Nothwehr et al., 2000). The recognition of cargo proteins by Vps26 might be expected as its structure was found to be similar to that of β -arrestin – an endocytic adaptor protein that sorts GPCRs into clathrin-coated pits (Shi et al., 2006; Collins et al., 2008).

Interestingly, in mammals, Vps26 is present as two paralogues, encoded by the highly related genes *Vps26a* and *Vps26b* (Kerr et al., 2005). Both forms comprise arrestin-like folds suggesting that both might be able to recognise cargo proteins. Recent

evidence has shown that the endosome-to-Golgi retrieval of CIMPR requires Vps26a but not Vps26b, revealing that they exert a preference for different cargoes (Bugarcic et al., 2011). Additionally, Vps26a and Vps26b might operate at distinct points in the endocytic pathway, although confirmation of this finding will require simultaneous determination of the localisation of both proteins at their endogenous expression levels.

One layer of regulation of retromer–cargo interactions could be provided through the reversible palmitoylation of cysteine residues present in the cytoplasmic tails of certain retromer cargo, such as CIMPR and sortilin (McCormick et al., 2008). The addition of palmitic acid to cytoplasmic tails is believed to alter the position of the motif that is recognised by retromer, thereby regulating the interaction. This mechanism cannot, however, be universally employed by retromer cargo proteins as not all cargoes, e.g. SorL1/SorLA, have cysteine residues in their cytoplasmic tail that can be palmitoylated.

Regulation of GPCR signalling by retromer

Retromer and the WASH complex along with Snx27 mediate the retrieval of the β 2-adrenergic receptor (Temkin et al., 2011), and in the case of another GPCR, the parathyroid hormone receptor (PTHr), retromer plays a similar role but additionally also regulates receptor signalling (Feinstein et al., 2011). Upon ligand binding and activation of the receptor, PTHr initiates cyclic AMP (cAMP) production and is then endocytosed through its interaction with β -arrestin and subsequent incorporation into clathrin-coated pits. Once the PTHr-arrestin complex reaches the endosome, the retromer complex associates with PTHr, thereby displacing β -arrestin to mediate the retrieval of PTHr to the cell surface. It is the displacement of β -arrestin by retromer that stops the production of cAMP, revealing a direct role for retromer in regulating a signalling event (Fig. 2). As the Vps26 subunit is structurally similar to arrestins (Shi et al., 2006; Collins et al., 2008), it seems likely that the Vps26 is responsible for displacing β -arrestin from PTHr.

Retromer and disease

The prominent role that SorL1/SorLA has in mediating the localisation and processing of APP (Andersen et al., 2005; Andersen et al., 2006) and the requirement for retromer to regulate the localisation of SorL1/SorLA (Nielsen et al., 2007; Fjorback et al., 2012) strongly suggests that retromer-mediated endosomal protein sorting contributes to the underlying pathology of AD. Loss of retromer function increases amyloidogenic processing of APP, and studies of tissue from AD patients indicates that retromer expression could be downregulated in neuronal tissue, although the underlying cause is presently unknown (Small et al., 2005; Muhammad et al., 2008; Lane et al., 2010; Wen et al., 2011). It has also been shown recently that specific variants of the *Snx3* and *Rab7a* genes are genetically linked to late-onset AD (Vardarajan et al., 2012). As both Snx3 and Rab7a are required for the membrane association of the cargo-selective retromer complex, it is likely that the identified polymorphisms alter the expression of the respective proteins, thus leading to compromised retromer-mediated endosomal protein sorting.

In addition to a role for retromer function in AD pathogenesis, the WASH complex component strumpellin is mutated in HSP, a progressive neurodegenerative disease that results in loss of long cortico-spinal tract axons, as noted above (Valdmanis et al., 2007).

Furthermore, the WASH complex subunit KIAA1033/SWIP is mutated in a hereditary intellectual disability (Ropers et al., 2011). Although complete loss-of-function mutations of retromer complex subunits are expected to be lethal, as has been shown for *Vps26a* in transgenic knockout experiments in mice (Radice et al., 1991; Lee et al., 1992), there is evidence that point mutations can be responsible for other forms of neurodegenerative disease. A rare mutation of *Vps35* has been shown to be causal in an inherited form of Parkinson disease, although the precise effect of this mutation on retromer function has yet to be determined (Zimprich et al., 2011; Vilariño-Güell et al., 2011).

Along with a role in neurodegenerative disease, retromer function is hijacked by a number of pathogens. For example, retromer is required for the endosome-to-Golgi transport of the bacterial Shiga toxin (Bujny et al., 2007). *Snx3* has been implicated in the maturation of Salmonella-containing endosomes, although it is not yet clear whether in this role *Snx3* is associated with retromer proteins (Braun et al., 2010). Additionally, the Tip protein of Herpes virus can bind to *Vps35* to inhibit retromer function, leading to reduced CIMPR levels and downregulation of CD4 (Kingston et al., 2011), and entry of Vaccinia virus has been shown to require the Fam21 subunit of the WASH complex (Huang et al., 2008).

