# Morton Lecture

# Phosphoinositides and the regulation of tubular-based endosomal sorting

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## Abstract

**From the pioneering work of Mabel and Lowell Hokin in the 1950s, the biology of this specific isomer of hexahydroxycyclohexane and its phosphorylated derivatives, in the form of inositol phosphates and phosphoinositides, has expanded to fill virtually every corner of cell biology, whole-organism physiology and development. In the present paper, I give a personal view of the role played by phosphoinositides in regulating the function of the endosomal network, and, in so doing, highlight some of the basic properties through which phosphoinositides regulate cell function.**

## Introduction

How can a simple turtle have a fundamental affect on one's research career? Although few outside of marine biology will have even contemplated such a question, those familiar with Bernard Agranoff [1] and the biology of D-*myo*-inositol will immediately become reflective. From the pioneering work of Mabel and Lowell Hokin in the 1950s [2], the biology of this specific isomer of hexahydroxycyclohexane and its phosphorylated derivatives, in the form of inositol phosphates and phosphoinositides, has expanded to fill virtually every corner of cell biology, whole-organism physiology and development [3–10]. For those working in the field, the foundations of our understanding lie firmly with those inositol aficionados who defined the enzymology of inositol kinases and phosphatases and, in so doing, focused research on specific inositol phosphates and phosphoinositides whose turnover was manipulated by the cellular environment. This allowed the identification of receptors specific for individual phosphorylated inositol derivatives and the realization that binding occurs through a number of evolutionarily conserved modular protein domains. In turn, this led to an explosion in our molecular understanding of how inositol turnover is coupled to cell function, which, combined with modern molecular genetics and the identification of disease-associated mutations, is opening up new and exciting avenues in inositol research. A number of truly excellent reviews have described at some length specific aspects of inositol phosphate and phosphoinositide biology, and to these I refer the more specialist reader [3–10]. In the present paper, I give a personal view that focuses on the role of phosphoinositides in regulating the function of the endosomal network, but, in so doing, I hope to highlight some of the basic properties through which phosphoinositides regulate cell function.

**Key words:** Bin/amphiphysin/Rvs domain (BAR domain), endosome, phosphoinositide, retromer, sorting nexin (SNX).

**Abbreviations used:** Arp2/3, actin-related protein 2/3; BAR, Bin/amphiphysin/Rvs; CI-MPR, cation-independent mannose 6-phosphate receptor; DAG, 1-stearoyl 2-arachidonoyl diacylglycerol; EEA1, early endosomal antigen 1; EGFR, epidermal growth factor receptor; ERC, endocytic recycling compartment; ETC, endosome–*trans*-Golgi network carrier; FYVE, Fab1, YOTB, Vac1, EEA1 homology; ILV, intraluminal vesicle; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinase; PX, phox homology; SNX, sorting nexin; TGN, *trans*-Golgi network; VPS, vacuolar protein sorting; WASP, Wiskott–Aldrich syndrome protein; WASH, WASP and Scar homologue.

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## Inositol nomenclature: shaking hands with the turtle

The beauty of D-*myo*-inositol is that it contains six hydroxy residues, one on each of its six carbons, all of which have the potential to be phosphorylated (indeed, over 30 inositol phosphates have so far been identified in eukaryotes) [5,6] (Figure 1). For those outside the field, understanding the naming of these phosphorylated derivatives can be a daunting task, especially when one considers Howarth projections drawn on a two-dimensional page. However, remember the turtle and a few basic rules and everything becomes relatively straightforward (Figure 1).

The analogy, first described by Bernard Agranoff [1], and now accepted by the Nomenclature Committee of the


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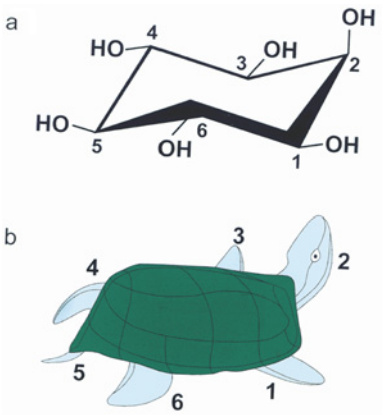
**Figure 1 | Beauty lies in the eye of the beholder: D-*myo*-inositol**

(**A**) Structure of D-*myo*-inositol showing the staggered chair conformation and the equatorial position of all hydroxy groups with the notable exception of the axial 2-OH. (**B**) The Agranoff turtle. Reproduced from [109] with permission.




International Union of Biochemistry [11], is as follows. A turtle has six appendages: head, tail and four flippers. When crawling along a beach, the turtle's tail and four flippers are moving parallel with the beach, they are effectively in an equatorial position. In order to see where it is going, the turtle must raise its head to a position that can be viewed as being at right angles to the beach; effectively, the head reorients to become axial. Looking at a Howarth projection, one can see that five of the hydroxy residues within the D-*myo*-inositol ring are equatorial, while the remaining hydroxy residue is axial. In polite society, etiquette dictates that on first meeting a turtle, one should greet it by shaking its right front flipper. The right front flipper is therefore considered carbon D1. By numbering the axial turtle's head as carbon D2, it follows that the left front flipper is carbon D3, left hind flipper D4, tail D5 and right hind flipper D6. D-*myo*-inositol 1,4,5-trisphosphate therefore has phosphate groups place on the turtle's right front flipper, left hind flipper and tail.

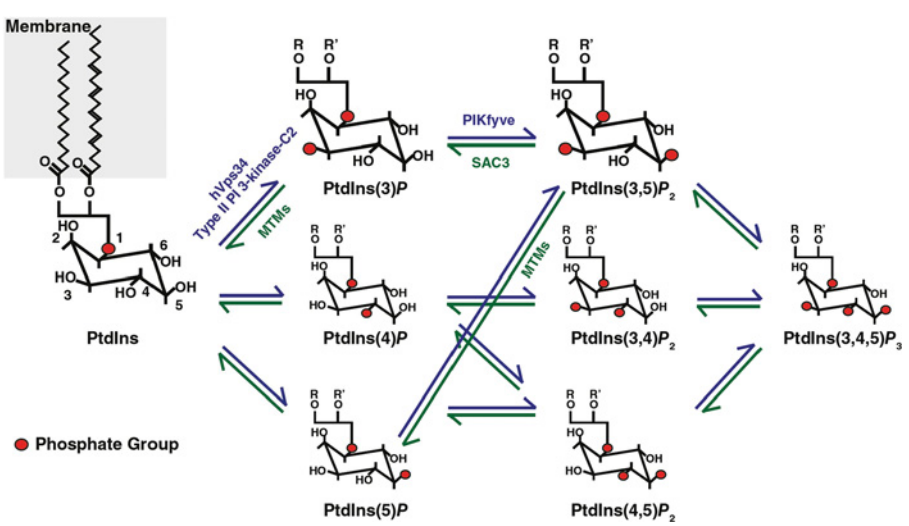
## Phosphoinositides

A major class of phosphorylated D-*myo*-inositol-containing compounds are the membrane-associated phosphoinositides. Here the parent lipid is PtdIns (phosphatidylinositol), where the inositol 1-monophosphate headgroup is coupled (shakes hands), almost exclusively to DAG (1-stearoyl 2-arachidonoyl diacylglycerol). This allows PtdIns to partition into the inner leaflet of cellular membranes, oriented such that its inositol headgroup is exposed to the cytosol. Although the remaining five hydroxy groups are available for phosphorylation, only the D3, D4 and D5 are actually phosphorylated *in vivo*, either singly or in combinations. This generates a family of seven stereospecific phosphorylated derivatives: the phosphoinositides (Figure 2).

Phosphoinositides are minor membrane lipids. PtdIns is the most abundant, comprising less than 10% of total cell phospholipids. PtdIns is synthesized principally in the endoplasmic reticulum before being transferred to other cellular compartments by membrane trafficking or via the action of specific lipid-transfer proteins. Relative to PtdIns, PtdIns4*P* (phosphatidylinositol 4-monophosphate) is present

**Figure 2 | The phosphoinositide family**

PtdIns consists of a membrane-embedded lipid moiety composed of DAG linked to a cytosolic headgroup comprising D-*myo*-inositol 1-phosphate. Reversible phosphorylation of 3-, 4- and/or 5-OH positions, either singly or in combination, yields individual members of the phosphoinositide family. The endolysosomal species, PtdIns(3)*P* and PtdIns(3,5)$P_2$ form the focus of the present review. MTMs, myotubularin phosphatases. Reproduced from [110] with permission.

