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Endosome protein sorting: motifs and machinery

M. N. J. Seaman

University of Cambridge, Cambridge Institute for Medical Research/Dept. of Clinical Biochemistry, Wellcome Trust/MRC Building, Addenbrookes Hospital, Hills Road, Cambridge, CB2 0XY (United Kingdom),
Fax. +44 1223 762640, e-mail: mnjs100@cam.ac.uk

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Abstract. The steady-state localisation of membrane proteins in the endocytic system is the result of many sorting events that occur at various points throughout the endosomal pathway. A protein that has been endocytosed from the plasma membrane or sorted at the trans-Golgi network (TGN) and transported to an endosome will ultimately be delivered to one of three destinations: the plasma membrane, the TGN or the lysosome. Where a membrane protein is trafficked to depends on the interactions between sorting motifs present in the membrane protein and the machinery that can decode these motifs. Much of the protein machinery that recognises sorting motifs is conserved from yeast to man, and in this review I will discuss this machinery and the motifs that govern endosomal protein sorting. (Part of a Multi-author Review)

Keywords. Sorting, coat proteins, motifs, endosome, vesicle, tubule.

Introduction

The compartments that comprise the endosomal system perform the essential role of sorting proteins that have been delivered from both the biosynthetic route and endocytosed from the plasma membrane. Sorting of membrane proteins occurs through a complex interplay between sorting motifs intrinsic in the membrane proteins, and the sorting machinery that recognises the motifs to direct the membrane protein to one of three distinct destinations. These sorting events play a vital role in macromolecular/nutrient uptake, regulating signalling from receptors, lysosome biogenesis, the response to infection and the generation of immunity, and therefore the machinery and mechanisms that govern endosomal protein sorting are sometimes subverted by pathogens or are affected by hereditary disease. A protein that has been endocytosed from the plasma membrane or sorted at the trans-Golgi network (TGN) and delivered to an endosome will ultimately be directed to the plasma membrane, the TGN or delivered to a lysosome, usually for degradation.

The fundamental mechanisms that mediate endosomal protein sorting are conserved from simple eukaryotes such as yeast to complex metazoans such as man [1], and much has been learned from studies in yeast where the ability to genetically manipulate yeast has been employed to probe the functioning of the endosomal system [2]. Whilst the endosomal system comprises a number of distinct compartments, the trafficking pathways that functionally link these sorting stations enable the endocytic system to be considered to be essentially a continuum in which protein localisation to the various endosomal compartments is a dynamic process that results in proteins transiently residing in a particular compartment before being sorted and trafficked on to the next destination. Assigning a specific function to any one endosomal compartment is difficult due to the dynamic nature of endosomal protein sorting. Endosomes have, however, traditionally been named according to the temporal/spatial relationship with the plasma membrane, i.e. early endosomes are those generated from fusion of endocytic vesicles, whilst late endosomes have undergone various sorting events to remove proteins destined for either the TGN or the plasma membrane and will ultimately fuse with lysosomes, the terminus in the endocytic pathway. More recently, additional distinctions have been used to denote function, such as ‘sorting endosome’ or ‘recycling endosome’. The picture becomes more

complex in polarised epithelial cells where sorting to apical or basolateral plasma membrane domains requires specialised endosomes that function in sorting to one or other of the two plasma membranes. A simplified diagram of the various compartments of the endocytic pathway along with some of the key machinery present in an 'ordinary' mammalian cell is shown in Figure 1.

Morphologically, the endosomal pathway comprises many vesicular structures 200–500 nm in diameter, often with threadlike tubules (~20 nm in diameter) emanating from them. The vesicular endosomes usually have internal vesicles or other membranes and are often referred to as multivesicular bodies (MVBs). The spatial position of endosomes within the cell is regulated by interactions with the microtubule cytoskeleton and the action of kinesin and dynein motors along with many other accessory proteins [3]. Early endosomes are usually spread throughout the cytoplasm, whilst late endosomes are concentrated towards the perinuclear region. As endosomes move down microtubules (in a dynein-dependent manner) towards the microtubule organising centre (MTOC), proteins are sorted to their respective destinations. Movement towards the MTOC is accompanied by increasing acidification, which is mediated by the proton-pumping action of the vacuolar ATPase [4]. Disturbance of the pH gradient across the endosomal membrane can block retrieval to the TGN and other sorting events, although it is mechanistically unclear how the pH gradient regulates endosomal sorting [5]. What is more clear is the role of small GTPases of the rab family in the organisation of the endosomal pathway and the importance of phosphatidyl inositol phosphate in regulating the membrane association of many of the critical proteins involved in endosomal protein sorting [6–8]. As the respective roles of small GTPases and phosphotidyl inositol phosphate in membrane traffic are reviewed in detail elsewhere in this edition of *Cellular and Molecular Life Sciences*, I will therefore discuss the machinery, motifs and mechanisms that govern endosomal protein sorting to the three distinct destinations mentioned: the plasma membrane, the TGN and the lysosome. Studies over the years have illuminated the mechanisms of endosomal protein sorting but have also created a veritable jungle of acronyms for the various sorting machinery. The goal of this review is to produce a roadmap for the endocytic trafficking pathways, emphasing and highlighting the key players. For the most part, membrane proteins in the endocytic system are recognised by cytoplasmic proteins that transiently associate with endosomes, interact with a membrane protein through a specific sorting motif and thereby direct that membrane protein into a discrete membrane domain that will then bud off the endosome to form a vesicular or tubular transport intermediate that will ultimately deliver its cargo of membrane proteins to the next destination in the pathway. Endosomal protein sorting is therefore mechanistically similar to other protein sorting/membrane trafficking events that occur at the endoplasmic reticulum, the Golgi complex and the plasma membrane. An exception to this general concept is the sorting of activated signalling receptors for degradation in the lysosome. This occurs by an inward invagination of the endosomal membrane to create intra-lumenal vesicles that are able to sequester certain proteins from the cytoplasm, thereby serving to downregulate the signalling pathways that are initiated by the activated receptor. The generation of the intra-lumenal endosomal vesicles is topologically opposite to other membrane trafficking events such as endocytosis from the plasma membrane or the budding of vesicles from the TGN, but is similar to the budding of virus particles from the plasma membrane of an infected cell [9].

