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Retromer and sorting nexins in development

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1. ABSTRACT

Trafficking and signaling processes involve common molecular components. The machinery that controls intracellular trafficking is vital in ensuring that signaling mechanisms take place correctly. An illustrative example of this relationship is the sustained signaling of endocytosed membrane receptors, such as receptor Tyr kinases and G-protein coupled receptors, after ligand-induced activation. An intriguing role in controlling the fate of these and other receptors at the endosome has been attributed to members of the sorting nexin protein family. The best characterized sorting nexins are subunits of a multimeric complex, termed retromer. It was first found in yeast that retromer mediates endosome-to-Golgi retrieval of receptors after they have delivered soluble hydrolase precursors into the vacuole, the organelle equivalent to the mammalian lysosome. Work in cultured mammalian cells later demonstrated that retromer performs an analogous function in higher eukaryotes. Data from genetically modified mice, and from a simpler organism such as the nematode *Caenorhabtidis elegans*, has revealed that retromer performs an essential role during embryogenesis. This review will discuss implications of recent work on this subject.

2. INTRODUCTION

Subcellular organelles contain a distinct set of resident proteins required for the proper functioning of each compartment. The continuous exchange of proteins and lipids between intracellular compartments is largely mediated by transport vesicles, which are formed by the cooperative action of cytosolic protein coats (1). These coats shape the membrane into regions of high curvature and capture cargo proteins for their proper sorting (2-5). Protein sorting and trafficking operate under the regulation of well-conserved mechanisms. In a simple organism such as yeast, a pathway particularly important is protein delivery to the vacuole, the lytic compartment equivalent to the mammalian lysosome. Many Vacuolar protein sorting (Vps) proteins participate in this pathway (6, 7), and a small subset of these are the components of a multimeric protein complex, termed retromer, and composed of five Vps subunits: Vps35p, Vps26p, Vps29p, Vps5p and Vps17p (8, 9).

At the yeast late-Golgi, like in its functional equivalent in mammalian cells, the *trans*-Golgi network (TGN), specific receptors deliver soluble enzyme precursors to the vacuole. The dissociation of ligand from

receptor takes place in the acidic environment of an endosomal prevacuolar compartment (PVC), from which the empty receptor must be recycled back to the Golgi for reuse. In yeast, retromer is an essential protein complex that performs endosome-to-Golgi retrograde transport of Vps10p, the receptor for the soluble vacuolar hydrolase precursor pro-carboxypeptidase Y (CPY) (8-11). The role of retromer is conserved in higher eukaryotes for the retrieval of the cation-independent mannose 6-phosphate receptor (CI-MPR) once delivery of lysosomal hydrolase precursors by the receptor has been completed (12, 13). Retromer also associates with the polymeric immunoglobulin receptor (pIgR) and promotes transcytosis of pIgR and its ligand, the polymeric immunoglobulin A (pIgA), in polarized epithelial cells, preventing their recycling and degradation (14). Retromer could therefore have a more general role of retrieving certain receptors from missorting to lysosomal degradation or to other destinations, which agrees with the scheme that endosomal cargo follows multiple recycling or retrieval pathways (15-17).

Based on early studies in the yeast model, retromer assembly and functioning involves the cooperation of two subcomplexes. One subcomplex consists of two sorting nexins (SNXs), Vps5p and Vps17p, which are in charge of membrane deformation (9, 18, 19), and a second subcomplex, consisting of Vps35p, Vps26p and Vps29p, which provides cargo recognition (8, 9, 20-22). Retromer assembles in a similar way in higher eukaryotes, with Vps35, as the complex core (23, 24), associating directly with cargo (12). Recent structural and binding studies have provided valuable information on the interaction sites among retromer subunits and on how these subunits integrate in the complex (24-26). Orthologs of the yeast *VPS5*, *VPS26*, *VPS29* and *VPS35* genes have been identified in several organisms, including humans (8, 18, 23, 27-29), and data from genetically modified mice sustain that retromer performs an essential role during mammalian embryogenesis (30-33). The findings indicate that these proteins act in the same pathway and provide evidence for the existence of a mammalian retromer structurally similar to the yeast retromer. While the mechanism of retromer function during development awaits further elucidation, recent data in the nematode *C. elegans* demonstrate the requirement of retromer to generate Wnt proteins competent for long-range signaling during establishment of neuronal polarity and migration (34, 35).

A major goal of this review is to emphasize the role of retromer and SNXs in controlling membrane trafficking, focusing on the implications in animal development.

3. RETROMER: CONSERVED FUNCTION FROM YEAST TO HIGHER EUKARYOTES

The vacuole of the budding yeast *Saccharomyces cerevisiae* is a central organelle in the physiology of this organism. Among its many roles, the vacuole functions in pH homeostasis and osmotic regulation, protein degradation, and as a storage organelle. To carry out all of these functions, it is essential that the vacuole contains its full complement of proteins, which reach the vacuole through different mechanisms, by pathways originated in secretory or endocytic organelles, in the cytosol or by inheritance of vacuolar material by daughter cells during cell division. Genetic screens designed to identify components involved in each pathway showed that these pathways are intimately related. Around 60 genes required for proper trafficking to the yeast vacuole have been identified by genetic and biochemical approaches. A great deal of knowledge on these Vps pathways has been obtained from the use of *VPS* and *PEP* (peptidase-deficient) mutants displaying different degrees of alteration in trafficking to the vacuole. The *VPS* mutants were isolated as a collection of mutants that fail to sort pro-CPY or a CPY-invertase fusion protein to the vacuole, secreting the soluble hydrolase precursor because of a block in the vacuolar biogenesis pathway (36-38). The *PEP* mutants were isolated as being defective in CPY enzymatic activity (39). Subsequent complementation analysis revealed extensive overlap between these two mutant groups (40). These studies altogether demonstrated that the transport of vacuolar hydrolases from the late-Golgi is a key protein pathway to the yeast vacuole (reviewed in 41, 42).

Hydrolase precursors are sorted at the late-Golgi in yeast essentially in the same way as at the TGN in mammalian cells (Figure 1). In yeast, the late-Golgi type I transmembrane protein Vps10p is a major sorting receptor for pro-CPY (8-11). In mammalian cells, sorting is mediated by MPRs, through recognition of a mannose-6-phosphate tag added to the newly synthesized enzymes in the Golgi. There are two distinct MPRs in mammalian cells: the 46 kDa cation-dependent MPR (CD-MPR) and the 300 kDa CI-MPR. Both receptors are type I transmembrane proteins that share some sequence homology in their respective lumenal domains (43). MPRs are sorted into clathrin-coated vesicles at the TGN through interactions with Golgi-associated, gamma-ear containing, ADP-ribosylation factor (ARF)-binding (GGA) proteins, which bind to acidic-cluster-dileucine signals in the cytoplasmic tails of the MPRs (44). Vps10p is also sorted at the yeast late-Golgi by clathrin and GGA proteins (45). While Vps10p shares no significant sequence homology with the MPRs, they both perform essentially an identical function in enzyme transport and, like the MPRs, Vps10p rapidly cycles between the late-Golgi and endosomes (10, 11, 46). These similarities emphasize that protein sorting and trafficking pathways are regulated by well-conserved mechanisms (see 16, 42, 47 for reviews).

Analysis of the collection of *VPS* mutants in Scott Emr's laboratory initially revealed that three mutants, *VPS29*, *VPS30* and *VPS35*, had the same phenotypes as the *VPS10* mutant. Thus, these gene products were likely candidates for mediating Vps10p trafficking. Subsequent experiments indicated that Vps29p, Vps30p and Vps35p are indeed required to retrieve Vps10p from endosomes to the Golgi, since loss of their function resulted in mislocalization of Vps10p to the yeast vacuole, from where it could not return to the Golgi (8). Mislocalization of the

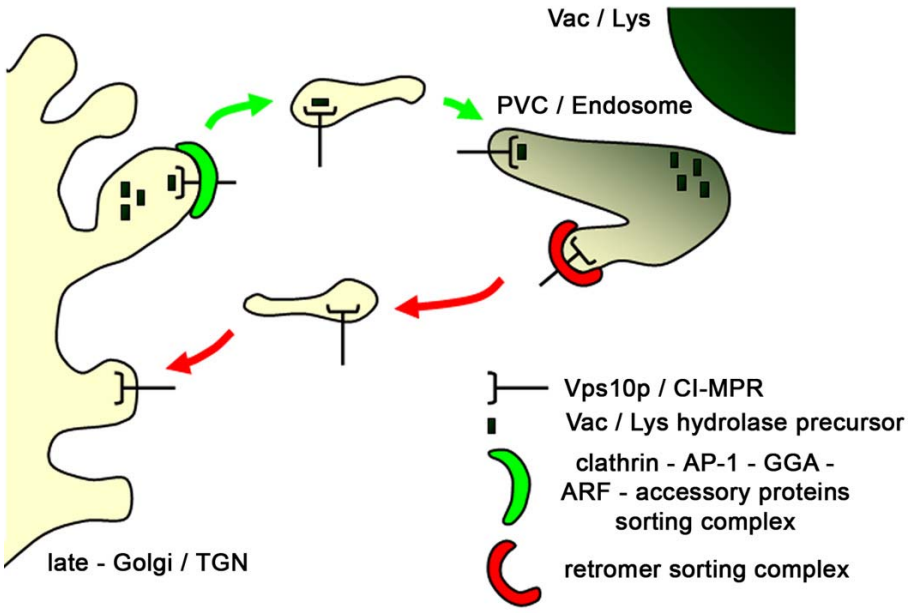

Figure 1. Sorting of hydrolase receptors in yeast and in mammalian cells by retromer. Vacuolar or lysosomal hydrolase precursors are sorted by specific receptors at the yeast late-Golgi, which is equivalent to the mammalian *trans*-Golgi network (TGN). In yeast, Vps10p is a major sorting receptor, while in mammalian cells hydrolases sorting is mediated by mannose-6-phosphate receptors (MPRs). Golgi-associated, gamma-ear containing, ADP-ribosylation factor (ARF)-binding (GGA) proteins are recruited at the late-Golgi / TGN membrane through interaction with ARF and then cooperate with the adaptor protein complex AP-1 to package Vps10p or MPRs into clathrin-coated carriers. Additional accessory proteins are recruited to function in vesicle assembly, release and delivery to the endosomal system (*green arrows*). Under the acidic environment of the yeast prevacuolar compartment (PVC), or in the mammalian endosome, the hydrolase precursors dissociate from their receptors and follow their fate to the yeast vacuole (Vac), or to the mammalian lysosome (Lys), where they are cleaved to produce their active forms. The empty receptors are then recycled back to the Golgi for reuse (*red arrows*), undergoing new rounds of hydrolases delivery. In yeast, the retromer complex, formed by the sorting nexins Vps5p / Vps17p and by the Vps35p-Vps26p-Vps29p subcomplex, retrieves Vps10p from endosome-to-Golgi. Retromer assembly and function are conserved throughout evolution, and thus it recycles in a similar manner one type of MPR, the cation-independent MPR (CI-MPR). Additional budding / sorting mechanisms involved in CI-MPR retrieval from early or late endosomes, as well as protein complexes described in yeast or in mammalian systems and performing vesicle tethering / fusion at the Golgi apparatus, are omitted for simplicity.

receptor thus caused the defect in pro-CPY sorting, underlining the importance of a retrieval pathway to ensure the correct functioning of the forward Golgi-to-vacuole pathway. Other studies from the same laboratory showed that two other *VPS* gene products, the SNXs Vps5p and Vps17p, form a dimer that also functions in maintaining the proper localization of Vps10p (18). Biochemical experiments on recombinant Vps5p indicated that this protein could also self-assemble and oligomerize (9). Localization studies using both immunofluorescence and immuno-electron microscopy placed Vps5p at discrete sites on endosomal membranes with the appearance of nascent budding vesicles, although these experiments were done in a class E *VPS* mutant that has an enlarged PVC (48), because of the difficulties in recognizing the PVC in yeast (9, 29). At that moment, the data supported a model in which the Vps5p / Vps17p dimer functions in retrieval of Vps10p and other Golgi membrane proteins from the PVC back to the late-Golgi. In the absence of Vps5p function, Golgi membrane proteins are not able to cycle from the prevacuole back to the late-Golgi and instead they are directly transported to the vacuole via the default pathway (18, 29). Through the use of chemical crosslinkers, Vps35p and Vps29p were subsequently shown to interact with each other as part of a pentameric complex also including Vps5p, Vps17p and a fifth component, Vps26p (9). For its proposed function in endosome-to-Golgi retrieval, this complex was called “retromer”.

As observed in these early experiments, membrane stripping of retromer with salts partially disassembled retromer, which divided into two subcomplexes. One was composed of the SNXs Vps5p / Vps17p, and formed a stable dimer. The other subcomplex was composed of Vps29p / Vps35p (9). By size-exclusion chromatography, Vps26p eluted predominantly in later fractions, as a monomeric form interacting with Vps35p or with the other subunits in a more transient or more dynamic way (22). Interestingly, this division into subcomplexes mirrors the phenotypic differences between the respective mutant strains. Thus, yeast strains deleted for the *VPS29* or *VPS35* genes are class A mutants, which display morphologically normal vacuoles, whereas deletions of *VPS5* or *VPS17* produce class B mutants, with severely fragmented vacuoles. *VPS26* mutants are class F mutants,

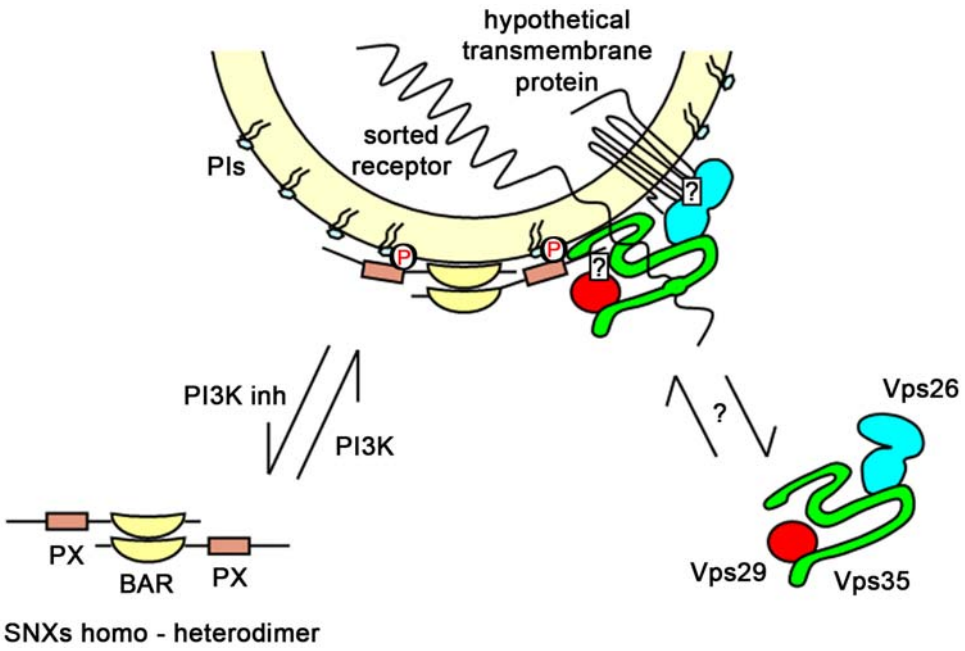

Figure 2. Working model for retromer assembly and functioning. Assembly of retromer involves the cooperation of two subcomplexes. One consists of two sorting nexins (SNXs). In yeast, these are Vps5p and Vps17p. In mammalian cells, one is SNX1, the Vps5p ortholog, which could work as a homodimer or heterodimerize with the closely related SNX2, or with a different SNX, such as SNX5 or SNX6 (see Table 1). SNXs dimerize through their C-terminal coiled-coil regions forming a functional banana shaped Bin/Amphiphysin/Rvs (BAR) domain. Both BAR and Phox homology (PX) domains preferentially target the SNXs dimer to tubular endosome microdomains rich in phosphatidylinositol 3-phosphate (PI 3-P), or other 3-phosphoinositides (PIs), hence the requirement of phosphoinositide 3-kinase (PI3K) for proper membrane association of SNXs and for retromer function, which are affected by PI3K inhibitors (inh). It is thought that the SNXs act as a scaffold for the correct membrane association of the Vps35-Vps26-Vps29 subcomplex, although the latter subcomplex probably assembles and associates independently to the membrane. Vps35p serves as the platform for the formation of this subcomplex, provides cargo specificity and associates directly with cargo proteins (referred to here as 'sorted receptor').Vps26 may undergo an arrestin-like conformational change, allowing its interaction with a hypothetical transmembrane protein. This would promote or stabilize the association of Vps35 to the membrane, thus affecting Vps35 interaction with the receptor's cytosolic tail. Vps29, which may also associate with the N-terminal SNX region, seems to have a catalytic role, either upon Vps35, as proposed in yeast, or upon the receptor itself, as suggested from *in vitro* studies. This activity could serve to promote clustering of the subcomplex or even the receptor's retrieval process itself.

which have an intermediate phenotype, exhibiting a partially fragmented vacuole (48). In spite of this apparent independence of Vps26p within the retromer, additional binding and functional studies made clear that assembly of retromer involves the cooperation of these five subunits in two protein subcomplexes. Based on the first description in yeast, one subcomplex consists of Vps5p and Vps17p, which self-assemble and cooperate for membrane deformation and coat formation (9, 18, 19). The second subcomplex consists of Vps35p, Vps26p and Vps29p. Of these subunits, Vps35p provides cargo specificity and associates directly with cargo proteins (20, 21); Vps29p serves a catalytic role, activating Vps35p and promoting its oligomerization to facilitate concentration of cargo in specific membrane domains (8, 9); and Vps26p promotes or stabilizes the interaction between the SNXs subcomplex and Vps35p, thus bringing together the structural and cargo-selective components of retromer (22). Vps26p may also contribute to Vps35p membrane association, thus affecting the interaction between Vps35p and cargo proteins, such as Vps10p (22) (Figure 2).

Remarkably, the mentioned Vps30p was not found to be part of retromer. However, its role in endosome-to-Golgi retrieval was later characterized. It was shown that Vps30p forms a complex with Vps38p, a protein unique in yeast (49). Vps30p / Vps38p bind to a second complex formed by Vps34p, the only phosphoinositide 3-kinase (PI3K) in yeast (50), and its activator Vps15p. This interaction promotes the production of phosphatidylinositol 3-phosphate (PI 3-P). Studies in both yeast and mammalian cells have shown that membrane PI 3-P is recognized by specific phosphoinositide binding motifs found in proteins acting as downstream effectors in the regulation of membrane trafficking (51-54). In this way, spatially restricted synthesis of PI 3-P could concentrate PI 3-P effectors at the membrane, thereby allowing the effector proteins to interact with specific membrane-bound molecules via other protein-protein interaction domains. As members of the SNX family of proteins, Vps5p and Vps17p contain a conserved phagocyte NADPH oxidase or Phox homology (PX) domain that binds to PI 3-P (55) (see Section 4). PI 3-

P was indeed found required for proper membrane localization of Vps5p / Vps17p (56). As a requisite for efficient sorting function, this interaction likely provides the site for proper membrane location of the Vps35p-Vps26p-Vps29p subcomplex (19, 56). However, a role of PI 3-P or other 3-phosphoinositides on SNXs recruitment remains a matter of controversy (57; reviewed in 58-60), because deletion of Vps34p, or deletion of the PX domain of either Vps5p or Vps17p, only caused a modest effect on Vps5p membrane association (19).