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at approximately 0.05 % and PtdIns(4,5)$P_2$ (phosphatidylinositol 4,5-bisphosphate) is present at approximately 0.005 %. PtdIns3*P* (phosphatidylinositol 3-monophosphate) and PtdIns5*P* (phosphatidylinositol 5-monophosphate) are at approximately 0.002 % and PtdIns(3,4)$P_2$ (phosphatidylinositol 3,4-bisphosphate), PtdIns(3,5)$P_2$ (phosphatidylinositol 3,5-bisphosphate) and PtdIns(3,4,5)$P_3$ (phosphatidylinositol 3,4,5-trisphosphate) are all approximately at 0.0001 % [12,13].

A vital element in the biology of phosphoinositides is their rapid inter-conversion through the actions of specific kinases and phosphatases (see [14–19] for detailed reviews of the extensive literature concerning these enzymes). Briefly, mammalian cells express families of kinases and phosphatases which add or remove phosphates from the D3, D4 and D5 position of the inositol headgroup of specific phosphoinositide isomers. Thus Class I PI3Ks (phosphoinositide 3-kinases) generate PtdIns(3,4,5)$P_3$ by catalysing the addition of a phosphate to the 3-position of PtdIns(4,5)$P_2$, whereas PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a 3-phosphatase that dephosphorylates PtdIns(3,4,5)$P_3$, regenerating PtdIns(4,5)$P_2$ [19,20]. Broadly speaking, the molecular architecture of phosphoinositide kinases and phosphatases are organized so as to target the catalytic region to different subcellular membranes through a mechanism that, for the majority of enzymes, remains to be completely defined. This has the effect of spatially restricting the activity of individual kinases and phosphatases to the membrane cytosol interface of specified compartments. If one adds to this the fact that these enzymes are also designed to respond to changes in the cellular state, for example receptor activation or changes to the cell cycle, then a picture emerges in which the metabolic turnover of specific phosphoinositides can be spatially and temporal restricted to unique membrane compartments.

## The cellular phosphoinositide map

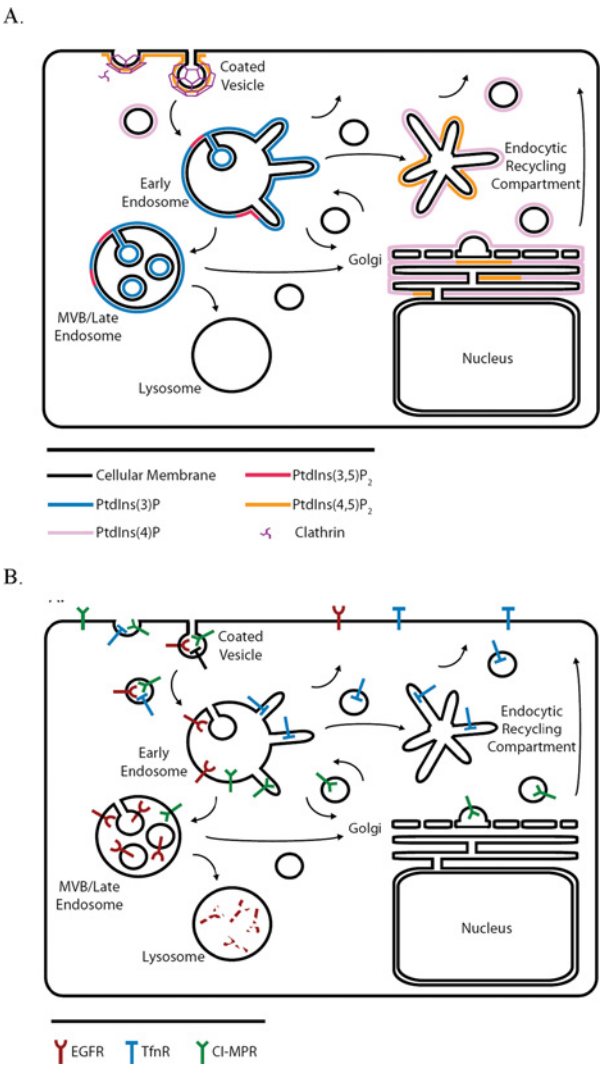
Although the exact phosphoinositide composition of individual membrane compartments is not comprehensively understood, imaging-based studies employing fluorescently tagged isomer-specific phosphoinositide biosensors have described a general map of phosphoinositide distribution [21,22]. The major site of enrichment for PtdIns(4,5)$P_2$, PtdIns(3,4)$P_2$ and PtdIns(3,4,5)$P_3$ is the plasma membrane, PtdIns(4)*P* and PtdIns(4,5)$P_2$ are found on the ERC (endocytic recycling compartment) and the TGN (*trans*-Golgi network), and PtdIns(5)*P* appears to be present within nuclei. As for the endosomal network, early endosomes are characterized by the presence of PtdIns(3)*P*, whereas PtdIns(3,5)$P_2$ has been suggested to localize to late elements of the endocytic network (Figure 3).

Such a phosphoinositide map should always be taken as a generality. It is clear that, under certain physiological conditions, specific phosphoinositides can be generated on membranes other than those described within the map. For example, the early endosomal phosphoinositide PtdIns(3)*P* can be generated on the inner leaflet of the plasma membrane during insulin stimulation [23], appears to be associated with the outer plasma membrane leaflet [24] and is present in the endoplasmic reticulum during the formation of autophagosomes [25].

Broadly speaking, three basic roles have been proposed for phosphoinositides, all of which occur solely or partially at the membrane–cytosol interface: first, as substrates for phosphoinositide-specific phospholipases leading to the generation of the second class of inositol-containing compounds, the water-soluble inositol phosphates; secondly, as regulators of integral membrane proteins such as ion

**Figure 3 | The phosphoinositide map and its relationship with cargo sorting through the endolysosomal network**

(**A**) Steady-state distribution of phosphoinositides within the network: the 'phosphoinositide map'. MVB, multivesicular body. (**B**) Interconnected membranous compartments that constitute the endolysosomal network and the various routes for sorting of specific cargoes (see the text for more details). TfnR, transferrin receptor.

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channels; and thirdly, to act as membrane-localized sites for the recruitment and/or activation of cytosolic proteins.

## Sensing the phosphoinositide identity code

The realization that modular PH (pleckstrin homology) domains constitute evolutionarily conserved motifs that by binding phosphoinositides target their host proteins to membranes enriched in the corresponding phosphoinositide provided a breakthrough in phosphoinositide research [26]. The identification of other modular phosphoinositide-binding domains, including FYVE [Fab1, YOTB, Vac1, EEA1 (early endosomal antigen 1) homology], PX (phox homology), ENTH (epsin N-terminal homology) and ANTH (AP180 N-terminal homology) domains, enormously extended the molecular dissection of phosphoinositide-mediated cell biology (for structural details of these and other phosphoinositide-binding domains, plus broader discussion of their roles in signalling and membrane trafficking, see [27–29]).

Phosphoinositide-binding sites and surfaces are associated with integral proteins such as ion channels or, more commonly, peripheral cytosolic proteins. For the latter, binding is often characterized by relatively low (often micromolar) affinities [21,27,30]. This favours a rapid reversible mode of binding where the phosphoinositide-binding protein is constantly sampling the membrane surface for the presence of the corresponding phosphoinositide, enriching at sites where the lipid resides [21,27,30]. By itself, such binding may not be of sufficient strength to lead to stable membrane association. Here, the affinity for phosphoinositide recognition needs to be enhanced by combination with another effect to drive productive membrane localization. This additional localization signal can simply be interaction with a pre-localized binding partner [21,27,30] or can be the avidity-mediated enhancement in affinity that occurs through generation of multivalent interactions with membranes. Thus, although phosphoinositides are commonly regarded as membrane-association cues that target peripheral proteins to membrane surfaces, it is increasingly clear that other interactions besides recognition of phosphoinositides function to restrict the association of phosphoinositide-binding proteins to cellular membranes. Such avidity-dependent phosphoinositide-mediated membrane association can take multiple forms. For example, the PH domain of dynamin has a low-millimolar affinity for membranes containing PtdIns(4,5)$P_2$ [31]. However, upon self-assembly into oligomers, the apparent affinity shifts towards the micro/nano-molar range, leading to stabilization of dynamin oligmers to PtdIns(4,5)$P_2$-enriched regions of the plasma membrane during the process of endocytic scission [32].