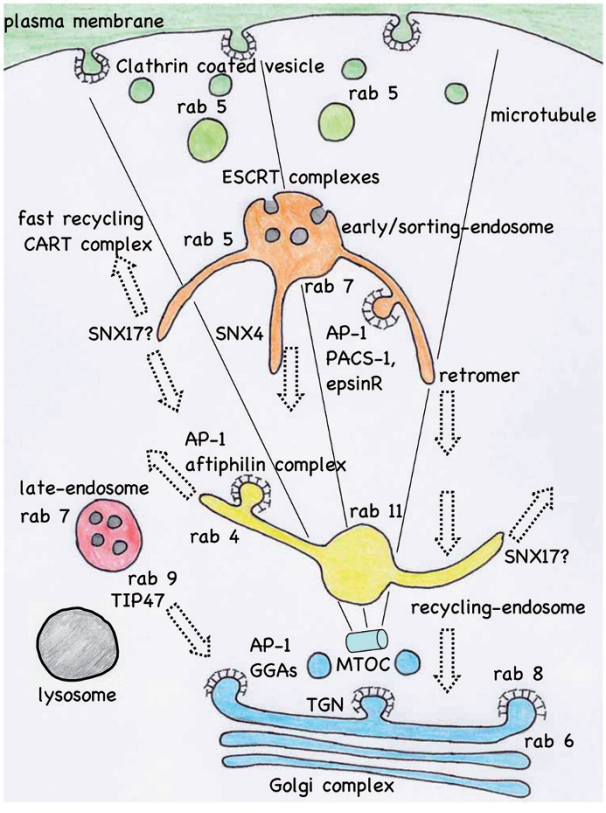

Figure 1. A simplified diagram of the endocytic pathway in a 'typical' mammalian cell. Sorting of proteins within the endocytic system is accompanied by increasing acidification of endosomes as they move down microtubules towards the perinuclear region. Small GTPases of the rab family act as key regulators defining subdomains within the endocytic pathway where specific sorting events occur.

Sorting into MVBs

Much of what is now known about the functioning of the endocytic pathway has come from studies of yeast mutants that are defective in biosynthetic transport to the yeast vacuole, the yeast equivalent of the lysosome. Screens to identify the genes that are required for the delivery of carboxypeptidase Y (CPY) to the vacuole have yielded more than 60 different vacuole protein sorting (*VPS*) genes that are required for efficient CPY delivery to the vacuole [2]. When the mutants were phenotypically analysed based upon criteria such as severity of the CPY sorting defect, vacuole morphology and growth, the many *VPS* genes were grouped into six classes, A-F [10]. The six classes of *VPS* genes have turned out to be a remarkably accurate predictor of which genes encode proteins that function together at a certain sorting/trafficking step. For example, the *VPS* genes that comprise the class E group all encode proteins that function at the endosome to sort membrane proteins into the lumen of the endosome for delivery to the vacuole and subsequent degradation [2].

The class E group of *VPS* genes is the most numerous of the six classes comprising 18 genes. Studies to characterise the function and interactions of the many class E genes have made rapid progress, allowing the class E genes to be grouped together based on certain phenotypic properties and physical interactions [11–14]. There are now four distinct protein complexes known as the ESCRT (endosomal sorting complex required for transport) complexes that act to mediate the sorting of proteins into the intra-lumenal vesicles [15]. Critical to the process of sorting into the intra-lumenal vesicles is the covalent modification of target proteins by the addition of ubiquitin. The importance of ubiquitin to this sorting process was revealed through studies in yeast on the sorting and transport to the vacuole of carboxypeptidase S (CPS) and the endocytosis of the mating pheromone receptor, Ste2p, and the downregulation of the epidermal growth factor receptor (EGFR) in mammalian cells [11, 16, 17]. Unlike CPY, which is soluble, CPS is a type II membrane protein. Both CPS and Ste2p are ubiquitylated through the action of Rsp5p, which is a HECT (Homologous to the E6-AP Carboxyl Terminus)-domain-containing ubiquitin ligase [18, 19]. The ubiquitylation of the cytoplasmic tail of CPS is a complex process involving a third protein called Bsd2p, which acts as an adaptor protein to recruit Rsp5p to CPS [20]. The Bsd2p cytoplasmic domain contains PPxY motifs (also known as PY motifs) that bind to WW domains in Rsp5p. PPxY motifs (or variants that are similar in sequence) therefore act as recognition motifs for Rsp5p and are present in other adaptor proteins such as Tre1p which, in addition to Bsd2p, is involved in ubiquitin-mediated downregulation of the divalent metal cation transporter Smf1p, which is targeted for degradation under metal-replete conditions [21–23]. The Bsd2p-CPS interaction occurs through the transmembrane domains of Bsd2p and CPS, which both contain charged residues that promote the interaction between Bsd2p and CPS [20, 23]. The role of the transmembrane domain in targeting CPS for ubiquitylation and subsequent sorting into intra-lumenal vesicles is a noteworthy exception to the general rule that a sorting motif in the cytoplasmic domain is the critical determinant in sorting in the endosomal system.

The prevailing model for the action of the ESCRT complexes is that they function sequentially to sort ubiquitylated cargo proteins (e.g. CPS) into intra-lumenal endosomal vesicles in a manner akin to a conveyer belt with ESCRT-0 at the start and ESCRT-III at the end [24, 25]. In this model, when a ubiquitylated protein such as CPS arrives in the endosome, the ubiquitin is recognised by the ESCRT-0 complex. The Vps27p component of ESCRT-0 contains a ubiquitin interaction motif (UIM) [26, 27]. Vps27p is first targeted to the endosomal membrane through its phosphotidyl inositol 3-phosphate (Ptd-Ins 3-P) binding FYVE (Fab1, YOTB, Vac19, EEA1) domain [28]. Additionally, Vps27p interacts with Hse1p, which itself can interact with ubiquitin through a UIM. The mammalian counterparts to Vps27p and Hse1p are HRS and STAM1, respectively [27, 29, 30]. STAM2, a homologue of STAM1 [31], may also function with HRS, although this remains to be formally demonstrated. HRS can bind to clathrin, which has been found as part of a flat lattice coating regions of the endosomal membrane. The precise purpose of clathrin at this site remains unknown, but it is possible that the clathrin lattice acts to constrain the HRS/STAM complex and its ubiquitylated cargo protein in a discrete domain of the endosome [32, 33].

Once ESCRT-0 has bound to a ubiquitylated protein such as CPS, the ESCRT-I complex performs its function. ESCRT-I in yeast comprises Vps23p, Vps28p, Vps37p and Mvb12p [11, 34–36]. Vps23p and its mammalian ortholog, TSG101, contain a variant of the E2 domain present in many ubiquitin ligases. This unusual E2 variant (UEV) domain can bind to ubiquitin, enabling the ESCRT-I complex to perform its role [11, 37, 38]. Studies in mammalian cells have identified two Mvb12p orthologs and four homologs of Vps37p [39–41]. In addition to interacting with ubiquitin, Vps23p can also bind Vps27p, functionally linking ESCRT-0 with ESCRT-I [42, 43]. After ESCRT-I, there is ESCRT-II, which is made up

of Vps22p, Vps36p and two copies of Vps25p [12]. Vps36p can bind to ubiquitin via a different ubiquitin-binding domain called an NZF (Npl4p zinc finger) domain [44]. The mammalian Vps36p ortholog utilises a GLUE domain (GRAM-like ubiquitin-binding domain in EAP45) to perform this function [45]. Vps36p can also interact with Vps28p, linking the activities of ESCRT-I and -II [14]. Interestingly, overexpression of all of the ESCRT-II components can bypass the requirement for the ESCRT-I component, Mvb12p, consistent with the hypothesis that ESCRT-II acts downstream of ESCRT-I [36]. Last in the ESCRT complex 'conveyer belt' is the ESCRT-III complex [13]. In yeast this comprises Snf7p, Vps20p, Vps2p and Vps24p. Vps20p is myristoylated, which can anchor it to the endosomal membrane and can additionally interact with the ESCRT-I component, Vps28p, and the ESCRT-II protein, Vps25p [14, 46]. Vps24p can bind to phosphotidyl inositol 3,5-P_2 (PtdIns 3,5-P_2), providing another interaction with the membrane [47]. The interactions between the different ESCRT complexes therefore creates a sophisticated network of protein-protein interactions that is required for sorting of proteins into MVBs.