Beyond endosomal protein sorting

The role and importance of retromer in endosomal protein sorting is now well established. It is therefore to be expected that retromer function and the function of retromer-associated proteins might contribute to additional physiological processes that involve the endocytic system. TBC1D5 has recently been shown to bind to the autophagy marker protein LC3, and functional siRNA-based screens have indicated that it might have a role in mitophagy – the clearance of mitochondria through autophagy (Popovic et al., 2012; Orvedahl et al., 2011). A detailed analysis of the role of TBC1D5 in autophagy has yet to be reported, although it is tempting to speculate that TBC1D5 regulates Rab7a activity. As Rab7a is required for the fusion of autophagosomes with lysosomes (Jäger et al., 2004; Gutierrez et al., 2004), the regulation of the activity of Rab7a by TBC1D5 might provide a checkpoint in autophagy.

The endosome-to-Golgi retrieval function of retromer might also be more directly required for autophagy. Under normal conditions, the autophagosome protein Atg9 cycles between the Golgi and endosomes. Upon induction of autophagy however, Atg9 recycling is reduced, resulting in it localising firstly to endosomes and then autophagosomes (Young et al., 2006). The retrieval of CIMPR is also reduced upon induction of autophagy. Whether this shift in the localisation of Atg9 and CIMPR occurs through the downregulation of retromer-mediated retrieval remains to be determined, but might be an interesting avenue to explore.

Conclusions and future perspectives

Retromer mediates endosome-to-Golgi retrieval and, through interactions with accessory proteins such as the WASH complex, also has a role in endosome-to-plasma membrane transport. Retromer can regulate GPCR signalling and has a key role in mediating the localisation and processing of APP, thus implicating retromer function in AD. Mutation of retromer components (e.g. *Vps35*) or proteins that operate with retromer (e.g. the strumpellin subunit of the WASH complex) results in

other forms of progressive neurodegenerative disease, including Parkinson disease and HSP.

What developments might occur in the future? First, the number of cargo proteins that depend on retromer for their correct localisation is increasing month-by-month and it seems likely that additional membrane proteins, possibly physiologically important proteins or membrane proteins implicated in disease, will be identified that require retromer for their appropriate localisation. I would anticipate that additional retromer-associated proteins will also be discovered that will have interesting roles in regulating endosomal protein sorting. Given that retromer operates at endosomes, where many signals initiated in response to extracellular stimuli are processed, it is likely that future research will reveal how retromer activity impinges on various signalling pathways and will identify signalling events that themselves regulate retromer function, possibly through post-translational modifications such as phosphorylation. Several of the retromer proteins or retromer-associated proteins can be phosphorylated, including the *Snx*-BAR dimer and Fam21 (see <http://www.uniprot.org/>), but it is currently unknown what role phosphorylation or other post-translational modifications might play in regulating retromer. It is 14 years since retromer was first described, but its story has only just begun.

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References

- Andersen, O. M., Reiche, J., Schmidt, V., Gotthardt, M., Spoelgen, R., Behlke, J., von Arnim, C. A., Breiderhoff, T., Jansen, P., Wu, X. et al. (2005). Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* **102**, 13461-13466.
- Andersen, O. M., Schmidt, V., Spoelgen, R., Gliemann, J., Behlke, J., Galatis, D., McKinstry, W. J., Parker, M. W., Masters, C. L., Hyman, B. T. et al. (2006). Molecular dissection of the interaction between amyloid precursor protein and its neuronal trafficking receptor SorLA/LR11. *Biochemistry* **45**, 2618-2628.
- Arighi, C. N., Hartnell, L. M., Aguilar, R. C., Haft, C. R. and Bonifacino, J. S. (2004). Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. *J. Cell Biol.* **165**, 123-133.
- Attar, N. and Cullen, P. J. (2010). The retromer complex. *Adv. Enzyme Regul.* **50**, 216-236.
- Belenkaya, T. Y., Wu, Y., Tang, X., Zhou, B., Cheng, L., Sharma, Y. V., Yan, D., Selva, E. M. and Lin, X. (2008). The retromer complex influences Wnt secretion by recycling wntless from endosomes to the trans-Golgi network. *Dev. Cell* **14**, 120-131.
- Blackstone, C., O'Kane, C. J. and Reid, E. (2011). Hereditary spastic paraplegias: membrane traffic and the motor pathway. *Nat. Rev. Neurosci.* **12**, 31-42.
- Borner, G. H., Harbour, M., Hester, S., Lilley, K. S. and Robinson, M. S. (2006). Comparative proteomics of clathrin-coated vesicles. *J. Cell Biol.* **175**, 571-578.
- Borner, G. H., Antrobus, R., Hirst, J., Bhumbra, G. S., Kozik, P., Jackson, L. P., Sahlender, D. A. and Robinson, M. S. (2012). Multivariate proteomic profiling identifies novel accessory proteins of coated vesicles. *J. Cell Biol.* **197**, 141-160.
- Braun, V., Wong, A., Landekic, M., Hong, W. J., Grinstein, S. and Brumell, J. H. (2010). Sorting nexin 3 (SNX3) is a component of a tubular endosomal network induced by Salmonella and involved in maturation of the Salmonella-containing vacuole. *Cell. Microbiol.* **12**, 1352-1367.
- Bryant, D. M., Kerr, M. C., Hammond, L. A., Joseph, S. R., Mostov, K. E., Teasdale, R. D. and Stow, J. L. (2007). EGF induces macropinocytosis and SNX1-modulated recycling of E-cadherin. *J. Cell Sci.* **120**, 1818-1828.
- Bugarcic, A., Zhe, Y., Kerr, M. C., Griffin, J., Collins, B. M. and Teasdale, R. D. (2011). *Vps26A* and *Vps26B* subunits define distinct retromer complexes. *Traffic* **12**, 1759-1773.
- Bujny, M. V., Popoff, V., Johannes, L. and Cullen, P. J. (2007). The retromer component sorting nexin-1 is required for efficient retrograde transport of Shiga toxin from early endosome to the trans Golgi network. *J. Cell Sci.* **120**, 2010-2021.
- Burda, P., Padilla, S. M., Sarkar, S. and Emr, S. D. (2002). Retromer function in endosome-to-Golgi retrograde transport is regulated by the yeast *Vps34* PtdIns 3-kinase. *J. Cell Sci.* **115**, 3889-3900.