Why incorporate phosphoinositide binding into a membrane-association system that is based on avidity? Low affinity gives rise to a highly plastic system, defined by dynamic instability, that allows rapid remodelling in response to, for example, fluctuations in the level of phosphoinositides. Moreover, the fact that membrane association depends upon multivalent interactions builds into the system a property referred to as coincidence detection [30]. Here, two or more signals, one of which is phosphoinositide recognition, are required to be present at the same time within the same membrane in order to achieve membrane association of the phosphoinositide-binding protein. In turn, coincidence detection affords a greater element of regulation as manipulation of signals, other than the level of phosphoinositides, can have pronounced effects on the membrane association and hence the function of phosphoinositide-binding proteins. Avidity-based membrane association thereby gives rise to a greater spatial and temporal regulation of phosphoinositide-mediated cellular regulation.

## Phosphoinositides and the endolysosomal network

Using classic centrifugal fractionation of rat liver homogenates, Christian de Duve and colleagues described in 1955 [33] that the activity of acid phosphatase, and various other acid pH optimum hydrolyases, was enclosed within granules biochemically distinct from mitochondria (defined by cytochrome oxidase activity) and microsomes (an endoplasmic reticulum-enriched fraction defined by glucose-6-phosphatase). Given their apparent digestive properties, these granules were given the name ‘lysosomes’ [34]. Almost a decade later, in examining the uptake of intravenously administered horseradish peroxidase by rat kidney epithelium, Werner Straus [35] observed that internalized peroxidase initially passed through a peripheral pre-lysosomal vacuole before appearing in lysosomes located in the perinuclear area. While Straus termed these pre-lysosomal vacuoles ‘phagosomes’, subsequent work exploring a variety of cell types and receptor systems led to pre-lysosomal vacuoles being termed pinosomes, receptosomes, intermediate vacuoles, CURLs (compartments of uncoupling of receptor and ligand) and endosomes [36,37]. With growing evidence that pre-lysosomal vacuoles comprised a heterogeneous population of vacuoles, not all of which contained receptors, the more general term ‘endosome’ became the preferred nomenclature [36]. Thus the central axis of the endolysosomal network was established.

The intervening years have witnessed a growing appreciation of the complex organization of the endolysosomal network [38,39] (Figure 3). After endocytosis, internalized cargoes enter the early endosome, a compartment characterized, at least morphologically, by interconnected vacuolar and tubular elements [40]. Here, cargo sorting occurs [39]. Cargoes destined for degradation, such as the EGFR (epidermal growth factor receptor), undergo sorting from the limiting membrane of the endosomal vacuole into ILVs (intraluminal vesicles) [41]. In parallel, other cargoes are retrieved away from this pathway. Specific signalling receptors, nutrient-sensing receptors [e.g. TfnRs (transferrin receptors)] and a wide array of other cargo are recycled back to the plasma membrane via a direct fast recycling route or,

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more slowly, through the juxtanuclear ERC [42,43]. Yet other cargoes, such as CI-MPRs (cation-independent mannose 6-phosphate receptors) are sorted for retrieval to the TGN [44,45]. Co-ordinated with cargo sorting, the early endosome undergoes a process of maturation, forming a late endosome, also termed, because of the presence of multiple ILVs, the MVB (multivesicular body). This becomes competent to fuse with the digestive lysosome, thereby degrading cargoes associated with the ILVs [46] (Figure 1).

Pivotal to deciphering the molecular mechanisms that allow cargo sorting through the endosomal network was the observation that perturbing the formation of PtdIns(3)*P* had a major impact on the biology of early endosomes. Treating cells with wortmannin, a drug that, by inhibiting PI3K activity, reduces the level of PtdIns(3)*P* on early endosomes, resulted in perturbed lysosomal degradation of internalized growth factor receptors and to morphological changes characterized by endosomal swelling and the formation of an extended array of endosomal tubules that contained lysosomally directed and recycling molecules [47]. Adding to this, inhibiting the generation of PtdIns(3,5)$P_2$, by either RNAi (RNA interference)-mediated suppression or small-molecular inhibition of the PtdIns(3)*P* 5-kinase PIKfyve/Fab1, leads to gross effects on late endosomal biology, characterized functionally by defects in the late endosome–lysosome axis, endosome–Golgi retrograde transport and endosomal acidification, and morphologically through the appearance of large swollen late endosomes ([48,49] and reviewed in [50]). Without question, these phosphoinositides play a pivotal role in conducting the complex orchestration of molecular events that underlie the many functions of the endolysosomal network.

## Deciphering the endosomal biology of PtdIns(3)*P*

Evidence first that FYVE domains and subsequently PX domains function as PtdIns(3)*P*-binding domains constituted the key events that catalysed a surge in understanding of the mechanistic details through which this phosphoinositide regulates endosomal function [51–55]. For example, identification of the FYVE domain-containing Rab5 effectors EEA1 and Rabenosyn5 and the elucidation of their role in regulating homotypic early endosomal fusion and the formation of PtdIns(3)*P* microdomains led to a description of early endosome biogenesis [56–58]. The combined power of yeast genetics and lipid biochemistry established the evolutionarily conserved role of PtdIns(3)*P* [and PtdIns(3,5)$P_2$] in regulating the endosomal association of ESCRT (endosomal sorting complex required for transport) -0 [a component of which, Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate), binds PtdIns(3)*P* via a FYVE domain], -I, -II [the VPS (vacuolar protein sorting) 36 element of which binds PtdIns(3)*P* via its GLUE domain] and -III complexes [CHMP3 (charged multivesicular body protein 3) associates with PtdIns(3,5)$P_2$], and how their sequential assembly regulates sorting of selected cargo into the endolysosomal degradative pathway [41,59].

Our own studies have focused on analysing the roles of the major PX domain-containing family in the mammalian genome, the SNXs (sorting nexins) [39,60,61]. The first SNX to be identified, SNX1, was isolated through a reported ability to associate with the cytosolic tail of the EGFR, an interaction required for the SNX1-mediated sorting of the receptor into the endolysosomal degradative pathway [62]. The mammalian family now comprises 33 distinct proteins divided into three subfamilies, SNX-BARs (BAR is Bin/amphiphysin/Rvs), SNX-PXs and SNX-others, which are united by virtue of the common sorting nexin-PX domain [39,60,61]. For such a large protein family, it is perhaps not surprising that the general term 'sorting nexin' is somewhat misleading, implying that all family members are involved in sorting: for some, this may well not to be the case.

SNXs are cytosolic proteins, targeted to the cytosolic face of the endolysosomal network, in part through PX domain-mediated binding to phosphoinositides. Where the specificity of binding has been analysed, most SNXs preferentially bind PtdIns(3)*P*. There are exceptions, however. For example, SNX1 and SNX2 bind PtdIns(3)*P* and PtdIns(3,5)$P_2$ equally well [63,64], SNX9 rather promiscuously binds PtdIns(4,5)$P_2$ [65–67], and SNX5 binds PtdIns(3)*P* and PtdIns(3,4)$P_2$ [68]. Obviously, analysing phosphoinositide binding *in vitro* is not a trivial matter, given issues such as ligand presentation (Biacore versus liposomes versus 'fat-blots'), so extrapolating physiological relevance from apparently distinct phosphoinositide-binding profiles is never straightforward [63]. That said, one thing is clear: a functional phosphoinositide-binding PX domain is a key element in targeting cytosolic SNXs to the endolysosomal network.

## The SNX-BAR subfamily and endosomal tubulation

From early ultrastructural analysis of the endosomal network, it was observed that, after internalization, receptor–ligand complexes entered a "complex arrangement of anastomosing tubular and larger vesicular structures which were often highly elongated" [69]. With the advent of double-label immunoelectron microscopy, it became evident that the tubular portion of this network constituted an escape route through which receptors could be retrieved away from the degradative lysosomal pathway (e.g. see the stunning images shown in Figures 5C and 5D of [70]). Unfortunately, relating these observations to a molecular understanding of how the complex tubular arrangement is generated and maintained, and how this is co-ordinated with the process of cargo sorting has remained unclear. The identification and functional characterization of the SNX-BAR subfamily has, however, begun to illuminate this area of fundamental cell biology.