The current view on 3-phosphoinositides requirement for retromer function mainly derives from studies in higher eukaryotes (Figure 2). Thus, we have observed redistribution to cytosol of SNXs 1 and 2 (the mammalian Vps5p orthologs) upon PI3K inhibition, leaving the subunits of the Vps35-Vps26-Vps29 subcomplex largely unaffected (61). This effect, however, seems to be more or less striking depending on the PI3K inhibitor used, and while even Vps26 disassociated in some cases (12, 62), SNX1 appeared unaffected in others, with SNX2 concentrating on enlarged early endosomes formed by effect of the inhibitor (63). Nevertheless, colocalization of the two retromer subcomplexes (61) and between each subcomplex and cargo (61, 64) greatly decreases upon PI3K inhibition. Therefore, more than simply a redistribution of SNXs to the cytosol, it seems clear that the primary role of PI3K on SNXs is to determine their proper membrane location, which is affected by depletion of PI 3-P from membranes. Under these circumstances, the Vps35-Vps26-Vps29 subcomplex, although efficiently recruited to the membrane, becomes unable to cluster cargo for its efficient sorting from appropriate locations that must be defined by the SNXs subcomplex (61).

Recent findings provide evidence that assembly of each retromer subcomplex is regulated independently. Thus, SNX1, or the closely related SNX2, have not been found in co-immunoprecipitated Vps35-Vps26-Vps29 subcomplexes (14, 61, 62). In agreement with these data, SNX1 was not detected in pull-down assays of mammalian Vps29 (24). Moreover, antisense depletion of both SNX1 and SNX2 did not prevent targeting of Vps26 to membranes (65), although it did in other cases (62), perhaps depending on the endogenous levels of SNXs. In a reciprocal way, knocking down Vps35, Vps26 or Vps29 (12-14, 62, 66) depletes the whole Vps35-Vps26-Vps29 subcomplex but it does not affect the levels of SNX1 or SNX2. In spite of these negative data, it has been demonstrated, by immunoprecipitations performed with exogenously expressed proteins, and by yeast-two hybrid assays suitable for detecting weak interactions, that SNX1 (23, 62, 63) as well as SNX2 (62) physically interact with the Vps35-Vps26-Vps29 subcomplex. This suggests that the association between these subcomplexes is transient and dynamic. Alternatively, it is possible that different SNX family members associate more strongly than SNX1 / SNX2 with the Vps35-Vps26-Vps29 subcomplex in some systems. In this regard, a very recent work demonstrates that SNX5 and SNX6 may constitute functional equivalents of the yeast Vps17p and therefore they emerge as potential components of the mammalian retromer (67) (see Section 4).

From the current data in yeast and in mammalian cells, a general model of possible retromer functioning can be envisaged (Figure 2). By this model, SNXs are preferentially targeted, via their Bin/Amphiphysin/Rvs (BAR) and PX domains, to tubular endosome microdomains rich in 3-phosphoinositides (57, 68). SNXs would thus act as a scaffold for association of the Vps35-Vps26-Vps29 subcomplex, which then selects cargo for sorting based on the geometric efficiency of these tubular elements (68). Correct membrane association of the Vps35-Vps26-Vps29 subcomplex requires of SNXs (61, 62), which does not exclude that interactions between the two subcomplexes already take place in the cytoplasm (23). Vps29 appears important to mediate the interaction between these subcomplexes, at least in the yeast retromer (22). Vps26 structure reveals an arrestin fold (26). Similarly as arrestins binding to G-protein coupled receptors (GPCR) to regulate receptor endocytosis and signaling (69), Vps26 may undergo a conformational change, allowing its interaction with a putative multipass transmembrane protein. Thus, Vps26 appears involved in targeting Vps35 to the membrane (26). Once at the membrane, Vps35 associates directly with cargo (12, 21). Vps29 structure has also been revealed and it has a phosphoesterase fold with no apparent enzymatic activity (24, 25). Recent *in vitro* data suggest, however, that receptor dephosphorylation by Vps29 may be involved in the endosome-to-Golgi retrieval process (70).

The similarity in the sequences and interactions among orthologs of retromer subunits quickly suggested that retromer function was conserved in higher eukaryotes (23, 71-73). Indeed, it was found that retromer mediates endosome-to-Golgi sorting of the mammalian CI-MPR (12, 13, 15, 16). The study of retromer role in various model organisms has shown, however, that retromer plays additional roles in protein sorting along the endocytic pathway. These include the following. First, a retromer-like complex in the enteric protozoan parasite *Entamoeba histolytica*, composed of Vps35, Vps26 and Vps29, regulates transport to phagosomes of cysteine proteases by interaction with the small GTPase Rab7A and probably an as-yet-unidentified protease receptor(s) (74). It is important to point out that this microorganism lacks the CI-MPR, as well as the tail-interacting protein of 47 kD (TIP47) and Rab9 (74, 75), both of which have also been involved in late-endosomal retrieval of the CI-MPR (76). Secondly, Vps35, as part of an analogous retromer complex in plants, interacts with the vacuolar sorting receptor (VSR) (64), a molecule similar to Vps10p and the mammalian Vps10-domain protein sortilin (10, 77). While Vps35 and VSR colocalized on multivesicular bodies (MVB), functional evidence for a role of the plant retromer in VSR sorting was not provided in this study (64). Nevertheless, *Arabidopsis thaliana* Vps29 was found to function in the VSR retrograde transport needed for sorting of storage protein precursors during seed maturation (78). In addition, another report demonstrates the role of *A. thaliana* SNX1 in endosomal trafficking of the phytohormone auxin, implicating retromer in growth and developmental processes in plants (79). Third, we have shown that the Vps35-Vps26-Vps29 subcomplex associates with the pIgR

in a post-endocytic step and promotes transcytosis of pIgR and its ligand pIgA in polarized epithelial cells (14). Fourth, Vps26B, a Vps26 paralog, was shown to interact with Vps35 and integrate in the retromer complex (80). Unlike the original Vps26 (referred to as Vps26A in this report), Vps26B does not associate with endosomes, and instead is targeted to the plasma membrane, becoming concentrated at actin-rich lamellipodia, which intriguingly also contain some Vps26A and Vps35 (80). The possibility that this cell surface pool of retromer is playing a role in cell migration and/or adhesion clearly extends retromer functions beyond its originally proposed action in the endosomal system. Finally, a role of retromer has been proposed for regulating endosome-to-Golgi traffic of proteins involved in human neurological diseases. One is the β-site amyloid precursor protein (APP) cleaving enzyme (BACE) (81, 82), which hydrolyzes β-APP and leads to production of the neurotoxic amyloid-β (Aβ) peptide and progression of Alzheimer disease (AD) (83). The other is the Batten disease (BD) related protein Btn2p, a yeast protein upregulated upon deletion of Btn1p, ortholog of human ceroid lipofuscinosis neuronal-3 (*CLN3*), a gene mutated in a lysosomal storage disease (LSD) known as BD or juvenile neuronal ceroid lipofuscinosis (JNCL) (84, 85). However, the functional implication of retromer in neurodegeneration remains to be confirmed (see Section 6).

Most of the reports discussed above were based on data from cell culture systems. Data from genetically modified mice (30-33), and more recent publications using transgenic and deletion mutant strains of *C. elegans* (34, 35), provide strong evidence that retromer performs an essential role during embryonic development that is independent of MPR trafficking (33). These aspects will be discussed in detail in Section 5.

4. THE LARGE FAMILY OF SORTING NEXIN PROTEINS – REGULATION OF TRAFFICKING AND SIGNALING

Receptor sorting at the endosomal compartment is mediated by interactions of the receptor's cytosolic domain with peripheral membrane proteins or protein complexes. As we have discussed in the previous section, a remarkable example of these sorting events is performed by the retromer complex. Given the many potential trafficking itineraries through the complex endosomal pathway (16, 86), it has been proposed that receptor's fate is governed by its ability to interact with, or to avoid, a number of these endosomal sorting complexes. In an attempt to understand further endosomal retrieval of hydrolases receptors in higher eukaryotes, adaptor complexes and adaptor-like proteins participating in MPR trafficking were identified and studied. Initially, the findings pointed to important mechanistic differences in endosome-to-Golgi transport between yeast and mammals (47, 76). Alternatively, they suggested the existence of evolutionarily conserved components of the MPR retrieval machinery that remained to be identified. Soon after, it was demonstrated that retromer function in endosome retrieval of hydrolase receptors is well-conserved from yeast to man (12, 13, 15, 16). Of the five Vps protein subunits composing the retromer complex, mammalian orthologs for the yeast Vps5p, Vps26p, Vps29p and Vps35p have been identified (8, 18, 23, 27-29). Except in fungi, no ortholog of the SNX protein Vps17p has been found in higher eukaryotes (23, 87). Thus, both SNX1 and SNX2, as Vps5p orthologs, can assemble as homodimers or heterodimers (62), although it is possible that a different SNX heterodimerizes with SNX1 or SNX2 in the retromer (23, 88, 89), as proposed recently for SNX5 and SNX6 (67).

The screening for protein interactions has proven an excellent way of identifying components with a functional relevance. Thus, the numerous screenings performed to identify proteins associated with the cytosolic tail of endosomal receptors, or with other proteins involved in controlling membrane traffic and differentiation, has led to an increasing understanding, and at the same time complication, of the role played by SNXs in higher eukaryotes (90-105). In view of their involvement in protein sorting events, the SNX family of proteins has attracted considerable attention as potential candidates for determining protein itinerary in different cell types and developmental conditions. It is important to remark that their role in controlling protein export from endosomes potentially contributes to modulate signaling, perhaps after activated receptors have been internalized (59, 106-108). The essential role of SNXs in development has been revealed by gene-deletion experiments in mice, demonstrating the genetic interaction of SNXs 1 and 2 with retromer (33). The implication of SNXs in diverse trafficking pathways has been further illustrated by the recent description of the SNX13 knockout phenotype in mouse, which is also embryonic lethal (109).

4.1. Definition of sorting nexins

The reader is referred to references 58-60 for comprehensive reviews on this large protein family, which currently includes 10 members in yeast (59) and 30 in humans (110), plus other 17 PX domain-containing human proteins of related structure albeit diverse functions (60). The SNXs are defined in part by the presence of a PX domain belonging to a subgroup of the PX domain superfamily and with at least 50 % similarity to the PX domain of SNX1 (111). The SNXs are oligomeric proteins found distributed between membranes and cytosol, and the different family members contain a variety of protein-protein and protein-lipid interaction domains in addition to their PX domain. One is the BAR domain, present in over one third of the SNXs. By this definition, it appears that several members included in the family are not involved in cargo-sorting, and instead they have a role in oxidative processes, cytoskeleton arrangement or protein translocation. Therefore, they are not expected to be related to any process controlled by retromer, or even to any other membrane trafficking associated mechanism, and thus some proteins may have been erroneously included in the SNX group. Thus, it has been suggested that the criteria for defining a protein as a SNX require revision and that only those with a BAR domain and a few others should actually belong to this group (59).

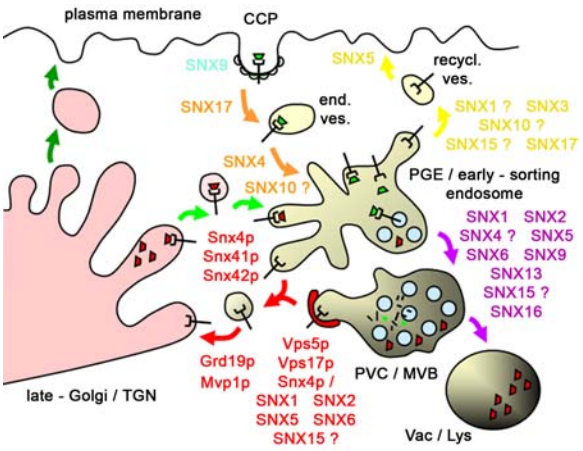

Figure 3. Functions of sorting nexins in yeast and in mammalian cells. The image shows major trafficking pathways where SNX family members have been involved in yeast or in a nonpolarized mammalian cell. Newly synthesized proteins are first sorted in the late-Golgi / *trans*-Golgi network (TGN) for delivery to the plasma membrane (*dark green arrows*). Proteins, such as a transmembrane receptor bound to its ligand (*green trapezoid*), can be endocytosed from the plasma membrane (*orange arrows*) via clathrin coated pits (CCP) and become incorporated into endocytic vesicles (end. ves.). From the post-Golgi endosome (PGE) / early-sorting endosome, a possible pathway involves ligand disassociation and receptor recycling back to the cell surface (*yellow arrows*) by recycling vesicles or tubules (recycl. ves.). Alternatively, endocytosed receptors and / or ligands can be degraded in the vacuole (Vac) / lysosomes (Lys; *purple arrows*) by the action of hydrolases (*red trapezoids*). In their precursor form, the hydrolases are also delivered by specific receptors to the endosomal system (*light green arrows*). These receptors are then recycled back to the Golgi for new rounds of enzyme delivery (*red arrows*), either from the PGE / early-sorting endosome or from the prevacuolar compartment (PVC) / late endosome-multivesicular body (MVB). This pathway is also followed by other integral membrane proteins that are retrieved to the Golgi. According to studies in yeast, the group of Snx4p/41p/42p (SNX4 orthologs) mediates retrieval from the PGE independently of retromer, while retromer and associated SNXs mediate retrieval from the PVC; Snx4p can also associate with retromer at the PVC. It is not known whether this applies to mammalian cells, where SNX1 and SNX2 are the Vps5p orthologs, while there is no ortholog for Vps17p, and SNX5, SNX6 or another SNX work in place of Vps17p in retromer action. Grd19p and Mvp1p are, respectively, the SNX3 and SNX8 orthologs and their possible integration in retromer for endosome-to-Golgi retrograde transport has not been reported. The SNXs color code matches that of the pathway where they have been implicated. A question mark is placed where the evidence is not solid.

The PX domain acts as phosphoinositide-binding motif that contributes to the localization of SNXs at membrane domains enriched in 3-phosphoinositides, such as PI 3-P (57). Since PI 3-P is found throughout membranes of the endocytic-lysosomal pathway (51), it seems plausible that other components, or other sequences apart from the PX domain, determine the precise localization of individual SNXs. Interestingly, it was observed that the PX domains of certain SNXs do not share some key residues, i.e. Arg58 and Arg105, that are essential for PI 3-P binding in $p40^{Phox}$, the prototype of PX domain proteins (58), and that are conserved in SNX1 (57). One example is SNX5, which binds to other phosphoinositide lipids apart from PI 3-P (112, 113). The preferential binding to other phosphoinositide lipids, and hence to specific membrane subdomains, may help to explain the various physiological roles of the SNX family members. In addition, membrane association of SNXs may be further strengthened by stabilization from protein-protein interactions through adjacent domains and by the BAR domain itself. The BAR domain, located at the C-terminus in some SNXs, is a dimerization and membrane binding module. As sensor of membrane curvature, it provides an explanation for how membrane geometry can regulate protein localization (114). The localization of SNXs to tubular elements of the sorting endosome containing PI 3-P is consistent with their BAR domain functioning as a binding-module and curvature-sensor. Thus, the SNXs would function as a coincidence detector in a way that, although several proteins associate with endosomes through PI 3-P, the SNXs becomes preferentially enriched in endosomal microdomains defined by high membrane curvature and the presence of PI 3-P or other 3-phosphoinositides (68). The ability to sense membrane curvature may contribute to retrieval of the CI-MPR or other transmembrane proteins into elongated membrane and budding vesicles. This hypothesis of coincidence detection can probably be applied to any BAR domain containing SNX, including SNX9 in its cooperation with dynamin for clathrin-coated vesicle budding at the plasma membrane (105, 115). Thus, while the BAR domain targets SNX homo or heterodimers to the membranes, the PX domain is necessary for proper membrane / endosomal localization (116). How both the PX and BAR domains are coordinated for SNX membrane targeting is not yet clear.

4.2. The functions of sorting nexins

In early studies, it was found that none of the members of the growing SNX family contained putative signal peptides or transmembrane domains (111), although an N-terminal short region of hydrophobic residues now suggests the presence of a transmembrane domain in three SNXs (60). It was also found that many SNXs have 1-3 stretches of 21 or more aminoacids within their C-terminal region that can form coiled-coil structures (111), later predicted to form BAR domains (65). Coiled-coil domains, as potential sites for protein-protein interactions, have been established as crucial for the function of other membrane transport proteins, such as syntaxins (117). Given their strong predisposition to form coiled-coil mediated protein complexes, SNXs emerged as possible candidates to control a number of membrane trafficking pathways or events. The findings showing that they self-associate forming homodimers or heterodimers (23, 118), and their differential interaction with transmembrane receptors in mammalian cells (88, 90), further increased the interest of these proteins as putative regulators of protein transport performing specific sorting roles depending on cell type and development stage (Figure 3; Table 1).

