The retromer complex – from genesis to revelations

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Abstract

The retromer complex has a well-established role in endosomal protein sorting, being necessary for maintaining the dynamic localization of hundreds of membrane proteins that traverse the endocytic system. Retromer function and dysfunction is linked with neurodegenerative diseases including Alzheimer's and Parkinson's disease and many pathogens - both viral and bacterial - exploit or interfere in retromer function for their own ends. In this review, the history of retromer is distilled into a concentrated form that spans the identification of retromer to recent discoveries that have shed new light on how retromer functions in endosomal protein sorting and why retromer is increasingly being viewed as a potential therapeutic target in neurodegenerative disease.

The discovery of retromer

The endomembrane system is a defining feature of eukaryotic cells and is conserved across the eukaryotic kingdom [1]. The **retromer** complex (see Glossary) has its genesis in genetic screens conducted in yeast *Saccharomyces cerevisiae* that identified >40 **vacuolar protein sorting** (***VPS***) genes necessary for the delivery of a hydrolase, **carboxypeptidase Y** (**CPY**), to the vacuole [2], [3]. A key gene in trafficking of CPY to the yeast vacuole is the *VPS10* gene which encodes a transmembrane receptor protein (Vps10p) for CPY [4].

Identification of the retromer complex came about through the analysis of *vps* mutants phenotypically similar to a *vps10*Δ mutant and *vps29*, *vps30* and *vps35* were identified [5]. The similarity of the phenotypes displayed by the *vps29*, *vps30* and *vps35* mutants suggested that they might function together – possibly as part of a large protein complex. Biochemical analyses revealed that Vps29p and Vps35p do indeed interact and with three other proteins - Vps5p, Vps17p and Vps26p - form a complex that was named retromer [6]. Vps30p was not detected in association with Vps29p or Vps35p and its role in regulating Vps10p trafficking was elucidated later in separate studies. Following *in vivo* and *in vitro* experiments, it was postulated that Vps5p and Vps17p assemble onto endosomal membranes to drive formation of a vesicle whilst Vps35p with Vps29p and Vps26p perform a cargo-selective role and direct proteins such as Vps10p into vesicles. Thus retromer was adjudged to comprise two functionally distinct subcomplexes – the cargo-selective trimer of Vps35p-Vps29p-Vps26p and a membrane bending dimer of Vps5p-Vps17p. Further studies conducted in both yeast and mammalian cells provided the first insights into how retromer assembles (Box 1).

The sorting nexin connection

For retromer to be membrane associated in yeast, the Vps5p-Vps17p dimer binds to phosphatidylinositol 3-phosphate (PtdIns3P) that is generated by the Vps34 phosphatidylinositol 3-kinase – a process regulated by Vps30p. The p40 phox-homology (PX) domains in Vps5p and Vps17p bind PtdIns3P to enable retromer membrane association [7]. PX domain-containing proteins are often referred to as **sorting nexins** – abbreviated to **SNX** [8], [9]. In mammals, there are two Vps5p homologues; SNX1 and SNX2. Vps17p lacks an obvious homologue in mammals but SNX5 and SNX6 function orthologously and form heterodimers with SNX1 or SNX2 [10]–[12]. Other sorting nexin proteins are functionally linked with the Vps35-Vps29-Vps26 trimer including SNX27 and SNX3, both of which are implicated in recognizing membrane proteins and are discussed in more detail below.

Conservation in evolution and insights from structural studies

The Vps26p component of retromer is highly conserved in mammals and has undergone a gene duplication to two distinct but highly related versions; Vps26a and Vps26b [13]. The two Vps26 proteins may have slightly different preferences with respect to endosomal protein sorting [14] although there is also data indicating functional redundancy [15]. X-ray crystallography has revealed that Vps26 is related to the arrestin family of proteins that operate in **cargo** selection for clathrin-mediated endocytosis hinting at a similar role for the Vps26 proteins [16], [17]. Analysis of *vps35* mutants identified a key region of the N-terminus of Vps35p necessary for association with Vps26p [18]–[20]. The C-terminal third of Vps35p binds to Vps29p which acts as a scaffold around which Vps35 folds [21]. Vps35 has not yet been crystalized in its entirety possibly because it is comprised of many α-helical HEAT repeats and adopts a solenoidal conformation which endows the Vps35 protein with intrinsic flexibility.

Studies using cryo-electron microscopy (EM) with tomographic imaging have proposed an arrangement for membrane-associated retromer from a thermophilic fungus (*Chaetomium thermophilum*) bound to recombinant Vps5p. The Vps35p-Vps29p-Vps26p trimer, when assembled on liposomes and imaged by cryo-EM formed 'arches' where two trimers met at a vertex of the C-terminal domain of Vps35p [22]. Vps26p, bound to Vps35p contacted the C-terminal region of Vps5p protein that was arranged in dimers on the liposomes (Figure 1). Vps17p was absent because it could not be successfully expressed in bacteria. The addition of the retromer proteins to the liposomes induced formation of tubules dimensionally similar to tubules observed *in vivo*. However, the cryo-EM data possibly downplays the role of Vps29p in assembly [23] and in-vivo experiments also strongly favour a role for the N-terminal region of Vps5p in mediating the association with the Vps35p-Vps29p-Vps26p trimer [24] but cryo-EM could not image the Vps5p N-terminal region due to its unstructured nature. The absence of Vps17p and its replacement by Vps5p could essentially double the available interaction sites for the trimer of Vps35p-Vps29p-Vps26p with the Vps5p dimer possibly contributing to the density and profusion of retromer arches observed.

A more recent cryo-EM-based study [25] indicates that the murine retromer trimer of Vps35-Vps29-Vps26 form a greater variety of oligomeric structures than was observed when retromer from the thermophilic fungus was imaged. Additionally, studies of retromer proteins bound onto supported lipid bilayers did not reveal a significant propensity to

generate tubular structures [26]. It is possible that the choice of a thermophilic fungus as the source of retromer proteins could have resulted in the retromer arches being especially rigid as thermophilic organisms must contain highly stable proteins and protein complexes that can endure at higher temperatures. In contrast, mammalian retromer proteins are quite labile at higher temperatures [27] and *in vivo* EM localization of retromer have not reported any arch-like structures, or even electron-dense membrane-associated coats although retromer is often present on endosomal tubules [28], [29]. Thus it remains to be determined how retromer assembles on membranes *in vivo* and to what extent the arches formed by the Vps35-Vps29-Vps26 trimer can contribute to membrane tubulation.