Soon after the identification of SNX1, bioinformatics established the presence of a number of related mammalian

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proteins that, in addition to the presence of the PX domain, possessed a series of coiled coils at their C-termini [71]. In collaboration with Dr Harvey McMahon, we established that these coiled-coil regions actually constitute functional BAR domains, thereby allowing the classification of the SNX-BAR family as comprising 12 proteins: SNX1, SNX2, SNX4–SNX9, SNX18, SNX30, SNX32 and SNX33 [72,73]. BAR domains are foremost dimerization motifs that by dimerizing form a rigid banana-shaped structure, the concave face of which contains a positively charged surface that allows association with the cytosolic face of membranes (reviewed in [74]). Importantly, the physical shape of the BAR domain conveys preferential binding to membranes of high positive curvature, one characteristic of membrane tubules. SNX-BARs effectively contain two membrane-binding properties: phosphoinositide binding via their PX domain and curvature sensing through the BAR domain, binding which they integrate (a form of coincidence detection [30,72]) in order to associate with high-curvature subdomains of the endolysosomal network enriched with their cognate phosphoinositide [72].

An additional feature of certain BAR domains is the ability to switch from a local curvature-sensing mode into a global curvature-inducing mode, characterized by the formation of higher-order helical arrays that, in coating the membrane, impose curvature which is accommodated by the membrane deforming into membrane tubules [74]. Packing within the helical array is supported by tip-to-tip and lateral contacts between neighbouring BAR domains [75,76]. Certain SNX-BAR proteins, for example SNX1, SNX9 and SNX18, have been shown to elicit vesicle–tubule transition in *in vitro* assays using recombinant proteins and artificial liposomes [66,72,77]. In such assays, it is argued that, on liposome binding, the effective concentration of the BAR domain increases, resulting in the co-ordinated assembly of the helical complex and formation of membranes tubules [73]. One vital issue with the interpretation of these data, which still remains to be resolved, is whether the effective BAR domain concentration required for *in vitro* tubule formation is ever reached in a physiological *in vivo* setting. In other words, is the physiological function of the BAR domain of SNX-BARs to sense and stabilize pre-existing membrane tubules rather than actually drive tubule formation itself. That said, evidence is emerging consistent with a model in which SNX-BAR proteins are important elements in eliciting the formation/stabilization of endosomal tubules, co-ordinating this with the process of cargo sorting [73]. The best-characterized example being the retromer complex.

## Retromer: an evolutionarily conserved sorting complex

Originally identified in the Emr laboratory, yeast retromer is a pentameric complex composed of two subcomplexes: a membrane-bound coat composed of heterodimers of the SNX-BARs Vps5p and Vps17p, and a distinct stable trimer comprising Vps26p, Vps29p and Vps35p [78] (Figure 4). This is often referred to as the cargo-selective subcomplex because Vps35p associates directly with the cytosolic tails of specific cargo proteins, which include the yeast carboxypeptidase Y receptor, Vps10p [79]. Functionally, the assembly of the retromer mediates the retrieval of Vps10p from a pre-vacuole endosomal compartment back to the TGN [78].

The mammalian retromer is more complex (reviewed in [80–84]). Gene duplication has resulted in two Vps5ps, SNX1 and SNX2 [64,72,85], and three Vps17ps, SNX5, SNX6 and SNX32 [86,87], whereas the VPS26–VPS29–VPS35 trimer includes two isoforms of VPS26 [88]. As discussed above, these sorting nexins contain BAR domains that drive the formation of a specific series of heterodimers, which appear to obey the rule that one Vps5p equivalent dimerizes with one Vps17p equivalent [87]. We have argued that assembly of these SNX-BARs drives the remodelling of endosomal membranes leading to the formation of tubular carriers called ETCs (endosome–TGN carriers) [89]. Co-ordinated with these events, the VPS26–VPS29–VPS35 trimeric complex selects specific cargo for enrichment into forming ETCs, thereby sorting cargo for endosome–TGN retrieval (Figure 5). Cargo includes sortilin receptors (equivalent to yeast Vps10p), CI-MPR, the iron transporter DMT1-II (bivalent metal transporter 1 II), polymeric immunoglobulin receptor, the Wnt morphogenic chaperone Wntless, Cholera and Shiga toxins, and proteins that regulate processing of amyloid precursor protein [83,84].

From studies utilizing live-cell confocal imaging combined with low-level lentiviral delivery of GFP (green fluorescent protein)-tagged retromer proteins, it has become evident that retromer-labelled endosomes are extremely dynamic, displaying short- and long-range-directed movements and vesicle–tubule transitions [87]. The dynamics of retromer tubules are themselves highly complex, being characterized as productive tubules, these undergo scission from the endosomal vacuole, leading to the formation of a tubular carrier, and abortive tubules, which collapse back into the endosomal vacuole before generating an isolated tubular carrier [87]. Vesicle–tubule transitions and the formation of tubular carriers therefore appear highly orchestrated and processive events that almost certainly contain a series of checkpoints designed to ensure efficient retromer-mediated sorting. Although we currently have no understanding of what constitutes a retromer checkpoint, we do have a growing appreciation of those events that lead to the formation of productive retromer-labelled ETCs.

Besides orchestrating cargo selection and membrane remodelling, retromer recruits a number of additional proteins required for efficient processing of cargo-enriched tubular carriers. Of particular note is association with the WASH [WASP (Wiskott–Aldrich syndrome protein) and Scar homologue] complex, which comprises FAM21, SWIP, Strumpellin and CCDC53 (coiled-coil domain-containing protein 53) [90]. WASH is a member of the WASP family, which, through binding the Arp2/3 (actin-related protein

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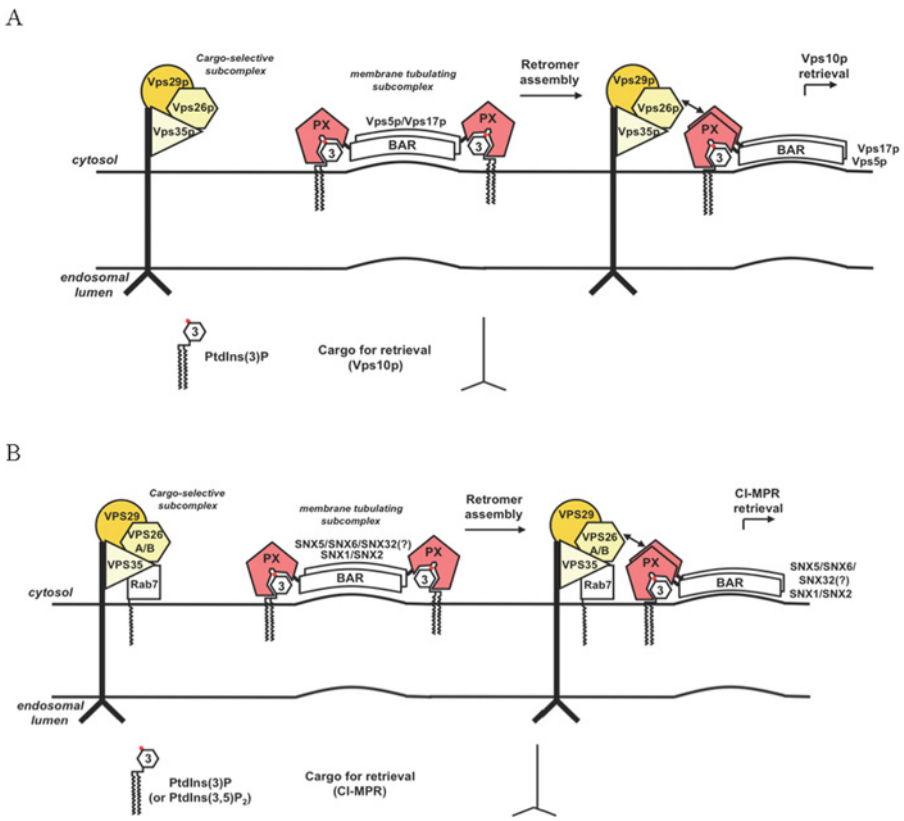
**Figure 4** | **The yeast and mammalian retromer complexes**

(**A**) The Vps5p and Vps17p SNX-BAR membrane-deforming and Vps26p–Vps29p–Vps35p cargo-selective subcomplexes of the pentameric yeast retromer. (**B**) Gene duplication and the resultant increased complexity of the mammalian retromer.