Table 1. Functions of sorting nexins in yeast and in mammalian cells

Sorting nexin	Pathway	References
SNX1	Endosome-to-Golgi retrieval of CI-MPR (retromer)	23, 62, 67, 68
	Downregulation of EGFR and other Tyr kinase receptors	63, 88, 90, 98, 134
	Downregulation of the GPCR PAR-1 (independent of retromer)	66, 120
	Transferrin receptor pathway	88
	Macropinosome maturation	228
SNX2	Endosome-to-Golgi retrieval of CI-MPR (retromer)	62, 65
	Downregulation of EGFR and other Tyr kinase receptors	63, 88
Vps5p	Endosome-to-Golgi retrieval of Vps10p, Kex2p, DPAP A and Pep12p at the PVC (retromer)	8, 9, 18, 19, 29, 132
Vps17p	Endosome-to-Golgi retrieval of Vps10p (retromer)	9, 19
SNX3	Endosome recycling	121, 141
Grd19p	Endosome-to-Golgi retrieval of Kex2p, DPAP A and Pep12p, but not Vps10p, at the PVC (retromer?)	132, 229
SNX4	Endosome vesicles fate	97
Snx4p/41p/42p	Retrieval of Snc1p at the PGE (independent of retromer)	132
	Cytoplasm-to-vacuole pathway at pre-autophagosomes	133
	Endosome-to-Golgi retrieval of Yif1p, but not Vps10p, at the PVC (Snx4p / retromer)	85
SNX5	Endosome-to-Golgi retrieval of CI-MPR (retromer)	67
	EGFR downregulation	113
	EGFR signaling at the plasma membrane	112
	Macropinosome maturation	228
SNX6	Endosome-to-Golgi retrieval of CI-MPR (retromer)	67
	Trafficking of TGF-β family of receptor Ser-Thr kinases	119
Mvp1p	Endosome-to-Golgi retrieval of Vps10p and Kex2p at the PVC (retromer?)	230, 231
	Endosome-to-plasma membrane recycling of the GSE complex at the PVC (independent of retromer)?	17, 232, 233
SNX9	EGFR downregulation	95
	Regulation of dynamin assembly and clathrin-mediated endocytosis	105, 115
	Trafficking and signaling of insulin receptors	234
SNX10	Endosome homeostasis	142
SNX13	EGFR downregulation	122, 123
SNX15	Association to retromer?	143
	Protein sorting to the MVB by interaction with the ESCRT-III complex?	235
SNX16	EGFR downregulation	124, 125
	PI 3-P signaling effector in endosome-to-cytosol export of viral nucleocapsids	126
SNX17	Endocytosis and / or recycling of LDLR family members (LDLR, VLDLR, ApoER2 and LRP)	96, 100, 129
	Endocytosis and degradative pathway of P-selectin	130, 131

Abbreviations: ApoER2: ApoE receptor 2; CI-MPR: cation-independent mannose 6-phosphate receptor; DPAP A: dipeptidyl aminopeptidase A; EGFR: EGF receptor; ESCRT: endosomal sorting complex required for transport; GPCR: G-protein coupled receptor; Grd: Golgi retention defective; GSE: GTPase-containing complex for Gap1p (general amino-acid permease) sorting in the endosomes; Kex2p: killer expression-2; LDLR: LDL receptor; LRP: LDLR-related protein; MVB: multivesicular body; Mvp1p: Vps1p-interacting protein; PAR-1: protease activated receptor-1; Pep12p: syntaxin, formerly Vps6p; PGE: post-Golgi endosome; PI 3-P: phosphatidylinositol 3-phosphate; PVC: prevacuolar compartment; Snc1p: suppressor of the full allele of CAD-1; SNX: sorting nexin; TGF-β: transforming growth factor-β; VLDLR: VLDL receptor; Vps: Vacuolar protein sorting; Yif1p: Yip1p (yeast Golgi integral membrane protein) -interacting factor

Thus far, the retromer subunit which identification in mammals has led to a deeper investigation is SNX1, the Vps5p ortholog (18, 29). SNX1 was first shown to interact with the cytosolic domain of the EGF receptor (EGFR) (90). Overexpression of SNX1 increased the receptor's turnover, leading to the suggestion that SNX1 is required for endosome-to-lysosome transport and downregulation of the EGFR and probably other receptor Tyr kinases (90). Further work in the field extended this intriguing role of SNX1 throughout the SNX family of proteins and it was proposed that they might modulate signals sent by receptors, perhaps after they have been internalized, which suggests that SNXs could act as truly signaling platforms (59). In this regard, SNX1, SNX1A (a presumed splice variant of SNX1), SNX2 and, to a lesser extent, SNX4 were reported to interact with the receptor Tyr kinases for EGF, the platelet-derived growth factor and insulin (88). Another example is SNX6, which interacts with the transforming growth factor-β (TGF-β) family of receptor Ser-Thr kinases and interferes with their signaling pathways (119). Moreover, SNX1 also associates with the protease activated receptor-1 (PAR-1), a GPCR for the coagulant protease thrombin (120). Here, overexpression of the C-terminal domain of SNX1, which includes its coiled-coil domains, specifically inhibited agonist-induced degradation of PAR-1, thus involving SNX1 in PAR-1 downregulation (120). Other SNXs involved in EGFR downregulation include SNX3 (121), SNX5 (113), SNX9 (95), SNX13 (122, 123), and SNX16 (124, 125). The whole picture becomes complicated when observing at the multiple effects described on EGFR trafficking upon overexpression of wild-type or mutant forms of these SNXs. For instance, in one study SNX5 overexpression inhibited EGFR degradation (113), while in another report it ensured that the receptor becomes quickly available at the cell surface (112). EGF degradation was also inhibited in cells overexpressing SNX3 (121), and ligand-dependent degradation of EGFR was delayed in transfected SNX13 cells (122). On the contrary, EGFR degradation was accelerated in SNX16 overexpressing cells (125), although only the SNX16 coiled-coil deletion mutant, and not the wild-type form, had such effect, as reported by a different group (124). Finally, while overexpression of SNX9 alone had no effect on EGFR degradation, EGFR degradation was promoted upon co-expression of a cooperative SNX9 partner, the activated Cdc42-associated kinase-2 (95). In

summary, while it is clear that SNX1 and other SNXs are involved in endosomal sorting of signaling receptors, how this process takes place remains to be elucidated. A possible mechanism could be based on SNXs acting as 3-phosphoinositide effectors in processes of endosome dynamics. This has been shown for SNX16, which appears required for back fusion of internal vesicles in late endosomes, a process utilized by certain viral nucleocapsids to move from endosomes to cytosol (126, 127).

To the above discussion, one could add the studies on the well-characterized SNX17, to which a number of interacting partners and roles have been ascribed. For such promiscuity are probably responsible a truncated band 4.1-ezrin-radixin-moesin (FERM) domain, a feature unique in the SNX family (except for the uncharacterized SNX31), and a C-terminus lacking the BAR or coiled-coil domains, which maintain the protein in oligomeric form (60, 128). SNX17 roles include binding and modulation of endocytosis and/or recycling of LDL receptor (LDLR) family members, such as the receptors for LDL and VLDL, the ApoE receptor 2 (ApoER2) and the LDLR-related protein (LRP) (96, 100, 129). SNX17 also interacts with the endothelial cell receptor P-selectin (responsible of leukocyte recruitment at the sites of inflammation) to regulate its internalization and degradation (130, 131). Finally, SNX17 has been shown to associate with krev1 interaction trapped 1, a protein involved in integrin signaling during cell adhesion and pathogenesis of an inherited vascular disorder known as cerebral cavernous malformation (128).

Studies in yeast, taking advantage of the relatively easy genetic manipulation of this simple eukaryote, have provided a simpler picture of the SNXs role in membrane trafficking. In general, SNXs in yeast participate in the recycling of certain membrane proteins, preventing them from reaching the vacuole. While some SNXs act, in concert with retromer, in the PVC, others operate independently in the so-called post-Golgi endosome (PGE) (132). A slight complexity worth mentioning here is the presence of the Snx4p group members (Snx4p/41p/42p) on specific perivacuolar structures called pre-autophagosomes, organelles distinct albeit probably derived from endosomes (132); in pre-autophagosomes, the Snx4p group plays a role in the cytosol-to-vacuole pathway, induced under nutrient starvation (133). In addition, a recent study found yeast Snx4p associated with retromer at the PVC (85), although previous evidence indicated that it functions in a retromer independent way at the PGE (132).

In contrast to the apparent simplicity found in the yeast model, mammalian SNXs have been suggested to function in internalization, pro-degradative sorting, endosomal recycling, or simply in endosomal sorting (58-60), as discussed above (Figure 3; Table 1). Since most of the evidence for SNXs function in mammalian cells derives from overexpression experiments, and antisense approaches have often given contradictory results, some of the roles proposed for members of this large family remain under debate. To illustrate this point, it is worth mentioning that antisense knockdown of SNX1 had no effect on EGFR degradation (63, 68) or on transferrin receptor trafficking (68). This contrasts with findings from previous studies utilizing overexpression approaches in which SNX1 (90, 134) or other SNXs (95, 113, 121, 122, 124, 125) were implicated in EGFR downregulation. According to others, however, overexpression of SNX1, SNX2, SNX3, or SNX4 did not increase the turnover of EGFR or insulin receptor (88). These contradictory results may be due to the disruption of a protein complex by excess amounts of one of its subunits upon overexpression, or to the titration of other interacting proteins by the overproduced component. Therefore, they may reflect the common difficulty of finding out whether overexpression has a positive or a dominant-negative effect. These discrepancies have lead to the suggestion that the apparent differences in SNXs functions between yeast and mammalian cells could be accounted for by differences in experimental approaches rather than by the notion that these proteins really display more varied properties in higher eukaryotes (135).

Does the interaction with SNXs determine receptor segregation into endosome tubules? It was once proposed that recycling is the default pathway for internalized membrane proteins. According to this idea, endosomal sorting involves repeated fusion of endocytosed vesicles with the endosomal compartment, which simultaneously exports components for recycling and accumulates material for degradation. This process leads to a greater accumulation in the compartment's lumen of ligands directed to the lysosome than receptor-ligand complexes destined for recycling, which remain at the membrane and, given the larger surface-to-volume ratio in endosome tubules, they accumulate relatively more in these. Such iterative fractionation process would provide a simple and efficient mechanism for recycling proteins and lipids back to the cell surface. Therefore, it was suggested that membrane proteins require specific signals to be routed to lysosomes, rather than to recycle (136). Indeed, biochemical analysis of various endocytic receptors has shown that these were nonselectively incorporated into endosome tubules for recycling or transcytosis, although receptor segregation was evident in the MVB *en route* to lysosomes (137). In this nonselective model, the geometry of the endocytic compartment, as well as its fusion and budding properties, must have a major influence on controlling efficiencies and rates of sorting membrane components from the luminal fluid phase (138). This scheme represented an alternative to the model of active segregation of receptors into endosome tubules, from which receptors could either be included or excluded (139). Here, alterations in the phospholipid composition, or changes in the physical properties of membranes caused by lipid modifications, membrane associated proteins or the cytoskeleton, were mechanisms proposed to explain such receptor segregation (139). Thus, it was later suggested that several proteins containing lipid-binding domains might function in organizing functional regions of endosome membranes; these include proteins containing phospholipid-binding domains, such as the FYVE motif, or components of cholesterol and sphingolipid-rich raft

microdomains, but also the SNXs, acting through their PX domains (140). The observations that overexpression of SNX3 (121, 141), SNX10 (142) or SNX15 (143) alters endosome morphology and perturbs the endocytic pathway in general is clearly suggestive that members of this family could perform both specific and general roles along membrane networks. Thus, a consensual feature of SNXs function seems to be the regulation of membrane trafficking for endosomal protein export (Figure 3; Table 1).

4.3. Sorting nexins and retromer

SNX1, and the closely related SNX2, are the mammalian orthologs of yeast Vsp5p. In the absence of a Vps17p ortholog, it was initially proposed that SNX2 or another SNX plays the role of Vps17p in higher eukaryotes (23). Given the high sequence similarities among several of the SNX members, in particular in their common PX domains (58-60), and their somehow analogous role in controlling receptors sorting, it is plausible that some other SNX interacts with the Vps35-Vps26-Vps29 subcomplex to perform specific functions in mammalian cells. Indeed, a recent study provides evidence that SNX5 and SNX6 are potential retromer subunits. In a loss-of-function screen, each one of the 30 SNX members was antisense targeted. Affected retromer function was determined by looking at the relative percentage of cells with CI-MPR dispersed in peripheral early endosomes, as opposite to TGN localized. Suppression of either SNX5 or SNX6 gave the strongest phenotype, far more dramatic than that observed by SNX1 depletion and, interestingly, SNX2 depletion did not produce significant changes (67). Further morphological and biochemical analysis showed colocalization of both SNX5 and SNX6 with SNX1 on early endosomes, but only SNX6 physically interacted with SNX1 (67), in apparent contrast to the association of SNX5 with SNX1 reported by others (113). In addition, because depletion of SNX5, but not of SNX6 or SNX1, induced fragmentation of the Golgi complex, the authors proposed that the alteration in CI-MPR distribution seen upon SNX5 depletion could be a consequence of a more general defect in endosome and Golgi trafficking (67).

The study discussed above did not provide data on interaction between SNX5 or SNX6 and the Vps35-Vps26-Vps29 subcomplex, as seen for SNX1 (23, 62, 63) as well as for SNX2 (62). However, it is noteworthy to point out that a link between retromer and SNXs 1 and 2 is not evident in all studies. Various reports have in fact suggested an independent regulation of retromer and SNX1 or SNX2. In analogy to the link between SNX1 and the EGFR (90), SNX1 also plays a role in agonist-induced PAR1 degradation independently of the Vps35-Vps26-Vps29 subcomplex (66). A different localization of SNX1 and SNX2 has also been reported, with SNX2 localizing mostly at early endosomes, while SNX1 was found associated to small vesicles distributed throughout the cytoplasm and devoid of the early endosome marker EEA1 (63). In contrast, another study found extensive colocalization of SNX1 and SNX2 on tubular elements of the early endosomes (65). Contrary to SNX1 (68), SNX2 was found not essential to mediate endosome-to-TGN transport of the CI-MPR (65), which again suggested that a different SNX heterodimerizes with SNX1 in mammalian cells (89, 143). Nonetheless, a partial colocalization of SNX2 with subunits of the Vps35-Vps26-Vps29 subcomplex appears clear in various studies (12, 61, 62, 65). Moreover, antisense depletion of both SNX1 and SNX2 was needed to affect markedly levels and intracellular distribution of the CI-MPR (62). Further evidence showing the relevance of SNX2 in retromer comes from genetic studies in mice. Mice lacking SNX1 or SNX2 are viable and fertile, whereas embryos deficient in both proteins arrest at midgestation. The observed phenotypes indicate that these two proteins act redundantly, but they perform a necessary function in mouse development (32). Interestingly, the phenotype of *Snx1*$^{-/-}$ *Snx2*$^{-/-}$ embryos is very similar to that of embryos lacking Vps26, a protein that in the initial studies in mice development was named Hβ58 (30, 31). These genetic experiments in mice suggest that SNX1 / SNX2 and Vps26 function in the same pathway. Considering the high similarity between these SNXs, the presence of both proteins may provide an evolutionary advantage in mammals. Recent data also indicate that SNX2 is surprisingly more critical than SNX1 for retromer function during development, although SNX1 participates in some retromer activity (33). Intriguingly, the localization and stability of CI-MPR was unaltered in mouse embryonic fibroblasts (MEF) (33), contrary to what others found in SNX1 or Vps26-antisense depleted HeLa cells (12, 13, 68) or in undifferentiated embryonic stem (ES) cells depleted for Vps26 (13). Finally, *Snx13*$^{-/-}$ embryos have recently been generated. While their embryonic lethal phenotype overall resembles that of the *Snx1*$^{-/-}$ *Snx2*$^{-/-}$ embryos, differences in organelle ultrastructure and in the distribution of endosome-lysosomal markers were seen in cells of extraembryonic tissue. This study supports the idea that SNXs play different roles in membrane traffic (109), without excluding their interaction with retromer. (These results will we discussed in more detail in Section 5.)

4.4. Role of sorting nexins in signaling

Retromer retrieves the CI-MPR from endosomes back to the Golgi in mammalian cells (12, 13, 15, 16). Retromer also associates with pIgR (14), which transports pIgA across polarized epithelial cells by a mechanism coupled to a signaling pathway (144). Manipulation of Vps35 levels, along with morphological evidence, showed that retromer plays a role in a post-endocytic compartment, promoting transcytosis of pIgR and its ligand IgA, thus preventing pIgR-pIgA degradation (14). The accumulated evidence sustains that retromer retrieves receptors from an endosomal compartment, thus preventing their degradation. This “rescue” mechanism carried out by retromer seems a very efficient system by which certain receptors could prevent their mis-sorting to lysosomes and degradation. Several reports discussed above implicate SNXs in controlling traffic of receptors involved in cell proliferation processes. Hyperactive receptor signaling leads to deregulation of cell growth control and to alteration of developmental programs, with the consequent initiation of malignancy. Their intracellular pathways, towards degradation or recycling, therefore play a key role in

processes of cell growth control, as proliferation or apoptosis, and thus in important human diseases, including cancer (see 107, 108, 145 for reviews). Considering the interactions described, retromer and SNXs might regulate the cell response to signaling factors and thus become implicated in mechanisms of cell proliferation.

Taking the picture as a whole, the contribution of retromer and SNXs to the control of protein trafficking should not be considered separately. By controlling receptor sorting, retromer and SNXs necessarily play an important role in maintaining the cellular equilibrium. Therefore, one could speculate that a high activity of retromer / SNXs contributes towards blocking downregulation, and therefore degradation, of signaling receptors in situations where the cell must keep a proliferative state. This presumably takes place, in a generalized way, in early stages of the embryogenesis. Likewise, excessively active retromer / SNXs may indirectly induce the cell to uncontrolled proliferation by preventing that proliferative signals are turned off. The overall effect could largely be analogous to the overgrowth and tumors observed in animal models and in humans by mutations in class E *VPS* orthologs (146, 147). In this latter case, however, the impairment is instead in the machinery required for receptor downregulation, i.e., in components of the endosomal sorting complex required for transport (ESCRT) machinery. With these considerations, it would be interesting to test the possibility that molecules interconnecting retromer / SNXs with signaling pathways control mechanisms of cellular proliferation. Hypothetically, this link might lead to the finding of potential therapeutic targets towards which drugs could be directed to treat diseases affecting cell proliferation or development.

5. ROLE OF RETROMER DURING DEVELOPMENT

The phenotype analysis of null mutations, as well as knockdowns obtained by antisense approaches, has established the key role of various Vps proteins during embryogenesis in mice (148-150), *C. elegans* (151), and *Drosophila* (147, 152). These studies emphasize the importance of the lysosomal pathways in development of higher eukaryotes. The first studies of mouse orthologs of retromer subunits supported that the essential components of this pathway are highly conserved in all eukaryotic cells (28, 30, 31). Undoubtedly, these early studies also emphasized the importance of controlling membrane trafficking in particular cell lineages during initial development stages. This section will cover the implications of recent work in genetically modified mice and in the worm *C. elegans* establishing an essential function of retromer during embryogenesis apparently unrelated to its conserved role in CI-MPR sorting.

5.1. Retromer's role in mouse embryogenesis

5.1.1. Vps26

The first indication of an ortholog of a yeast retromer subunit in higher eukaryotes comes from a study of the phenotype caused by an insertional mutation affecting early development of the mouse embryo (30, 153). This mutation affected the chromosome 10 at the *Hβ58* locus, which gave name to the gene, later shown to be equivalent to the yeast *PEP8* gene (27) and now also called *VPS26* (23). We will use throughout the following paragraphs the Vps nomenclature derived from research in yeast, except when specific references either to the mouse *Hβ58* locus or to the yeast *PEP8* gene are required.

Vps26$^{-/-}$ embryos developed normally through the late egg cylinder stage (embryonic day E6.5), but by the gastrulation stage (E7-7.5), they began to display a growth retardation of the embryonic ectoderm, the tissue giving rise to the epidermis, associated epidermal structures, the brain and the nervous system. These embryos survived for ~ two more days, but never progressed, in size or complexity, further than a normal E8.5 embryo (30). Interestingly, it was possible to derive permanent ES cell lines with normal growth and differentiation from *Vps26*$^{-/-}$ blastocysts, which indicates that Vps26 is not required for cell proliferation *in vitro* (154). Considering also the extensive growth and differentiation seen *in vivo* in homozygous mutant embryos, these authors concluded that Vps26 does not perform a housekeeping role but rather serves in a more specialized function (30). The widespread expression of Vps26 mRNA throughout various development stages of the wild-type embryo and in many differentiated tissues of fetal and adult mice suggested that Vps26 does not function solely during early embryogenesis. Instead, Vps26 appears to be redundant with other gene products (from zygotic or maternal origin) at early stages, but unique and essential after the egg cylinder stage (31). Very high mRNA levels in the chorioallantoic placenta (31), along with a failure of the mutant mouse to develop this organ (30), suggested a role of Vps26 in placental formation, which was later pursued by other researchers. Thus, it was proposed that Vps26 (or at least a protein with similar sequence to the *Hβ58* product) may function in both maternal and fetal tissues during placental formation and maturation in pregnant rats (155). Vps26 distribution in erythroid cells of the developing fetal vasculature suggested a role in hematopoietic development, which is a key process to bring the maternal and fetal systems into close proximity and ensure embryo survival. However, the strong nuclear staining observed in those analysis questions the specificity of the antibody used (155). A similar morphological study performed in a viviparous reptile confirmed the role of Vps26 in chorioallantoic placental formation and development (156). Interestingly, strong staining was found here at the subapical region in some epithelial cells of the uterine epithelium (156), but how the conclusions from these studies in animal models could fit with the data on Vps26 and retromer gathered from research in yeast was not clear.