In yeast, each of the ESCRT complexes is essential for proper sorting of ubiquitylated cargo protein. In mammalian cells, however, there is some evidence to suggest that loss of ESCRT-II through siRNA-mediated depletion of the mammalian ESCRT-II proteins does not abolish the sorting and turnover of the EGFR [48]. This may hint that there is some degree of flexibility or redundancy in the ESCRT system or that the different ESCRT complexes may exhibit a preference for certain ubiquitylated cargoes.

The ESCRT-0 through -III complexes are believed to act sequentially to sort ubiquitylated proteins into nascent budding intra-lumenal vesicles in the endosomal membrane, although the mechanics of this process have yet to be determined [24, 25]. The 'conveyer belt' model of ESCRT complex function is a simple and attractive concept, but it does not provide a clear mechanism for the generation of the intra-lumenal vesicle. A recently proposed alternative model predicts that the ESCRT complexes form a concentric ring structure in which ubiquitylated proteins traverse the rings to be concentrated in the middle and then 'pulled' down into an inward budding vesicle [49]. In this model the sequence of the function of the ESCRT complexes is reversed with a hexameric ESCRT-0 complex at the centre of the concentric rings which are formed by ESCRT-I, -II, -III complexes from the middle to the outside. Both models are conceptually elegant and there is data to support both. The protein-protein interaction data, in which the Vps28p component of ESCRT-I can interact with the Vps20p component of ESCRT-III, argues that the three ESCRT complexes (I-III) are able to interact in a more complex manner than a linear conveyer belt would suggest [14]. The concentric ring model proposes the existence of a structure that could be large enough to be visible by electron microscopy (EM) in the same way that nuclear pore complexes are visible by EM, but so far, no such structures have been observed. Whatever the precise sequence of events that govern the assembly, interactions and functions of the ESCRT-0 -I, -II and -III complexes, it is clear that the process is highly dynamic and complex. There will doubtless be many more fascinating insights made from studies of mutants in yeast and other model systems.

An obvious critical question that needs to be addressed is, How is the intra-lumenal vesicle formed? One possibility is that lipids act to deform the membrane and drive the budding process. In mammalian cells the lipid lysobisphosphatidic acid (LBPA) has been localised to intra-lumenal vesicles. When LBPA-containing liposomes are generated *in vitro* with an appropriate pH gradient across the membrane, internal vesicles similar to those observed in multivesicular bodies are produced [50, 51]. A protein called ALIX binds to LBPA-loaded liposomes and has been shown to be required for LBPA to generate the internal vesicles. In yeast, the homolog to ALIX is Bro1p, which binds to the ESCRT-III component Snf7p and also to the de-ubiquitinating enzyme Doa4p thereby linking the ESCRT-III complex with the de-ubiquitylation machinery required to recycle ubiquitin into the cytosol (see below) [14, 52]. In yeast, however, there does not appear to be any LBPA present, so either an analogous lipid can perform the same role or yeast employ very different mechanisms to generate intra-lumenal vesicles to mammalian cells.

The studies in mammalian cells on the downregulation of the EGFR have identified a number of proteins that are phosphorylated in response to EGF stimulation. One of these is Annexin-1, which has been shown to promote inward budding of vesicles in a distinct population of MVBs that are EGFR-positive but LBPA-negative [53, 54]. This study therefore suggests that there are at least two distinct mechanisms that mediate formation of internal vesicles within the MVB, one involving Annexin-1 and another requiring LBPA.

Although the process of budding vesicles into the endosome lumen remains mysterious, it is clear that before this occurs, the ubiquitin is released from the membrane protein (e.g. CPS) by ubiquitin hydrolase enzymes to be recycled into the cytoplasm. In yeast, the ubiquitin hydrolase is Doa4p [55, 56], whilst in mammalian cells the functional orthologs are AMSH

[associated molecule with the Src homology 3 (SH3) domain of STAM] and UBPY (ubiquitin-specific protease Y), although AMSH and UBPY do not perform identical roles. UBPY is required for degradation of ubiquitylated EGFR via internalisation into multivesicular bodies, but AMSH is not [48, 57, 58]. Disassembly of the ESCRT complexes is driven by the ATPase activity of Vps4p, a member of the AAA-ATPase family of proteins that also includes the N-ethyl maleimide sensitive factor (NSF) that functions to disassemble SNARE complexes [59–61]. Disassembly appears to be the reverse of assembly and is initiated by the interaction between Vps4p and the ESCRT-III complex. How is the function of the ESCRT complexes and the process of generating intra-lumenal vesicles regulated? This question has yet to be answered beyond the clear requirement for ATP hydrolysis by Vps4p to regulate the assembly/disassembly of the ESCRT complexes. One possibility for regulation is the role of the only transmembrane protein among the class E genes – namely Vps44p/Nhx1p [62] - which functions as a sodium/proton exchanger, releasing protons from the lumen of the endosome and sending sodium ions in the opposite direction. It is possible then that Vps44p could regulate the activity of the ESCRT complexes by 'sensing' the lumenal pH and stimulate/inhibit the ESCRT complexes accordingly. Although at this point it is not known whether Vps44p/Nhx1p can directly interact with any of the members of the ESCRT complexes. Alternatively, the Vps44p/Nhx1p-driven influx of sodium ions into the endosomal lumen could activate or trigger the function of lumenal proteins or lipids that drive intra-lumenal vesicle formation. These possibilities remain to be tested.

Retrieval to the TGN

The TGN is itself a major site of protein sorting, directing biosynthetic proteins towards the plasma membrane for secretion or to an endosomal compartment for further sorting, including possible retrieval to the TGN [63]. There is, therefore, bi-directional traffic between the endosome and the TGN, and these pathways are essential for the sorting of newly synthesised lysosomal/vacuolar hydrolases for ultimate delivery to the lysosome/vacuole. In recent years, much progress has been made in identifying the sorting machinery that operates in this cyclical pathway and understanding the mechanisms that control protein localisation to the TGN. I will focus on the machinery and mechanisms that occur at the endosomal membrane, but sorting events at the endosome can and do impinge on the sorting machinery at the TGN.