Similarities and differences from yeast to mammals

It is important to note that there are clear differences between the retromer complex as it was first reported in the yeast *S. cerevisiae* and the retromer complex in higher eukaryotes such as mammalian cells. Whereas in yeast, retromer is a stable heteropentamer that can readily be recovered by immunoprecipitation [6], [24], [30], in mammalian cells the trimer comprising Vps35-Vps29-Vps26 does not readily associate with the dimer containing the mammalian SNX proteins [31], [32] and the two subcomplexes can actually be localized to discrete regions of endosomal membranes [33]. Indeed, for mammalian cells, the term "retromer" has evolved to mean just the trimer of Vps35-Vps29-Vps26 and this is also sometimes called the "core" retromer complex or the "**cargo-selective complex**" [34], [35]. The dimer of SNX1 or SNX2 with SNX5 or SNX6 is then referred to simply as the SNX dimer or sometimes the SNX-BAR dimer to convey the fact that the C-terminal coiled-coils regions comprise Bin-Amphiphysin-Rvs (BAR) domains that can drive membrane tubulation [36]. In mammals, the lack of a robust association between the retromer trimer of Vps35-Vps29-Vps26 and the SNX-BAR dimer has implications for the membrane association of the retromer trimer (discussed below) and may also hint that the arches formed by the retromer trimer could make a substantial contribution to tubule formation – although this currently is perhaps somewhat speculative.

The studies of retromer function in yeast clearly established a role for it in the endosome-to-Golgi retrieval of the CPY receptor Vps10p [5], [6], [37]. Initial analyses of retromer in mammalian cells indicated broad agreement with the observations from yeast. Mammalian retromer was shown to localize to endosomes and was necessary for the endosome-to-Golgi retrieval of the **cation-independent mannose 6-phosphate receptor** (**CI-MPR**), a membrane protein that functions analogously to Vps10p. Loss of retromer

function results in reduced trafficking of lysosomal hydrolases due to impaired endosome-to-Golgi retrieval of the CI-MPR. Other membrane proteins were also found to depend on retromer for their retrieval to the Golgi including a protein called sortilin that is homologous to Vps10p [28], [29].

Accessory factors for retromer

In mammalian cells (and many other higher eukaryotes) retromer and the SNX dimer associate with a number of accessory proteins that facilitate the sorting of membrane proteins on endosomes, many of which are not present in simple eukaryotes such as yeast. Perhaps best known of the mammalian retromer accessory proteins is the **WASH complex** – a set of five proteins that contains the actin nucleating promoting factor (WASP and SCAR homologue) Wash1 and regulates formation of filamentous (F)-actin on endosomes [32], [38], [39]. The WASH complex, although ancient in its evolutionary origins [40], is completely absent in fungi including *S. cerevisiae*. The other proteins of the WASH complex (KIAA1033, strumpellin, Fam21 and CCDC53) assemble and function with Wash1 in endosomal F-actin production but their individual roles have yet to be fully defined. For the WASH complex to associate with retromer, a direct interaction between Fam21 and Vps35 is required which ensures that the WASH complex is localized to endosomes in mammalian cells where retromer is present [41]–[43] (Figure 2).

The analysis of trafficking defects observed in cells lacking WASH complex function indicated wide-ranging effects on multiple membrane proteins including the transferrin receptor, the CI-MPR, and proteins such as Glut-1 (a glucose transporter) and the β2-adrenergic receptor (β2AR) [38], [39], [44], [45]. The majority of studies however appear to favour a role for the WASH complex in trafficking from endosome-to-the cell surface and it is notable that the WASH complex is not conserved in yeast where retromer mediates endosome-to-Golgi retrieval. One of the reasons why understanding the role of the WASH complex in endosomal protein sorting is a challenge is that there are a number of proteins that interact with the WASH complex to either regulate its function or affect endosomal protein sorting in another manner. For example, the SNX27 protein interacts with the Fam21 protein and Vps26 and is essential for the endosome-to-cell surface recycling of several cargo proteins including Glut-1 and the β2Adrenergic receptor (β2AR) [45], [46]. Like the WASH complex, SNX27 is not conserved in yeast supporting the view that the WASH complex with SNX27 is more important for recycling to the cell surface than retrieval to the Golgi. The

recently identified CCC complex is another set of proteins that associate with the WASH complex via Fam21 and play an important role in endosomal protein sorting [47].

The SNX-BAR dimer (i.e. SNX1, SNX2 with SNX5/SNX6) also has notable accessory proteins, namely the dynactin-dynein complex. The dynein protein is a minus end-directed microtubule motor that functions in the transport of large structures (e.g. vesicles or tubules) along microtubules towards the microtubule organising centre (MTOC) where the Golgi complex resides [48], [49].

Retromer and Parkinson's disease

In some cases of familial autosomal dominant Parkinson's disease (PD), a mutation to Vps35 (D620N) results in PD with symptoms that are typical of idiopathic PD. The effect of the mutation is to impair the association of Vps35 with the WASH complex resulting in reduced WASH complex recruitment to endosomes [44], [50]. This leads to defects in endosomal protein sorting including reduced CI-MPR retrieval, mislocalization of Glut-1 to endosomes and effects on the trafficking of glutamate receptors in neuronal cells although currently it is not known how this mutation actually alters endosomal F-actin [44], [51], [52]. The effect of the Vps35 D620N mutation extends to reduced lysosomal function and consequences for processes such as autophagy that depend on functional lysosomes [50]. Interestingly, the D620N mutation lies close to the interface between two Vps35 proteins at the apex of the 'arch' observed through cryoEM. Currently however, it is not known if the D620N mutation impacts on the formation or stability of the retromer 'arch.' The position of the D620N mutation does however likely preclude directly impacting on the cargo-sorting activity of retromer.