At the time when the first observations in Vps26 knockout mice were made, the *VPS* gene products were being characterized in yeast (38, 48, 157) and their corresponding homologs were only beginning to be identified in higher eukaryotes (158), although research on mutant strains of the equivalent *VPL* (Vacuolar protein

localization) (37), *VPT* (Vacuolar protein targeting) (36) and *PEP* genes had a somewhat older history (39, 159). Those that had described the *PEP* mutants were actually those that first found a role of Pep8p / Vps26p in protein delivery to the yeast vacuole (27). They examined the processing of three soluble vacuolar proteases, CPY and the proteinases A and B, in *PEP8* deletion mutants, and found defects in enzyme processing, which were only striking for CPY. Based on localization data, it was suggested that Pep8p has a role at a postsorting stage in protein delivery to the yeast vacuole (27). This was later confirmed when Vps26 was found to be part of retromer and thus involved in endosome-to-Golgi retrieval (9). In analogy with the ES cells isolated from *Vps26*$^{-/-}$ blastocysts (154), disruption of the *PEP8* gene in yeast did not affect growth or viability. In addition, the yeast mutant was reported to display normal vacuoles (27), although a previous phenotypic characterization of the yeast *VPS* mutants had shown that *VPS26* deleted cells, as other class F *VPS* mutants, display a large vacuole and fragmented vacuolar-like structures around (48). The reason for these different interpretations is unknown.

The relatively mild defects in protein processing to the vacuole described for the *PEP8* / *VPS26* yeast mutants (9, 27) are in striking contrast with the embryonic lethality seen in mice harboring the *Hβ58* mutation (30, 31). It was surprising, and at the same time paradoxical, to find that while major effects in these mutant mice were found in the embryonic ectoderm, Vps26 expression is much lower in this cell lineage than in extraembryonic lineages, such as the visceral endoderm / yolk-sac lineage, which contained the highest mRNA levels although they were morphologically unaffected by the mutation (30, 31). These authors speculated that Vps26 would behave as a secreted protein of which normal expression in the extraembryonic tissue may be required for proper development of the embryonic ectoderm, but this hypothesis was not tested directly (31). Since Vps26 lacks a recognizable signal sequence, an alternative and more interesting explanation was that Vps26 would instead have a role at an early step of synthesis, transport or secretion of other molecule(s) – nutrient or growth factor – thus indirectly affecting the ability of the visceral endoderm to support embryonic growth (31). Indeed, it has become clear that while the visceral endoderm, and the yolk-sac as a whole, have a role in nutrient uptake and transport, they also have an active role in guiding early embryo development (reviewed in 160-162). This idea still remained untested after the function of Vps26p in yeast lysosomal enzyme processing (27) and its incorporation in the retromer were defined (9). Intriguingly, Vps26p appears to have a relatively minor role in stabilization of the complex (22). However, in agreement with the studies on the mouse *Hβ58* mutation, it has recently been observed that SNX2 (but not SNX1) is also highly expressed in the yolk-sac of embryos at midgestation (33). A possible explanation to these observations in mouse models can now be found in recent studies on developing nematodes, as it will be discussed later in this section.

5.1.2. Vps35

Another retromer subunit found implicated in mammalian embryogenesis is Vps35. To identify genes expressed during early mouse embryogenesis, cDNA libraries were constructed from unfertilized eggs and embryos until the blastocyst stage (163). Based on its high expression levels in unfertilized egg and preimplantation embryo, a novel maternal transcript was named maternal-embryonic 3 (MEM3) and turned out to be the Vps35p mammalian ortholog (28). MEM3 mRNA levels decreased in the 2-cell embryo and increased again in the 8-cell embryo, decreasing again during embryo postimplantation and remaining low in adult tissues (28). From this expression profile, and the already reported role of this protein in yeast intracellular sorting (164), it was suggested that MEM3 follows the expression pattern of a housekeeping gene (28), which contradicted the interpretation previously made for the *Hβ58* gene product (30, 31).

5.1.3. Sorting nexins 1 and 2

As the extensive SNX family was beginning to be explored in higher eukaryotes, their role *in vivo* was addressed in mice lacking those SNX members initially proposed to form part of a putative mammalian retromer (23). SNXs 1 and 2, as Vps5p orthologs, were proposed to assemble as homo or heterodimers in a conserved mammalian retromer (see Sections 3 and 4) and mice that lack either one of these proteins were generated. While *Snx1*$^{-/-}$ and *Snx2*$^{-/-}$ mice were viable and fertile, and displayed no obvious phenotype, *Snx1*$^{-/-}$ *Snx2*$^{-/-}$ embryos arrested at midgestation, indicating that SNX1 and SNX2 are functionally redundant and perform an essential role in embryogenesis (32). The phenotype of these double-knockout embryos was very similar to that of embryos lacking Vps26 (30, 31), showing growth retardation by E7.5 and arresting between E9.5 and E11.5. They also displayed a disproportionate growth of extraembryonic structures, such as the yolk-sac, in comparison to the embryonic ectoderm (32). Ultrastructural analysis showed an accumulation of apical electron dense structures of unknown nature in visceral endoderm cells of *Snx1*$^{-/-}$ *Snx2*$^{-/-}$ embryos at E9.5. These morphological alterations supported the hypothesis that alterations in nutrient transport to the developing embryo could be the cause of the fatal phenotype, as proposed previously for *Vps26*$^{-/-}$ embryos (31). They also contrasted with an apparently normal endosome-to-lysosome pathway in primary embryonic fibroblasts, as seen by staining of endocytosed molecules. The latter also implies that SNX1 and SNX2 appear not essential for this pathway, at least in some cells (32).

To address if absence of SNXs 1 and 2 in embryonic tissues also contributes to lethality, tetraploid aggregation rescue experiments were carried out. This system is based on aggregation of ES cells with developmentally compromised tetraploid embryos. In the chimeric embryos, the tetraploid component is selected against in all lineages where ES cells properly differentiate. ES cells will thus form the embryo proper, while the tetraploid component only contributes to the extraembryonic tissues, allowing the production of mice entirely derived from ES cells (165, 166). By this technique, it was possible to generate diploid / tetraploid

chimeras formed by *Snx1*$^{-/-}$ *Snx2*$^{-/-}$ embryos with partially wild-type extraembryonic tissues. Interestingly, these mutant embryos were much larger than the regular double-knockouts, although they still arrested at E12.5. While defects in extraembryonic tissues were partially corrected by the presence of wild-type cells, the prevailed lethality indicates that defects are also present in embryonic tissues (32). Nevertheless, it is possible that wild-type SNX1 and SNX2 in extraembryonic tissues were insufficient to fulfill the proposed supportive role to the developing embryo, since the yolk-sac mesoderm and the amnion are completely ES-derived and thus composed by SNX1 and SNX2 depleted cells (165).

These genetic analyses in mice suggest that SNX1, SNX2 and Vps26 act in the same pathway and provide *in vivo* evidence of a mammalian retromer structurally similar to the yeast retromer. To address a possible link with the EGFR pathway, in which both SNXs have been implicated (63, 90), *Snx1*$^{-/-}$ or *Snx2*$^{-/-}$ mice were crossed with mice wearing the EGFR waved-2 mutation (*EGFR*$^{Wa-2}$), a Val-to-Gly substitution at residue 743 which nearly abolishes its Tyr kinase activity (167) and causes impaired maternal lactation without affecting newborn viability (168). While genetic interactions have previously been found between the waved-2 mutation and other gene products of the EGFR signaling pathway (169), no alterations in phenotype or viability were observed here in *Snx1*$^{-/-}$ *EGFR*$^{Wa-2/Wa-2}$ or *Snx2*$^{-/-}$ *EGFR*$^{Wa-2/Wa-2}$ double homozygous (32). In principle, these data seem to exclude an interaction between SNXs 1 or 2 with the EGFR pathway during development. However, the relatively mild phenotype caused by this hypomorphic mutation may be appropriate to identify components required for EGFR signaling (169), but unsuitable to study molecules involved in subsequent downregulation events.

The first evidence that SNX1 and SNX2 are not genetically equivalent comes from the observation that, whereas *Snx1*$^{-/-}$ *Snx2*$^{+/-}$ embryos reached term in expected numbers, 40 % of *Snx1*$^{+/-}$ *Snx2*$^{-/-}$ embryos died, and those that survived showed clear signs of growth retardation continuing into later life, since remained 10-20 % smaller than their *Snx1*$^{+/+}$ *Snx2*$^{-/-}$ littermates (32). These differences were further studied in knockouts for SNX1, SNX2 and Vps26. By RT-PCR, it was shown that, while SNX1 levels were similar in the extraembryonic yolk-sac as in the developing embryo, SNX2 and Vps26 levels were proportionally more abundant in the yolk-sac (33). This finding supports the previously observed high expression of Vps26 in the visceral endoderm (31). These data also emphasize a stronger relevance of SNX2 vs. SNX1 in the retromer complex. The observation that *Snx1*$^{+/-}$ *Snx2*$^{-/-}$ *Vps26*$^{+/-}$ embryos displayed 100 % lethality, whereas *Snx1*$^{-/-}$ *Snx2*$^{+/-}$ *Vps26*$^{+/-}$ embryos were born in expected ratios, is even more conclusive on this aspect. Since only 10 % of *Snx2*$^{-/-}$ *Vps26*$^{+/-}$ embryos survived (33), one may conclude that SNX1 integration in retromer during development is practically negligible. Analysis of the phenotypic alterations in the various developing mutant embryos showed three distinct checkpoints involving retromer function, and thus critical for embryonic survival. The observed phenotypes included developmental delay (E8.5), as previously described (30-32); exencephaly (E13.5); and hemorrhage in head or abdomen, with accompanying craniofacial abnormalities and occasional exencephaly (E18.5) (33). This suggests that retromer may serve multiple roles during development.

These recent studies in mammalian development show that, although both SNXs can cooperate in the retromer, SNX2 appears more effective than SNX1 (32, 33). As discussed in Section 4, studies in culture cells have focused more on SNX1 and, except for a recent study showing that both SNXs are equally effective (62), they suggest overall the opposite view, providing evidence for a key participation of SNX1 in retromer-dependent sorting of the CI-MPR (60, 65, 67, 68). From these studies, it is also unclear whether both SNXs colocalize (62, 65) or not (63) on the same endosomal structures. In addition, different mechanisms in regulating agonist-induced EGFR (63) or PAR1 degradation (66) have been proposed for these two proteins. Thus, in spite of the currently accepted view that retromer assembly and function are largely conserved throughout evolution (12, 13, 15, 16), cell type and developmental differences may account for some of the discrepancies, albeit not always. As an example of this, it was seen that Vps26 antisense depletion in cultured HeLa cells, which depletes the whole Vps35-Vps26-Vps29 subcomplex, significantly reduced CI-MPR levels by increased receptor degradation in lysosomes (12). In contrast, a contemporary study reported that antisense knockdown of this subcomplex did not affect the stability of the CI-MPR in the same cell type, and the receptor was instead redistributed to early endosomes and the plasma membrane (13), as seen later by others (67). A higher CI-MPR turnover was however observed in ES cells derived from *Vps26*$^{-/-}$ embryos (13). This inconsistency was interpreted by differences in the rate of the receptor's trafficking pathways, which are responsible for its diverse subcellular localization among Golgi, endosomes and the cell surface, as previously reported (see 43, 47, 170 for reviews on MPRs distribution and traffic). The reason for these discrepancies described in cultured HeLa cells is unknown, although probably both the CI-MPR distribution and levels are altered upon affecting retromer's function, as observed upon combined depletion of SNX1 and SNX2 (62). In either case, interaction and localization data convincingly demonstrate the retromer's role in endosome-to-Golgi retrieval of the CI-MPR (15, 16).

CI-MPR localization was also investigated in the mouse models. Evidence for CI-MPR mislocalization or instability in MEF isolated from single or double-knockout embryos for the SNXs 1 and 2 or from *Vps26*$^{-/-}$ embryos could not be provided (33). The discrepancy of these observations with the previous data showing altered distribution of the CI-MPR in ES cells derived from undifferentiated *Vps26*$^{-/-}$ embryos (13) was explained by the authors as owed to differences in cell type and degree of differentiation. It was also reminded that one cannot exclude a possible effect on other linked genes by the insertional mutation technique used to initially obtain the mice with the *Hβ58* mutation, from which those ES cells

were derived (30, 153). On the contrary, the *Vps26*$^{-/-}$ MEF were derived from embryos carrying a targeted mutation of the gene (33). Further immunocytochemical analysis in SNXs 1 and 2 double-knockout embryos and in *Vps26*$^{-/-}$ embryos showed that CI-MPR was also found correctly localized in the perinuclear area of yolk-sac endoderm cells (33), where Vps26 normally displays a high expression (31). These negative data suggest that altered trafficking of the CI-MPR is not responsible for the lethal phenotype in retromer-depleted embryos. Finally, concerning the more critical role in retromer activity during embryogenesis proposed for SNX2 vs. SNX1, one may speculate that SNX2 may have become specialized in retromer assembly or stability, whereas SNX1 in interactions with certain cargo proteins. That way, SNX2 gene deletion may have a more striking effect in vivo. On the contrary, SNX1 antisense depletion in cells might particularly affect certain pathway regulated by retromer and not necessarily essential for development or survival. Thus, depending on the level of SNXs depletion by antisense, and on the assay used to measure the effect, alterations on e.g. CI-MPR trafficking in cultured cells could seem more (62) or less striking (65, 67).

5.1.4. Other sorting nexins

Other SNXs recently proposed to be retromer subunits are SNX5 and SNX6 (67), for which knockout mice have not yet been reported. Additional importance on the role of SNXs in embryo development has been provided by a recent study on SNX13. As in the knockouts for retromer subunits, targeted disruption of the mouse SNX13 gene resulted in embryonic lethality, further demonstrating the importance of regulating endocytic trafficking in early stages of development (109). Besides the PX domain, the SNX13 sequence includes a regulator of G protein signaling (RGS) domain. Hence, SNX13 is also known as RGS-PX1 and it belongs to both the SNX and the RGS protein families (60). SNX13 was involved in lysosomal degradation of the EGFR (122), functioning as a GTPase activating protein for the heterotrimeric G protein Gαs in a complex with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs), a component of the ESCRT machinery (123).

Similar to *Snx1*$^{-/-}$ *Snx2*$^{-/-}$ and *Vps26*$^{-/-}$ embryos, *Snx13*$^{-/-}$ embryos arrest at midgestation, showing growth retardation and, in particular, displaying abnormalities in the yolk-sac endoderm (109). However, ultrastructural analysis of visceral endoderm cells suggests that different intracellular pathways are affected. *Snx13*$^{-/-}$ embryos lacked the large apical electron dense structures negative for both endosome and lysosome markers that were seen in cells from *Snx1*$^{-/-}$ *Snx2*$^{-/-}$ embryos (109). Instead, abundant and elaborate dense tubules resembling an enlarged recycling endosome system (16, 86) were seen. Autophagic vacuoles were also observed, and this was interpreted as an effect of cell stress by nutrient shortage, probably caused by deficient nutrient uptake and further lysosome processing (109). These observations agree with the hypothesis that nutrients' processing in the yolk-sac endoderm is needed to supply the embryo with the resultant products (160), as suggested to be affected also in the Vps26 and SNX1/2 knockouts (30-33). Thus, knockouts for SNX1/2 and SNX13 develop comparable defects that largely result in the same phenotype. However, this study implies that SNX13 acts at different intracellular compartments than SNX1 and SNX2 in the extraembryonic tissue, and probably through interactions with molecules not involving the retromer.

The recent studies in mice knockout for various retromer subunits (33) and this newer report on SNX13 knockouts (109) suggest that more evidence on the participation of these proteins in embryonic development is expected to emerge shortly. Another recent example in this context is the involvement of SNX5 in hematopoiesis and blood vessel formation in zebrafish early development (171). Intriguingly, this is reminiscent of a role proposed for Vps26 in controlling hematopoietic development in rat placenta (155). Overall, these approaches shed light on the mechanism(s) of retromer and SNXs functioning during development.

5.1.5. The cation-independent mannose 6-phosphate receptor

The studies on retromer-depleted embryos suggest that altered trafficking of the CI-MPR is not responsible for their lethal phenotype (33). If an increased turnover of the CI-MPR was the cause, the *CI-MPR*$^{-/-}$ embryos would then be expected to share a similar phenotype as retromer-depleted embryos, which is not the case, nor is the case of humans suffering I-cell disease (ICD). These individuals are defective in the phosphotransferase that generates the mannose-6-phosphate tags on lysosomal enzymes and still survive through early adolescence (172-174). The CI-MPR was however reported to be essential for late embryonic development and growth regulation, although the phenotype of *CI-MPR*$^{-/-}$ embryos clearly differs with that of knockouts for retromer subunits (175). *CI-MPR*$^{-/-}$ homozygosis is generally lethal at birth, and mutants display increased size. This phenotype was explained by receptor interactions with the insulin-like growth factor II (IGF2), hence the receptor is also known as IGF2/MPR (43, 170). While the role of CI-MPR in adult tissues would primarily be to maintain enzyme levels in the lysosome, in the embryo, however, the receptor is mainly involved in internalizing IGF2 in excess, thus inhibiting growth; an impairment of this function is reported to be the primary cause of death in *CI-MPR*$^{-/-}$ embryos (175, 176). Therefore, the complex embryonic phenotype displayed by the IGF2/MPR knockout mice could result from this multiligand activity. Absence of CI-MPR would lead to high serum concentrations of active proliferative ligands such as IGF2 (175, 176), accumulation of inactive ligands such as latent TGF-β (176) and proliferin (177), as well as impaired growth-inhibitory effects by retinoids (178) (see 47, 179 for a complete list of CI-MPR ligands, containing or not a mannose 6-phosphate residue). Finally, it should be kept in mind that similar defects in steady-state levels of lysosomal enzymes are present in the CD-MPR null mice, with no obvious effect in viability (180, 181), making very unlikely that affected CI-MPR sorting is responsible of the fatal phenotype in retromer-depleted embryos.

5.1.6. The polymeric immunoglobulin receptor

Retromer also associates with the pIgR and promotes transcytosis of pIgR and its ligand pIgA in polarized epithelial cells. This interaction prevents recycling or degradation of pIgR-pIgA in a somehow analogous way as CI-MPR trafficking is regulated by retromer (14). Mice knockout for pIgR have also been reported (182, 183). While these mice lacked active epithelial and hepatic pIgA transport, they were born in expected ratios and therefore disruption of the pIgR gene is not lethal. Complete absence of pIgR in humans has not been identified (184), although it is clear that the receptor is not essential neither for development nor for the health of mice kept in a conventional laboratory facility. Although IgA secretion appears already operative in early stages of the embryogenesis (185), from these studies in pIgR-deleted mice one can conclude that the essential role of retromer during embryogenesis is unrelated to pIgR-pIgA trafficking.

5.2. Retromer's role in Wnt signaling

We have seen that the retromer's role, while evolutionary conserved from yeast, may vary considerably in different cell types or developmental stages. Importantly, the key participation of retromer during embryogenesis has remained unsolved until very recently. Two independent studies in the worm *C. elegans* have come up with a possible explanation. With the use of transgenic and deletion mutant strains, they demonstrate the requirement of retromer to produce Wnt proteins efficient for long-range signaling in the establishment of anteroposterior (A/P) neuronal polarity (34, 35).

The Wnt signaling pathway (wingless in *Drosophila*) is highly conserved throughout evolution and extensively used during animal development, from the simplest Cnidaria to humans. Activation of Wnt signal transduction pathways upon ligand binding can regulate multiple aspects during embryogenesis, including cell proliferation, migration, fate specification, polarity, differentiation and axon outgrowth. In addition, an overactivation of Wnt signaling by mutation is a major factor of oncogenesis in various human tissues (186). An understanding of the Wnt action mechanisms has emerged from several experimental systems, including genetics in *Drosophila* and *C. elegans*, biochemistry in cell culture models and ectopic gene expression in *Xenopus* embryos (see 187-191 for reviews).

