more general phenomenon is open to discussion and further investigation.

Clearly our knowledge and understanding of the chemical systems underpinning growth and patterning remain far in advance of the mechanical systems acting in biological systems to co-ordinate cellular activities. However, the rapid progress being made raises the prospect that the next few years will see further integration of chemical and physical viewpoints, possibly leading, after a century of research, to a reconciled Thompson/Turing view of growth and form.

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Endosomal Trafficking: Retromer and Retriever Are Relatives in Recycling

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Transmembrane proteins are sorted from endosomes to avoid lysosomal degradation. A recent study has identified a new multimeric complex called retriever that is essential for recycling numerous cell-surface cargoes from endosomes and is structurally and functionally related to the well-characterised retromer complex.

Most proteins internalised from the cell surface initially enter an early endosome and follow a maturation pathway, which culminates in the fusion of late endosomes with lysosomes and degradation of the endosomal contents. To avoid this fate, a subset of transmembrane proteins can be rescued from this pathway after internalisation. This process is primarily mediated by the retromer complex (consisting of the vacuolar protein sorting family members VPS26, VPS29, and VPS35) and its associated proteins. Cargo sorting by retromer is thought to occur in four

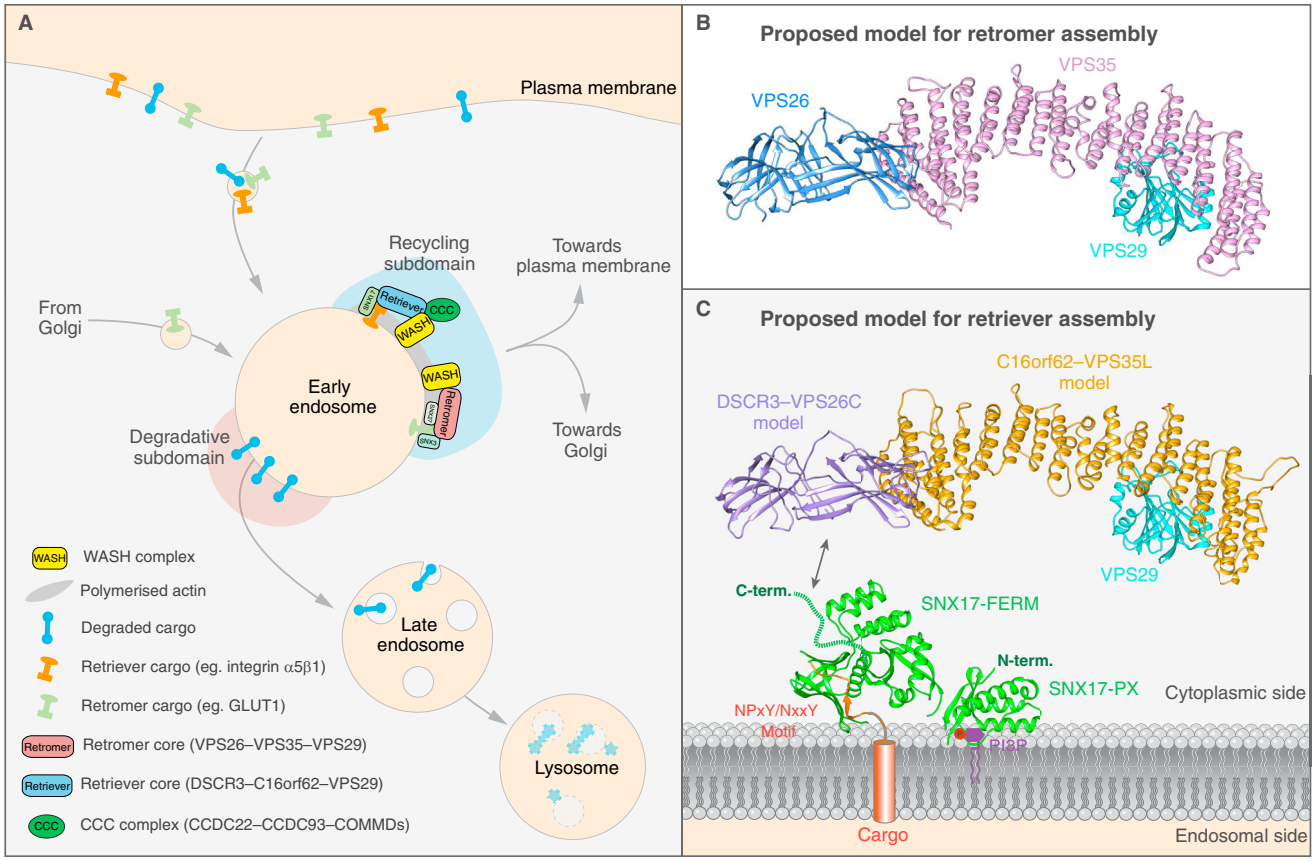

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Figure 1. Overview of transmembrane protein sorting in endosomes.
(A) Proposed model of protein sorting from the degradative pathway. Endocytosed proteins enter the endocytic pathway. After entry into an endosome, proteins segregate into 'degradative' and 'recycling' domains. Transmembrane proteins are sorted into the recycling domain by the retromer or retriever complexes and their associated proteins. After the recycling, cargoes are removed from the endosome into tubular/vesicular carriers and returned to the plasma membrane or transported to the Golgi apparatus. The remaining endosome matures into a late endosome, a process that includes the internalisation of its outer membrane into intraluminal vesicles. Once matured, the late endosome fuses with a lysosome, resulting in degradation of the internal contents by lysosomal enzymes. (B) Proposed model for retromer assembly based on the crystal structures of VPS26–VPS35N (5F0J) and VPS35C–VPS29 (2R17) and small angle X-ray scattering (SAXS) experimental data [9]. (C) Proposed model for retriever assembly obtained combining the known structure of VPS29 (2R17) with the structural models of DSCR3 and C16orf62. The models were calculated with MODELLER using as template the structures of VPS26 (5F0J) and VPS35 (5F0J, 2R17) as described in [10]. The architecture of the retriever complex (DSCR3–C16orf62–VPS29) was derived from superimposing the individual structures on the retromer model. SNX17 consists of an amino-terminal PX domain (3LUI), which binds phosphatidylinositol-3-phosphate (PI3P) found on early endosomal membranes, a FERM domain (4GXB), which binds cargo proteins that contain a NPxY/NxxY-sorting motif, and a carboxy-terminal tail predicted to be unstructured (depicted by a green dashed line). Immunoprecipitation assays from McNally *et al.* [4] suggest that the carboxy-terminal tail of SNX17 associates with DSCR3 of the retriever complex. However, the molecular details of the interaction between SNX17 and retriever remain unknown.