Along with the D620N mutation in Vps35, other mutations in either Vps35 or Vps26a have been reported to be linked with Parkinson's disease. The R524W mutation in Vps35 impairs membrane association [53] but has not been shown to be fully penetrant, unlike the D620N mutation. Mutations in Vps26a also result in reduced retromer function but cause atypical PD suggesting a somewhat different mode of action to the Vps35 D620N mutation [54], [55].

Similarly to SNX27, the RME-8 protein also binds to Fam21 and may provide a link between the WASH complex and SNX1 [56], [57]. Loss of RME-8 function leads to a pronounced tubulation of endosomal membranes and this results in wide-ranging trafficking defects [56], [58]. RME-8 has also been reported to be mutated in inherited forms of PD and patients with PD-causing RME-8 mutations have clinical symptoms very similar to patients

of Vps35 D620N mutations [59]. Thus, with respect to some of the inherited forms of PD, there appears to be convergence of disease-causing mutations centered on retromer, the WASH complex and endosomal protein sorting [60].

Recruitment to endosomes

The fact that the SNX-BAR dimer and the trimer of Vps35-Vps29-Vps26 do not readily associate in mammalian cells creates a problem in terms of the recruitment of the trimer because, unlike the SNX-BAR dimer, none of the proteins of the Vps35-Vps29-Vps26 trimer have intrinsic membrane-binding activity. In mammalian cells, the retromer trimer binds to Rab7a and SNX3 for its association with endosomal membranes [31], [61]–[65]. The Rab7a protein is a small GTPase conserved across all eukaryotes. Rab7a is active when GTP-bound and a GDP-locked mutant of Rab7a exerts a dominant negative effect on retromer recruitment [31], [61]. Interestingly, a member of the Rab GTPase activating protein (GAP) family, TBC1D5, was identified as interacting with retromer. The TBC1D5 protein, when overexpressed, causes the retromer trimer to dissociate from the membrane similar to the GDP-locked Rab7a mutant [31]. Subsequent studies revealed that TBC1D5 acts as a GAP for Rab7a and thus is a negative regulator of recruitment for retromer [66]–[68].

The TBC1D5 protein binds via a direct interaction with Vps29 and recruitment of TBC1D5 to endosomes requires association with Vps29 [32]. Given that the Vps35-Vps29-Vps26 trimer also recruits the WASH complex, it appears that this conserved protein complex functions as something of a hub to recruit other proteins to endosomes. This view is confirmed by the observation that the Vps35-Vps29-Vps26 trimer also recruits a protein called VARP to endosomes. The VARP protein serves as a guanine nucleotide exchange factor (GEF) for the Rab21 GTPase and is necessary for the endosome-to-cell surface recycling of Glut-1 and other cargo proteins that cycle between endosomes and the cell surface [69].

The SNX3 protein, although a sorting nexin does not contain a **BAR domain** and therefore does not drive membrane tubulation. In addition to mediating recruitment of the retromer Vps35-Vps29-Vps26 trimer with Rab7a, SNX3 may also have an important role to play in selecting cargo [70]. In yeast, the SNX3 protein is called Grd19p and is required for endosome-to-Golgi retrieval of Ftr1p, an iron transporter [71]. Furthermore, genetic screens conducted in the nematode *C. elegans* showed that SNX3 is necessary for retromer-mediated endosome-to-Golgi retrieval of the Wntless protein, a membrane protein involved in the secretion of the morphogen Wnt (summarised in [72]).

Cargo recognition and notable cargo proteins

The mechanisms through which cargo proteins are recognized and sorted by retromer are key to how retromer operates and remain to be formally established although much progress has been made in recent years. For both the CI-MPR and sortilin, hydrophobic sequences in their cytoplasmic tails are necessary for their endosome-to-Golgi retrieval [73]. The sequence W-L-M (tryptophan-leucine-methionine) in the CI-MPR tail and F-L-V (phenylalanine-leucine-valine) in the sortilin tail are key to their retrieval. Later studies showed that Y-L-L (tyrosine-leucine-leucine) in the cytoplasmic domain of an iron transporter (Slc11a2, also known as DMT1-II) is necessary for its retromer-mediated endosome-to-Golgi retrieval thereby creating a consensus of aromatic-leucine-hydrophobic as a motif for endosome-to-Golgi retrieval [74].

It has been established that sorting motifs such as the W-L-M sequence in the CI-MPR play a key role but how are such motifs recognized? Recent data indicates that sorting nexins may mediate the recognition of these aromatic hydrophobic motifs. For the CI-MPR, the SNX5 and SNX6 protein can bind to the cytoplasmic tail requiring the W-L-M motif and their PX domains to do so [75][76]. For other retromer cargo proteins, e.g. the DMTII-1 iron transporter that has a similar motif (Y-L-L), the SNX3 protein, complexed with the retromer Vps35-Vps29-Vps26 trimer provides the means to recognize and sort such cargo proteins [70]. It is not yet clear how sortilin, which has a F-L-V motif is recognized but given that its sorting motif is biochemically very similar to those of the CI-MPR and DMTII-1, it seems likely that it could be recognized by either the SNX5 or SNX6 proteins, or the SNX3 protein in conjunction with the Vps35-Vps29-Vps26 trimer (Figure 3). Thus the understanding of how cargo proteins are sorted has evolved considerably with the role of sorting nexin proteins becoming more prominent.

The Vps26 protein, possibly due to its similarity with arrestins, can also directly associate with cargo, e.g. the SorL1 protein [77] although other factors such as SNX27 may also contribute to sorting SorL1 in mammalian cells [78]. In yeast however, SNX27 is not present and a recent study revealed that the yeast Vps26 protein recognizes specific motifs in different cargo proteins to ensure their retrieval from endosomes [79]. Although some controversy exists as to how important the Vps35-Vps29-Vps26 trimer is for the endosome-to-Golgi retrieval of the CI-MPR [33], [80], [81], it seems likely that the relative importance of the SNX-BAR proteins and the Vps35-Vps29-Vps26 trimer in CI-MPR retrieval may vary between cell types and a recent study of CI-MPR recycling indicates that there are several

distinct carriers that can transport the CI-MPR from endosomes to the Golgi, some are SNX-BAR dependent and some require the Vps35-Vps29-Vps26 trimer [82].