As with the elucidation of the role of the ESCRT complexes in sorting ubiquitylated cargo, studies in yeast have led the way to understanding the endosome-to-TGN pathway. The yeast vacuolar hydrolase receptor Vps10p is essential for sorting CPY to the vacuole [64, 65]. Vps10p binds CPY in the late-Golgi and is then sorted by the Golgi-associated, γ-ear containing, ARF binding (GGA) proteins into clathrin-coated vesicles for delivery to a prevacuolar endosome [66]. For efficient sorting of CPY to be maintained, Vps10p must be retrieved from the endosome to the Golgi [67]. The initial identification of the proteins that are responsible for sorting of Vps10p for retrieval came about through the realisation that mutants with phenotypes similar to the *vps10* mutant would be candidates for functioning in trafficking Vps10p. This hypothesis was proved true when the *VPS35*, *VPS30* and *VPS29* genes were shown to be required for retrieval of Vps10p from the endosome to the Golgi [68]. Loss of function of these genes results in Vps10p becoming mislocalised to the vacuolar membrane, producing a strong CPY sorting defect. Biochemical studies using cross-linking reagents revealed that Vps35p directly interacts with Vps29p and forms a heteropentameric complex which also contains Vps26p, Vps5p and Vps17p [69]. Vps5p and Vps17p are members of the sorting nexin family of proteins and act as a heterodimer through their C-terminal domains, which contain coiled-coil regions required for dimerisation [70].

The complex formed by the Vps35, Vps29, Vps26, Vps17 and Vps5 proteins was named retromer to denote its role in retrieval. Interestingly, Vps30p does not associate with the retromer complex but instead binds to the Vps38 protein, and together they play a critical regulatory role in endosome-to-Golgi retrieval by stimulating the production of Ptd-Ins 3-P by the lipid kinase Vps34p [71]. Ptd-Ins 3-P is required for the membrane association of Vps5p and Vps17p, which dimerise and are recruited to Ptd-Ins 3-P-rich regions on the endosomal membrane by their Ptd-Ins 3-P binding p40-phox homology (PX) domains [72]. Biochemically, and phenotypically, the heteropentameric retromer complex can be dissected into two subcomplexes which fulfil distinct roles in mediating the endosome-to-Golgi retrieval of Vps10p [69]. Vps35p/Vps29p/Vps26p together form the cargo-selective subcomplex, whilst the Vps5p/Vps17p dimer has a structural role deforming the endosomal membrane to generate tubular or vesicular transport intermediates. The Vps35 protein can directly interact with the cytoplasmic tail of Vps10p [73, 74].

In mammalian cells, the functional counterparts to Vps10p are the mannose-6-phosphate receptors (MPRs). There are two types of MPRs, the cation-dependent (CD-MPR) and the cation-independent (CI-MPR) [75]. Like Vps10p, both are type-I transmembrane proteins. Both bind to lysosomal hydrolases in the TGN and are sorted by the GGA proteins into clathrin-coated vesicles for delivery to an endosome [76, 77]. After dissociating from its ligand, both are retrieved to the TGN for further rounds of hydrolase sorting, although it is possible that different sorting machinery is required for the endosome-to-TGN retrieval of the CI-MPR and CD-MPR. Retrieval of the CI-MPR requires the mammalian retromer complex [78, 79]. With the exception of Vps17p, each of the members of yeast retromer is conserved in mammalian cells. Although there is no obvious Vps17p ortholog, the Vps5p homolog, SNX1, forms a dimer with SNX2, thereby emulating the Vps5p/Vps17p dimer in yeast [80]. In mammalian cells, the picture is further complicated by the involvement of additional sorting nexin proteins, SNX5 and SNX6, that interact with SNX1 and SNX2 in the endosome-to-TGN retrieval of the CI-MPR [81]. SNX1 can tubulate membranes *in vitro* and *in vivo* through the action of its C-terminal Bin, Amphiphysin, Rvs (BAR) domain, and therefore the SNX1 protein can act in a similar manner to Vps5p in yeast [82]. Mammalian VPS35 can interact with the cytoplasmic tail of the CI-MPR, but the interaction is apparently weak, requiring the yeast two-hybrid system to detect [79].

There has been very significant progress made in determining the structures of the mammalian retromer complex proteins. The structure of the VPS29 protein was solved first, revealing that VPS29 has a phosphoesterase fold similar to those present in the family of phosphatases typified by PP2A [83]. Biochemical studies have now shown that VPS29 can desphosphorylate the serine preceding the C-terminal acidic dileucine in the tail of the CI-MPR [84]. As phosphorylation of this serine enhances the binding of the GGA proteins to the acidic dileucine [85], the role of VPS29 (and thereby retromer) in dephosphorylating this sorting motif neatly illustrates how the activities of the GGA sorting machinery at the TGN are coupled to the function of retromer at the endosomal membrane, thereby creating a cyclical pathway. The structure of VPS26 revealed an unexpected similarity to the arrestin family of proteins [86]. Arrestins act at the plasma membrane to sort specific cargos into endocytic clathrin-coated vesicles [87]. The similarity of VPS26 to the arrestins hints that perhaps VPS26 might fulfil a similar cargo-selective role within retromer. This hypothesis has yet to be proved, but if the interactions between retromer and cargo proteins such as the CIMPR were to involve not only VPS35 but also VPS26 and VPS29, then the heterotrimeric complex would perhaps be able to interact more strongly *in vivo* than the relatively weak interaction observed for VPS35 and the CI-MPR tail using the two-hybrid system.

Recent studies using chimaeric reporter proteins have identified a conserved sorting motif in the cytoplasmic tail of the CI-MPR that is required for its endosome-to-Golgi retrieval and for the interaction with retromer *in vivo*. This motif comprises hydrophobic residues WLM and is similar to a conserved sequence in the cytoplasmic tail of another protein that is retrieved to the TGN by retromer, namely sortilin. The motif FLV in the sortilin tail is essential for the retrieval of a reporter protein carrying the sortilin tail [88]. It is noteworthy that the CI-MPR and sortilin should have very similar motifs that are required for their endosome-to-Golgi retrieval as they also have identical acidic di-leucine motifs, which have been shown to promote the recruitment of GGA2 to the TGN membrane [89]. This is another example of how the sorting of proteins at the TGN is functionally linked with the sorting events at the endosomal membrane even though the two compartments are spatially separated.

How does retromer recognise the hydrophobic motifs present in the tails of the CI-MPR and sortilin? This question remains to be answered and provides one of the many challenges for researchers in this field. It is possible that additional accessory proteins could contribute to the mechanisms of cargo recognition by retromer. In yeast, the Grd19 protein associates with retromer to mediate the endosome-to-Golgi retrieval of Ftr1p, which is required for iron homeostasis [90]. Under iron-replete conditions, Ftr1p is degraded in the vacuole, but when iron becomes limiting, it is diverted away from the degradative pathway by Grd19p, which then employs the retromer-mediated endosome-to-Golgi retrieval pathway to deliver Ftr1p to the yeast Golgi for resecretion to the plasma membrane. This is an excellent example of how the sorting/trafficking of proteins within the endosomal system can be regulated in accordance with changing environmental conditions. As Grd19p has also been shown to interact with the cytoplasmic tail of the mating pheromone processing enzyme Kex2p to mediate the retrieval of Kex2p [91], Grd19p is likely to be constitutively active in endosome-to-Golgi retrieval. Therefore, the interaction between Grd19p and Ftr1p must be subject to some form of regulation so that it occurs under iron-limiting conditions when Ftr1p is directed into the endosome-to-Golgi path-

way. What form this regulation takes has yet to be determined.