- Canuel, M., Lefrancois, S., Zeng, J. and Morales, C. R. (2008). AP-1 and retromer play opposite roles in the trafficking of sortilin between the Golgi apparatus and the lysosomes. *Biochem. Biophys. Res. Commun.* **366**, 724-730.
- Caplan, S., Naslavsky, N., Hartnell, L. M., Lodge, R., Polishchuk, R. S., Donaldson, J. G. and Bonifacio, J. S. (2002). A tubular EHD1-containing compartment involved in the recycling of major histocompatibility complex class I molecules to the plasma membrane. *EMBO J.* **21**, 2557-2567.
- Carlton, J., Bujny, M., Peter, B. J., Oorschot, V. M., Rutherford, A., Mellor, H., Klumperman, J., McMahon, H. T. and Cullen, P. J. (2004). Sorting nexin-1 mediates tubular endosome-to-TGN transport through coincidence sensing of high-curvature membranes and 3-phosphoinositides. *Curr. Biol.* **14**, 1791-1800.
- Carnell, M., Zech, T., Calaminus, S. D., Ura, S., Hagedorn, M., Johnston, S. A., May, R. C., Soldati, T., Machesky, L. M. and Insall, R. H. (2011). Actin polymerization driven by WASH causes V-ATPase retrieval and vesicle neutralization before exocytosis. *J. Cell Biol.* **193**, 831-839.
- Cereghino, J. L., Marcussen, E. G. and Emr, S. D. (1995). The cytoplasmic tail domain of the vacuolar protein sorting receptor Vps10p and a subset of VPS gene products regulate receptor stability, function, and localization. *Mol. Biol. Cell* **6**, 1089-1102.
- Chen, D., Xiao, H., Zhang, K., Wang, B., Gao, Z., Jian, Y., Qi, X., Sun, J., Miao, L. and Yang, C. (2010). Retromer is required for apoptotic cell clearance by phagocytic receptor recycling. *Science* **327**, 1261-1264.
- Chia, P. Z., Gasnereau, I., Lieu, Z. Z. and Gleeson, P. A. (2011). Rab9-dependent retrograde transport and endosomal sorting of the endopeptidase furin. *J. Cell Sci.* **124**, 2401-2413.
- Chin, L. S., Raynor, M. C., Wei, X., Chen, H. Q. and Li, L. (2001). Hrs interacts with sorting nexin 1 and regulates degradation of epidermal growth factor receptor. *J. Biol. Chem.* **276**, 7069-7078.
- Collins, B. M., Norwood, S. J., Kerr, M. C., Mahony, D., Seaman, M. N., Teasdale, R. D. and Owen, D. J. (2008). Structure of Vps26B and mapping of its interaction with the retromer protein complex. *Traffic* **9**, 366-379.
- Cozier, G. E., Carlton, J., McGregor, A. H., Gleeson, P. A., Teasdale, R. D., Mellor, H. and Cullen, P. J. (2002). The phox homology (PX) domain-dependent, 3-phosphoinositide-mediated association of sorting nexin-1 with an early sorting endosomal compartment is required for its ability to regulate epidermal growth factor receptor degradation. *J. Biol. Chem.* **277**, 48730-48736.
- Cullen, P. J. and Korswagen, H. C. (2012). Sorting nexins provide diversity for retromer-dependent trafficking events. *Nat. Cell Biol.* **14**, 29-37.
- Daumke, O., Lundmark, R., Vallis, Y., Martens, S., Butler, P. J. and McMahon, H. T. (2007). Architectural and mechanistic insights into an EHD ATPase involved in membrane remodelling. *Nature* **449**, 923-927.
- Derivery, E., Sousa, C., Gautier, J. J., Lombard, B., Loew, D. and Gautreau, A. (2009). The Arp2/3 activator WASH controls the fission of endosomes through a large multiprotein complex. *Dev. Cell* **17**, 712-723.
- Eaton, S. (2008). Retromer retrieves wntless. *Dev. Cell* **14**, 4-6.
- Esk, C., Chen, C. Y., Johannes, L. and Brodsky, F. M. (2010). The clathrin heavy chain isoform CHC22 functions in a novel endosomal sorting step. *J. Cell Biol.* **188**, 131-144.
- Feinstein, T. N., Wehbi, V. L., Ardura, J. A., Wheeler, D. S., Ferrandon, S., Gardella, T. J. and Vilardaga, J. P. (2011). Retromer terminates the generation of cAMP by internalized PTH receptors. *Nat. Chem. Biol.* **7**, 278-284.
- Fjorback, A. W., Seaman, M., Gustafsen, C., Mehmedbasic, A., Gokool, S., Wu, C., Miltz, D., Schmidt, V., Madsen, P., Nyengaard, J. R. et al. (2012). Retromer binds the FANSHY sorting motif in SorLA to regulate amyloid precursor protein sorting and processing. *J. Neurosci.* **32**, 1467-1480.