Retromer function can impact on many distinct physiological processes that may occur over extended time periods (e.g. developmental changes via Wnt-mediated signaling) or relatively short term signaling processes, e.g. the role of retromer in regulating the localization and activity of the parathyroid hormone receptor (PTHR) – a G-protein coupled receptor [83]. When the PTHR binds its ligand at the cell surface it is internalised through the action of β-arrestin. Upon arrival at endosomes, the β-arrestin is displaced by Vps26 in the retromer trimer and directs the PTHR into an endosome-to-cell surface recycling pathway. Other signaling pathways that are regulated via retromer-mediated sorting include JAK/STAT signaling initiated through the interferon receptor [84].

The physiological importance of Vps26 in cargo-recognition is perhaps best exemplified by the role that Vps26 with the Vps35-Vps29-Vps26 trimer plays in mediating the trafficking and localization of the SorL1 membrane protein – a membrane protein related to yeast Vps10p. SorL1 is strongly linked to Alzheimer's disease (AD) [85] because SorL1 binds to amyloid precursor protein (APP) and directs the trafficking of APP away from endosomes [86]. SorL1 can associate with the retromer trimer through Vps26 and the APP-SorL1 complex is then trafficked away from endosomes and thus protected from cleavage by BACE1 [77]. It has been reported that expression levels of retromer are reduced in the brains of AD patients and that loss of retromer function can lead to elevated production of Aβ [87], [88]. Interestingly, a mutation in Vps35 which renders it unstable was detected in a patient with early onset AD [89]. Additionally, variants of the SNX3 and Rab7a genes necessary for recruitment of retromer have been linked to late-onset AD [59]. It is interesting to contrast the role of retromer in AD where it appears to be strongly associated with cargo-sorting and its role in PD where the interaction of retromer with accessory proteins (i.e. the WASH complex) may be pivotal in disease mechanisms.

Retromer as a potential therapeutic target

Given the prominent role of retromer in degenerative diseases such as PD and AD [90] that are increasing in prevalence, modulating retromer to increase its activity is being seen as a promising avenue for future therapeutic intervention. In a pioneering study, a small molecule 'chaperone' that binds at the Vps35-Vps29 interface was found to stabilize retromer, increase its levels in cells [27] thus reducing processing of APP to Aβ and

phosphorylation of Tau [91]. The chaperone also enhanced the function of a Vps35 mutant associated with PD [53].

Another approach where TBC1D5 expression was inhibited also increased retromer association with the membrane and could partially rescue the effects of the PD-causing Vps35 D620N mutation whilst reducing APP processing to Aβ [68]. Most recently, in a transgenic mouse model of AD, increased Vps35 expression was able to rescue several phenotypes observed, confirming that retromer activity and function is of key importance in diseases such as AD [92], [93].

Increasing the levels of membrane-associated retromer could enhance the fidelity of endosomal protein sorting or increase the capacity of the endosomal protein sorting pathways that employ retromer by generating additional tubules/vesicles (Figure 4). In either case, for me, it is a revelation that retromer, a fundamental element of the endosomal protein sorting machinery, could be a viable therapeutic target in diseases such as AD and PD that have appeared to be so intractable for a long time. It must be acknowledged however that a lot of work is required before therapies based on activating or enhancing retromer are developed for patients with AD or PD.

In addition to being strongly implicated in neurodegenerative disease, the function of the retromer complex and associated proteins is known to be disrupted or hijacked by pathogens, both bacterial and viral (Box 2).

Areas for future study

A key question relating to retromer-mediated sorting of cargo proteins for endosome-to-cell surface recycling in mammalian cells is whether SNX1/SNX2 with SNX5/SNX6 are involved. Currently there does not seem to be evidence that clearly establishes a role for these SNX-BAR proteins in the endosome-to-cell surface pathway whereas they are required for the endosome-to-Golgi pathway [12]. Perhaps then a different set of SNX-BAR proteins could operate in the endosome-to-cell surface pathway, for example, SNX4, SNX7 or SNX8. Indeed, the SNX4 protein in mammals is required for endosome-to-cell surface recycling of the transferrin receptor [94] and with SNX7 regulates Atg9a localization [95]. The SNX8 protein has been implicated in endosome-to-Golgi retrieval so perhaps is not a good candidate for mediating endosome-to-cell surface recycling [96].

Another aspect of retromer biology that demands further research is how retromer is regulated. It has been shown in yeast that Vps5p and Vps17p are phosphoproteins [97] but how phosphorylation might affect their function is unknown. In mammalian cells, many of

the retromer proteins are phosphoproteins (see https://www.phosphosite.org) but it is unknown what role phosphorylation plays in modulating retromer-based protein sorting. The Vps35 subunit is ubiquitylated by the Parkin protein – a ubiquitin (Ub) ligase that is mutated in some forms of inherited PD. The addition of Ub to Vps35 does not alter the kinetics of Vps35 degradation but rather appears to affect the retromer-WASH complex-mediated sorting of certain membrane proteins, e.g. Atg9a protein that is important for autophagy [98].

Concluding remarks

It is now 22 years since retromer was first described. Progress in understanding how retromer functions in endosomal protein sorting has been swift - especially in the last ten years. Important insights have been obtained through the identification of sorting motifs in cargo proteins, the mechanisms that govern membrane association, the assembly of retromer proteins on the membrane and the interaction with accessory factors. The role of retromer in diseases such as Parkinson's and Alzheimer's disease or infectious diseases has spotlighted the physiological importance of retromer-mediated endosomal protein sorting. And yet much remains to be learned and we may expect that progress will continue at a pace. Let's see what the next ten years bring for retromer.