stages: endosomal membrane recruitment of retromer by sorting nexin 3 (SNX3) and the GTPase Rab7; cargo binding by retromer via either SNX27 or SNX3; membrane deformation by SNX-BAR proteins (SNX1, 2, 5, 6); and tubulation driven by both SNX-BAR proteins and actin network polymerisation triggered by the WASH complex [1]. The resulting cargo-enriched tubules bud from endosomes and traffic to either the plasma membrane or the Golgi apparatus [2,3] (Figure 1A,B).

In a recent paper in *Nature Cell Biology*, McNally *et al.* [4] identify and characterise a new protein complex required for sorting of transmembrane proteins. The complex, which they term 'retriever', was identified using a proteomics screen for interactors of SNX17 and consists of the proteins C16orf62, DSCR3, and VPS29. The authors demonstrate that this complex is associated with endosomes, is present in the cell as a stable core complex, and is essential for the recycling of multiple cargoes.

SNX17 is a sorting nexin that associates with endosomes through a phosphoinositide-binding module and with the cytosolic tail of transmembrane proteins through a FERM-like domain. It has been known for nearly 15 years that SNX17 mediates cargo retrieval from the lysosomal degradation pathway in a retromer-independent manner [5,6]; however, the machinery that functioned with SNX17 in this process was largely unknown. McNally *et al.* [4] addressed this issue by carrying out a comparative proteomic analysis between cells expressing either a functional or a defective form of SNX17. This approach demonstrated that functional SNX17 is physically linked to the CCC complex (which contains CCDC22, CCDC93, and

seven COMMD family proteins) C16orf62, DSCR3, and VPS29.

DSCR3, associated with Down's syndrome in humans, is a homolog of the retromer component VPS26 [7]. In addition, DSCR3 is predicted to adopt an arrestin-like fold, as found in VPS26, and C16orf62 is predicted to contain α-helical repeats, a structural feature present in VPS35 [8,9] (Figure 1C). Based on this strong structural homology, the authors proposed that C16orf62, DSCR3, and VPS29 may be part of a core assembly unit similar to the retromer core heterotrimer, and they term this heterotrimer retriever. The existence of this new complex was recently hypothesised on the basis of mass spectrometry maps of mammalian interactomes and 3D modelling [10]. However, McNally *et al.* [4] are the first to biochemically substantiate the presence of the retriever heterotrimer by a combination of immunoprecipitation assays and characterisation of the purified complex.

The interaction of retriever with SNX17 is essential for cargo selection. Specifically, the authors demonstrated that the carboxy-terminal domain of SNX17 is necessary and sufficient for association with retriever. Interestingly, this interaction is not needed for endosomal association because depletion of SNX17 did not affect recruitment of retriever to the membrane. Instead, endosomal recruitment of retriever requires a functional CCC complex. The CCC complex itself does not associate with endosomes but interacts with the FAM21 subunit of the WASH complex present at the surface of endosomes [11]. Accordingly, FAM21 was shown to be recruited to the membrane independent of retromer and to be necessary for both endosomal recruitment and cargo sorting of retriever. Thus, the authors propose the following steps in the recruitment of retriever to endosomes: first, the CCC complex is recruited to endosomes by the WASH complex through the interaction of CCDC93 with FAM21, and then the retriever complex interacts with the CCC complex where it can engage SNX17 and the cargo (Figure 1A).
In this study, it is inferred that CCC and the retriever complexes are two interacting complexes rather than a single macro-complex because siRNA-mediated silencing of the CCC complex did not reduce the expression level of retriever.