- Franch-Marro, X., Wendler, F., Guidato, S., Griffith, J., Baena-Lopez, A., Itasaki, N., Maurice, M. M. and Vincent, J. P. (2008). Wingless secretion requires endosome-to-Golgi retrieval of Wntless/Evi/Sprinter by the retromer complex. *Nat. Cell Biol.* **10**, 170-177.
- Girard, M., Poupon, V., Blondeau, F. and McPherson, P. S. (2005). The DnaJ-domain protein RME-8 functions in endosomal trafficking. *J. Biol. Chem.* **280**, 40135-40143.
- Gokool, S., Tattersall, D. and Seaman, M. N. (2007). EHD1 interacts with retromer to stabilize SNX1 tubules and facilitate endosome-to-Golgi retrieval. *Traffic* **8**, 1873-1886.
- Gomez, T. S. and Billadeau, D. D. (2009). A FAM21-containing WASH complex regulates retromer-dependent sorting. *Dev. Cell* **17**, 699-711.
- Gutierrez, M. G., Munafó, D. B., Berón, W. and Colombo, M. I. (2004). Rab7 is required for the normal progression of the autophagic pathway in mammalian cells. *J. Cell Sci.* **117**, 2687-2697.
- Harbour, M. E. and Seaman, M. N. J. (2011). Evolutionary variations of VPS29, and their implications for the heteropentameric model of retromer. *Commun. Integr. Biol.* **4**, 619-622.
- Harbour, M. E., Breusegem, S. Y. A., Antrobus, R., Freeman, C., Reid, E. and Seaman, M. N. J. (2010). The cargo-selective retromer complex is a recruiting hub for protein complexes that regulate endosomal tubule dynamics. *J. Cell Sci.* **123**, 3703-3717.
- Harbour, M. E., Breusegem, S. Y. and Seaman, M. N. J. (2012). Recruitment of the endosomal WASH complex is mediated by the extended 'tail' of Fam21 binding to the retromer protein Vps35. *Biochem. J.* **442**, 209-220.
- Harterink, M., Port, F., Lorenowicz, M. J., McGough, I. J., Silhankova, M., Betist, M. C., van Weering, J. R., van Heesbeen, R. G., Middelkoop, T. C., Basler, K. et al. (2011). A SNX3-dependent retromer pathway mediates retrograde transport of the Wnt sorting receptor Wntless and is required for Wnt secretion. *Nat. Cell Biol.* **13**, 914-923.
- Hong, Z., Yang, Y., Zhang, C., Niu, Y., Li, K., Zhao, X. and Liu, J. J. (2009). The retromer component SNX6 interacts with dynactin p150(Glued) and mediates endosome-to-TGN transport. *Cell Res.* **19**, 1334-1349.
- Horazdovsky, B. F., Davies, B., Seaman, M. N. J., McLaughlin, S. A., Yoon, S.-K. and Emr, S. D. (1997). A sorting nexin-1 homologue, Vps5p, forms a complex with Vps17p and is required for recycling the vacuolar protein-sorting receptor. *Mol. Biol. Cell* **8**, 1529-1541.
- Huang, C. Y., Lu, T. Y., Bair, C. H., Chang, Y. S., Jwo, J. K. and Chang, W. (2008). A novel cellular protein, VPEF, facilitates vaccinia virus penetration into HeLa cells through fluid phase endocytosis. *J. Virol.* **82**, 7988-7999.
- Jäger, S., Bucci, C., Tanida, I., Ueno, T., Kominami, E., Saftig, P. and Eskelinen, E. L. (2004). Role for Rab7 in maturation of late autophagic vacuoles. *J. Cell Sci.* **117**, 4837-4848.
- Jia, D., Gomez, T. S., Metlagel, Z., Umetani, J., Otwinowski, Z., Rosen, M. K. and Billadeau, D. D. (2010). WASH and WAVE actin regulators of the Wiskott-Aldrich syndrome protein (WASP) family are controlled by analogous structurally related complexes. *Proc. Natl. Acad. Sci. USA* **107**, 10442-10447.
- Jia, D., Gomez, T. S., Billadeau, D. D. and Rosen, M. K. (2012). Multiple repeat elements within the FAM21 tail link the WASH actin regulatory complex to the retromer. *Mol. Biol. Cell* **23**, 2352-2361.
- Kerr, M. C., Bennetts, J. S., Simpson, F., Thomas, E. C., Flegg, C., Gleeson, P. A., Wicking, C. and Teasdale, R. D. (2005). A novel mammalian retromer component, Vps26B. *Traffic* **6**, 991-1001.