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655
656

Glossary

BAR domain – conserved domain present in many sorting nexin proteins that can promote tubulation of endosomal membranes

Cargo – general term applied to a membrane protein that is sorted by retromer or associated proteins such as SNX3 or SNX27

Cargo-selective complex – a term used over several years to describe the retromer trimer of Vps35-Vps29-Vps26

Cation-independent mannose 6-phosphate receptor (CI-MPR) – a lysosomal hydrolase receptor that cycles rapidly between endosomes and the trans-Golgi network (TGN)

Carboxypeptidase Y (CPY) – a well-studied yeast vacuolar hydrolase

Retromer – a protein complex first identified in yeast, functioning in the endosome-to-Golgi retrieval pathway.

Sorting nexin (SNX) – a group of functionally diverse proteins that all contain a p40 Phox homology (PX) domain that mediates binding to phosphatidly inositol 3-phosphate

Vacuolar protein sorting (VPS) – a collection of yeast mutants defective in sorting to the yeast vacuole that have been instrumental in identifying genes that encode proteins that function in that pathway

WASH complex – a protein complex present in most eukaryotes that mediates filamentous (F)-actin production at endosomes

Box 1 - How retromer assembles

Retromer in yeast is a stable heteropentamer with one copy of each protein and determined that whilst Vps26p facilitates the association of Vps35p-Vps29p with the Vps5p-Vps17p dimer, Vps29p is critical for assembly of the retromer complex [24], [30]. The elucidation of the structure of mammalian Vps29 revealed a globular conformation and identified a hydrophobic patch centred on a conserved leucine that mediates interaction with the Vps5p-Vps17p dimer confirming the key role that Vps29p plays in complex assembly [23].

A truncation-based analysis of the Vps5p and Vps17p showed that Vps5p and Vps17p dimerize through their respective C-terminal domains. Deletion of Vps17 does not prevent Vps5p assembling with Vps35p-Vps29p-Vps26p but does result in a severe CPY sorting defect. Thus, Vps17p is dispensable for the formation of the retromer complex but is essential for retromer function [24], [97]. The unstructured N-terminal region of Vps5p mediates the interaction between the Vps5p-Vps17p dimer and the Vps35p-Vps29p-Vps26p trimer but interestingly truncation of the N-terminal region of Vps5p that prevents formation of the retromer complex causes only partial loss of function [24]. Thus it appears that the two subcomplexes of retromer, although tightly associated in yeast, can function somewhat when apparently unable to physically associate. This observation is generally in line with data from studies in mammalian cells that have shown how the Vps35-Vps29-Vps26 trimer does not stably associate with the mammalian equivalent of the Vps5p-Vps17p dimer [32], [33], [99].

Box 2 - Retromer interaction with pathogens

The retromer complex along with many associated proteins are ancient in origin, conserved in evolution and ubiquitously expressed. Thus it is to be expected that several pathogens - viral and bacterial – will interact with retromer or exploit its activity and function for their own ends. In the case of bacteria, the interaction with retromer proteins is often associated establishing a productive infection and avoiding degradation in the endocytic pathway. For example, the Brucella bacteria requires retromer for infection [100], and the legionella bacteria, upon infection, secretes a protein - RidL - into the cytoplasm that binds to Vps29 at the site where TBC1D5 normally binds [101]–[104]. This has the effect of displacing TBC1D5 from retromer and impacts on the GTP state of Rab7a, possibly to enable Legionella to avoid degradation in the endocytic system. Salmonella secretes various proteins that interact with host proteins to sustain an infection including a protein called SseC that can bind to the retromer complex [105]. Additionally, Chlamydia can secrete a protein, IncE, which binds to SNX5 to impair the interaction with the CI-MPR, possibly reducing lysosomal function [102, 103].

The human papilloma virus exploits retromer-mediated endosomal protein sorting in order to achieve a successful infection and requires the activity of the TBC1D5 protein to do so [108]–[110]. In HIV infected cells, the Env protein (a component of the viral coat) is sorted by retromer into the endosome-to-Golgi retrieval pathway [111]. The hepatitis C virus also requires the function of retromer for infection whilst vaccinia has been reported to require retromer, the WASH complex and Rabs 11 and 22 [94-96].

Figure Legends

Figure 1. Arrangement for retromer assembled on the endosome membrane.

A The heteropentameric retromer complex as first identified in yeast. It is shown membrane-associated with the proteins arranged consistent with the cryo-electron microscopy (EM) data and also data from native immunoprecipitation experiments. The α-solenoidal Vps35 protein associates with Vps26 and Vps29 to form a stable trimer. The sorting nexin proteins, Vps5 and Vps17 dimerize and bind the endosomal membrane. Vps29 plays a key role in assembly of the heteropentameric complex and Vps26 contacts Vps5. **B**. Two retromer pentamers create an arch-like conformation on the membrane and through assembly of multiple arches could possibly promote or stabilise endosomal membrane tubules. The retromer arches have yet to be observed *in vivo* in higher eukaryotes however and a much looser arrangement/association of the Vps35-Vps29-Vps26 trimer with the sorting nexin dimer appears to be how retromer functions in mammalian cells.

Figure 2. Accessory proteins and sorting into distinct pathways.

A. In higher eukaryotes such as mammals, the retromer complex (specifically, the trimer of Vps35-Vps29-Vps26) associates with a notable array of accessory proteins such as the WASH complex and TBC1D5, neither of which are conserved in yeast. The WASH complex mediates production of endosomal F-actin that could aid protein sorting or play a more structural role at endosomes by facilitating membrane fission. The Fam21-Vps35 interaction occurs through the extended unstructured 'tail' of Fam21 binding the C-terminal third of Vps35. Loss of Vps35 expression (or Vps26) in mammalian cells causes the WASH complex to become cytoplasmic and a similar result occurs if the tail of Fam21 is overexpressed. The retromer trimer requires SNX3 and Rab7a for its membrane association and the activity of Rab7a is controlled through TBC1D5, a GTPase activating protein (GAP) for Rab7. The basic arrangement of Vps35, Vps29 and Vps26 is believed to be fundamentally the same in mammals as it is in yeast. **B.** In higher eukaryotes, retromer is necessary for sorting into two distinct pathways: endosome-to-cell surface and endosome-to-Golgi. These two pathways traffic distinct membrane (cargo) proteins with examples given. This may be possible because sorting nexin proteins such as SNX27 or SNX3 can provide specificity for the sorting into distinct pathways (i.e. SNX27 is required for endosome-to-cell surface retrieval of proteins such as the glucose transporter, Glut1).