Secreted Wnt proteins can form a concentration gradient during embryonic development and thus function as morphogens. They then interact with different cell surface receptors located on the target cells, particularly with the seven-transmembrane Frizzled and Ryk/Derailed Tyr kinases (191). Among Wnt proteins, the Egg-laying defective family member EGL-20 is expressed by a group of cells located at the posterior end of the nematode *C. elegans* during early larval development. EGL-20 controls long-range migrations of the neuroblasts QL and QR and their descendants along the A/P body axis (192). Other well studied neurons in *C. elegans* are a set of six touch-receptor or mechanosensory neurons that mediate touch sensitivity. These are the PLML, PLMR, and PVM neurons, present in the posterior region of the worm, and the ALML, ALMR, and AVM neurons, located anteriorly; all of them send processes in anterior direction (193). Recently, it has been shown that Wnt and Frizzled proteins control the development and organization of the mechanosensory neuron processes along the A/P body axis, playing a role in axonal growth cone migration by orienting polarity of the developing neurons (35, 194, 195). In one of these studies, the worms were mutated with ethylmethane sulfonate and screened for neurons with possible defects, which were visualized by expression of a GFP-tagged reporter gene (35). Surprisingly, a mutation in the *VPS35* gene, involving a deletion that probably eliminated most or all protein function, caused polarity defects as those seen in Wnt and Frizzled mutants. In particular, the polarity of ALM and PLM neurons was frequently altered in *VPS35* mutants. The mutants were slightly small and uncoordinated and most died during embryogenesis, although heterozygous animals appeared like wild-types (35). *VPS26* and *VPS29* deletion mutants were also analyzed to understand these alterations within the retromer context. Interestingly, *VPS26* mutants displayed a comparable phenotype, but no alterations were seen in the *VPS29* mutants, and thus Vps29 appears less essential for retromer function in Wnt signaling (35). This observation does not fully contradict previous data obtained in yeast, where Vps35p and Vps26p can still interact, albeit at reduced levels, in *VPS29* deleted cells (22), with Vps35p, but not Vps26p, becoming substantially less stable (24). It does not agree however with antisense experiments in mammalian cells, where Vps29 knockdown depletes the whole Vps35-Vps26-Vps29 retromer subcomplex (62). Effects in *C. elegans* mechanosensory neurons caused by mutations in SNXs were not reported in this study. Instead, these authors analyzed effects of mutations in subunits of the Vps35-Vps26-Vps29 subcomplex on PVM neuron migration (35). Resembling *EGL-20* mutants, PVM polarity was reversed in mutants for either subunit. Intriguingly, EGL-20 intracellular levels were unaffected in retromer mutants and the study underlined that the polarity defects in retromer mutants were often less severe than in Wnt-null mutants. In summary, this study demonstrates the requirement of retromer for establishing A/P neuronal polarity in *C. elegans* (35).

Comparable results were obtained in a contemporary study, which demonstrated a role of retromer in EGL-20 producing cells for long-range Wnt signaling along the *C. elegans* A/P body axis (34). In a high-throughput genome-wide screen, *C. elegans* genes were antisense targeted (196) and the worms analyzed for Q cell migration. By this technique, the authors found the Vps35p ortholog to be required for posterior location of the QL descendant neuroblasts, for EGL-20 dependent anterior migration of the QR descendants and the hermaphrodite specific neurons (HSN), and for the correct polarity of the posterior epidermal seam cell V5, which becomes a neuroblast. The phenotype of *VPS35* deletion mutants was also seen strikingly similar to that of *EGL-20* mutants (34). Vps35 was found involved in a critical step in EGL-20 expressing cells that allows EGL-20 to spread anteriorly.

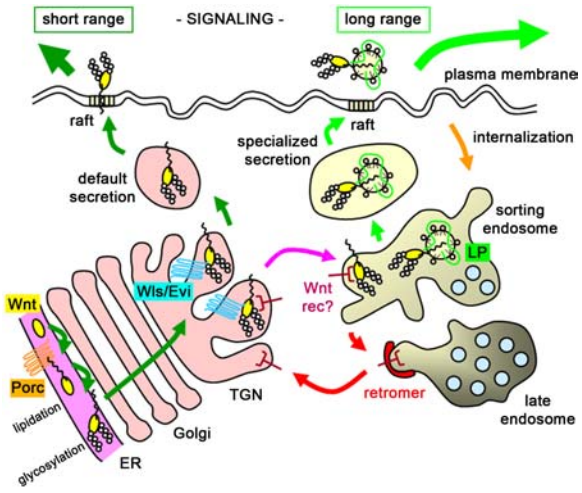

Figure 4. Hypothetical model for a retromer role in Wnt signaling. Newly synthesized Wnt proteins undergo post-translational modifications at the endoplasmic reticulum (ER) to become fully active morphogens. Wnt proteins are palmitoylated by the *O*-acyltransferase porcupine (Porc), a multipass transmembrane protein that also stimulates Wnt *N*-glycosylation and targets them to the ER membrane (*dark green arrows*). Probably at the *trans*-Golgi network (TGN), Wnt proteins interact with another multipass transmembrane protein, Wntless / Evenness interrupted (Wls/Evi), also named Sprinter (Srt). By an unknown mechanism, that may involve an additional post-translational modification, Wls/Evi ensures Wnt secretion. The partition of Wnt proteins into vesicles targeted to cholesterol-sphingolipid rich raft domains is also important for Wnt secretion (raft microdomains are only represented at the plasma membrane). In addition, a putative Wnt receptor (Wnt rec?) may deliver Wnt proteins to the endosomal system (*pink arrow*), where they could associate with lipoprotein particles (LP). Alternatively, this receptor may transport enzymes or other proteins involved in Wnt processing / secretion (not represented). Wnt proteins are thus sorted out of default secretion (*dark green arrows*) and are incorporated into specialized secretory vesicles (*light green arrows*). Lipoproteins could then be the vehicles facilitating Wnt extracellular transport and transfer to target cells at long distances; this will ensure long-range signaling. In retromer depleted mutants, the retrieval mechanism that returns a putative Wnt receptor back to the TGN (*red arrow*) would not be operative, and Wnt proteins would instead be secreted via the default pathway, becoming only suitable for short-range signaling. Wnt retention at the secretory pathway, or at the cell surface for subsequent Wnt internalization (*orange arrow*) and recycling, may also help to sustain signaling.

Here, embryonic lethality did not greatly increase in *VPS35* mutants. The same largely applied to *VPS26*, *VPS29* and *VPS5* mutants (34), contrasting with the high embryonic death rate of the corresponding mutants in the study discussed previously (35). *VPS29* mutants had also a milder phenotype than *VPS35* mutants, but polarity of Q cell migration was clearly affected. Interestingly, loss of VPS5 had no effect by itself, but VPS5 antisense depletion in *VPS26* or *VPS35* mutants enhanced their defects and caused lethality (34). An important aspect to remark is that this study provides evidence that the essential function of retromer during development is conserved in Wnt-producing cells of vertebrates. Thus, Vps35 antisense knockdown in Xenopus embryos or in cultured human cells also impaired Wnt signaling, albeit not Wnt secretion (34).

The evidence presented in these studies indicates that retromer does not participate in formation and secretion of active Wnt proteins, but rather in a specific mechanism ensuring production of Wnt proteins competent for long-range signaling. Therefore, rather than a housekeeping role, a specific function of retromer in embryogenesis has now been uncovered. A possible model to explain the critical role of retromer in Wnt producing cells was proposed as follows (Figure 4). Wnt proteins undergo post-translational modifications to become fully active morphogens at both short and long-range distances during animal development. In particular, Wnt proteins are palmitoylated at the endoplasmic reticulum by the *O*-acyltransferase porcupine/more mesoderm-1 (Porc, as named in *Drosophila*, or MOM-1, in *C. elegans*) (197). This lipid modification stimulates N-glycosylation and membrane-anchoring of Wnt proteins, localizing before secretion into specialized lipid raft / detergent-resistant microdomains (198). Lipid-linked morphogens, like Wnt and Hedgehog (Hh) proteins, associate with lipoproteins before secretion, thus ensuring efficient long-range diffusion and subsequent interaction with their target cells for signal transmission (199, 200). One could imagine retromer regulating trafficking of Wnt processing enzymes, such as Porc/MOM-1, or trafficking of proteins required for adequate Wnt secretion, such as lipoproteins (34, 35). Association of Wnt proteins with lipoproteins might take place in an endosomal compartment containing the lipoprotein particles. This would require Wnt proteins to be sorted out of a default secretory pathway, perhaps by a specific receptor, and to be transported from Golgi to endosomes prior to secretion. As for recycling of Vps10p in yeast, one could speculate that retromer retrieves a putative Wnt receptor from the endosome and redirects it back to the TGN for new rounds of Wnt delivery to the endosomal compartment. This retrieval mechanism would not be operative in the absence of retromer, and in consequence, Wnt proteins would be secreted via the default pathway, making them suitable for short-term but not for long-term signaling (34). Although in this model an analogy between Wnt and Hh proteins has been made, these studies did not address whether Hh proteins were affected in retromer mutants and it is possible that retromer action is specific for Wnt production (see below). Thus, it could be that retromer action is part of a mechanism governing processing of certain signaling proteins in the producing cells, so that the receiving cell will properly interpret spatial gradients or temporal changes in protein concentration.

What is the receptor for Wnt proteins sorted by retromer in Wnt producing cells? While many components required for reception and processing of the Wnt signal in target cells have been identified and characterized, the

process of Wnt secretion is considered a "black box". Thus, apart from Porc/MOM-1 role in Wnt posttranslational modifications and the subsequent association of Wnt to lipoproteins, very little is known on the regulation of synthesis, secretion and release of the Wnt signal out of the cell. However, a piece of information on the process of Wnt secretion has been recently provided by independent studies (201-204). By using different approaches of genetic screening in *Drosophila*, these research groups identified a new component specifically controlling Wnt secretion, which was named Wntless (Wls) (201), Evenness interrupted (Evi) (202) or Sprinter (Srt) (204). We will refer to here as Wls/Evi, since this is how it simultaneously appeared in the literature (203). Wls/Evi is a multipass transmembrane protein evolutionary and functionally conserved from worms to humans. Absence of Wls/Evi led to abnormal Wnt folding and retention in cells, without affecting Hh secretion or expression of Hh downstream target genes (201, 202, 204). Although no obvious enzymatic activity can be inferred from its sequence, Wls/Evi might be an enzyme that specifically modifies Wnt proteins, similarly to Porc/MOM-1, but acting subsequently in the pathway. Alternatively, Wls/Evi could be part of a specialized machinery for regulating Wnt secretion, ensuring proper intracellular transport of Wnt proteins to the cell surface. It probably acts at the TGN or in specialized secretory vesicles *en route* to the subapical cell surface of polarized epithelial cells and plays a role in packaging, localizing or fusing Wnt-containing vesicles with the plasma membrane (201, 202, 204). Related to this idea, earlier findings demonstrated that a significant proportion of Wnt proteins are found in recycling endocytic vesicles, by which they are sent back to the surface for secretion (205). This provides additional support for the existence of a transmembrane protein functioning as a putative receptor that allows Wnt proteins to follow a privileged pathway to the cell surface. Whether Wls/Evi is specifically sorted by retromer remains to be tested, although from what we know it seems unlikely, since contrary to the observations in Wls/Evi mutants, Wnt secretion is not affected in retromer mutants (34, 35). At any rate, the function proposed, almost fifteen years ago, for the *Hβ58* gene product, of regulating synthesis, transport or secretion of some other molecule required for proper embryo development seemed a clever anticipation of retromer's essential role during embryogenesis (31).

6. RETROMER IN DISEASE: THE IMPORTANCE OF ACCURATE INTRACELLULAR PROTEIN SORTING

Many human disorders arise from defects in intracellular trafficking. Over the years, researchers have studied how defects in the ability to sort or transport molecules to their appropriate cellular destinations can greatly affect cell functionality and therefore cause disease (206-210). Organ-specific diseases can arise by alterations in the sorting signals of individual proteins, or in the trafficking machinery delivering these proteins to their proper destinations. Some of the well-known diseases resulting from altered trafficking or cell polarity include cystic fibrosis, Wilson disease (copper toxicity) or familial hypercholesterolemia. In polarized epithelial cells, for instance, altered localization of molecules can dramatically affect barrier and transport functions, thereby causing disease. In these cells, basolateral sorting, endocytosis, recycling and transcytosis of cell-surface molecules are key processes for maintaining epithelial polarity, and pathological conditions can arise because of the inappropriate removal or insertion of itinerant proteins (209). Similarly significant is tight junction dysfunction, which deregulates cell polarity and causes kidney disease (209, 211).

Given their diversity and complexity, a particular interest has been given to defects in delivery of lysosomal proteins, most of them enzymes, which cause LSD. Because of the variety of undegraded substrates that accumulate in lysosomes, patients with LSD display multiple and complex phenotypes that include, among others, psychomotor retardation, mental / intellectual retardation / deterioration and enlarged internal organs, such as liver and spleen (see 173, 174 for recent reviews on the biology and pathology of lysosomal disorders). A relatively large subgroup of LSDs is caused by the accumulation of lipids in late endosomes or lysosomes. Among these diseases, a well studied one is the Niemann-Pick (NP) syndrome, caused by accumulation of constituents of lipid raft microdomains and for which three forms have been described. NP types A and B are characterized by sphingomyelin accumulation, whereas NP type C is caused by cholesterol accumulation (208). Two interesting aspects must be mentioned for the type C form, a rare autosomal recessive neurovisceral disorder caused by mutations in one of two genetic loci, *NPC1* or *NPC2* (mutations in *NPC1* account for 95% of the cases). First, the cholesterol accumulation in degradative compartments of the endocytic pathway is apparently due to a failure in the mechanism responsible for redistribution of cholesterol taken up by LDL endocytosis, in contrast to most of the LSDs, which are caused by defective metabolic enzymes. Since both NPC1 and NPC2 may participate in delivering LDL-cholesterol from late endosomes to proper cellular sites, retrieval mechanisms at the endosome appear affected in this pathology (reviewed in 210). Secondly, there is a mouse model exhibiting a clinical phenotype very similar to that seen in the human disease (208, 210). More recently, a model in *Drosophila* has also been created by mutating *NPC1a*, the fly *NPC1*-like gene, which intriguingly encodes a transmembrane protein related to the Hh receptor Patched (212). This is also an example that animal models involving altered trafficking mechanisms could reveal new links between signaling pathways that may help to deal with diseases.

Retromer is involved in CI-MPR retrieval (15, 16), and this receptor ensures arrival of enzymes to lysosomes (43, 47, 181). Although alterations in retromer function as a cause of LSD have not been described thus far, a relationship cannot be excluded. An aspect that has increased the difficulty of understanding the variability of LSD phenotypes is that cells in certain tissues have developed effective compensatory mechanisms to maintain their lysosomal function. In hepatocytes of CI-MPR (172)

or CD-MPR (180) knockout mice, newly synthesized enzymes are secreted, which prevents them of reaching the lysosome. However, cells can still acquire the lysosomal enzymes via secretion-recapture or direct cell-to-cell transfer mechanisms (172, 174). Alternative, MPR-independent mechanisms for transport of newly synthesized enzymes to the lysosomes are likely to exist also in patients with ICD. While fibroblasts from these patients have indeed a marked deficiency in lysosomal enzymes, enzyme levels are normal in most tissues, including liver, spleen, kidney and brain (172). Interestingly, the phenotype of mice triple-deficient for CI-MPR, CD-MPR and IGF2 that survive resembles that of ICD patients, presenting dwarfism, facial dysmorphism, elevated activities of lysosomal enzymes in serum and abnormal lysosomal storage in connective tissue cells (213). A parallelism between deficiencies in the CI-MPR and in retromer is clearly not obvious in view of the animal models, and from the current evidence, we conclude that the essential role of retromer during development is unrelated to its conserved role in CI-MPR sorting, as discussed in Section 5 (33). Thus, rather than having a general function in controlling membrane trafficking, retromer specifically sorts certain transmembrane proteins at the TGN-endosome interface.

As advanced at the end of Section 3, a link between retromer and disease was recently described for two neurological disorders, one of them very well known. First, retromer was proposed to regulate endosome-to-TGN traffic of BACE (81, 82), the enzyme that cleaves β-APP, leading to the release of the neurotoxic Aβ peptide and progression of AD (83). In the model proposed, retromer dysfunction, suggested to take place by reduction of retromer levels, would increase the concentration of BACE in the endosome. Since BACE activity is maximal in the acidic environment of the endosome, Aβ will be produced and subsequently secreted in large quantities (81, 82). An interaction between BACE and retromer might be explained via a Vps10-domain containing protein, increasing the relevance of this aspect in the context of this review, because retromer depletion by antisense results in mistrafficking to the endosome of the Vps10p homolog sortilin (13). Sortilin is believed to have a similar trafficking itinerary as the CI-MPR and to be engaged in protein sorting at the TGN (77). Importantly, the brain appears to be the organ with the highest expression of this family of Vps10-domain containing proteins. Another receptor of particular interest in this family is SorLA (LR11), a multiligand hybrid receptor containing 11 LDLR class A repeats and a Vps10p/sortilin domain (214, 215). Among other brain regions, SorLA is abundant in the hippocampal formation and particularly in the entorhinal cortex (215, 216), the subregion of the hippocampus that appears most vulnerable to AD (82). In a microarray screening, it was found a loss of SorLA expression in lymphoblasts derived from patients with AD and in histologically normal-appearing neurons of AD brains (217). Indeed, subsequent research confirmed the interaction of SorLA with APP (218) and BACE (219). These findings also support a possible relationship between retromer and trafficking of LDLR family members in neurons. Related to these aspects, the retromer subunit Vps26 also emerged as a positional candidate gene involved in AD. Thus, the human *VPS26* gene was located at locus 10q21.1 (220), and more recently it was found that this chromosome 10 region contains a quantitative trait locus displaying susceptibility to late-onset AD (221, 222). Although a recent study did not find an association of single nucleotide polymorphisms of the *VPS26* gene with sporadic forms of AD (223), these data do not exclude a possible functional involvement of Vps26 in AD pathogenesis.

Even more recently, retromer has been involved in another neurological disorder. This is the case of JNCL, a LSD also known as BD, for which are responsible mutations in the human *CLN3* gene, that codifies for an endosome/lysosomal protein of elusive function (224). BD is an autosomal recessive disease leading to progressive neurodegeneration and premature death in humans. A characteristic of BD and related diseases is the abnormal accumulation of autofluorescence storage material in the lysosome (225, 226). This altered membrane trafficking has been studied in yeast, in which two BD related proteins have been identified. One is Btn1p, the CLN3 ortholog, and the other is Btn2p, which is upregulated upon Btn1p deletion and has a role in cell growth and homeostasis. This model of BD in yeast (84) has encouraged further research. Thus, a recent study shows a direct interaction between Btn2p and Vps26p in a complex also including Snx4p and regulating endosome-to-Golgi retrieval of Yif1p (Yip1p-interacting factor; Yip1p: yeast Golgi integral membrane protein) (85), a component of the endoplasmic reticulum-Golgi fusion machinery (227) that needs to be moved to the Golgi. This study also supports that retromer subunits can assemble with other sorting components and thereby transport other molecules apart from Vps10p, although an association between Snx4p and retromer in yeast contradicts previous data (132). Nonetheless, the possible implications of these data in BD are worth being considered for future studies. While the functional link between retromer and neurodegeneration remains to be confirmed, these examples emphasize that alterations in the endosome-to-Golgi pathway can lead to serious human diseases.