- Kingston, D., Chang, H., Ensser, A., Lee, H. R., Lee, J., Lee, S. H., Jung, J. U. and Cho, N. H. (2011). Inhibition of retromer activity by herpesvirus saimiri tip leads to CD4 downregulation and efficient T cell transformation. *J. Virol.* **85**, 10627-10638.
- Koumandou, V. L., Klute, M. J., Herman, E. K., Nunez-Miguel, R., Dacks, J. B. and Field, M. C. (2011). Evolutionary reconstruction of the retromer complex and its function in *Trypanosoma brucei*. *J. Cell Sci.* **124**, 1496-1509.
- Kurten, R. C., Eddington, A. D., Chowdhury, P., Smith, R. D., Davidson, A. D. and Shank, B. B. (2001). Self-assembly and binding of a sorting nexin to sorting endosomes. *J. Cell Sci.* **114**, 1743-1756.
- Lane, R. F., Raines, S. M., Steele, J. W., Ehrlich, M. E., Lah, J. A., Small, S. A., Tanzi, R. E., Attie, A. D. and Gandy, S. (2010). Diabetes-associated SorCS1 regulates Alzheimer's amyloid-beta metabolism: evidence for involvement of SorL1 and the retromer complex. *J. Neurosci.* **30**, 13110-13115.
- Lee, J. J., Radice, G., Perkins, C. P. and Costantini, F. (1992). Identification and characterization of a novel, evolutionarily conserved gene disrupted by the murine H beta 58 embryonic lethal transgene insertion. *Development* **115**, 277-288.
- Lieu, Z. Z. and Gleeson, P. A. (2010). Identification of different itineraries and retromer components for endosome-to-Golgi transport of TGN38 and Shiga toxin. *Eur. J. Cell Biol.* **89**, 379-393.
- Lu, N., Shen, Q., Mahoney, T. R., Liu, X. and Zhou, Z. (2011). Three sorting nexins drive the degradation of apoptotic cells in response to PtdIns(3)P signaling. *Mol. Biol. Cell* **22**, 354-374.
- Lumb, J. H., Leung, K. F., Dubois, K. N. and Field, M. C. (2011). Rab28 function in trypanosomes: interactions with retromer and ESCRT pathways. *J. Cell Sci.* **124**, 3771-3783.
- Mamo, A., Jules, F., Dumaresq-Doiron, K., Costantino, S. and Lefrancois, S. (2012). The role of ceroid lipofuscinosis neuronal protein 5 (CLN5) in endosomal sorting. *Mol. Cell Biol.* **32**, 1855-1866.
- McCormick, P. J., Dumaresq-Doiron, K., Pluviose, A. S., Pichette, V., Tosato, G. and Lefrancois, S. (2008). Palmitoylation controls recycling in lysosomal sorting and trafficking. *Traffic* **9**, 1984-1997.
- McGough, I. J. and Cullen, P. J. (2011). Recent advances in retromer biology. *Traffic* **12**, 963-971.
- Muhammad, A., Flores, I., Zhang, H., Yu, R., Staniszewski, A., Planel, E., Herman, M., Ho, L., Kreber, R., Honig, L. S. et al. (2008). Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration, and Abeta accumulation. *Proc. Natl. Acad. Sci. USA* **105**, 7327-7332.
- Mukhopadhyay, A., Pan, X., Lambright, D. G. and Tissenbaum, H. A. (2007). An endocytic pathway as a target of tubby for regulation of fat storage. *EMBO Rep.* **8**, 931-938.
- Nakada-Tsukui, K., Saito-Nakano, Y., Ali, V. and Nozaki, T. (2005). A retromerlike complex is a novel Rab7 effector that is involved in the transport of the virulence factor cysteine protease in the enteric protozoan parasite *Entamoeba histolytica*. *Mol. Biol. Cell* **16**, 5294-5303.
- Naslavsky, N., McKenzie, J., Altan-Bonnet, N., Sheff, D. and Caplan, S. (2009). EHD3 regulates early-endosome-to-Golgi transport and preserves Golgi morphology. *J. Cell Sci.* **122**, 389-400.
- Nielsen, M. S., Gustafsen, C., Madsen, P., Nyengaard, J. R., Hermey, G., Bakke, O., Mari, M., Schu, P., Pohlmann, R., Dennes, A. et al. (2007). Sorting by the cytoplasmic domain of the amyloid precursor protein binding receptor SorLA. *Mol. Cell Biol.* **27**, 6842-6851.
- Nisar, S., Kelly, E., Cullen, P. J. and Mundell, S. J. (2010). Regulation of P2Y1 receptor traffic by sorting Nexin 1 is retromer independent. *Traffic* **11**, 508-519.
- Nothwehr, S. F. and Hines, A. E. (1997). The yeast VPS5/GRD2 gene encodes a sorting nexin-1-like protein required for localizing membrane proteins to the late Golgi. *J. Cell Sci.* **110**, 1063-1072.