Another protein that could increase the repertoire of cargo binding for yeast retromer is Btn2p which functions to maintain the Golgi localisation of the Yif1 protein [92]. Btn2p is weakly homologous to the protein Hook1, which in mammalian cells associates with the cytoskeleton and in *Drosophila* interacts with endocytic vesicles. Btn2p was found to associate with retromer and also Snx4p (which is discussed in more detail below) and the endocytic SNARE proteins Snc1p, Snc2p, Tlg1p, Tlg2p and Vti1p. As *BTN2* deletion does not result in the mislocalisation of Vps10p, the role of Btn2p is likely to be to facilitate the sorting of specific Golgi-resident proteins such as Yif1p.

In addition to interacting with cargo proteins, mammalian retromer has recently been shown to interact with EHD1 [93]. The EHD1 protein is a member of a family of proteins that contain Eps15p homology (EH) domains and has been shown in several studies to be involved in the endosome-to-plasma membrane trafficking pathway [94, 95]. Studies using GFP-tagged EHD1 have revealed that it is localised to threadlike tubules that serve as transport intermediates from endosomes. The interaction between retromer and EHD1 argues that, in addition to a role in endosome-to-plasma membrane transport, EHD1 also acts in the endosome-to-TGN pathway. What might EHD1 do in these pathways? As EHD1 is detected on endosomal tubules, it is possible that it plays a role in forming or stabilising these tubules. In the retromer-mediated endosome-to-TGN pathway, the sorting nexin protein SNX1 can induce membrane tubulation both *in vitro* and *in vivo* [82]. Tubules would serve as excellent transport intermediates for the retrieval of membrane proteins from endosomes to either the TGN or the plasma membrane due to their high surface area-to-volume ratio. Membrane tubules are, however, intrinsically less stable than small vesicles and will therefore require proteins to act as scaffolding to prevent the tubule from vesiculating. EHD1 may be one such 'scaffolding' protein.

Interestingly, loss of EHD1 function by RNAi results in a dramatic reduction of SNX1-positive tubules consistent with a role for EHD1 in stabilising the tubules generated by SNX1 action [93]. The crystallographic structure of EHD2, a closely related paralog of EHD1, suggests another role for the EHD proteins – as a tubule scission enzyme similar to dynamin in function [96]. EHD1 does have an ATPase domain that is required for its function, and this could provide the mechanochemical force necessary to pinch off a tubule from the endosomal membrane. *In vitro* studies using recombinant protein have shown that EHD2 can form 'collars' around a thin membrane tubule consistent with a role in scission [96]. Alternatively, an EHD 'collar' could function to constrain the endosomal tubule at the end close to the endosome and thereby prevent the tubule from being pulled back into the vesicular endosome. A third possibility is that an EHD 'collar' could act as a physical barrier to prevent proteins that have been sorted into the tubule from flowing back into the vesicular endosome. The interaction between EHD1 and retromer would be consistent with the third hypothesis, but resolving how EHD1 (and the other EHD proteins) function in these trafficking pathways will require more work.

Aside from retromer, the cytoplasmic tail of the CI-MPR interacts with a protein called TIP47 [97]. *In vitro* studies using a cell-free system that reconstitutes late endosome-to-Golgi transport has demonstrated the importance of TIP47 in this transport step. TIP47 recognises a di-aromatic motif in the tail of the CD-MPR and binds to a proline-rich region in the CI-MPR tail [98]. TIP47 also binds the small GTPase rab9, which is required for the efficient retrieval of the CI-MPR [99]. There is, however, some controversy over the role of TIP47 in this pathway as TIP47 is both homologous and structurally similar to lipid droplet-binding proteins [100]. TIP47, which is not conserved in lower eukaryotes, may therefore have dual functions in both endosome-to-Golgi retrieval and lipid droplet formation.

The importance of retromer in endosome-to-Golgi retrieval has been demonstrated through studies in both yeast and mammalian cells, but retromer is not the only protein complex that functions in endosome-to-Golgi retrieval. In yeast, the sorting nexin proteins Snx4p, Snx41p and Snx42p function together in the retrieval of the yeast SNARE protein Snc1p, which is required for the fusion of secretory vesicles with the plasma membrane [101]. Snx4/41/42p form a complex that can be chemically cross-linked to the yeast SNARE protein Snc1p, suggesting a direct interaction, although the molecular basis for this interaction is not yet known. Snx4p, Snx41p and Snx42p are all members of the sorting nexin family and therefore contain Ptd-Ins 3-P-binding PX domains and coiled-coiled domains in their C-terminal halves which are likely to be required for tubule/vesicle formation. The membrane association of the Snx4/41/42 protein complex depends upon Vps38p which is required to stimulate the Ptd Ins 3-kinase Vps34p. Interestingly, Vps38p is also required for membrane association of the retromer sorting nexin dimer Vps5p/Vps17p [72, 101]. Given that both retromer and the Snx4/41/42p complex depend on Vps38p to stimulate Vps34p activity, and both retromer and Snx4/41/42p associate with the Btn2 protein, a potential conclusion is that

retromer and Snx4/41/42p could be acting in the same retrieval pathway but sorting different cargo proteins: Vps10p for retromer and the Snc1p SNARE protein for Snx4/41/42p. This hypothesis could possibly be proved or disproved using the resolving power of electron microscopy (EM) although these detailed immunoEM studies are notoriously difficult to do in yeast due to the transient nature of the structures being visualised. A schematic diagram detailing some of the sorting events and machinery involved in endosome-to-Golgi retrieval in yeast is shown in Figure 2.

In mammalian cells, SNX4 is necessary to sort the transferrin receptor (TfnR) from early endosomes into the recycling endosome from where the TfnR will be directed back to the plasma membrane. Ablation of SNX4 expression using siRNA results in the degradation of the TfnR in lysosomes. SNX4 function in mammalian cells is facilitated by interacting with KIBRA, a dynein light chain-binding protein [102]. Whether there is a direct interaction between SNX4 and the cytoplasmic tail of the TfnR has yet to be determined, and therefore any sorting motif recognised by SNX4 is also presently unknown. It is interesting, however, that the yeast Snx4/41/42p complex and mammalian SNX4 both act to sort proteins that function at the plasma membrane [101, 102], hinting that there may be a common motif that specifies interaction with Snx4/41/42p or SNX4. The importance of the cytoskeleton in delivery from early endosomes to the recycling endosome is underscored by recent evidence implicating myosin VI along with LMTK2 (Lemur tyrosine kinase 2), a membrane-bound tyrosine kinase, in regulating tubule formation and transport to the rab11-positive recycling endosome [103]. Myosin VI, like other myosins, moves along actin cables (but in the minus-end direction) and has been shown to be important for endocytosis from the plasma membrane, and sorting at the TGN [104]. The substrate for the kinase activity of LMTK2 has yet to be identified, but one intriguing possibility that demands further investigation is a role for LMTK2 in regulating SNX4 function. SNX4 is closely related to SNX1, which is able to tubulate membranes *in vitro* and *in vivo*, so perhaps SNX4 acts in a similar manner, possibly regulated by LMTK2. This is speculative at present, but the similar effects of loss of function of SNX4 or LMTK2 argues that they may be functioning at the same step.