2/3) complex, nucleates the formation of branched actin filaments [91,92]. The VPS26–VPS29–VPS35 subcomplex associates with WASH through direct binding of VPS35 to FAM21: additional binding of VPS35 and SNX1/SNX2 to WASH and FAM21 may stabilize the interaction [91–93]. In the absence of WASH, retromer-mediated endosome–TGN transport is perturbed [91–93]. This correlates with the formation of extended retromer-labelled tubular profiles, which appear to arise from a decreased efficiency in the processing and scission of retromer tubules [92,93]. A model therefore emerges where retromer recruits WASH to sites of endosomal tubulation (Figure 6). From here, WASH stimulates Arp2/3 actin nucleation activity, leading to actin polymerization and the generation of longitudinal force that in turn aids the efficiency of tubule scission. Interestingly, WASH may not act in isolation to generate longitudinal force. SNX5 and SNX6 directly bind p150$^{glued}$, a component of the dynein–dynactin minus-end-directed microtubule motor [87]. This allows the long-range movement of retromer-labelled endosomes and carriers from the cell periphery to the TGN (an organelle that lies juxtaposed to the microtubule-organizing centre). Unsurprisingly, suppression of p150$^{glued}$, or disruption of its association with SNX5/SNX6, leads to perturbed trafficking and a reduced long-range movement of retromer-labelled endosomes [87]. Moreover, in the absence of p150$^{glued}$, retromer tubules become exaggerated and elongated, suggesting that, like the situation with the WASH complex, coupling of retromer to the dynein motor is required to generate longitudinal force necessary for efficient scission of retromer tubules [87].

## Drawing parallels with other SNX-BAR proteins

For SNX9, SNX18 and SNX33, the dimerization of their BAR domains is restricted to homodimers [77,94]. Coincidence sensing of PtdIns(4,5)$P_2$ and membrane curvature targets these SNX-BARs to high-curvature subdomains of clathrin-coated pits during late stages of clathrin-mediated endocytosis ([66,67], but see also [77]). Importantly, through an N-terminal SH3 (Src homology 3) domain, SNX9, SNX18 and SNX33 bind to and stimulate the basal GTPase activity of dynamin [77,95–98]. In addition, they also interact with a variety of other proteins, including the Arp2/3 actin nucleators WASP and N-WASP (neuronal WASP) [98–101]. One model therefore proposes that these proteins function by recruiting dynamin to high-curvature invaginated regions of clathrin-coated pits (reviewed in [102]) NB: the

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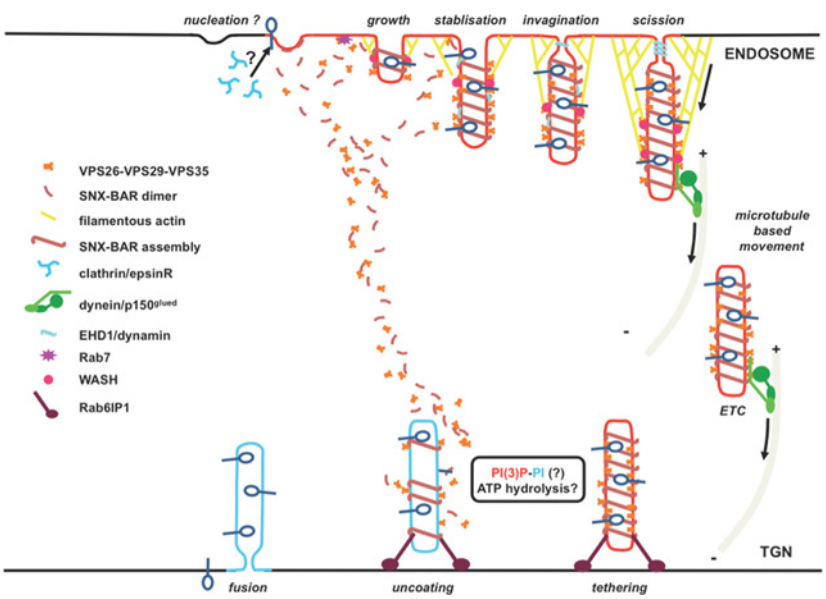
**Figure 5 | Mechanistic details of mammalian retromer-mediated endosome–TGN sorting**

The Figure illustrates the central dogma of all membrane trafficking pathways, from membrane deformation, sorting and scission at the donor membrane (endosome) to movement, tethering, uncoating and fusion with the recipient compartment (TGN). For the retromer, specific combinations of SNX1/SNX2/SNX5/SNX6 (and possibly SNX32) dimers utilize co-incidence sensing to associate with curved regions of the PtdIns(3)*P*-enriched endosome. Here, as their effective concentration increases, they form a helical assembly that drives/stabilizes membrane tubulation which is coupled with cargo sorting through the association of VPS26–VPS29–VPS35 with cytosolic tails of appropriate cargo (e.g. CI-MPR). Maturation of the tubular profile and its ultimate scission, which may require dynamin-like proteins of the EHD family, is assisted by longitudinal force generated through association with microtubule motors and/or the WASH-mediated polymerization of actin. Association of SNX5/SNX6 with the minus-end-directed dynactin–dynein microtubule motor allows for long-range movement towards the TGN where the interaction of SNX1/SNX2 with the proposed TGN-localized tether Rab6IP1 allows for carrier recognition. Carrier uncoating occurs before SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor)-mediated fusion to allow for CI-MPR retrieval back to the TGN. The role of clathrin and clathrin-interacting proteins such as epsinR in the clustering of cargo and initial nucleation of retromer carrier formation remains unclear. We speculate that uncoating will require an input of energy to destabilize the retromer coat complex, and may be aided through turnover of PtdIns(3)*P* (see the text and [84] for more details).




SNX-BARs are argued to function as curvature sensors rather than as curvature inducers in this model) (Figure 6). Here, the assembly of dynamin leads to the formation of a narrow tubule, which through the SNX-BAR stimulation of the dynamin GTPase activity, becomes destabilized. The co-ordinated recruitment of WASP results in a burst of actin polymerization that, by generating longitudinal tension, aids the final release of the clathrin-coated vesicle [102]. Although there is little functional evidence to suggest that SNX9, SNX18 and SNX33 function either directly and/or indirectly in the sorting of cargo proteins into forming clathrin-coated pits, *Drosophila* SNX9 and mammalian SNX18 have, however, been reported to respectively bind the cargo adaptor proteins AP-2 and AP-1 [77,103]. There are clear parallels with the function of SNX-BARs within the retromer complex: (i) a restricted pattern of BAR domain-mediated dimerizations; (ii) targeting to a high-curvature subdomain of the endolysosomal network as a result of coincidence sensing; (iii) scaffolding of protein complexes that aid cargo selection, elicit further membrane remodelling and/or drive membrane scission, and (iv) generation of longitudinal tension through a localized burst of actin polymerization and/or association with molecular motors. For the remaining SNX-BARs, SNX4, SNX7, SNX8 and SNX30, one would therefore predict a similar *modus operandi*. Indeed, SNX4 utilizes coincidence sensing to associate with tubular elements of the early endosome: the PX domain of this SNX-BAR specifically binds to PtdIns(3)*P* [103]. SNX4 also associates with the dynein motor complex, which is required for this SNX-BAR to regulate tubular-based sorting to the juxtanuclear ERC and Golgi apparatus [103–105]. Defining the function of the remaining SNX-BAR therefore looks certain to lead to further molecular insight into the role played by phosphoinositides in defining the architecture and function of the tubulovesicular endosomal network.

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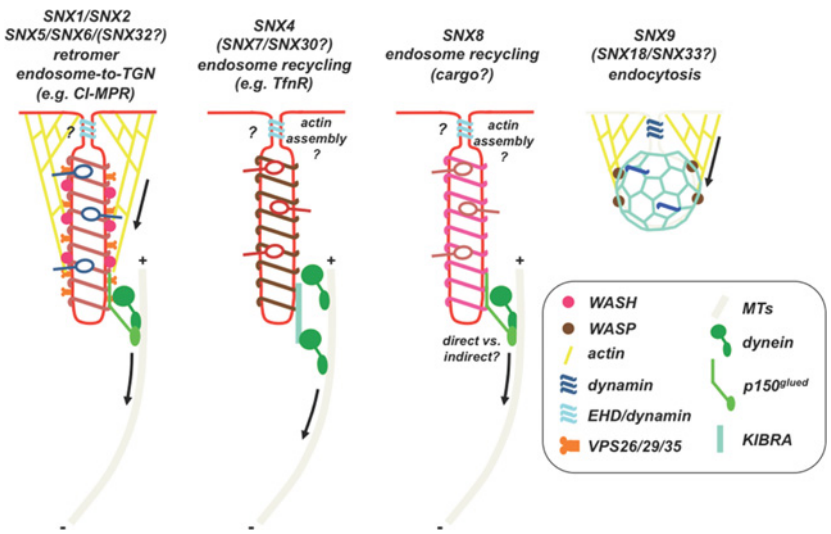
**Figure 6 | SNX-BAR proteins and endosomal sorting**

On the basis of mechanistic details of the role played by mammalian SNX-BARs in retromer- and SNX9-mediated sorting, we speculate as to common themes for other SNX-BAR family members (see the text for more details). KIBRA, kidney and brain protein; MT, microtubule; TfnR, transferrin receptor.