- Nothwehr, S. F., Ha, S. A. and Bruinsma, P. (2000). Sorting of yeast membrane proteins into an endosome-to-Golgi pathway involves direct interaction of their cytosolic domains with Vps35p. *J. Cell Biol.* **151**, 297-310.
- Orvedahl, A., Sumpter, R., Jr, Xiao, G., Ng, A., Zou, Z., Tang, Y., Narimatsu, M., Gilpin, C., Sun, Q., Roth, M. et al. (2011). Image-based genome-wide siRNA screen identifies selective autophagy factors. *Nature* **480**, 113-117.
- Pan, C. L., Baum, P. D., Gu, M., Jorgensen, E. M., Clark, S. G. and Garriga, G. (2008). *C. elegans* AP-2 and retromer control Wnt signaling by regulating mig-14/Wntless. *Dev. Cell* **14**, 132-139.
- Peter, B. J., Kent, H. M., Mills, I. G., Vallis, Y., Butler, P. J., Evans, P. R. and McMahon, H. T. (2004). BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science* **303**, 495-499.
- Pocha, S. M., Wassmer, T., Niehage, C., Hofflack, B. and Knust, E. (2011). Retromer controls epithelial cell polarity by trafficking the apical determinant Crumbs. *Curr. Biol.* **21**, 1111-1117.
- Popoff, V., Mardones, G. A., Tenza, D., Rojas, R., Lamaze, C., Bonifacino, J. S., Raposo, G. and Johannes, L. (2007). The retromer complex and clathrin define an early endosomal retrograde exit site. *J. Cell Sci.* **120**, 2022-2031.
- Popoff, V., Mardones, G. A., Bai, S. K., Chambon, V., Tenza, D., Burgos, P. V., Shi, A., Benaroch, P., Urbé, S., Lamaze, C. et al. (2009). Analysis of articulation between clathrin and retromer in retrograde sorting on early endosomes. *Traffic* **10**, 1868-1880.
- Popovic, D., Akutsu, M., Novak, I., Harper, W., Behrends, C. and Dikic, I. (2012). Rab GAPs in Autophagy: regulation of endocytic and autophagy pathways by direct binding to human ATG8 modifiers. *Mol. Cell Biol.* **32**, 1733-1744.
- Port, F., Kuster, M., Herr, P., Furger, E., Bänziger, C., Hausmann, G. and Basler, K. (2008). Wingless secretion promotes and requires retromer-dependent cycling of Wntless. *Nat. Cell Biol.* **10**, 178-185.
- Prosser, D. C., Tran, D., Schooley, A., Wendland, B. and Ngee, J. K. (2010). A novel, retromer-independent role for sorting nexins 1 and 2 in RhoG-dependent membrane remodeling. *Traffic* **11**, 1347-1362.
- Puthenveedu, M. A., Lauffer, B., Temkin, P., Vistein, R., Carlton, P., Thorn, K., Taunton, J., Weiner, O. D., Parton, R. G. and von Zastrow, M. (2010). Sequence-dependent sorting of recycling proteins by actin-stabilized endosomal microdomains. *Cell* **143**, 761-773.
- Radice, G., Lee, J. J. and Costantini, F. (1991). H beta 58, an insertional mutation affecting early postimplantation development of the mouse embryo. *Development* **111**, 801-811.
- Rapaport, D., Auerbach, W., Naslavsky, N., Pasmanik-Chor, M., Galperin, E., Fein, A., Caplan, S., Joyner, A. L. and Horowitz, M. (2006). Recycling to the plasma membrane is delayed in EHD1 knockout mice. *Traffic* **7**, 52-60.
- Rojas, R., Kametaka, S., Haft, C. R. and Bonifacino, J. S. (2007). Interchangeable but essential functions of SNX1 and SNX2 in the association of retromer with endosomes and the trafficking of mannose 6-phosphate receptors. *Mol. Cell Biol.* **27**, 1112-1124.
- Rojas, R., van Vlijmen, T., Mardones, G. A., Prabhu, Y., Rojas, A. L., Mohammed, S., Heck, A. J., Raposo, G., van der Sluijs, P. and Bonifacino, J. S. (2008). Regulation of retromer recruitment to endosomes by sequential action of Rab5 and Rab7. *J. Cell Biol.* **183**, 513-526.
- Ropers, F., Derivery, E., Hu, H., Garshabi, M., Karbasiyan, M., Herold, M., Nürnberg, G., Ullmann, R., Gautreau, A., Sperling, K. et al. (2011). Identification of a novel candidate gene for non-syndromic autosomal recessive intellectual disability: the WASH complex member SWIP. *Hum. Mol. Genet.* **20**, 2585-2590.