Last, but by no means least of the endosome-to-Golgi retrieval machinery is PACS-1, a protein that binds to acidic cluster motifs present in proteins such as furin and the CI-MPR [105]. Furin is an endopeptidase that functions as pro-homone convertase in the TGN, similar to the Kex2 protein in yeast. Like many TGN-resident proteins, furin periodically escapes the TGN and is then retrieved from an endosome to the TGN. Studies using transiently expressed dominant-negative mutants of PACS-1 indicate that this retrieval requires PACS-1 and the clathrin adaptor complex AP-1. PACS-1 binds to an acidic patch in the tail of furin to direct furin into vesicles for retrieval to the TGN [103]. PACS-1 along with AP-1 is also required for retrieval from immature secretory granules to remove proteins such as furin as the granule matures before fusion with the plasma membrane [106]. Studies using transgenic mice have shown that AP-1 is also important for the retrieval of the CD-MPR and the CI-MPR [107], although siRNA ablation of AP-1 expression does not prevent the endosome-to-TGN retrieval of a chimaeric protein carrying the cytoplasmic tail of the CI-MPR [88].

AP-1, like the other adaptor complexes, AP-2, AP-3 and AP-4, recognises the tyrosine-containing motif, YxxΦ (where Φ is a hydrophobic residue), which is bound by the medium chains (μ-subunits) of the adaptor complexes [108, 109]. Structural studies on the related adaptor complex, AP-2, have shown that phosphorylation of the AP complex regulates YxxΦ binding by inducing a conformation change in the μ-subunit to generate an 'open' conformation that facilitates the interaction between μ and the YxxΦ motif. This change in conformation is also necessary for binding to phosphotidylinositol phosphate in the membrane [109]. It has yet to be determined whether precisely the same mechanisms govern the binding of YxxΦ motifs to AP-1, but *in vitro* studies using recombinant CI-MPR tails have indicated that membrane-associated AP-1 (which is phosphorylated) is able to strongly interact with YxxΦ motifs whilst cytosolic AP-1 (non-phosphorylated) only weakly interacts with YxxΦ motifs [110, 111]. AP-1 is able to recognise and sort not only YxxΦ motifs but also acidic di-leucine motifs, although the molecular mechanisms that govern the interaction between AP-1 and acidic di-leucine motifs have yet to be determined [110]. AP-1 also interacts with a growing number of accessory proteins that can serve to increase the cargo-binding capacity of AP-1. One such accessory protein is EpsinR [112]. Recent studies have shown that EpsinR can interact with the SNARE protein Vti1b, though the interaction does not occur through the recognition of a short linear sorting motif but rather through the interaction of a large number (~15) of surface residues on Vti1b and EpsinR [113, 114]. Mutations that block the interaction between EpsinR and Vti1b result in Vti1b becoming mislocalised to peripheral endosomal structures, implicating EpsinR in maintaining Vti1b in a perinuclear TGN compartment. The EpsinR-Vti1b interaction is there-

fore an exception to the rule that sorting motifs are usually short linear sequences.

In addition to EpsinR, AP-1 also interacts with a trimeric protein complex comprising proteins called γ-synergin, aftiphilin and p200 [115]. The γ-synergin protein is an EH domain-containing protein that binds to γ-adaptin in the globular 'ear' or appendage domain. Together with aftiphilin and p200, γ-synergin can interact with SCAMP1, a member of a family of vesicle-associated proteins. The γ-synergin-SCAMP1 interaction is mediated by the EH domain of γ-synergin-binding NPF motifs in SCAMP1. Interestingly, SCAMP1 can also interact with proteins called Intersectins that function at the plasma membrane [116]. Does the sorting of SCAMPS by the γ-synergin/aftiphilin/p200 complex with AP-1 suggest that AP-1 may be involved in directing proteins into a pathway that leads back to the plasma membrane? This is a very real possibility as AP-1 is also known to be required for generation of vesicles containing the small GTPase rab4, which is believed to be a key regulator of endosome recycling to the plasma membrane [117]. Studies in polarised cells have also shown that a variant of AP-1 which contains an epithelial-specific μ-subunit (μ1b) is required for sorting to the basolateral domain [118]. The role of AP-1 in protein sorting is further complicated by a body of evidence that places AP-1 at the TGN-sorting MPRs with the GGA proteins [119]. The evidence so far suggests that AP-1 could be involved in multiple pathways (endosome-to-TGN, TGN-to-endosome and endosome/TGN-to-plasma membrane), which might be considered unusual for a cytoplasmic coat complex but there are other examples of proteins functioning at more than one step, one of which is the actin motor protein myosin VI [104]. Perhaps the role of AP-1 is modulated by interactions with the various accessory proteins (e.g. EpsinR) that in turn interact with other 'cargo molecules' (e.g. SNAREs such as Vti1b), thereby specifying the directionality of the pathway. For example, AP-1 interacting with PACS-1 could specify retrieval from an endosome [106], whilst AP-1 interacting with the γ-synergin/aftiphilin/p200 complex might be required for AP-1 to sort cargo (e.g. SCAMP1) for delivery to the plasma membrane [115, 116]. What is clear is that the region of the cell where AP-1 is concentrated, namely the perinuclear region, is a complex collection of both TGN and endosomal compartments from where proteins are sorted to multiple destinations. Resolving how AP-1 and other sorting machinery operate to maintain the specificity of protein localisation will require additional studies.

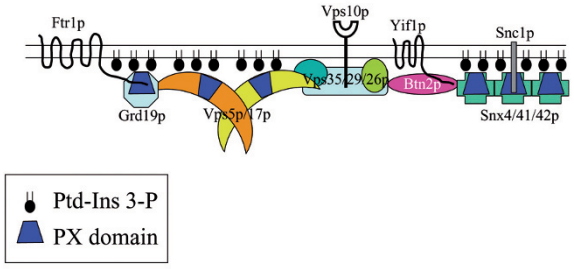

Figure 2. A schematic diagram of the endosome-to-Golgi sorting machinery in yeast. Protein complexes such as retromer and the Snx4/41/42p complex mediate the retrieval of distinct proteins such as Vps10p and Snc1p respectively. Additional accessory proteins Grd19p, and Btn2p serve as cargo-specific adaptors in the retrieval of Ftr1p and Yif1p respectively. The process is regulated by the production of Ptd-Ins 3-P which is bound by PX domains in the sorting nexins proteins, Grd19p, Vps5p, Vps17p, and Snx4/41/42p. The simultaneous interaction of Btn2p with both retromer and Snx4/41/42p is speculative but supported by data from Gerst and colleagues [92].

Endosome-to-plasma membrane transport

The retrieval to the plasma membrane of endocytosed proteins such as the transferrin receptor is required to maintain an active population of receptors at the cell surface to mediate macromolecule uptake and other physiologically important processes. Studying this pathway presents several challenges, not least of which is that recycling of endocytosed receptors to the plasma membrane is rapid, occurring in less than 10 min, suggesting that an endosomal compartment close to the plasma membrane could act as one site of retrieval. Indeed, studies of the recycling of the transferrin receptor (TfnR) utilising cytoplasts in which perinuclear recycling endosomes have been microsurgically removed indicate that a significant proportion of TfnR recycling occurs via peripheral, early endosomes [120]. This 'fast' recycling pathway requires the function of the CART (cytoskeleton-associated recycling or transport) complex in mammalian cells [121]. The CART complex comprises the protein HRS, which is also part of ESCRT-0, and the cytoskeletal proteins actinin-4, BERP (brain-expressed RING finger protein) and myosin V. Loss of CART function results in the TfnR being routed into the 'slower' recycling pathway via perinuclear recycling endosomes. It is not yet clear, however, whether the CART complex mediates TfnR recycling by interacting directly with the TfnR cytoplasmic tail and, if so, which motif within the tail is recognised by the CART complex.