Figure 3. Interaction with cargo proteins.

752 The retromer trimer of Vps35-Vps29-Vps26, sometimes in conjunction with sorting nexins
753 such as SNX3 or SNX27, can sort membrane (cargo) proteins by recognizing motifs in the
754 cytoplasmic tails of proteins such as the CI-MPR or SorL1. Several retromer cargo proteins
755 employ motifs containing aromatic and hydrophobic residues, e.g. Trp-Leu-Met (WLM) in the
756 CI-MPR or Tyr-Leu-Leu (YLL) in the DMT2-1 iron transporter. These motifs can be
757 recognized by sorting nexin proteins but different motifs may be present in other cargo
758 proteins. The SNX3 protein can recognize the YLL motif whereas SNX5 (with either SNX1 or
759 SNX2) can recognize WLM. The biochemical similarity of these two motifs suggests that
760 some overlap between the different sorting nexins and their cargo may occur, e.g. SNX3
761 could recognize WLM perhaps.
762
763 **Figure 4. Enhancing retromer function – a therapeutic avenue for neurodegenerative**
764 **disease ?**
765 **A.** Retromer sorts proteins at the endosome into two distinct pathways. Levels of retromer
766 on endosomes could result in retromer-mediated endosomal protein sorting being a rate-
767 limiting step in the endosome-to-cell surface and endosome-to-Golgi pathways – perhaps
768 due to a role for retromer in tubule formation. **B.** Enhancing retromer function by increasing
769 retromer localization to endosomes. This could be achieved either by increasing expression
770 of Vps35, or by retaining Rab7a in an active state by down-regulating TBC1D5. Both
771 approaches can increase levels of retromer at the endosome and has been shown to rescue
772 phenotypes associated with Alzheimer's and Parkinson's disease.

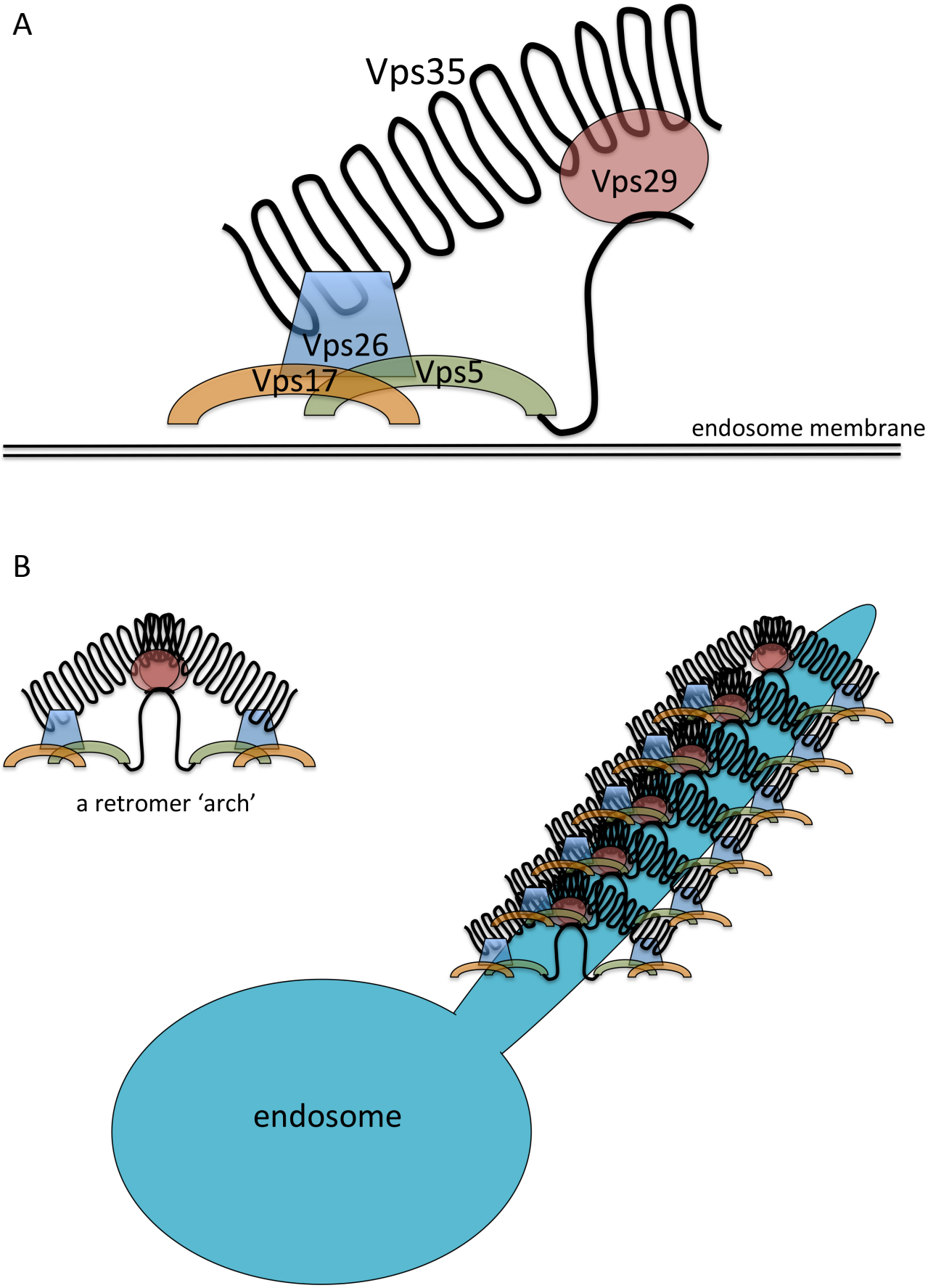
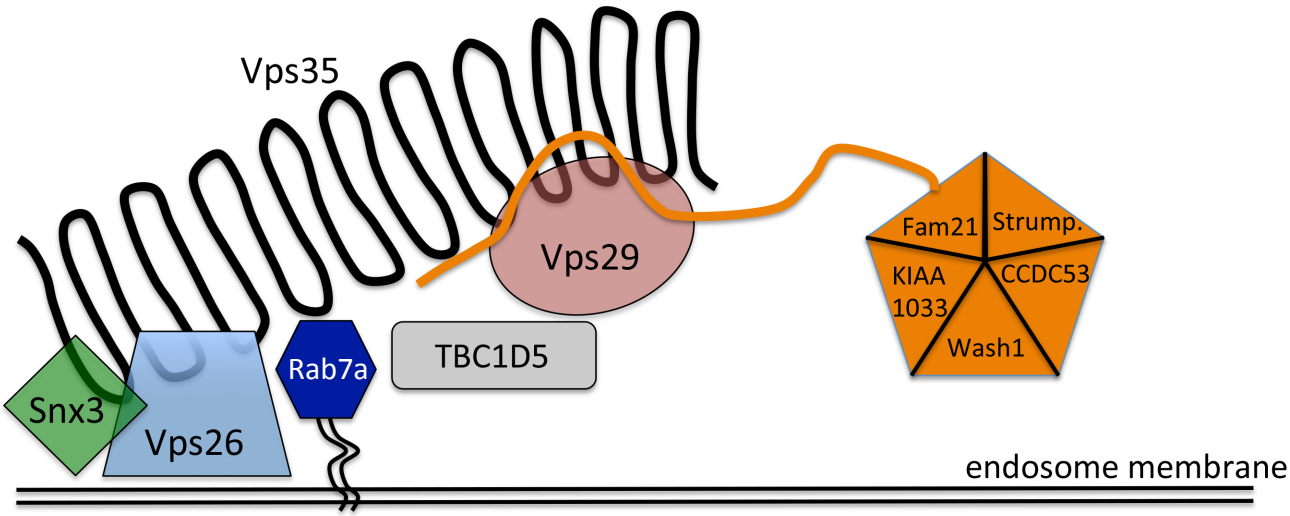