- Ruck, A., Attonito, J., Garces, K. T., Núñez, L., Palmisano, N. J., Rubel, Z., Bai, Z., Nguyen, K. C., Sun, L., Grant, B. D. et al. (2011). The Atg6/Vps30/Becclin 1 ortholog BEC-1 mediates endocytic retrograde transport in addition to autophagy in *C. elegans*. *Autophagy* **7**, 386-400.
- Schwarz, D. G., Griffin, C. T., Schneider, E. A., Yee, D. and Magnuson, T. (2002). Genetic analysis of sorting nexins 1 and 2 reveals a redundant and essential function in mice. *Mol. Biol. Cell* **13**, 3588-3600.
- Seaman, M. N. J. (2004). Cargo-selective endosomal sorting for retrieval to the Golgi requires retromer. *J. Cell Biol.* **165**, 111-122.
- Seaman, M. N. J. (2007). Identification of a novel conserved sorting motif required for retromer-mediated endosome-to-TGN retrieval. *J. Cell Sci.* **120**, 2378-2389.
- Seaman, M. N. J., Marcussone, E. G., Cereghino, J.-L. and Emr, S. D. (1997). Endosome to Golgi retrieval of the vacuolar protein sorting receptor, Vps10p, requires the function of the VPS29, VPS30, and VPS35 gene products. *J. Cell Biol.* **137**, 79-92.
- Seaman, M. N. J., McCaffery, J. M. and Emr, S. D. (1998). A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast. *J. Cell Biol.* **142**, 665-681.
- Seaman, M. N. J., Harbour, M. E., Tattersall, D., Read, E. and Bright, N. (2009). Membrane recruitment of the cargo-selective retromer subcomplex is catalysed by the small GTPase Rab7 and inhibited by the Rab-GAP TBC1D5. *J. Cell Sci.* **122**, 2371-2382.
- Shi, H., Rojas, R., Bonifacino, J. S. and Hurley, J. H. (2006). The retromer subunit Vps26 has an arrestin fold and binds Vps35 through its C-terminal domain. *Nat. Struct. Mol. Biol.* **13**, 540-548.
- Shi, A., Sun, L., Banerjee, R., Tobin, M., Zhang, Y. and Grant, B. D. (2009). Regulation of endosomal clathrin and retromer-mediated endosome to Golgi retrograde transport by the J-domain protein RME-8. *EMBO J.* **28**, 3290-3302.
- Silhankova, M., Port, F., Harterink, M., Basler, K. and Korswagen, H. C. (2010). Wnt signalling requires MTM-6 and MTM-9 myotubularin lipid-phosphatase function in Wnt-producing cells. *EMBO J.* **29**, 4094-4105.
- Skänland, S. S., Wälchli, S., Brech, A. and Sandvig, K. (2009). SNX4 in complex with clathrin and dynein: implications for endosome movement. *PLoS ONE* **4**, e5935.
- Small, S. A., Kent, K., Pierce, A., Leung, C., Kang, M. S., Okada, H., Honig, L., Vonsattel, J. P. and Kim, T. W. (2005). Model-guided microarray implicates the retromer complex in Alzheimer's disease. *Ann. Neurol.* **58**, 909-919.
- Stack, J. H., Herman, P. K., Schu, P. V. and Emr, S. D. (1993). A membrane-associated complex containing the Vps15 protein kinase and the Vps34 PI 3-kinase is essential for protein sorting to the yeast lysosome-like vacuole. *EMBO J.* **12**, 2195-2204.
- Strochlic, T. I., Setty, T. G., Sitaram, A. and Burd, C. G. (2007). Grd19/Snx3p functions as a cargo-specific adapter for retromer-dependent endocytic recycling. *J. Cell Biol.* **177**, 115-125.
- Swarbrick, J. D., Shaw, D. J., Chhabra, S., Ghai, R., Valkov, E., Norwood, S. J., Seaman, M. N. J. and Collins, B. M. (2011). VPS29 is not an active metallophosphatase but is a rigid scaffold required for retromer interaction with accessory proteins. *PLoS ONE* **6**, e20420.
- Tabuchi, M., Yanatori, I., Kawai, Y. and Kishi, F. (2010). Retromer-mediated direct sorting is required for proper endosomal recycling of the mammalian iron transporter DMT1. *J. Cell Sci.* **123**, 756-766.
- Teasdale, R. D. and Collins, B. M. (2012). Insights into the PX (phox-homology) domain and SNX (sorting nexin) protein families: structures, functions and roles in disease. *Biochem. J.* **441**, 39-59.
- Temkin, P., Lauffer, B., Jäger, S., Cimermanic, P., Krogan, N. J. and von Zastrow, M. (2011). SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors. *Nat. Cell Biol.* **13**, 715-721.