A key component of the perinuclear recycling endosome is the small GTPase rab11, which has a host of interacting proteins, including the exocyst complex, which mediates fusion with the plasma membrane, the

actin motor protein myosin Vb and the Fip2 protein, which in turn interacts with EHD1 and EHD3 [122]. Along with a role in retromer-mediated endosome-to-Golgi retrieval, EHD1 also functions in endosome-to-plasma membrane traffic and has been shown to be important for maintaining the pool of MHC class I and β-integrin at the plasma membrane [94, 123]. The precise function of EHD1 in this pathway has yet to be determined, but it seems likely that EHD1 will play a role in regulating tubule dynamics, possibly by stabilising long endosomal tubules.

How are proteins sorted into the endosome-to-plasma membrane recycling pathway? This question remains to be answered fully, but progress is being made in identifying the machinery that mediates this sorting step, one component of which is the sorting nexin protein SNX17. Studies on the recycling of the LDL receptor-related protein LRP have shown that SNX17 can bind to the cytoplasmic tail of LRP and that the binding requires a membrane proximal NPxY motif, which is one of two NPxY motifs in the tail of LRP [124]. There is some debate over whether SNX17 mediates a direct endosome-to-plasma membrane pathway or perhaps is involved in an endosome-to-TGN pathway that ultimately results in resecretion of LRP to the plasma membrane. Studies on the leukocyte receptor protein P-selectin have shown that SNX17 binds to the cytoplasmic tail of P-selectin and can promote its endocytosis, diverting it away from the degradative/lysosomal pathway, possibly by trafficking P-selectin to an intracellular site such as the TGN [125]. Does SNX17 act alone in this pathway? This question remains to be answered, but it seems likely that other proteins will function alongside SNX17 to mediate endosome-to-plasma membrane recycling.

Another sorting nexin that can recognise sorting motifs is SNX27. A recent study has revealed an interaction between SNX27 and a G-protein-gated potassium channel, Kir3.3, that cycles between endosomes and the plasma membrane to regulate excitability in neuronal cells [126]. The interaction between SNX27 and Kir3.3 occurs through a PDZ [post synaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (DlgA), and zonula occuldens-1 protein (zo-1)] domain in SNX27 and a short linear motif (ESKV) in the C-terminus (cytoplasmic) domain of Kir3.3. Overexpression of SNX27 results in increased lysosomal degradation of Kir3.3, suggesting a role for SNX27 in directing Kir3.3 towards the degradative pathway, although loss-of-function studies using siRNA to ablate SNX27 expression are needed to confirm this hypothesis as overexpression of sorting nexins has been shown to cause dominant negative effects on the endosomal sorting of some proteins, such as the EGFR [127].

Regulation of the plasma membrane localisation of channels and transporters will clearly have profound consequences for the activity of the various channels and transporters that cycle between the plasma membrane and endosomal compartments. One of the most studied is the glucose transporter GLUT4 [128]. In muscle and fat cells, GLUT4 is translocated to the plasma membrane from an intracellular pool after the cells are stimulated by insulin. The binding of insulin by its receptor triggers a signalling cascade, part of which activates the sorting/trafficking machinery required to direct GLUT4 to the plasma membrane. One of the key components of the signalling cascade is Akt, which in turn phosphorylates AS160. The AS160 protein is required for insulin-stimulated GLUT4 translocation to the plasma membrane [129, 130], performing this function by interacting with Rip11, which also binds to rab11, a key mediator of recycling to the plasma membrane [131]. Other studies have indicated that AS160 can also interact with rab10, rab8a and rab14 [132, 133]. Intensive studies of GLUT4 have identified sorting motifs present in the cytoplasmic domain which are required for the proper trafficking of GLUT4. One of these is a di-leucine motif that is recognised by GGA proteins at the TGN. The other is a motif comprising the amino acids FQQI that is necessary for AS160-dependent GLUT4 translocation to the plasma membrane [134]. The FQQI motif is biochemically similar to the YxxΦ motifs recognised by the clathrin adaptor complexes, perhaps hinting at a role for one of the AP complexes to be involved in GLUT4 translocation. The structural studies on the recognition of YxxΦ motifs by the μ-subunits of AP argue against the FQQI motif being able to act like a YxxΦ motif, as hydrogen bonds from the hydroxyl group on tyrosine residues in the YxxΦ motif are critical to the interaction between YxxΦ motifs and clathrin adaptor μ-subunits [135]. There are, however, μ-adaptin-related homologs of unknown function [136] that perhaps could act in sorting of the GLUT4 via the FQQI motif, although this is currently just speculation.

The translocation of GLUT4 to the plasma membrane is a good example of how cells can respond to external stimuli and regulate sorting/trafficking from the endosome to the plasma membrane. Simple eukaryotes such as yeast can also do this. In *S. cerevisiae*, the general amino acid permease (Gap1p) is normally rapidly degraded via the Rsp5p ubiquitin-mediated degradative pathway when yeast are in nitrogen-rich media [137]. However, under nitrogen starvation conditions, Gap1p is sorted by the endo-

somally localised GSE (GTPase-containing complex required for Gap1p sorting at endosome) complex for delivery to the plasma membrane to scavenge any available amino acids and transport them into the yeast [138]. This sorting and trafficking to the plasma membrane does not appear to involve retrieval to the Golgi and subsequent resecretion to the plasma membrane, and is therefore different to the trafficking of Ftr1p, which was discussed earlier. The GSE complex comprises the five distinct proteins, Gtr1p, Gtr2p, Gse1p, Gse2p and Ltv1p, and can directly bind to a di-aromatic motif in the cytoplasmic domain of Gap1p. Curiously, two of the components of the GSE complex, Gtr1p and Gtr2p, are GTPases that regulate the activity of the GSE complex according to their nucleotide-bound state. Gtr2p is able to bind to Gap1p to stimulate Gap1p translocation to the plasma membrane but only when in the GDP-bound state, whilst Gtr1p is active in the GTP-bound state. The Gtr1p and Gtr2p members of the GSE complex are conserved in mammalian cells, but it is not yet clear whether they perform an analogous role to that in yeast.

Endosomal protein sorting and disease

The endosomal system plays a vital role in modulating the response to infection and the generation of immunity not least by controlling the surface expression of the antigen-presenting MHC class I and class II proteins. It is not surprising therefore that various pathogens have evolved mechanisms to intervene in the endosomal system to subvert the trafficking of immunologically important proteins. A good example of viral intervention in the endosomal system is the karposi sarcoma-associated herpes virus (KSHV) encoded K3 protein that is able to induce the downregulation of MHC class I by ubiquitylation. The KSHV K3 protein encodes a ubiquitin ligase of the E3 family that induces the endocytosis of MHC class I and subsequent lysosomal degradation following TSG101-dependent sorting into intra-lumenal vesicles of MVBs [139, 140]. The KSHV K3 protein therefore employs the ESCRT complex machinery to its own ends to downregulate cell surface MHC class I and enable the virus to evade the immune system.