7. PERSPECTIVE

In the relatively short period of eight years since the word “retromer” first appeared in the scientific literature (9), over half of the reports on retromer are found in the last two years. Similarly, since the role of SNX1 was first described ten years ago (90), the number of SNX family members quickly grew up and their involvement in membrane trafficking and signaling pathways has grown increasingly. The mouse *Hβ58* locus, which disruption blocks early embryonic development, was described fifteen years ago (30). New evidence of retromer and sorting nexins controlling development processes is now accumulating from studies in model organisms. These studies also provide new data on how alterations in the strict control of trafficking pathways can lead to disease. With no doubt, there is more soon to come on the essential function of these proteins in development and their relevance in human disease.

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9. REFERENCES

1. Rothman, J.E.: Mechanisms of intracellular protein transport. *Nature* 372, 55-63 (1994)
2. Schmidt, A., M. Wolde, C. Thiele, W. Fest, H. Kratzin, A.V. Podtelejnikov, W. Witke, W.B. Huttner & H.D. Soling: Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. *Nature* 401, 133-141 (1999)
3. Takei, K., V.I. Slepnev, V. Haucke & P. De Camilli: Functional partnership between amphiphysin and dynamin in clathrin-mediated endocytosis. *Nat Cell Biol* 1, 33-39 (1999)
4. Ford, M.G., I.G. Mills, B.J. Peter, Y. Vallis, G.J. Praefcke, P.R. Evans & H.T. McMahon: Curvature of clathrin-coated pits driven by epsin. *Nature* 419, 361-366 (2002)
5. Lee, M.C., L. Orci, S. Hamamoto, E. Futai, M. Ravazzola & R. Schekman: Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. *Cell* 122, 605-617 (2005)
6. Rothman, J.H., C.T. Yamashiro, P.M. Kane & T.H. Stevens: Protein targeting to the yeast vacuole. *Trends Biochem Sci* 14, 347-350 (1989)
7. Stack, J.H., B. Horazdovsky & S.D. Emr: Receptor-mediated protein sorting to the vacuole in yeast: roles for a protein kinase, a lipid kinase and GTP-binding proteins. *Annu Rev Cell Dev Biol* 11, 1-33 (1995)
8. Seaman, M.N., E.G. Marcusson, J.L. Cereghino & S.D. Emr: 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 (1997)
9. Seaman, M.N., J.M. McCaffery & S.D. Emr: A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast. *J Cell Biol* 142, 665-681 (1998)
10. Marcusson, E.G., B.F. Horazdovsky, J.L. Cereghino, E. Gharakhanian & S.D. Emr: The sorting receptor for yeast vacuolar carboxypeptidase Y is encoded by the VPS10 gene. *Cell* 77, 579-586 (1994)
11. Cooper, A.A. & T.H. Stevens: Vps10p cycles between the late-Golgi and prevacuolar compartments in its function as the sorting receptor for multiple yeast vacuolar hydrolases. *J Cell Biol* 133, 529-541 (1996)
12. Arighi, C.N., L.M. Hartnell, R.C. Aguilar, C.R. Haft & J.S. Bonifacino: Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor. *J Cell Biol* 165, 123-133 (2004)
13. Seaman, M.N.: Cargo-selective endosomal sorting for retrieval to the Golgi requires retromer. *J Cell Biol* 165, 111-122 (2004)
14. Vergés, M., F. Luton, C. Gruber, F. Tiemann, L.G. Reinders, L. Huang, A.L. Burlingame, C.R. Haft & K.E. Mostov: The mammalian retromer regulates transcytosis of the polymeric immunoglobulin receptor. *Nat Cell Biol* 6, 763-769 (2004)
15. Seaman, M.N.: Recycle your receptors with retromer. *Trends Cell Biol* 15, 68-75 (2005)
16. Bonifacino, J.S. & R. Rojas: Retrograde transport from endosomes to the trans-Golgi network. *Nat Rev Mol Cell Biol* 7, 568-579 (2006)
17. Seaman, M.N.: Endosome sorting: GSE complex minds the Gap. *Nat Cell Biol* 8, 648-649 (2006)
18. Horazdovsky, B.F., B.A. Davies, M.N. Seaman, S.A. McLaughlin, S. Yoon & S.D. Emr: 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 (1997)
19. Seaman, M.N. & H.P. Williams: Identification of the functional domains of yeast sorting nexins Vps5p and Vps17p. *Mol Biol Cell* 13, 2826-2840 (2002)
20. Nothwehr, S.F., P. Bruinsma & L.A. Strawn: Distinct domains within Vps35p mediate the retrieval of two different cargo proteins from the yeast prevacuolar/endosomal compartment. *Mol Biol Cell* 10, 875-890 (1999)
21. Nothwehr, S.F., S.A. Ha & P. Bruinsma: 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 (2000)
22. Reddy, J.V. & M.N. Seaman: Vps26p, a component of retromer, directs the interactions of Vps35p in endosome-to-Golgi retrieval. *Mol Biol Cell* 12, 3242-3256 (2001)
23. Haft, C.R., M. de la Luz Sierra, R. Bafford, M.A. Lesniak, V.A. Barr & S.I. Taylor: Human orthologs of yeast vacuolar protein sorting proteins Vps26, 29, and 35: assembly into multimeric complexes. *Mol Biol Cell* 11, 4105-4116 (2000)
24. Collins, B.M., C.F. Skinner, P.J. Watson, M.N. Seaman & D.J. Owen: Vps29 has a phosphoesterase fold that acts as a protein interaction scaffold for retromer assembly. *Nat Struct Mol Biol* 12, 594-602 (2005)
25. Wang, D., M. Guo, Z. Liang, J. Fan, Z. Zhu, J. Zang, X. Li, M. Teng, L. Niu, Y. Dong & P. Liu: Crystal structure of human vacuolar protein sorting protein 29 reveals a phosphodiesterase/nuclease-like fold and two protein-protein interaction sites. *J Biol Chem* 280, 22962-22967 (2005)
26. Shi, H., R. Rojas, J.S. Bonifacino & J.H. Hurley: The retromer subunit Vps26 has an arrestin fold and binds Vps35 through its C-terminal domain. *Nat Struct Mol Biol* 13, 540-548 (2006)
27. Bachhawat, A.K., J. Suhan & E.W. Jones: The yeast homolog of H < beta > 58, a mouse gene essential for embryogenesis, performs a role in the delivery of proteins to the vacuole. *Genes Dev* 8, 1379-1387 (1994)
28. Hwang, S., L.E. Benjamin, B. Oh, J.L. Rothstein, S.L. Ackerman, R.S. Beddington, D. Solter & B.B. Knowles: Genetic mapping and embryonic expression of a novel, maternally transcribed gene Mem3. *Mamm Genome* 7, 586-590 (1996)
29. Nothwehr, S.F. & A.E. Hindes: 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 (1997)
30. Radice, G., J.J. Lee & F. Costantini: H beta 58, an insertional mutation affecting early postimplantation development of the mouse embryo. *Development* 111, 801-811 (1991)
31. Lee, J.J., G. Radice, C.P. Perkins & F. Costantini: Identification and characterization of a novel, evolutionarily conserved gene disrupted by the murine H beta 58 embryonic lethal transgene insertion. *Development* 115, 277-288 (1992)
32. Schwarz, D.G., C.T. Griffin, E.A. Schneider, D. Yee & T. Magnuson: Genetic analysis of sorting nexins 1 and 2 reveals a redundant and essential function in mice. *Mol Biol Cell* 13, 3588-3600 (2002)
33. Griffin, C.T., J. Trejo & T. Magnuson: Genetic evidence for a mammalian retromer complex containing sorting nexins 1 and 2. *Proc Natl Acad Sci USA* 102, 15173-15177 (2005)
34. Coudreuse, D.Y., G. Roel, M.C. Betist, O. Destree & H.C. Korswagen: Wnt gradient formation requires retromer function in Wnt-producing cells. *Science* 312, 921-924 (2006)
35. Prasad, B.C. & S.G. Clark: Wnt signaling establishes anteroposterior neuronal polarity and requires retromer in *C. elegans*. *Development* 133, 1757-1766 (2006)
36. Bankaitis, V.A., L.M. Johnson & S.D. Emr: Isolation of yeast mutants defective in protein targeting to the vacuole. *Proc Natl Acad Sci USA* 83, 9075-9079 (1986)
37. Rothman, J.H. & T.H. Stevens: Protein sorting in yeast: mutants defective in vacuole biogenesis mislocalize vacuolar proteins into the late secretory pathway. *Cell* 47, 1041-1051 (1986)
38. Robinson, J.S., D.J. Klionsky, L.M. Banta & S.D. Emr: Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. *Mol Cell Biol* 8, 4936-4948 (1988)
39. Jones, E.W.: Proteinase mutants of *Saccharomyces cerevisiae*. *Genetics* 85, 23-33 (1977)
40. Rothman, J.H., I. Howald & T.H. Stevens: Characterization of genes required for protein sorting and vacuolar function in the yeast *Saccharomyces cerevisiae*. *EMBO J* 8, 2057-2065 (1989)
41. Bryant, N.J. & T.H. Stevens: Vacuole biogenesis in *Saccharomyces cerevisiae*: protein transport pathways to the yeast vacuole. *Microbiol Mol Biol Rev* 62, 230-247 (1998)
42. Bowers, K. & T.H. Stevens: Protein transport from the late Golgi to the vacuole in the yeast *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 1744, 438-454 (2005)
43. Kornfeld, S.: Structure and function of the mannose 6-phosphate/insulinlike growth factor II receptors. *Annu Rev Biochem* 61, 307-330 (1992)
44. Robinson, M.S. & J.S. Bonifacino: Adaptor-related proteins. *Curr Opin Cell Biol* 13, 444-453 (2001)
45. Costaguta, G., C.J. Stefan, E.S. Bensen, S.D. Emr & G.S. Payne: Yeast Gga coat proteins function with clathrin in Golgi to endosome transport. *Mol Biol Cell* 12, 1885-1896 (2001)
46. Cereghino, J.L., E.G. Marcusson & S.D. Emr: 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 (1995)
47. Ghosh, P., N.M. Dahms & S. Kornfeld: Mannose 6-phosphate receptors: new twists in the tale. *Nat Rev Mol Cell Biol* 4, 202-212 (2003)
48. Raymond, C.K., I. Howald-Stevenson, C.A. Vater & T.H. Stevens: Morphological classification of the yeast vacuolar protein sorting mutants: evidence for a prevacuolar compartment in class E vps mutants. *Mol Biol Cell* 3, 1389-1402 (1992)
49. Kihara, A., T. Noda, N. Ishihara & Y. Ohsumi: Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae*. *J Cell Biol* 152, 519-530 (2001)
50. Schu, P.V., K. Takegawa, M.J. Fry, J.H. Stack, M.D. Waterfield & S.D. Emr: Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science* 260, 88-91 (1993)
51. Gillooly, D.J., I.C. Morrow, M. Lindsay, R. Gould, N.J. Bryant, J.M. Gaullier, R.G. Parton & H. Stenmark: Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. *EMBO J* 19, 4577-4588 (2000)
52. Odorizzi, G., M. Babst & S.D. Emr: Phosphoinositide signaling and the regulation of membrane trafficking in yeast. *Trends Biochem Sci* 25, 229-235 (2000)
53. Corvera, S.: Phosphatidylinositol 3-kinase and the control of endosome dynamics: new players defined by structural motifs. *Traffic* 2, 859-866 (2001)
54. Simonsen, A., A.E. Wurmser, S.D. Emr & H. Stenmark: The role of phosphoinositides in membrane transport. *Curr Opin Cell Biol* 13, 485-492 (2001)
55. Yu, J.W. & M.A. Lemmon: All phox homology (PX) domains from *Saccharomyces cerevisiae* specifically recognize phosphatidylinositol 3-phosphate. *J Biol Chem* 276, 44179-44184 (2001)
56. Burda, P., S.M. Padilla, S. Sarkar & S.D. Emr: Retromer function in endosome-to-Golgi retrograde transport is regulated by the yeast Vps34 PtdIns 3-kinase. *J Cell Sci* 115, 3889-3900 (2002)
57. Zhong, Q., M.J. Watson, C.S. Lazar, A.M. Hounslow, J.P. Waltho & G.N. Gill: Determinants of the endosomal localization of sorting nexin 1. *Mol Biol Cell* 16, 2049-2057 (2005)
58. Worby, C.A. & J.E. Dixon: Sorting out the cellular functions of sorting nexins. *Nat Rev Mol Cell Biol* 3, 919-931 (2002)
59. Carlton, J., M. Bujny, A. Rutherford & P. Cullen: Sorting nexins-unifying trends and new perspectives. *Traffic* 6, 75-82 (2005)
60. Seet, L.F. & W. Hong: The Phox (PX) domain proteins and membrane traffic. *Biochim Biophys Acta* 1761, 878-896 (2006)
61. Vergés, M., I. Sebastián & K.E. Mostov: Phosphoinositide 3-kinase regulates the role of retromer in transcytosis of the polymeric immunoglobulin receptor. *Exp Cell Res (2006), doi:10.1016/j.yexcr.2006.11.010*
62. Rojas, R., S. Kametaka, C.R. Haft & J.S. Bonifacino: 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* (2006)
63. Gullapalli, A., T.A. Garrett, M.M. Paing, C.T. Griffin, Y. Yang & J. Trejo: A role for sorting nexin 2 in epidermal

growth factor receptor down-regulation: evidence for distinct functions of sorting nexin 1 and 2 in protein trafficking. *Mol Biol Cell* 15, 2143-2155 (2004)
64. Oliviusson, P., O. Heinzerling, S. Hillmer, G. Hinz, Y.C. Tse, L. Jiang & D.G. Robinson: Plant retromer, localized to the prevacuolar compartment and microvesicles in *Arabidopsis*, may interact with vacuolar sorting receptors. *Plant Cell* 18, 1239-1252 (2006)
65. Carlton, J.G., M.V. Bujny, B.J. Peter, V.M. Oorschot, A. Rutherford, R.S. Arkell, J. Klumperman, H.T. McMahon & P.J. Cullen: Sorting nexin-2 is associated with tubular elements of the early endosome, but is not essential for retromer-mediated endosome-to-TGN transport. *J Cell Sci* 118, 4527-4539 (2005)
66. Gullapalli, A., B.L. Wolfe, C.T. Griffin, T. Magnuson & J. Trejo: An essential role for SNX1 in lysosomal sorting of protease-activated receptor-1: evidence for retromer-, Hrs-, and Tsg101-independent functions of sorting nexins. *Mol Biol Cell* 17, 1228-1238 (2006)
67. Wassmer, T., N. Attar, M.V. Bujny, J. Oakley, C.J. Traer & P.J. Cullen: A loss-of-function screen reveals SNX5 and SNX6 as potential components of the mammalian retromer. *J Cell Sci* 120, 45-54 (2007)
68. Carlton, J., M. Bujny, B.J. Peter, V.M. Oorschot, A. Rutherford, H. Mellor, J. Klumperman, H.T. McMahon & P.J. Cullen: Sorting nexin-1 mediates tubular endosome-to-TGN transport through coincidence sensing of high-curvature membranes and 3-phosphoinositides. *Curr Biol* 14, 1791-1800 (2004)
69. Lefkowitz, R.J. & E.J. Whalen: beta-arrestins: traffic cops of cell signaling. *Curr Opin Cell Biol* 16, 162-168 (2004)
70. Damen, E., E. Krieger, J.E. Nielsen, J. Eygensteyn & J.E. Van Leeuwen: The human Vps29 retromer component is a metallo-phosphoesterase for a cation-independent mannose 6-phosphate receptor substrate peptide. *Biochem J* 398, 399-409 (2006)
71. Edgar, A.J. & J.M. Polak: Human homologues of yeast vacuolar protein sorting 29 and 35. *Biochem Biophys Res Commun* 277, 622-630 (2000)
72. Zhang, P., L. Yu, J. Gao, Q. Fu, F. Dai, Y. Zhao, L. Zheng & S. Zhao: Cloning and characterization of human VPS35 and mouse Vps35 and mapping of VPS35 to human chromosome 16q13-q21. *Genomics* 70, 253-257 (2000)
73. Pfeffer, S.R.: Membrane transport: retromer to the rescue. *Curr Biol* 11, R109-R111 (2001)
74. Nakada-Tsukui, K., Y. Saito-Nakano, V. Ali & T. Nozaki: 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 (2005)
75. Saito-Nakano, Y., B.J. Loftus, N. Hall & T. Nozaki: The diversity of Rab GTPases in *Entamoeba histolytica*. *Exp Parasitol* 110, 244-252 (2005)
76. Carroll, K.S., J. Hanna, I. Simon, J. Krise, P. Barbero & S.R. Pfeffer: Role of Rab9 GTPase in facilitating receptor recruitment by TIP47. *Science* 292, 1373-1376 (2001)
77. Petersen, C.M., M.S. Nielsen, A. Nykjaer, L. Jacobsen, N. Tommerup, H.H. Rasmussen, H. Roigaard, J. Gliemann, P. Madsen & S.K. Moestrup: Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography. *J Biol Chem* 272, 3599-3605 (1997)
78. Shimada, T., Y. Koumoto, L. Li, M. Yamazaki, M. Kondo, M. Nishimura & I. Hara-Nishimura: AtVPS29, a putative component of a retromer complex, is required for the efficient sorting of seed storage sroteins. *Plant Cell Physiol* 47, 1187-1194 (2006)
79. Jaillais, Y., I. Fobis-Loisy, C. Miege, C. Rollin & T. Gaude: AtSNX1 defines an endosome for auxin-carrier trafficking in Arabidopsis. *Nature* 443, 106-109 (2006)
80. Kerr, M.C., J.S. Bennetts, F. Simpson, E.C. Thomas, C. Flegg, P.A. Gleeson, C. Wicking & R.D. Teasdale: A novel mammalian retromer component, Vps26B. *Traffic* 6, 991-1001 (2005)
81. He, X., F. Li, W.P. Chang & J. Tang: GGA proteins mediate the recycling pathway of memapsin 2 (BACE). *J Biol Chem* 280, 11696-11703 (2005)
82. Small, S.A., K. Kent, A. Pierce, C. Leung, M.S. Kang, H. Okada, L. Honig, J.P. Vonsattel & T.W. Kim: Model-guided microarray implicates the retromer complex in Alzheimer's disease. *Ann Neurol* 58, 909-919 (2005)
83. Vassar, R., B.D. Bennett, S. Babu-Khan, S. Kahn, E.A. Mendiaz, P. Denis, D.B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M.A. Jarosinski, A.L. Biere, E. Curran, T. Burgess, J.C. Louis, F. Collins, J. Treanor, G. Rogers & M. Citron: Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286, 735-741 (1999)
84. Chattopadhyay, S., P.M. Roberts & D.A. Pearce: The yeast model for Batten disease: a role for Btn2p in the trafficking of the Golgi-associated vesicular targeting protein, Yif1p. *Biochem Biophys Res Commun* 302, 534-538 (2003)
85. Kama, R., M. Robinson & J.E. Gerst: Btn2, a hook1 ortholog and potential batten disease-related protein, mediates late endosome-Golgi protein sorting in yeast. *Mol Cell Biol* 27, 605-621 (2007)
86. Maxfield, F.R. & T.E. McGraw: Endocytic recycling. *Nat Rev Mol Cell Biol* 5, 121-132 (2004)
87. Kohrer, K. & S.D. Emr: The yeast VPS17 gene encodes a membrane-associated protein required for the sorting of soluble vacuolar hydrolases. *J Biol Chem* 268, 559-569 (1993)
88. Haft, C.R., M. de la Luz Sierra, V.A. Barr, D.H. Haft & S.I. Taylor: Identification of a family of sorting nexin molecules and characterization of their association with receptors. *Mol Cell Biol* 18, 7278-7287 (1998)
89. Phillips, S.A., V.A. Barr, D.H. Haft, S.I. Taylor & C.R. Haft: Identification and characterization of SNX15, a novel sorting nexin involved in protein trafficking. *J Biol Chem* 276, 5074-5084 (2001)
90. Kurten, R.C., D.L. Cadena & G.N. Gill: Enhanced degradation of EGF receptors by a sorting nexin, SNX1. *Science* 272, 1008-1010 (1996)
91. Howard, L., K.K. Nelson, R.A. Maciewicz & C.P. Blobel: Interaction of the metalloprotease disintegrins MDC9 and MDC15 with two SH3 domain-containing proteins, endophilin I and SH3PX1. *J Biol Chem* 274, 31693-31699 (1999)
92. Otsuki, T., S. Kajigaya, K. Ozawa & J.M. Liu: SNX5, a new member of the sorting nexin family, binds to the Fanconi anemia complementation group A protein. *Biochem Biophys Res Commun* 265, 630-635 (1999)