- Valdmanis, P. N., Meijer, I. A., Reynolds, A., Lei, A., MacLeod, P., Schlesinger, D., Zatz, M., Reid, E., Dion, P. A., Drapeau, P. et al. (2007). Mutations in the KIAA0196 gene at the SPG8 locus cause hereditary spastic paraplegia. *Am. J. Hum. Genet.* **80**, 152-161.
- van Weering, J. R., Verkade, P. and Cullen, P. J. (2012). SNX-BAR-mediated endosome tubulation is co-ordinated with endosome maturation. *Traffic* **13**, 94-107.
- Vardarajan, B., Bruesegem, S., Harbour, M., St George-Hyslop, P., Seaman, M. N. J. and Farrer, L. A. (2012). Identification of Alzheimer disease associated variants in genes that regulate retromer function. *Neurobiol. Aging* **33**, 2231.e15-e30.
- Vergés, M., Luton, F., Gruber, C., Tiemann, F., Reinders, L. G., Huang, L., Burlingame, A. L., Haft, C. R. and Mostov, K. E. (2004). The mammalian retromer regulates transcytosis of the polymeric immunoglobulin receptor. *Nat. Cell Biol.* **6**, 763-769.
- Vilariño-Güell, C., Wider, C., Ross, O. A., Dachselt, J. C., Kachergus, J. M., Lincoln, S. J., Soto-Ortolaza, A. I., Cobb, S. A., Wilhoite, G. J., Bacon, J. A. et al. (2011). VPS35 mutations in Parkinson disease. *Am. J. Hum. Genet.* **89**, 162-167.
- Wang, J. T., Kerr, M. C., Karunaratne, S., Jeanes, A., Yap, A. S. and Teasdale, R. D. (2010). The SNX-PX-BAR family in macropinocytosis: the regulation of macropinosome formation by SNX-PX-BAR proteins. *PLoS ONE* **5**, e13763.
- Wassmer, T., Attar, N., Bujny, M. V., Oakley, J., Traer, C. J. and Cullen, P. J. (2007). A loss-of-function screen reveals SNX5 and SNX6 as potential components of the mammalian retromer. *J. Cell Sci.* **120**, 45-54.
- Wassmer, T., Attar, N., Harterink, M., van Weering, J. R., Traer, C. J., Oakley, J., Goud, B., Stephens, D. J., Verkade, P., Korswagen, H. C. et al. (2009). The retromer coat complex coordinates endosomal sorting and dynein-mediated transport, with carrier recognition by the trans-Golgi network. *Dev. Cell* **17**, 110-122.
- Wen, L., Tang, F. L., Hong, Y., Luo, S. W., Wang, C. L., He, W., Shen, C., Jung, J. U., Xiong, F., Lee, D. H. et al. (2011). VPS35 haploinsufficiency increases Alzheimer's disease neuropathology. *J. Cell Biol.* **195**, 765-779.
- Yang, P. T., Lorenovic, M. J., Silhankova, M., Coudreuse, D. Y., Betist, M. C. and Korswagen, H. C. (2008). Wnt signaling requires retromer-dependent recycling of MIG-14/Wntless in Wnt-producing cells. *Dev. Cell* **14**, 140-147.
- Young, A. R., Chan, E. Y., Hu, X. W., Köchl, R., Crawshaw, S. G., High, S., Hailey, D. W., Lippincott-Schwartz, J. and Tooze, S. A. (2006). Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J. Cell Sci.* **119**, 3888-3900.
- Yu, J. W. and Lemmon, M. A. (2001). All phox homology (PX) domains from *Saccharomyces cerevisiae* specifically recognize phosphatidylinositol 3-phosphate. *J. Biol. Chem.* **276**, 44179-44184.
- Zech, T., Calaminus, S. D., Caswell, P., Spence, H. J., Carnell, M., Inshall, R. H., Norman, J. and Machesky, L. M. (2011). The Arp2/3 activator WASH regulates $\alpha 5 \beta 1$ -integrin-mediated invasive migration. *J. Cell Sci.* **124**, 3753-3759.
- Zhang, P., Wu, Y., Belenkaya, T. Y. and Lin, X. (2011). SNX3 controls Wingless/Wnt secretion through regulating retromer-dependent recycling of Wntless. *Cell Res.* **21**, 1677-1690.
- Zhang, J., Reiling, C., Reinecke, J. B., Prislán, I., Marky, L. A., Sorgen, P. L., Naslavsky, N. and Caplan, S. (2012). Rabankyrin-5 interacts with EHD1 and Vps26 to regulate endocytic trafficking and retromer function. *Traffic* **13**, 745-757.
- Zhou, B., Wu, Y. and Lin, X. (2011). Retromer regulates apical-basal polarity through recycling Crumbs. *Dev. Biol.* **360**, 87-95.
- Zimprich, A., Benet-Pagès, A., Struhal, W., Graf, E., Eck, S. H., Offman, M. N., Haubenberger, D., Spielberger, S., Schulte, E. C., Lichtner, P. et al. (2011). A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am. J. Hum. Genet.* **89**, 168-175.