The human immunodeficiency virus (HIV) not only has the ability to downregulate MHC class I from the cell surface but can also hijack the ESCRT machinery to generate more virus particles. In the case of HIV, the virally encoded Nef protein acts as an adaptor binding to MHC class I and the clathrin adaptor complex AP-1 to divert MHC class I away from a trafficking pathway to the cell surface [141, 142]. Nef is able to interact with AP-1 using an acidic di-leucine motif within the Nef protein. But intriguingly, whilst AP-1 is essential for Nef-induced MHC class I downregulation, mutation of the acidic di-leucine motif does not prevent this, hinting that there may be other mechanisms that allow Nef to interact with AP-1. HIV also encodes proteins that interact with the ESCRT machinery, one of which, HIV-1 p6 (also known as Gag), binds to the ESCRT-I component, TSG101 (the mammalian Vps23 homolog) [143] and also to ALIX (also known as AIP1), which has been functionally linked to LBPA-mediated internal vesicle formation [144]. Another HIV-encoded protein, EIAV p9, also binds to ALIX/AIP1. The ability of retroviral pathogens such as HIV to subvert the endosomal protein-sorting machinery for their own propagation enables these viruses not only to escape immune surveillance but also to have small genomes encoding only a minimal number of proteins required to make more virus particles.

Virus pathogens are not the only onslaught the endosomal system faces. Various bacteria can alter the activity of key regulators of endosomal protein sorting to promote their own survival. One of the best studied is *Helicobactor pylori,* which is the causative agent in many stomach ulcers. The VacA protein secreted by *H. pylori* can form anion-selective channels in the limiting membranes of lysosomes and late endosomes, resulting in an accumulation of vacuolated structures. Inhibition of the activity of the small GTPase rab7 prevents the formation of the vacuolated endo/lysosomes linking the activity of rab7 to the generation of vacuolated structures by the VacA toxin [145]. Other bacterial pathogens employ the endocytic machinery to mediate the cellular uptake of toxins. For example, the bacteria *Shigella,* which can cause diarrhoea, secretes Shiga toxin, which is endocytosed and subsequently delivered to the TGN via the retromer-mediated route [146, 147].

In addition to the effect of pathogens on the endosomal system, there are a number of genetic diseases that cause altered endosomal protein sorting, sometimes as the result of mutations to sorting motifs, and other times the result of mutations to sorting machinery. An example of a mutation to a sorting motif that causes disease is Liddle's syndrome, a form of inherited hypertension which is caused by mutation of a PY motif in the sodium channel protein ENaC [148]. In the presence of high levels of sodium ions, the ENaC protein is downregulated through ubquitylation, which is mediated by the NEDD4 protein, the mammalian equivalent of yeast Rsp5p. The PY motif is necessary to recruit the NEDD4 and hence mutation of the PY motif

Table 1.

Pathway	Cargo		Motif(s)		Machinery		Components	
	Yeast	mammals	Yeast	mammals	Yeast	mammals	Yeast	mammals
Into MVB	CPS, Ste2p	EGFR	PY	PY	Ub ligase	Ub ligase	Rsp5p	NEDD4
	CPS, Ste2p	EGFR	Ub	Ub	ESCRT-0	ESCRT-0	Vps27p Hse1p	HRS STAM1,2
	CPS, Ste2p	EGFR	Ub	Ub	ESCRT-I	ESCRT-I	Vps23p Vps28p Vps37p Mvb12p	TSG101 VPS28 VPS37A,B,C,D MVB12A,B
	CPS, Ste2p		Ub	Ub	ESCRT-II	ESCRT-II	Vps36p Vps25p Vps22p	EAP45 EAP20 EAP30
	CPS, Ste2p	EGFR			ESCRT-III	ESCRT-III	Vps2p Vps20p Vps24p Snf7p	CHMP2A,B CHMP6 CHMP3 CHMP4A,B,C
Endosome-to-Golgi	Vps10p	CI-MPR, sortilin	FYVF(?)	WLM, FLV	retromer	retromer	Vps35p	VPS35
	Kex2p		FxFxD				Vps29p	VPS29
	DPAPA		FxFxN				Vps26p Vps5p Vps17p	VPS26A,B SNX1 SNX2 SNX5, SNX6
	Ftr1p		?		Grd19p+retromer		Grd19p+retromer	SNX3+retromer(?)
	Snc1p		?		Snx4 complex		Snx4p Snx41p Snx42p	
		Furin		acidic patch		PACS-1+AP1		PACS-1 γ-adaptin, σ1-adaptin β1-adaptin, μ1-adaptin
	Vti1	Vti1b	surface residues?	surface residues	EpsinR+AP1(?)	EpsinR +AP1		EpsinR+AP-1
Endosome-to-plasma membrane recycling		TfnR		?		CART complex		HRS, BERP, Actinin-4, myosin V
						SNX4		SNX4 KIBRA
		LRP		NPxY		SNX17		SNX17
	Gap1p		WY		GSE complex		Gtr1p Gtr2p Gse1p Gse2p Ltv1p	
		SCAMP1		NPF		aftiphilin complex + AP1		aftiphilin γ-synergin p200 AP-1

results in a failure to ubquitylate and downregulate the sodium channel.

The mutations to the adaptor complex AP-3 that result in a form of Hermansky-Pudlack disease provide an example of a genetic disease where sorting machinery is affected. AP-3 is related to AP-1 and functions to sort membrane proteins to lysosomes and also lysosome-related organelles such as melanosomes. Loss of AP-3 function is not lethal but instead causes a form of albinism due to defects in pigment granule formation in melanocytes and also results in slow blood clotting due to deficiencies in platelet function [149, 150].

Summary and future perspectives

Sorting in the endocytic system is a complex and highly dynamic process in which a wide variety of sorting motifs are recognised by specific sorting machinery to direct the transport of a membrane protein to its destination. Some of the key sorting motifs and machinery have been described here (and are summarised in Table 1). But there are likely to be other sorting motifs which remain to be identified. Some will be short linear motifs such as the YxxΦ or acidic di-leucine motif; others may comprise many residues on the surface of a folded domain as is the case for the EpsinR-Vti1b interaction. There will also be additional sorting machinery discovered that will be able to recognise the novel sorting motifs, and there may be regulatory proteins that can act on either the sorting motifs or the sorting machinery to modulate protein sorting in the endocytic system. As the motifs and mechanisms that govern endosomal protein sorting become clearer, it is likely that there will be many more additional examples of disease resulting from aberrant protein localisation, and there are exciting times ahead as these studies are reported. The advent and application of siRNA and high-throughput technologies will doubtless be instrumental in delineating the endosomal protein-sorting pathways, and identifying and defining the networks of protein-protein interactions that underpin the mechanisms of endosomal protein sorting.

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