93. Florian, V., T. Schluter & R. Bohnensack: A new member of the sorting nexin family interacts with the C-terminus of P-selectin. *Biochem Biophys Res Commun* 281, 1045-1050 (2001)
94. Fuchs, U., G. Rehkamp, O.A. Haas, R. Slany, M. Konig, S. Bojesen, R.M. Bohle, C. Damm-Welk, W.D. Ludwig, J. Harbott & A. Borkhardt: The human formin-binding protein 17 (FBP17) interacts with sorting nexin, SNX2, and is an MLL-fusion partner in acute myelogeneous leukemia. *Proc Natl Acad Sci USA* 98, 8756-8761 (2001)
95. Lin, Q., C.G. Lo, R.A. Cerione & W. Yang: The Cdc42 target ACK2 interacts with sorting nexin 9 (SH3PX1) to regulate epidermal growth factor receptor degradation. *J Biol Chem* 277, 10134-10138 (2002)
96. Stockinger, W., B. Sailler, V. Strasser, B. Recheis, D. Fasching, L. Kahr, W.J. Schneider & J. Nimpf: The PX-domain protein SNX17 interacts with members of the LDL receptor family and modulates endocytosis of the LDL receptor. *EMBO J* 21, 4259-4267 (2002)
97. Leprince, C., E. Le Scolan, B. Meunier, V. Fraisier, N. Brandon, J. De Gunzburg & J. Camonis: Sorting nexin 4 and amphiphysin 2, a new partnership between endocytosis and intracellular trafficking. *J Cell Sci* 116, 1937-1948 (2003)
98. Pons, V., F. Hullin-Matsuda, M. Nauze, R. Barbaras, C. Peres, X. Collet, B. Perret, H. Chap & A. Gassama-Diagne: Enterophilin-1, a new partner of sorting nexin 1, decreases cell surface epidermal growth factor receptor. *J Biol Chem* 278, 21155-21161 (2003)
99. Vanoosthuyse, V., G. Tichtinsky, C. Dumas, T. Gaude & J.M. Cock: Interaction of calmodulin, a sorting nexin and kinase-associated protein phosphatase with the *Brassica oleracea S* locus receptor kinase. *Plant Physiol* 133, 919-929 (2003)
100. Burden, J.J., X.M. Sun, A.B. Garcia & A.K. Soutar: Sorting motifs in the intracellular domain of the low density lipoprotein receptor interact with a novel domain of sorting nexin-17. *J Biol Chem* 279, 16237-16245 (2004)
101. Heydorn, A., B.P. Sondergaard, B. Ersboll, B. Holst, F.C. Nielsen, C.R. Haft, J. Whistler & T.W. Schwartz: A library of 7TM receptor C-terminal tails. Interactions with the proposed post-endocytic sorting proteins ERM-binding phosphoprotein 50 (EBP50), N-ethylmaleimide-sensitive factor (NSF), sorting nexin 1 (SNX1), and G protein-coupled receptor-associated sorting protein (GASP). *J Biol Chem* 279, 54291-54303 (2004)
102. Towler, M.C., P.A. Gleeson, S. Hoshino, P. Rahkila, V. Manalo, N. Ohkoshi, C. Ordahl, R.G. Parton & F.M. Brodsky: Clathrin isoform CHC22, a component of neuromuscular and myotendinous junctions, binds sorting nexin 5 and has increased expression during myogenesis and muscle regeneration. *Mol Biol Cell* 15, 3181-3195 (2004)
103. Hu, Y.F., H.L. Zhang, T. Cai, S. Harashima & A.L. Notkins: The IA-2 interactome. *Diabetologia* 48, 2576-2581 (2005)
104. Schaaf, C.P., J. Benzing, T. Schmitt, D.H. Erz, M. Tewes, C.R. Bartram & J.W. Janssen: Novel interaction partners of the TPR/MET tyrosine kinase. *FASEB J* 19, 267-269 (2005)
105. Soulet, F., D. Yarar, M. Leonard & S.L. Schmid: SNX9 regulates dynamin assembly and is required for efficient clathrin-mediated endocytosis. *Mol Biol Cell* 16, 2058-2067 (2005)
106. von Zastrow, M. & K. Mostov: Signal transduction. A new thread in an intricate web. *Science* 294, 1845-1847 (2001)
107. Sorkin, A. & M. von Zastrow: Signal transduction and endocytosis: close encounters of many kinds. *Nat Rev Mol Cell Biol* 3, 600-614 (2002)
108. Bache, K.G., T. Slagsvold & H. Stenmark: Defective downregulation of receptor tyrosine kinases in cancer. *EMBO J* 23, 2707-2712 (2004)
109. Zheng, B., T. Tang, N. Tang, K. Kudlicka, K. Ohtsubo, P. Ma, J.D. Marth, M.G. Farquhar & E. Lehtonen: Essential role of RGS-PX1/sorting nexin 13 in mouse development and regulation of endocytosis dynamics. *Proc Natl Acad Sci USA* 103, 16776-16781 (2006)
110. Karkkainen, S., M. Hiipakka, J.H. Wang, I. Kleino, M. Vaha-Jaakkola, G.H. Renkema, M. Liss, R. Wagner & K. Saksela: Identification of preferred protein interactions by phage-display of the human Src homology-3 proteome. *EMBO Rep* 7, 186-191 (2006)
111. Teasdale, R.D., D. Loci, F. Houghton, L. Karlsson & P.A. Gleeson: A large family of endosome-localized proteins related to sorting nexin 1. *Biochem J* 358, 7-16 (2001)
112. Merino-Trigo, A., M.C. Kerr, F. Houghton, A. Lindberg, C. Mitchell, R.D. Teasdale & P.A. Gleeson: Sorting nexin 5 is localized to a subdomain of the early endosomes and is recruited to the plasma membrane following EGF stimulation. *J Cell Sci* 117, 6413-6424 (2004)
113. Liu, H., Z.Q. Liu, C.X. Chen, S. Magill, Y. Jiang & Y.J. Liu: Inhibitory regulation of EGF receptor degradation by sorting nexin 5. *Biochem Biophys Res Commun* 342, 537-546 (2006)
114. Peter, B.J., H.M. Kent, I.G. Mills, Y. Vallis, P.J. Butler, P.R. Evans & H.T. McMahon: BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science* 303, 495-499 (2004)
115. Lundmark, R. & S.R. Carlsson: Regulated membrane recruitment of dynamin-2 mediated by sorting nexin 9. *J Biol Chem* 279, 42694-42702 (2004)
116. Zhong, Q., C.S. Lazar, H. Tronchere, T. Sato, T. Meerloo, M. Yeo, Z. Songyang, S.D. Emr & G.N. Gill: Endosomal localization and function of sorting nexin 1. *Proc Natl Acad Sci U S A* 99, 6767-6772 (2002)
117. Bock, J.B., R.C. Lin & R.H. Scheller: A new syntaxin family member implicated in targeting of intracellular transport vesicles. *J Biol Chem* 271, 17961-17965 (1996)
118. Kurten, R.C., A.D. Eddington, P. Chowdhury, R.D. Smith, A.D. Davidson & B.B. Shank: Self-assembly and binding of a sorting nexin to sorting endosomes. *J Cell Sci* 114, 1743-1756 (2001)
119. Parks, W.T., D.B. Frank, C. Huff, C. Renfrew Haft, J. Martin, X. Meng, M.P. de Caestecker, J.G. McNally, A. Reddi, S.I. Taylor, A.B. Roberts, T. Wang & R.J. Lechleider: Sorting nexin 6, a novel SNX, interacts with the transforming growth factor-beta family of receptor serine-threonine kinases. *J Biol Chem* 276, 19332-19339 (2001)
120. Wang, Y., Y. Zhou, K. Szabo, C.R. Haft & J. Trejo: Down-regulation of protease-activated receptor-1 is regulated by sorting nexin 1. *Mol Biol Cell* 13, 1965-1976 (2002)

121. Xu, Y., H. Hortsman, L. Seet, S.H. Wong & W. Hong: SNX3 regulates endosomal function through its PX-domain-mediated interaction with PtdIns(3)P. *Nat Cell Biol* 3, 658-666 (2001)
122. Zheng, B., Y.C. Ma, R.S. Ostrom, C. Lavoie, G.N. Gill, P.A. Insel, X.Y. Huang & M.G. Farquhar: RGS-PX1, a GAP for G alpha S and sorting nexin in vesicular trafficking. *Science* 294, 1939-1942 (2001)
123. Zheng, B., C. Lavoie, T.D. Tang, P. Ma, T. Meerloo, A. Beas & M.G. Farquhar: Regulation of epidermal growth factor receptor degradation by heterotrimeric G alpha S protein. *Mol Biol Cell* 15, 5538-5550 (2004)
124. Hanson, B.J. & W. Hong: Evidence for a role of SNX16 in regulating traffic between the early and later endosomal compartments. *J Biol Chem* 278, 34617-34630 (2003)
125. Choi, J.H., W.P. Hong, M.J. Kim, J.H. Kim, S.H. Ryu & P.G. Suh: Sorting nexin 16 regulates EGF receptor trafficking by phosphatidylinositol-3-phosphate interaction with the Phox domain. *J Cell Sci* 117, 4209-4218 (2004)
126. Le Blanc, I., P.P. Luyet, V. Pons, C. Ferguson, N. Emans, A. Petiot, N. Mayran, N. Demaurex, J. Faure, R. Sadoul, R.G. Parton & J. Gruenberg: Endosome-to-cytosol transport of viral nucleocapsids. *Nat Cell Biol* 7, 653-664 (2005)
127. Uchil, P. & W. Mothes: Viral entry: a detour through multivesicular bodies. *Nat Cell Biol* 7, 641-642 (2005)
128. Czubayko, M., P. Knauth, T. Schluter, V. Florian & R. Bohnensack: Sorting nexin 17, a non-self-assembling and a PtdIns(3)P high class affinity protein, interacts with the cerebral cavernous malformation related protein KRIT1. *Biochem Biophys Res Commun* 345, 1264-12672 (2006)
129. van Kerkhof, P., J. Lee, L. McCormick, E. Tetrault, W. Lu, M. Schoenfish, V. Oorschot, G.J. Strous, J. Klumperman & G. Bu: Sorting nexin 17 facilitates LRP recycling in the early endosome. *EMBO J* 24, 2851-2861 (2005)
130. Williams, R., T. Schluter, M.S. Roberts, P. Knauth, R. Bohnensack & D.F. Cutler: Sorting nexin 17 accelerates internalization yet retards degradation of P-selectin. *Mol Biol Cell* 15, 3095-3105 (2004)
131. Knauth, P., T. Schluter, M. Czubayko, C. Kirsch, V. Florian, S. Schreckenberger, H. Hahn & R. Bohnensack: Functions of sorting nexin 17 domains and recognition motif for P-selectin trafficking. *J Mol Biol* 347, 813-825 (2005)
132. Hettema, E.H., M.J. Lewis, M.W. Black & H.R. Pelham: Retromer and the sorting nexins Snx4/41/42 mediate distinct retrieval pathways from yeast endosomes. *EMBO J* 22, 548-557 (2003)
133. Nice, D.C., T.K. Sato, P.E. Stromhaug, S.D. Emr & D.J. Klionsky: Cooperative binding of the cytoplasm to vacuole targeting pathway proteins, Cvt13 and Cvt20, to phosphatidylinositol 3-phosphate at the pre-autophagosomal structure is required for selective autophagy. *J Biol Chem* 277, 30198-30207 (2002)
134. Chin, L.S., M.C. Raynor, X. Wei, H.Q. Chen & L. Li: Hrs interacts with sorting nexin 1 and regulates degradation of epidermal growth factor receptor. *J Biol Chem* 276, 7069-7078 (2001)
135. Pelham, H.R.: Insights from yeast endosomes. *Curr Opin Cell Biol* 14, 454-462 (2002)
136. Dunn, K.W., T.E. McGraw & F.R. Maxfield: Iterative fractionation of recycling receptors from lysosomally destined ligands in an early sorting endosome. *J Cell Biol* 109, 3303-3314 (1989)
137. Vergés, M., R.J. Havel & K.E. Mostov: A tubular endosomal fraction from rat liver: biochemical evidence of receptor sorting by default. *Proc Natl Acad Sci USA* 96, 10146-10151 (1999)
138. Mayor, S., J.F. Presley & F.R. Maxfield: Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. *J Cell Biol* 121, 1257-1269 (1993)
139. Geuze, H.J., J.W. Slot & A.L. Schwartz: Membranes of sorting organelles display lateral heterogeneity in receptor distribution. *J Cell Biol* 104, 1715-1723 (1987)
140. Gruenberg, J.: The endocytic pathway: a mosaic of domains. *Nat Rev Mol Cell Biol* 2, 721-730 (2001)
141. Tai, G., L. Lu, T.L. Wang, B.L. Tang, B. Goud, L. Johannes & W. Hong: Participation of the syntaxin 5/Ykt6/GS28/GS15 SNARE complex in transport from the early/recycling endosome to the trans-Golgi network. *Mol Biol Cell* 15, 4011-4022 (2004)
142. Qin, B., M. He, X. Chen & D. Pei: Sorting nexin 10 induces giant vacuoles in mammalian cells. *J Biol Chem* 281, 36891-36896 (2006)
143. Barr, V.A., S.A. Phillips, S.I. Taylor & C.R. Haft: Overexpression of a novel sorting nexin, SNX15, affects endosome morphology and protein trafficking. *Traffic* 1, 904-916 (2000)
144. Luton, F., M. Verges, J.P. Vaerman, M. Sudol & K.E. Mostov: The SRC family protein tyrosine kinase p62yes controls polymeric IgA transcytosis *in vivo*. *Mol Cell* 4, 627-32 (1999)
145. Katzmann, D.J., G. Odorizzi & S.D. Emr: Receptor downregulation and multivesicular-body sorting. *Nat Rev Mol Cell Biol* 3, 893-905 (2002)
146. Li, L., X. Li, U. Francke & S.N. Cohen: The TSG101 tumor susceptibility gene is located in chromosome 11 band p15 and is mutated in human breast cancer. *Cell* 88, 143-154 (1997)
147. Herz, H.M., Z. Chen, H. Scherr, M. Lackey, C. Bolduc & A. Bergmann: vps25 mosaics display non-autonomous cell survival and overgrowth, and autonomous apoptosis. *Development* 133, 1871-1880 (2006)
148. Komada, M. & P. Soriano: Hrs, a FYVE finger protein localized to early endosomes, is implicated in vesicular traffic and required for ventral folding morphogenesis. *Genes Dev* 13, 1475-1485 (1999)
149. Miura, S., T. Takeshita, H. Asao, Y. Kimura, K. Murata, Y. Sasaki, J.I. Hanai, H. Beppu, T. Tsukazaki, J.L. Wrana, K. Miyazono & K. Sugamura: Hgs (Hrs), a FYVE domain protein, is involved in Smad signaling through cooperation with SARA. *Mol Cell Biol* 20, 9346-9355 (2000)
150. Ruland, J., C. Sirard, A. Elia, D. MacPherson, A. Wakeham, L. Li, J.L. de la Pompa, S.N. Cohen & T.W. Mak: p53 accumulation, defective cell proliferation, and early embryonic lethality in mice lacking tsg101. *Proc Natl Acad Sci USA* 98, 1859-1864 (2001)
151. Roudier, N., C. Lefebvre & R. Legouis: CeVPS-27 is an endosomal protein required for the molting and the endocytic trafficking of the low-density lipoprotein