In addition to the structural parallels, retriever and retromer are functionally analogous. McNally *et al.* [4] demonstrated that the retriever complex is essential for the recycling of the previously characterised SNX17 cargoes, such as the heterodimeric integrin α5β1 and low-density lipoprotein receptor-related protein 1 (LRP1) [5,6,12,13]. When retriever complex components were depleted by siRNA, integrin α5β1 showed decreased surface abundance and lower total protein levels. In addition, pharmacological inhibition of lysosomal activity led to the recovery of integrin α5β1 protein levels in retriever-depleted cells, suggesting that integrin α5β1 was being degraded in the lysosome. To identify new SNX17–retriever cargoes the authors performed a proteomics screen for proteins enriched on the surface of cells depleted of SNX17. In addition to SNX17-associated cargoes, such as integrins, amyloid precursor protein (APP), and epidermal growth factor receptor (EGFR), a number of new transmembrane cargoes containing the SNX17 interaction motif (NxxY) were identified. Novel putative cargoes include solute carrier (SLC) proteins, cell surface receptors and their ligands, and a host of proteins required for cell adhesion.

By comparing the datasets of cargoes enriched on the cell surface after SNX17 depletion with those of cargoes enriched following depletion of VPS35 or SNX27, the authors found that there are some cargoes that are specific for retromer (e.g. the glucose transporter GLUT1), some that are specific for retriever (e.g. integrin β1) and some cargoes that are affected by depletion of either retromer or retriever (e.g. CD97). These findings could suggest either that there is some co-operation between the two sorting complexes or that some cargoes can associate with both recycling complexes.

McNally *et al.* [4] also provided initial evidence that human pathogens hijack the retriever machinery. The L2 capsid protein of human papillomavirus (HPV) has previously been shown to interact with SNX17 [14], and indeed cells depleted of retriever subunits showed decreased entry of HPV pseudovirus. VPS29, a protein shared between retromer and retriever, is a target of the *Legionella* effector RidL [15]; it therefore seems likely that RidL interferes with the retriever recycling pathway. It will be interesting to investigate whether retriever components are targets for other human pathogens.

This study has revealed important aspects of the function of the retriever complex but several open questions remain to be addressed. What is the role of the CCC complex? The data demonstrate that this complex interacts with and is required for the endosomal localisation and cargo sorting of retriever. The human proteome has ten COMMD proteins, seven of which appear to associate with retriever. It is not known whether this demonstrates redundancy, functional flexibility or a simultaneous association into a mega-complex. Due to the size of the CCC complex, it seems likely that it has as yet unidentified roles in addition to bridging retriever and WASH.

What is the structure of the potential SNX17–retriever–CCC complex and the structural details of its interaction with the WASH complex? The structures of VPS29 [8], SNX17 [16] and the amino-terminal region of COMMD1 and COMMD9 [17] are known, but no structural information is available for the other subunits. Elucidating the architecture of this novel multiprotein complex is an exciting new challenge for structural biologists and will help us to understand the molecular mechanism of action of this pathway.

How does the cargo-bound retriever complex exit the endosome? There is some evidence of a tubular coat from immunoelectron microscopy studies, which revealed that overexpressed SNX17 is found on tubular structures, reminiscent of SNX-BAR/retromer tubules [18]. No BAR-domain proteins have been experimentally demonstrated to functionally interact with retriever. It is possible that the recycling SNX-BAR proteins have a dual role with retriever, or perhaps there are totally different SNX-BAR, BAR or curvature-inducing/coat proteins required for retriever budding into tubular/vesicular carriers. In addition, if cargo-bound retriever is exiting the endosome in tubular structures, it will be interesting to see whether these tubules contain both retromer and retriever or if there are two classes of tubules.

In conclusion, retriever is a novel trimeric complex that is structurally and functionally analogous to the retromer complex. Both complexes localise to the same endosomal domain, associate with sorting nexins for specific cargo selection, sort transmembrane cargoes from the endo-lysosomal pathway back to the cell surface, and depend on the WASH complex for cargo sorting. Accordingly, it has been suggested that the three components of retriever — C16orf62, DSCR3, and VPS29 — are renamed as VPS35L, VPS26C, and VPS29. There are, however, interesting differences, such as the different mode of recruitment to endosomes (retromer through sorting nexins and Rab7, and retriever through CCC–WASH) and the differential involvement of the CCC complex (essential for retriever, but not associated with retromer).

The discovery of retriever provides a step forward in our understanding of protein trafficking systems. Future studies will be essential to further dissect the relationship between retromer and retriever in endosomal cargo recycling. It has become increasingly clear that defective endosomal recycling is a causal factor of human diseases, such as Alzheimer's and Parkinson's disease [19,20]. Thus, the discovery of retriever has not only advanced research in recycling pathways but also provided the potential to gain insight into the mechanistic details of human pathologies.

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