A
Vps35
Vps29
Vps26
Vps17
Vps5
endosome membrane
B
a retromer 'arch'
endosome

Figure 1

A


Vps35
Vps29
Fam21
Strump.
KIAA 1033
CCDC53
Wash1
TBC1D5
Rab7a
Snx3
Vps26
endosome membrane

B

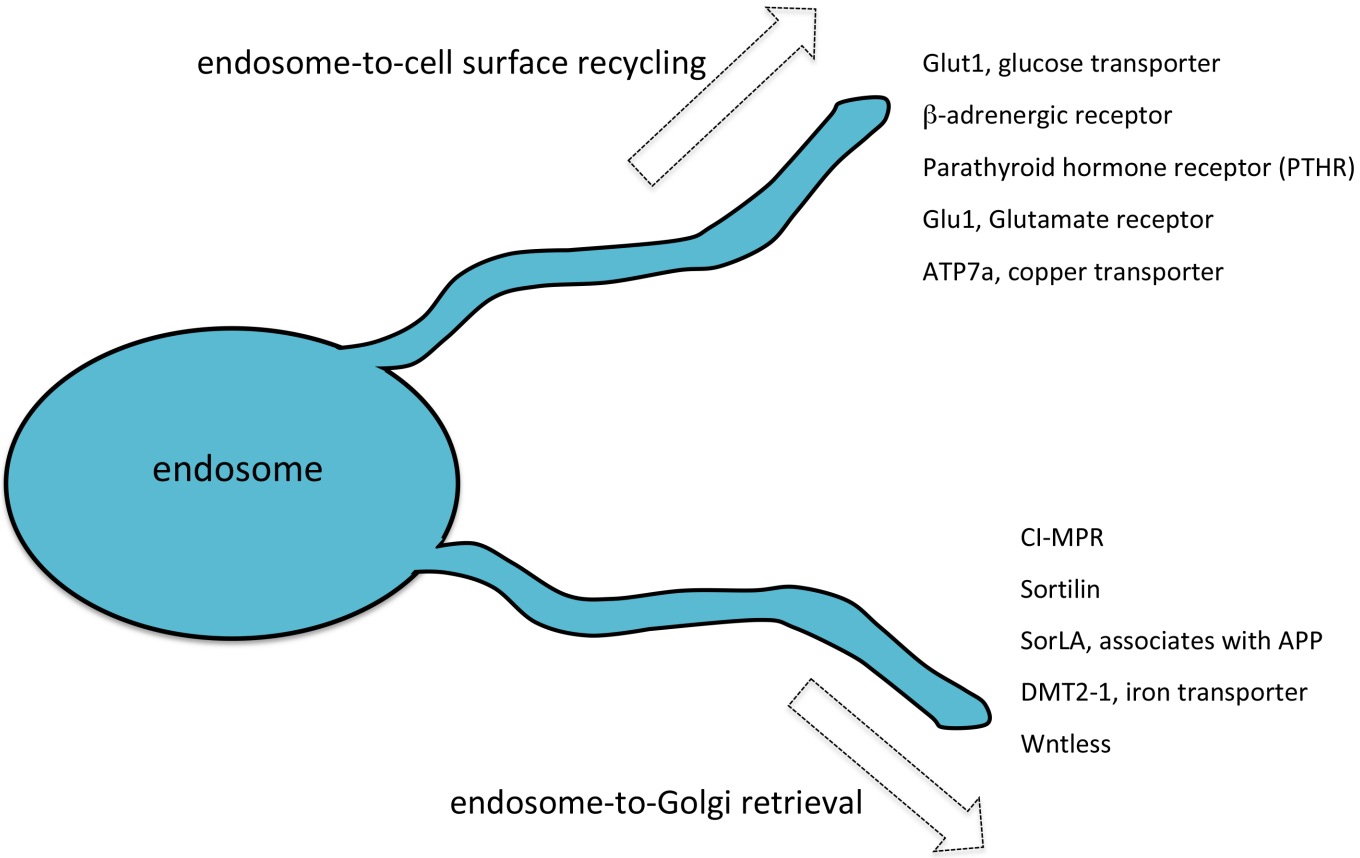

endosome-to-cell surface recycling
Glut1, glucose transporter
β-adrenergic receptor
Parathyroid hormone receptor (PTHR)
Glu1, Glutamate receptor
ATP7a, copper transporter
endosome
CI-MPR
Sortilin
SorLA, associates with APP
DMT2-1, iron transporter
Wntless
endosome-to-Golgi retrieval