receptor-related protein 1 in *Caenorhabditis elegans*. *Traffic* 6, 695-705 (2005)
152. Pulipparacharuvil, S., M.A. Akbar, S. Ray, E.A. Sevrioukov, A.S. Haberman, J. Rohrer & H. Kramer: *Drosophila* Vps16A is required for trafficking to lysosomes and biogenesis of pigment granules. *J Cell Sci* 118, 3663-3673 (2005)
153. Costantini, F., G. Radice, J.L. Lee, K.K. Chada, W. Perry & H.J. Son: Insertional mutations in transgenic mice. *Prog Nucleic Acid Res Mol Biol* 36, 159-169 (1989)
154. Robertson, E.J., F.L. Conlon, K.S. Barth, F. Costantini & J.J. Lee: Use of embryonic stem cells to study mutations affecting postimplantation development in the mouse. *Ciba Found Symp* 165, 237-250; discussion 250-255 (1992)
155. Rider, V., S.R. Jones, R.T. Foster & K. Imakawa: Changes in the temporal and spatial expression of H beta 58 during formation and maturation of the chorioallantoic placenta in the Rat. *Biol Reprod* 63, 1735-1746 (2000)
156. Paulesu, L., C. Cateni, R. Romagnoli, F. Chellini, F. Angelini, F.M. Guarino, V. Rider, K. Imakawa & E. Bigliardi: Evidence of H beta 58, a gene involved in mammalian placental development, in the three-toed skink, Chalcides chalcides (Squamata: Scincidae), a viviparous placentotrophic reptile. *Placenta* 22, 735-741 (2001)
157. Robinson, J.S., T.R. Graham & S.D. Emr: A putative zinc finger protein, *Saccharomyces cerevisiae* Vps18p, affects late Golgi functions required for vacuolar protein sorting and efficient alpha-factor prohormone maturation. *Mol Cell Biol* 11, 5813-5824 (1991)
158. van der Bliek, A.M. & E.M. Meyerowitz: Dynamin-like protein encoded by the *Drosophila* shibire gene associated with vesicular traffic. *Nature* 351, 411-414 (1991)
159. Jones, E.W., G.S. Zubenko & R.R. Parker: PEP4 gene function is required for expression of several vacuolar hydrolases in *Saccharomyces cerevisiae*. *Genetics* 102, 665-677 (1982)
160. Jollie, W.P.: Development, morphology, and function of the yolk-sac placenta of laboratory rodents. *Teratology* 41, 361-381 (1990)
161. Beddington, R.S. & E.J. Robertson: Anterior patterning in mouse. *Trends Genet* 14, 277-284 (1998)
162. Bielinska, M., N. Narita & D.B. Wilson: Distinct roles for visceral endoderm during embryonic mouse development. *Int J Dev Biol* 43, 183-205 (1999)
163. Rothstein, J.L., D. Johnson, J.A. DeLoia, J. Skowronski, D. Solter & B. Knowles: Gene expression during preimplantation mouse development. *Genes Dev* 6, 1190-1201 (1992)
164. Paravicini, G., B.F. Horazdovsky & S.D. Emr: Alternative pathways for the sorting of soluble vacuolar proteins in yeast: a vps35 null mutant missorts and secretes only a subset of vacuolar hydrolases. *Mol Biol Cell* 3, 415-427 (1992)
165. Nagy, A., E. Gocza, E.M. Diaz, V.R. Prideaux, E. Ivanyi, M. Markkula & J. Rossant: Embryonic stem cells alone are able to support fetal development in the mouse. *Development* 110, 815-821 (1990)
166. Nagy, A., J. Rossant, R. Nagy, W. Abramow-Newerly & J.C. Roder: Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc Natl Acad Sci USA* 90, 8424-8428 (1993)
167. Luetteke, N.C., H.K. Phillips, T.H. Qiu, N.G. Copeland, H.S. Earp, N.A. Jenkins & D.C. Lee: The mouse waved-2 phenotype results from a point mutation in the EGF receptor tyrosine kinase. *Genes Dev* 8, 399-413 (1994)
168. Fowler, K.J., F. Walker, W. Alexander, M.L. Hibbs, E.C. Nice, R.M. Bohmer, G.B. Mann, C. Thumwood, R. Maglitto, J.A. Danks, R. Chetty, A.W. Burgess & A.R. Dunn: A mutation in the epidermal growth factor receptor in waved-2 mice has a profound effect on receptor biochemistry that results in impaired lactation. *Proc Natl Acad Sci USA* 92, 1465-1469 (1995)
169. Chen, B., R.T. Bronson, L.D. Klaman, T.G. Hampton, J.F. Wang, P.J. Green, T. Magnuson, P.S. Douglas, J.P. Morgan & B.G. Neel: Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. *Nat Genet* 24, 296-299 (2000)
170. Kornfeld, S. & I. Mellman: The biogenesis of lysosomes. *Annu Rev Cell Biol* 5, 483-525 (1989)
171. Yoo, K.W., E.H. Kim, S.H. Jung, M. Rhee, B.K. Koo, K.J. Yoon, Y.Y. Kong & C.H. Kim: Snx5, as a Mind bomb-binding protein, is expressed in hematopoietic and endothelial precursor cells in zebrafish. *FEBS Lett* 580, 4409-4416 (2006)
172. Dittmer, F., E.J. Ulbrich, A. Hafner, W. Schmahl, T. Meister, R. Pohlmann & K. von Figura: Alternative mechanisms for trafficking of lysosomal enzymes in mannose 6-phosphate receptor-deficient mice are cell type-specific. *J Cell Sci* 112, 1591-1597 (1999)
173. Futerman, A.H. & G. van Meer: The cell biology of lysosomal storage disorders. *Nat Rev Mol Cell Biol* 5, 554-565 (2004)
174. Vellodi, A.: Lysosomal storage disorders. *Br J Haematol* 128, 413-431 (2005)
175. Wang, Z.Q., M.R. Fung, D.P. Barlow & E.F. Wagner: Regulation of embryonic growth and lysosomal targeting by the imprinted Igf2/Mpr gene. *Nature* 372, 464-467 (1994)
176. Filson, A.J., A. Louvi, A. Efstratiadis & E.J. Robertson: Rescue of the T-associated maternal effect in mice carrying null mutations in Igf-2 and Igf2r, two reciprocally imprinted genes. *Development* 118, 731-736 (1993)
177. Lee, S.J. & D. Nathans: Proliferin secreted by cultured cells binds to mannose 6-phosphate receptors. *J Biol Chem* 263, 3521-3527 (1988)
178. Kang, J.X., J. Bell, R.L. Beard & R.A. Chandraratna: Mannose 6-phosphate/insulin-like growth factor II receptor mediates the growth-inhibitory effects of retinoids. *Cell Growth Differ* 10, 591-600 (1999)
179. Hawkes, C. & S. Kar: The insulin-like growth factor-II/mannose-6-phosphate receptor: structure, distribution and function in the central nervous system. *Brain Res Rev* 44, 117-140 (2004)
180. Köster, A., P. Saftig, U. Matzner, K. von Figura, C. Peters & R. Pohlmann: Targeted disruption of the M(r) 46,000 mannose 6-phosphate receptor gene in mice results in misrouting of lysosomal proteins. *EMBO J* 12, 5219-5223 (1993)
181. Ludwig, T., R. Le Borgne & B. Hoflack: Roles for mannose-6-phosphate receptors in lysosomal enzyme sorting, IGF-II binding and clathrin-coat assembly. *Trends Cell Biol* 5, 202-206 (1995)
182. Johansen, F.E., M. Pekna, I.N. Norderhaug, B. Haneberg, M.A. Hietala, P. Krajci, C. Betsholtz & P.

Brandtzaeg: Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. *J Exp Med* 190, 915-922 (1999)
183. Shimada, S., M. Kawaguchi-Miyashita, A. Kushiro, T. Sato, M. Nanno, T. Sako, Y. Matsuoka, K. Sudo, Y. Tagawa, Y. Iwakura & M. Ohwaki: Generation of polymeric immunoglobulin receptor-deficient mouse with marked reduction of secretory IgA. *J Immunol* 163, 5367-5373 (1999)
184. Brandtzaeg, P., D.E. Nilssen, T.O. Rognum & P.S. Thrane: Ontogeny of the mucosal immune system and IgA deficiency. *Gastroenterol Clin North Am* 20, 397-439 (1991)
185. Ben-Hur, H., P. Gurevich, A. Elhayany, M. Moldavsky, L. Shvidel, E. Shezen, N. Shumlin & I. Zusman: Secretory immune system in human embryonic and fetal development: joining chain and immunoglobulin transport (Review). *Int J Mol Med* 14, 35-42 (2004)
186. Polakis, P.: Wnt signaling and cancer. *Genes Dev* 14, 1837-1851 (2000)
187. Cadigan, K.M. & R. Nusse: Wnt signaling: a common theme in animal development. *Genes Dev* 11, 3286-3305 (1997)
188. Wodarz, A. & R. Nusse: Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol* 14, 59-88 (1998)
189. Peifer, M. & P. Polakis: Wnt signaling in oncogenesis and embryogenesis--a look outside the nucleus. *Science* 287, 1606-1609 (2000)
190. Logan, C.Y. & R. Nusse: The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20, 781-810 (2004)
191. Cadigan, K.M. & Y.I. Liu: Wnt signaling: complexity at the surface. *J Cell Sci* 119, 395-402 (2006)
192. Whangbo, J. & C. Kenyon: A Wnt signaling system that specifies two patterns of cell migration in *C. elegans*. *Mol Cell* 4, 851-858 (1999)
193. Tavernarakis, N. & M. Driscoll: Mechanotransduction in *Caenorhabditis elegans*: the role of DEG/ENaC ion channels. *Cell Biochem Biophys* 35, 1-18 (2001)
194. Hilliard, M.A. & C.I. Bargmann: Wnt signals and frizzled activity orient anterior-posterior axon outgrowth in *C. elegans*. *Dev Cell* 10, 379-390 (2006)
195. Pan, C.L., J.E. Howell, S.G. Clark, M. Hilliard, S. Cordes, C.I. Bargmann & G. Garriga: Multiple Wnts and frizzled receptors regulate anteriorly directed cell and growth cone migrations in *Caenorhabditis elegans*. *Dev Cell* 10, 367-377 (2006)
196. Fraser, A.G., R.S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann & J. Ahringer: Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature* 408, 325-330 (2000)
197. Nusse, R.: Wnts and Hedgehogs: lipid-modified proteins and similarities in signaling mechanisms at the cell surface. *Development* 130, 5297-5305 (2003)
198. Zhai, L., D. Chaturvedi & S. Cumberledge: *Drosophila* wnt-1 undergoes a hydrophobic modification and is targeted to lipid rafts, a process that requires porcupine. *J Biol Chem* 279, 33220-33227 (2004)
199. Mann, R.S. & J. Culi: Developmental biology: morphogens hitch a greasy ride. *Nature* 435, 30-33 (2005)
200. Panáková, D., H. Sprong, E. Marois, C. Thiele & S. Eaton: Lipoprotein particles are required for Hedgehog and Wingless signalling. *Nature* 435, 58-65 (2005)
201. Bänziger, C., D. Soldini, C. Schutt, P. Zipperlen, G. Hausmann & K. Basler: Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell* 125, 509-522 (2006)
202. Bartscherer, K., N. Pelte, D. Ingelfinger & M. Boutros: Secretion of Wnt ligands requires Evi, a conserved transmembrane protein. *Cell* 125, 523-533 (2006)
203. Ching, W. & R. Nusse: A dedicated Wnt secretion factor. *Cell* 125, 432-433 (2006)
204. Goodman, R.M., S. Thombre, Z. Firtina, D. Gray, D. Betts, J. Roebuck, E.P. Spana & E.M. Selva: Sprinter: a novel transmembrane protein required for Wg secretion and signaling. *Development* 133, 4901-4911 (2006)
205. Pfeiffer, S., S. Ricardo, J.B. Manneville, C. Alexandre & J.P. Vincent: Producing cells retain and recycle Wingless in *Drosophila* embryos. *Curr Biol* 12, 957-962 (2002)
206. Kornfeld, S.: Trafficking of lysosomal enzymes in normal and disease states. *J Clin Invest* 77, 1-6 (1986)
207. Schlegel, A., R.G. Pestell & M.P. Lisanti: Caveolins in cholesterol trafficking and signal transduction: implications for human disease. *Front Biosci* 5, D929-D937 (2000)
208. Simons, K. & J. Gruenberg: Jamming the endosomal system: lipid rafts and lysosomal storage diseases. *Trends Cell Biol* 10, 459-462 (2000)
209. Stein, M., A. Wandinger-Ness & T. Roitbak: Altered trafficking and epithelial cell polarity in disease. *Trends Cell Biol* 12, 374-381 (2002)
210. Chang, T.Y., P.C. Reid, S. Sugii, N. Ohgami, J.C. Cruz & C.C. Chang: Niemann-Pick type C disease and intracellular cholesterol trafficking. *J Biol Chem* 280, 20917-20920 (2005)
211. Lee, D.B., E. Huang & H.J. Ward: Tight junction biology and kidney dysfunction. *Am J Physiol Renal Physiol* 290, F20-F34 (2006)
212. Huang, X., K. Suyama, J. Buchanan, A.J. Zhu & M.P. Scott: A Drosophila model of the Niemann-Pick type C lysosome storage disease: dnpc1a is required for molting and sterol homeostasis. *Development* 132, 5115-5124 (2005)
213. Dittmer, F., A. Hafner, E.J. Ulbrich, J.D. Moritz, P. Schmidt, W. Schmahl, R. Pohlmann & K.V. Figura: I-cell disease-like phenotype in mice deficient in mannose 6-phosphate receptors. *Transgenic Res* 7, 473-483 (1998)
214. Jacobsen, L., P. Madsen, S.K. Moestrup, A.H. Lund, N. Tommerup, A. Nykjaer, L. Sottrup-Jensen, J. Gliemann & C.M. Petersen: Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein. *J Biol Chem* 271, 31379-31383 (1996)
215. Yamazaki, H., H. Bujo, J. Kusunoki, K. Seimiya, T. Kanaki, N. Morisaki, W.J. Schneider & Y. Saito: Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member. *J Biol Chem* 271, 24761-24768 (1996)
216. Motoi, Y., T. Aizawa, S. Haga, S. Nakamura, Y. Namba & K. Ikeda: Neuronal localization of a novel

mosaic apolipoprotein E receptor, LR11, in rat and human brain. *Brain Res* 833, 209-215 (1999)
217. Scherzer, C.R., K. Offe, M. Gearing, H.D. Rees, G. Fang, C.J. Heilman, C. Schaller, H. Bujo, A.I. Levey & J.J. Lah: Loss of apolipoprotein E receptor LR11 in Alzheimer disease. *Arch Neurol* 61, 1200-1205 (2004)
218. Andersen, O.M., J. Reiche, V. Schmidt, M. Gotthardt, R. Spoelgen, J. Behlke, C.A. von Arnim, T. Breiderhoff, P. Jansen, X. Wu, K.R. Bales, R. Cappai, C.L. Masters, J. Gliemann, E.J. Mufson, B.T. Hyman, S.M. Paul, A. Nykjaer & T.E. Willnow: Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc Natl Acad Sci USA* 102, 13461-13466 (2005)
219. Spoelgen, R., C.A. von Arnim, A.V. Thomas, I.D. Peltan, M. Koker, A. Deng, M.C. Irizarry, O.M. Andersen, T.E. Willnow & B.T. Hyman: Interaction of the cytosolic domains of sorLA/LR11 with the amyloid precursor protein (APP) and beta-secretase beta-site APP-cleaving enzyme. *J Neurosci* 26, 418-428 (2006)
220. Mao, M., G. Fu, J.S. Wu, Q.H. Zhang, J. Zhou, L.X. Kan, Q.H. Huang, K.L. He, B.W. Gu, Z.G. Han, Y. Shen, J. Gu, Y.P. Yu, S.H. Xu, Y.X. Wang, S.J. Chen & Z. Chen: Identification of genes expressed in human CD34(+) hematopoietic stem/progenitor cells by expressed sequence tags and efficient full-length cDNA cloning. *Proc Natl Acad Sci USA* 95, 8175-8180 (1998)
221. Ertekin-Taner, N., N. Graff-Radford, L.H. Younkin, C. Eckman, M. Baker, J. Adamson, J. Ronald, J. Blangero, M. Hutton & S.G. Younkin: Linkage of plasma Abeta42 to a quantitative locus on chromosome 10 in late-onset Alzheimer's disease pedigrees. *Science* 290, 2303-2304 (2000)
222. Myers, A., P. Holmans, H. Marshall, J. Kwon, D. Meyer, D. Ramic, S. Shears, J. Booth, F.W. DeVrieze, R. Crook, M. Hamshere, R. Abraham, N. Tunstall, F. Rice, S. Carty, S. Lillystone, P. Kehoe, V. Rudrasingham, L. Jones, S. Lovestone, J. Perez-Tur, J. Williams, M.J. Owen, J. Hardy & A.M. Goate: Susceptibility locus for Alzheimer's disease on chromosome 10. *Science* 290, 2304-2305 (2000)
223. Riemenschneider, M., A. Schoepfer-Wendels, P. Friedrich, L. Konta, S.M. Laws, J.C. Mueller, A. Kurz & H. Forstl: No association of Vacuolar protein sorting 26 polymorphisms with Alzheimer's disease. *Neurobiol Aging (2006), doi:10.1016/j.neurobiolaging.2006.05.009*
224. Phillips, S.N., J.W. Benedict, J.M. Weimer & D.A. Pearce: CLN3, the protein associated with batten disease: structure, function and localization. *J Neurosci Res* 79, 573-583 (2005)
225. Goebel, H.H. & K.E. Wisniewski: Current state of clinical and morphological features in human NCL. *Brain Pathol* 14, 61-69 (2004)
226. Mole, S.E.: The genetic spectrum of human neuronal ceroid-lipofuscinoses. *Brain Pathol* 14, 70-76 (2004)
227. Barrowman, J., W. Wang, Y. Zhang & S. Ferro-Novick: The Yip1p.Yif1p complex is required for the fusion competence of endoplasmic reticulum-derived vesicles. *J Biol Chem* 278, 19878-19884 (2003)
228. Kerr, M.C., M.R. Lindsay, R. Luetterforst, N. Hamilton, F. Simpson, R.G. Parton, P.A. Gleeson & R.D. Teasdale: Visualisation of macropinosome maturation by the recruitment of sorting nexins. *J Cell Sci* 119, 3967-3980 (2006)
229. Voos, W. & T.H. Stevens: Retrieval of resident late-Golgi membrane proteins from the prevacuolar compartment of *Saccharomyces cerevisiae* is dependent on the function of Grd19p. *J Cell Biol* 140, 577-590 (1998)
230. Ekena, K. & T.H. Stevens: The *Saccharomyces cerevisiae* MVP1 gene interacts with VPS1 and is required for vacuolar protein sorting. *Mol Cell Biol* 15, 1671-1678 (1995)
231. Luo, W. & A. Chang: Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant. *J Cell Biol* 138, 731-746 (1997)
232. Gao, M. & C.A. Kaiser: A conserved GTPase-containing complex is required for intracellular sorting of the general amino-acid permease in yeast. *Nat Cell Biol* 8, 657-667 (2006)
233. Rubio-Texeira, M. & C.A. Kaiser: Amino acids regulate retrieval of the yeast general amino acid permease from the vacuolar targeting pathway. *Mol Biol Cell* 17, 3031-3050 (2006)
234. MaCaulay, S.L., V. Stoichevska, J. Grusovin, K.H. Gough, L.A. Castelli & C.W. Ward: Insulin stimulates movement of sorting nexin 9 between cellular compartments: a putative role mediating cell surface receptor expression and insulin action. *Biochem J* 376, 123-134 (2003)
235. Scott, A., J. Gaspar, M.D. Stuchell-Brereton, S.L. Alam, J.J. Skalicky & W.I. Sundquist: Structure and ESCRT-III protein interactions of the MIT domain of human VPS4A. *Proc Natl Acad Sci USA* 102, 13813-13818 (2005)

Abbreviations: A/P: anteroposterior; AD: Alzheimer disease; ALML, ALMR, AVM, PLML, PLMR, and PVM: touch-receptor / mechanosensory neurons; ApoER2: ApoE receptor 2; APP: amyloid precursor protein; ARF: ADP-ribosylation factor; Aβ: amyloid-β; BACE: β-site APP cleaving enzyme; BAR: Bin/Amphiphysin/Rvs; BD: Batten disease; Btn: BD related protein; CD-MPR: cation-dependent mannose 6-phosphate receptor; CI-MPR: cation-independent mannose 6-phosphate receptor; CLN3: ceroid lipofuscinosis neuronal-3; CPY: carboxypeptidase Y; E: embryonic day; EGFR: EGF receptor; EGL: Egg-laying defective; ES: embryonic stem; ESCRT: endosomal sorting complex required for transport; Evi: Evenness interrupted; FERM: band 4.1-ezrin-radixin-moesin; GGA: Golgi-associated, gamma-ear containing, ARF-binding; GPCR: G-protein coupled receptor; Hh: Hedgehog; HSN: hermaphrodite specific neurons; Hrs: hepatocyte growth factor-regulated tyrosine kinase substrate; ICD: I-cell disease; IGF2: insulin-like growth factor II; JNCL: juvenile neuronal ceroid lipofuscinosis; LDLR: LDL receptor; LRP: LDLR-related protein; LSD: lysosomal storage diseases; MEF: mouse embryonic fibroblasts; MEM3: maternal-embryonic 3; MOM: more mesoderm; MVB: multivesicular body; NP: Niemann-Pick; PAR-1: protease activated receptor-1; Pep (*PEP*): peptidase-deficient; PGE: post-Golgi endosome; PI 3-P: phosphatidylinositol 3-phosphate; PI3K: phosphoinositide 3-kinase; pIgA: polymeric immunoglobulin A; pIgR: polymeric

immunoglobulin receptor; Porc: porcupine; PVC: prevacuolar compartment; PX: Phox homology; RGS: regulator of G protein signaling; SNX: sorting nexin; Srt: Sprinter; TGF-β: transforming growth factor-β; TGN: *trans*-Golgi network; TIP47: tail-interacting protein of 47 kD; Vpl (*VPL*): Vacuolar protein localization; Vps (*VPS*): Vacuolar protein sorting; Vpt (*VPT*): Vacuolar protein targeting; VSR: vacuolar sorting receptor; *Wa-2*: waved-2; Wls: Wntless; Yif1p: Yip1p (yeast Golgi integral membrane protein) -interacting factor

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