## Outstanding questions

There are many outstanding questions. Do SNX-BAR proteins function primarily as curvature sensors or curvature inducers? We have suggested a model for retromer that focuses on the later, but one cannot exclude a role that relies solely on sensing and stabilizing pre-existing membrane curvature. How do SNX-BARs relate to other phosphoinositide-binding proteins which elicit tubulation from distinct endosomal compartments; for example, proteins from the EHD and Amot families [106,107]? Understanding these relationships may reveal interesting insight into sequential phosphoinositide-mediated sorting through specific endosomal compartments. What are the functions of other SNX family members, in particular the SNX-PX subgroup? Could some of these proteins function as cargo adaptors for sorting into SNX-BAR-mediated endosomal tubules [108]? What of the interchange between endosomal PtdIns(3)*P* and PtdIns(3,5)$P_2$: is this a checkpoint required for processivity during sorting at the early-to-late endosomal switch? What are the molecular entities that sense the generation of PtdIns(3,5)$P_2$, and how do they regulate sorting? Finally, and perhaps most importantly, by understanding phosphoinositide-regulated endosomal sorting, can we provide novel insights into why perturbing the metabolism of endosomal phosphoinositides leads to such a wide array of human diseases, and, in turn, can such molecular detail generate potential targets for a rationale of therapeutic intervention?

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## References

1 Agranoff, B.W. (2009) Turtles all the way: reflections on *myo*-inositol. J. Biol. Chem. **284**, 21121–21126
2 Hokin, L.E. and Hokin, M.R. (1955) Effects of acetylcholine on the turnover of phosphoryl units in individual phospholipids of pancreas slices and brain cortex slices. Biochim. Biophys. Acta **18**, 102–110
3 Barlow, C.A., Laishram, R.S. and Anderson, R.A. (2010) Nuclear phosphoinositides: a signaling enigma wrapped in a compartmental conundrum. Trends Cell Biol. **20**, 25–35
4 Berridge, M.J. (2009) Inositol trisphosphate and calcium signalling mechanisms. Biochim. Biophys. Acta **1793**, 933–940

©The Authors Journal compilation ©2011 Biochemical Society

5 Irvine, R.F. (2003) 20 years of Ins(1,4,5)$P_3$, and 40 years before. Nat. Rev. Mol. Cell Biol. **4**, 580–585
6 Michell, R.H. (2008) Inositol derivatives: evolution and functions. Nat. Rev. Mol. Cell Biol. **9**, 151–161
7 Shears, S.B. (2009) Diphosphoinositol polyphosphates: metabolic messengers? Mol. Pharm. **76**, 236–252
8 Skwarek, L.C. and Boulianne, G.L. (2009) Great expectations for PIP: phosphoinositides as regulators of signaling during development and disease. Dev. Cell **16**, 12–20
9 Vicinanza, M., D'Angelo, G., Di Campli, A. and De Matteis, M.A. (2008) Function and dysfunction of the PI system in membrane trafficking. EMBO J. **27**, 2457–2470
10 Wong, K.-K., Engelman, J.A. and Cantley, L.C. (2010) Targeting the PI3K signaling pathway in cancer. Curr. Opin. Genet Dev. **20**, 87–90
11 Nomenclature Committee of the International Union of Biochemistry Numbering of atoms in *myo*-inositol: recommendations 1988 (1989) Biochem. J. **258**, 1–2
12 De Matteis, M.A. and Godi, A. (2004) PI-loting membrane traffic. Nat. Cell Biol. **6**, 487–492
13 Stephens, L., McGregor, A. and Hawkins, P.T. (2000) Phosphoinositide 3-kinases: regulation by cell-surface receptors and function of 3-phosphorylated lipids. In Biology of Phosphoinositides (Cockcroft, S., ed.), pp. 32–108, Oxford University Press, Oxford
14 Robinson, F.L. and Dixon, J.E. (2006) Myotubularin phosphatases: policing 3-phosphoinositides. Trends Cell Biol. **16**, 403–412
15 Balla, A. and Balla, T. (2006) Phosphatidylinositol 4-kinases: old enzymes with emerging functions. Trends Cell Biol. **16**, 351–361
16 Nicot, A.S. and Laporte, J. (2008) Endosomal phosphoinositides and human diseases. Traffic **9**, 1240–1249
17 McCrea, H.J. and De Camilli, P. (2009) Mutations in phosphoinositide metabolizing enzymes and human disease. Physiology **24**, 8–16
18 Liu, Y. and Bankaitis, V.A. (2010) Phosphoinositide phosphatases in cell biology and disease. Prog. Lipid Res. **49**, 201–217
19 Vanhaesebroeck, B., Guillermet-Guilbert, J., Graupera, M. and Bilanges, B. (2010) The emerging mechanisms of isoform-specific PI3K signalling. Nat. Rev. Mol. Cell Biol. **11**, 329–341
20 Chalhoub, N. and Baker, S.J. (2009) PTEN and the PI3-kinase pathway in cancer. Annu. Rev. Pathol. **4**, 127–150
21 Di Paolo, G. and De Camilli, P. (2006) Phosphoinositides in cell regulation and membrane dynamics. Nature **443**, 651–657
22 Balla, T. (2009) Green light to illuminate signal transduction events. Trends Cell Biol. **19**, 575–586
23 Maffucci, T., Brancaccio, A., Piccolo, E., Stein, R.C. and Falasca, M. (2003) Insulin induces phosphatidylinositol-3-phosphate formation through TC10 activation. EMBO J. **22**, 4178–4189
24 Kale, S.D., Gu, B., Capelluto, D.G., Dou, D., Feldman, E., Rumore, A., Arredondo, F.D., Hanlon, R., Fudal, I., Rouxel, T. et al. (2010) External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. Cell **142**, 284–295
25 Axe, E.L., Walker, S.A., Manifava, M., Chandra, P., Roderick, H.L., Habermann, A., Griffiths, G. and Ktistakis, N.T. (2008) Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J. Cell Biol. **182**, 685–701
26 Harlan, J.E., Hajduk, P.J., Yoon, H.S. and Fesik, S.W. (1994) Pleckstrin homology domains bind to phosphatidylinositol-4,5-bisphosphate. Nature **371**, 168–170
27 Lemmon, M.A. (2008) Membrane recognition by phospholipid-binding domains. Nat. Rev. Mol. Cell Biol. **9**, 99–111
28 Balla, T. (2005) Inositol-lipid binding motifs: signal integrators through protein–lipid and protein–protein interactions. J. Cell Sci. **118**, 2093–2104
29 Cullen, P.J., Cozier, G.E., Banting, G. and Mellor, H. (2001) Modular phosphoinositide-binding domains: their role in signalling and membrane trafficking. Curr. Biol. **11**, R882–R893
30 Carlton, J.G. and Cullen, P.J. (2005) Coincidence detection in phosphoinositide signalling. Trends Cell Biol. **15**, 540–547
31 Klein, D.E., Lee, A., Frank, D.W., Marks, M.S. and Lemmon, M.A. (1998) The PH domains of dynamin isoforms require oligermisation for high affinity phosphoinositide binding. J. Biol. Chem. **273**, 27725–27733
32 Lemmon, M.A. (2007) PH domains and phosphoinositides. Biochem. Soc. Symp. **74**, 81–93
33 De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem. J. **60**, 604–617
34 De Duve, C. (2005) The lysosome turns fifty. Nat. Cell Biol. **7**, 847–849
35 Straus, W. (1964) Cytochemical observations on the relationship between lysosomes and phagosomes in kidney and liver by combined staining for acid phosphatase and intravenously injected horseradish peroxidase. J. Cell Biol. **20**, 497–507
36 Helenius, A., Mellman, I., Wall, D. and Hubbard, A. (1983) Endosomes. Trends Biochem. Sci. **8**, 245–249
37 Pastan, I. and Willingham, M.C. (1983) Receptor-mediated endocytosis: coated pits, receptosomes and the Golgi. Trends Biochem. Sci. **8**, 250–254
38 Soldati, T and Schliwa, M. (2006) Powering membrane traffic in endocytosis and recycling. Nat. Rev. Mol. Cell Biol. **7**, 897–908
39 Cullen, P.J. (2008) Endosomal sorting and signalling: an emerging role for sorting nexins. Nat. Rev. Mol. Cell Biol. **9**, 574–582
40 van Meel, E. and Klumperman, J. (2008) Imaging and imagination: understanding the endo-lysosomal system. Histochem. Cell Biol. **129**, 253–266
41 Hurley, J.H. and Hanson, P.I. (2010) Membrane budding and scission by the ESCRT machinery: it's all in the neck. Nat. Rev. Mol. Cell Biol. **11**, 556–566
42 Grant, B.D. and Donaldson, J.G. (2009) Pathways and mechanisms of endocytic recycling. Nat. Rev. Mol. Cell Biol. **10**, 597–608
43 Hsu, V.W. and Prekeris, R. (2010) Transport at the recycling endosome. Curr. Opin. Cell Biol. **22**, 528–534
44 Bonifacino, J.S. and Rojas, R. (2006) Retrograde transport from endosomes to the *trans*-Golgi network. Nat. Rev. Mol. Cell Biol. **7**, 568–579
45 Johannes, L. and Popoff, V. (2008) Tracing the retrograde route in protein trafficking. Cell **135**, 1175–1187
46 Saftig, P. and Klumperman, J. (2009) Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat. Rev. Mol. Cell Biol. **10**, 623–635
47 Shpetner, H., Joly, M., Hartley, D. and Corvera, S. (1996) Potential sites of PI-3 kinase function in the endocytic pathway revealed by the PI-3 kinase inhibitor, wortmannin. J. Cell Biol. **132**, 595–605
48 Rutherford, A.C., Traer, C.J., Wassmer, T., Pattni, K., Bujny, M.V., Carlton, J.G., Stenmark, H. and Cullen, P.J. (2006) The mammalian phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) regulates endosome-to-TGN retrograde transport. J. Cell Sci. **119**, 3944–3957
49 Jefferies, H.B., Cooke, F.T., Jat, P., Boucheron, C., Koizumi, T., Hayakawa, M., Kaizawa, H., Ohishi, T., Workman, P., Waterfield, M.D. and Parker, P.J. (2008) A selective PIKfyve inhibitor blocks PtdIns(3,5)$P_2$ production and disrupts endomembrane transport and retroviral budding. EMBO Rep. **9**, 164–170
50 Dove, S.K., Dong, K., Kobayashi, T., Williams, F.K. and Michell, R.H. (2009) Phosphatidylinositol 3,5-bisphosphate and Fab1p/PIKfyve underPPIn endo-lysosomal function. Biochem. J. **419**, 1–13
51 Gaullier, J.M., Simonsen, A., D'Arrigo, A., Bremnes, B., Stenmark, H. and Aasland, R. (1998) FYVE fingers bind PtdIns(3)*P*. Nature **394**, 432–433
52 Cheever, M.L., Sato, T.K., de Beer, T., Kutateladze, T.G., Emr, S.D. and Overduin, M. (2001) Phox domain interaction with PtdIns(3)*P* targets the Vam7 t-SNARE to vacuole membranes. Nat. Cell Biol. **3**, 613–618
53 Xu, Y., Hortsman, H., Seet, L., Wong, S.H. and Hong, W. (2001) SNX3 regulates endosomal function through its PX-domain-mediated interaction with PtdIns(3)*P*. Nat. Cell Biol. **3**, 658–666
54 Kanai, F., Liu, H., Field, S.J., Akbary, H., Matsuo, T., Brown, G.E., Cantley, L.C. and Yaffe, M.B. (2001) The PX domain of $p47^{phox}$ and $p40^{phox}$ bind to the lipid products of PI(3)K. Nat. Cell Biol. **3**, 675–678
55 Ellson, C.D., Gobert-Gosse, S., Anderson, K.E., Davidson, K., Erdlument-Bromage, H., Tempst, P., Thuring, J.W., Cooper, M.A., Lim, Z.Y. and Holmes, A.B. (2001) PtdIns(3)*P* regulates the neutrophil oxidase complex by binding to the PX domain of $p40^{phox}$. Nat. Cell Biol. **3**, 679–682
56 Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J.M., Brech, A., Callaghan, J., Toh, B.H., Murphy, C., Zerial, M. and Stenmark, H. (1998) EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. Nature **394**, 494–498