Figure 2

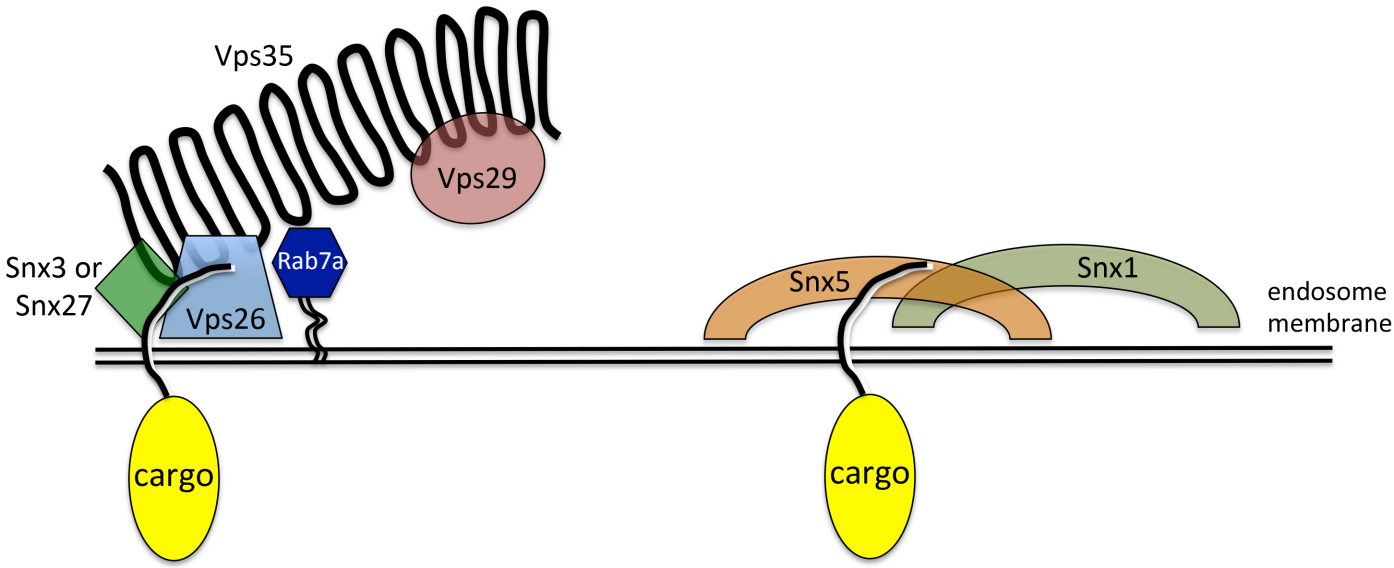
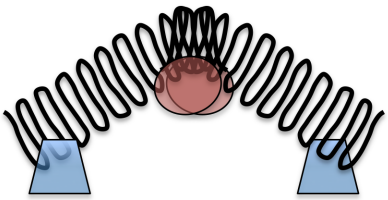
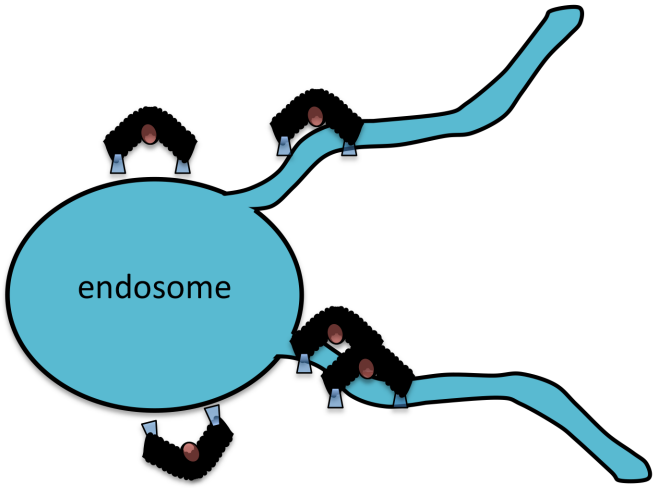

Vps35
Vps29
Snx3 or
Snx27
Vps26
Rab7a
Snx5
Snx1
endosome
membrane
cargo
cargo

Figure 3

A



Retromer trimer duplex



'normal' conditions

B

enhanced retromer function

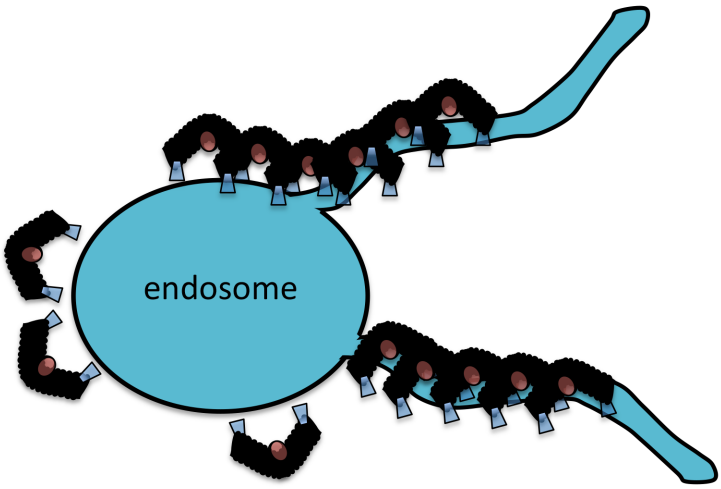


Figure 4