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57 Christoforidis, S., McBride, H.M., Burgoyne, R.D. and Zerial, M. (1999) The Rab5 effector EEA1 is a core component of endosome docking. Nature **397**, 621–625
58 Nielsen, E., Christoforidis, S., Uttenweiler-Joseph, S., Miaczynska, M., Dewitte, F., Wilm, M., Hoflack, B. and Zerial, M. (2000) Rabenosyn-5, a novel Rab5 effector, is complexed with hVPS45 and recruited to endosomes through a FYVE finger domain. J. Cell Biol. **151**, 601–612
59 Williams, R.L. and Urbé, S. (2007) The emerging shape of the ESCRT machinery. Nat. Rev. Mol. Cell Biol. **8**, 355–368
60 Carlton, J.G., Bujny, M.V., Rutherford, A.C. and Cullen, P. (2005) Sorting nexins: unifying trends and new perspectives. Traffic **6**, 75–82
61 Seet, L.F. and Hong, W. (2006) The Phox (PX) domain proteins and membrane traffic. Biochim. Biophys. Acta **1761**, 878–896
62 Kurten, R.C., Cadena, D.L. and Gill, G.N. (1996) Enhanced degradation of EGF receptor by a sorting nexin, SNX1. Science **272**, 1008–1010
63 Cozier, G.E., Carlton, J.G., McGregor, A.H., Gleeson, P.A., Teasdale, R.D., Mellor, H. and Cullen, P.J. (2002) The phox homology (PX) domain-dependent, 3-phosphoinositide-mediated association of sorting nexin-1 with an early sorting endosomal compartment is required for its ability to regulate EGF receptor degradation. J. Biol. Chem. **277**, 48730–48736
64 Carlton, J.G., Bujny, M.V., Peter, B.J., Oorschot, V.M., Rutherford, A., Arkell, R.S., Klumperman, J., McMahon, H.T. and Cullen, P.J. (2005) 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
65 Lundmark, R. and Carlsson, S.R. (2003) Sorting nexin-9 participates in clathrin-mediated endocytosis through interactions with the core components. J. Biol. Chem. **278**, 46772–46781
66 Yarar, D., Waterman-Storer, C.M. and Schmid, S.L. (2007) SNX9 couples actin assembly to phosphoinositide signals and is required for membrane remodelling during endocytosis. Dev. Cell **13**, 43–56
67 Yarar, D., Surka, M.C., Leonard, M.C. and Schmid, S.L. (2008) SNX9 activities are regulated by multiple phosphoinositides through both PX and BAR domains. Traffic **9**, 133–146
68 Merino-Trigo, A., Kerr, M.C., Houghton, F., Lindberg, A., Mitchell, C., Teasdale, R.D. and Gleeson, P.A. (2000) Sorting nexin-5 is localised to a subdomain of the early endosomes and is recruited to the plasma membrane following EGF stimulation. J. Cell Sci. **117**, 6413–6424
69 Wall, D.A., Wilson, G. and Hubbard, A.L. (1980) The galactose-specific recognition system of mammalian liver: the route of ligand internalisation in rat hepatocytes. Cell **21**, 79–93
70 Geuze, H.J., Slot, J.W., Strous, G.J., Lodish, H.F. and Schwartz, A.L. (1983) Intracellular site of asialoglycoprotein receptor-ligand uncoupling: double-label immunoelectron microscopy during receptor-mediated endocytosis. Cell **32**, 277–287
71 Teasdale, R.D., Loci, D., Houghton, F., Karlsson, L. and Gleeson, P.A. (2001) A large family of endosome-localized proteins related to sorting nexin-1. Biochem. J. **358**, 7–16
72 Carlton, J.G., Bujny, M.V., Peter, B.J., Oorschot, V.M., Rutherford, A., Mellor, H., Klumperman, J., McMahon, H.T. and Cullen, P.J. (2004) Sorting nexin-1 mediates tubular endosome-to-TGN transport through co-incidence sensing of high-curvature membranes and 3-phosphoinositides. Curr. Biol. **14**, 1791–1800
73 van Weering, J.R., Verkade, P. and Cullen, P.J. (2010) SNX-BAR proteins in phosphoinositide-mediated, tubular-based endosomal sorting. Semin. Cell Dev. Biol. **21**, 371–380
74 Frost, A., Unger, V.M. and De Camilli, P. (2009) The BAR domain superfamily: membrane-molding macromolecules. Cell **137**, 191–196
75 Shimada, A., Niwa, H., Tsujita, K., Suetsugu, S., Nitta, K., Hanawa-Suetsugu, K., Akasaka, R., Nishino, Y., Toyama, M., Chen, L. et al. (2007) Curved EFC/F-BAR-domain dimers are joined end to end into a filament for membrane invagination in endocytosis. Cell **129**, 761–772
76 Frost, A., Perera, R., Roux, A., Spasov, K., Destaing, O., Egelman, E.H., De Camilli, P. and Unger, V.M. (2008) Structural basis of membrane invagination by F-BAR domains. Cell **132**, 807–817
77 Haberg, K., Lundmark, R. and Carlsson, S.R. (2008) SNX18 is an SNX9 paralog that acts as a membrane tubulator in AP-1-positive endosomal trafficking. J. Cell Sci. **121**, 1495–1505
78 Seaman, M.N., McCaffery, J.M. and Emr, S.D. (1998) A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast. J. Cell Biol. **142**, 665–681
79 Seaman, M.N., Marcusson, E.G., Cereghino, J.L. and Emr, S.D. (1997) Endosome to Golgi retrieval of the vacuolar protein sorting receptor, Vps10p, requires the functional of the VPS29, VPS30 and VPS35 gene products. J. Cell Biol. **137**, 79–82
80 Seaman, M.N. (2005) Recycling your receptors with retromer. Trends Cell Biol. **15**, 68–75
81 Bonifacino, J.S. and Hurley, J.H. (2008) Retromer. Curr. Opin. Cell Biol. **20**, 427–436
82 Collins, B.M. (2008) The structure and function of the retromer protein complex. Traffic **9**, 1811–1822
83 Attar, N. and Cullen, P.J. (2010) The retromer complex. Adv. Enzyme Regul. **50**, 216–236
84 McGough, I.J. and Cullen, P.J. (2011) Recent advances in retromer biology. Traffic, doi:10.1111/j.1600-0854.2011.01210.x
85 Rojas, R., Kametaka, S., Haft, C.R. and Bonifacino, J.S. (2007) Interchangeable but essential functions of SNX1 and SNX2 in the association of retromer with endosomes and the trafficking of mannose 6-phosphate receptors. Mol. Cell. Biol. **27**, 1112–1124
86 Wassmer, T., Attar, N., Bujny, M.V., Oakley, J., Traer, C.J. and Cullen, P.J. (2007) A loss-of-function screen reveals SNX5 and SNX6 as potential components of the mammalian retromer. J. Cell Sci. **120**, 45–54
87 Wassmer, T., Attar, N., Harterink, M., van Weering, J.R., Traer, C.J., Oakley, J., Goud, B., Stephens, D.J., Verkade, P., Korswagen, H.C. and Cullen, P.J. (2009) The retromer coat complex coordinated endosomal sorting and dynein-mediated transport with carrier recognition by the *trans*-Golgi network. Dev. Cell **17**, 110–122
88 Kerr, M.C., Bennetts, J.S., Simpson, F., Thomas, E.C., Flegg, C., Gleeson, P.A., Wicking, C. and Teasdale, R.D. (2005) A novel mammalian retromer component, Vps26B. Traffic **6**, 991–1001
89 Mari, M., Bujny, M.V., Zeuschner, D., Geerts, W.J., Griffith, J., Petersen, C.M., Cullen, P.J., Klumperman, J. and Geuze, H.J. (2008) SNX1 defines an early endosomal recycling exit for sortilin and mannose 6-phosphate receptors. Traffic **9**, 380–393
90 Linardopoulou, E.V., Parghi, S.S., Friedman, C., Osborn, G.E., Parkhurst, S.M. and Trask, B.J. (2007) Human subtelomeric WASH genes encode a new subclass of the WASP family. PLoS Genet. **3**, e237
91 Gomez, T. and Billadeau, D.D. (2009) A FAM21-containing WASH complex regulates retromer-dependent sorting. Dev. Cell **17**, 699–711
92 Derivery, E., Sousa, C., Gautier, J.J., Lombard, B., Loew, D. and Gautreau, A. (2009) The Arp2/3 activator WASH control the fission of endosomes through a large multiprotein complex. Dev. Cell **17**, 712–723
93 Harbour, M.E., Breusegem, S.Y., Antrobus, R., Freeman, C., Reid, E. and Seaman, M.N. (2010) The cargo-selective retromer complex is a recruiting hub for protein complexes that regulate endosomal tubule dynamics. J. Cell Sci. **123**, 3703–3717
94 Dislich, B., Than, M.E. and Lichtenthaler, S.F. (2010) Specific amino acids in the BAR domain allow homodimerization and prevent heterodimerization of sorting nexin 33. Biochem. J. **433**, 75–83
95 Lundmark, R. and Carlsson, S.R. (2003) Sorting nexin 9 participates in clathrin-mediated endocytosis through interaction with the core components. J. Biol. Chem. **278**, 46772–46781
96 Lundmark, R. and Carlsson, S.R. (2004) Regulated membrane recruitment of dynamin-2 mediated by sorting nexin 9. J. Biol. Chem. **279**, 42694–42702
97 Soulet, F., Yarar, D., Leonard, M. and Schmid, S.L. (2005) SNX9 regulates dynamin assembly and is required for efficient clathrin-mediated endocytosis. Mol. Biol. Cell **16**, 2058–2067
98 Shin, N., Lee, S., Ahn, N., Kim, S.A., Ahn, S.G., YongPark, Z. and Chang, S. (2007) Sorting nexin 9 interacts with dynamin 1 and N-WASP and coordinates synaptic vesicle endocytosis. J. Biol. Chem. **282**, 28939–28950
99 Badour, K., McGavin, M.K., Zhang, J., Freeman, S., Vieira, C., Filipp, D., Julius, M., Mills, G.B. and Siminovitch, K.A. (2007) Interaction of the Wiskott–Aldrich syndrome protein with sorting nexin-9 is required for CD28 endocytosis and co-signalling in T cells. Proc. Natl. Acad. Sci. U.S.A. **104**, 1593–1598
100 Zhang, J., Zhang, X., Guo, Y., Xu, L. and Pei, D. (2009) Sorting nexin 33 induces mammalian cell micronucleated phenotype and actin polymerisation by interacting with Wiskott–Aldrich syndrome protein. J. Biol. Chem. **284**, 1659–1669


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101 Shin, N., Ahn, N., Chang-Lleto, B., Park, J., Takei, K., Ahn, S.G., Kim, S.A., Di Paolo, G. and Chang, S. (2008) SNX9 regulates tubular invagination of the plasma membrane through interaction with actin cytoskeleton and dynamin 2. J. Cell Sci. **121**, 1252–1263
102 Lundmark, R. and Carlsson, S.R. (2009) SNX9: a prelude to vesicle release. J. Cell Sci. **122**, 5–11
103 Traer, C.J., Rutherford, A.C., Plamer, K.J., Wassmer, T., Oakley, J., Attar, N., Carlton, J.G., Kremerskothen, J., Stephens, D.J. and Cullen, P.J. (2007) SNX4 coordinates endosomal sorting of TfnR with dynein-mediated transport into the endocytic recycling compartment. Nat. Cell Biol. **9**, 1370–1380
104 Skanland, S.S., Walchli, S., Utskarpen, A., Wandinger-Ness, A. and Sandvig, K. (2007) Phosphoinositide-regulated retrograde transport of ricin: crosstalk between hVps34 and sorting nexins. Traffic **8**, 297–309
105 Skanland, S.S., Walchli, S., Brech, A. and Sandvig, K. (2009) SNX4 in complex with clathrin and dynein: implications for endosome movement. PLoS ONE **4**, e5935
106 Naslavsky, N. and Caplan, S. (2011) EHD proteins: key conductors if endocytic transport. Trends Cell Biol. **21**, 122–131
107 Heller, B., Adu-Gyamfi, E., Smith-Kinnaman, W., Babbey, C., Vora, M., Xue, Y., Bittman, R., Stahelin, R.V. and Wells, C.D. (2010) Amot recognizes a juxtanuclear endocytic recycling compartment via a novel lipid binding domain. J. Biol. Chem. **285**, 12308–12320
108 Strochlic, T.I., Setty, T.G., Sitaram, A. and Burd, C.G. (2007) Grd19/Snx3p functions as a cargo-specific adaptor for retromer-dependent endocytic recycling. J. Cell Biol. **177**, 115–125
109 Shears, S.B. (2004) How versatile are inositol phosphate kinases? Biochem. J. **377**, 265–280
110 Rutherford, A.C. and Cullen, P.J. (2009) Phosphoinositides: navigation through the endosomal maze. . Biochemist **31** 5, 20–25

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