ENDOCYTIC RECYCLING

Frederick R. Maxfield and Timothy E. McGraw

After endocytosis, most membrane proteins and lipids return to the cell surface, but some membrane components are delivered to late endosomes or the Golgi. We now understand that the pathways taken by internalized molecules that eventually recycle to the cell surface can be surprisingly complex and can involve a series of sorting events that occur in several organelles. The molecular basis for many of these sorting processes is only partly understood.

TRANSCYTOSIS
The process in which materials are transported across a polarized cell by internalization from one membrane domain (for example, the basolateral membrane), passage through the endosomal/recycling system and eventual delivery to the other domain (for example, the apical membrane).

Department of Biochemistry, Weill Medical College of Cornell University, 1300 York Avenue, New York, NewYork 10021, USA.
e-mails: frmaxfie@med.cornell.edu; temcgraw@med.cornell.edu
doi:10.1038/nrm1315

Endocytic membrane traffic in mammalian cells has an essential role in delivering membrane components, receptor-associated ligands and solute molecules to various intracellular destinations. Until recently, the models that described endocytic routes were relatively simple, with the main pathways thought to lead to degradation in lysosomes or recycling back to the plasma membrane. Polarized epithelial cells also had another branch point that led to TRANSCYTOSIS across the cell. We now understand that endocytic trafficking pathways are more complex and multifunctional than the earlier simple models. Understanding the details of these pathways is essential for analysing many normal cellular processes. These complex itineraries also have important roles in the intracellular targeting of therapeutic agents and in determining the intracellular fates of pathogens and toxins. In this review, we describe the main pathways and functions of endocytic recycling, and summarize briefly the molecular mechanisms that underlie these processes.

There are several mechanisms for internalizing molecules from the cell surface (FIG. 1). The most well-understood endocytic process — receptor-mediated endocytosis — involves the internalization of receptors and their ligands by clathrin-coated pits[1]. Many of the ligands are subsequently degraded in late endosomes or lysosomes, whereas many of the receptors are re-used up to several hundred times. This recycling of receptors back to the plasma membrane was one of the first characterized examples of recycling in a membrane-trafficking pathway — a phenomenon that has now been documented for many other steps of membrane trafficking in both biosynthetic and endocytic pathways. These recycling pathways are essential for maintaining the proper composition of various organelles and for returning essential molecules that carry out specific functions to the appropriate compartments. It is now appreciated that there are many different forms of endocytosis and that there are complex and interconnected pathways that can carry molecules to various destinations within the endosomal system. This abundance of compartments and pathways challenges our ability to understand how the system operates to regulate the amount of various molecules in each compartment. A schematic diagram of endocytic membrane transport pathways that occur after receptor-mediated endocytosis in non-polarized cells is shown in FIG. 2. In this review, we focus on the recycling pathways that start with clathrin-coated-pit internalization. However, it should be noted that several of the non-clathrin-coated-pit internalization pathways fuse with these pathways[2–5]. A new type of membrane recycling that is based on functional sequestration in invaginations of the plasma membrane is also discussed briefly at the end of the review.

In addition to maintaining the homeostatic regulation of molecules in each of the compartments, the transport rates of membrane trafficking can be altered in response to signalling mechanisms to increase or decrease the surface expression of molecules. Examples of this regulation include a decrease in the surface expression of many signalling receptors in response to stimulation by their ligands (receptor downregulation), or an increase in the surface expression of glucose transporters in response to insulin. As discussed below, the changes in vesicular transport rates that underlie the changes in surface expression can be brought about either by changing the trafficking of

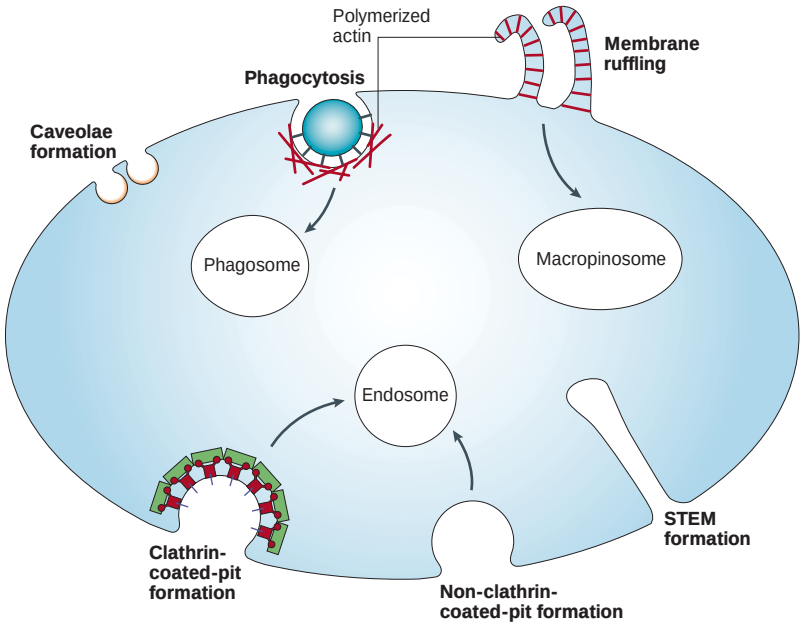


Figure 1 | **Modes of internalization.** Several types of plasma-membrane deformation give rise to the formation of sealed endocytic compartments, and the most well-characterized of these begins with clathrin-coated-pit formation (BOX 1). However, in many cultured cells, as much as half of the uptake of membrane and fluid can be by non-clathrin mechanisms. These non-clathrin processes are generally more difficult to study, because they might not be concentrative and often lack specific markers. Ruffling of membranes can lead to the formation of large endocytic compartments (macropinosomes) when the tip of the ruffle fuses back with the plasma membrane. This process generally occurs infrequently, but it can be stimulated by growth factors and other agents[129]. Caveolae are 55–60-nm invaginations at the cell surface[130] that are often coated with the protein caveolin. Under certain conditions, caveolae can pinch off to form vesicles, but this process also seems to be infrequent in unstimulated cells[131]. Phagocytosis is the uptake of relatively large particles (300 nm to several μm in diameter) by an actin-dependent process[132]. This process occurs most robustly in specialized cells such as macrophages, but most cells are capable of low levels of phagocytosis. In macrophages, large diameter (up to 2 μm) tubules (STEMs; surface-connected tubules entering macrophages) bud into the cytoplasm, and these have a role in the uptake of large lipoprotein particles[133]. It should be noted that there are further uptake mechanisms, such as non-clathrin-coated-pit uptake, for which markers are lacking at present[134]. Modified with permission from REF. 1 © (1997) the American Physiological Society.

individual molecules or by changing the behaviour of entire organelles.

Endocytic recycling is also essential for maintaining the distinction between apical and basolateral membranes in polarized cells[6]. In fact, in hepatocytes, most — but not all — newly synthesized apical membrane proteins are first delivered to the basolateral membrane and are subsequently delivered to the apical membrane by endocytic routes[7–9]. In all epithelia, the correct return of most proteins to the membrane from which they were removed is essential for maintaining the membrane-protein composition of the apical and basolateral plasma membranes. At the same time, a few molecules — such as immunoglobulin receptors — must be transcytosed efficiently after endocytosis. These extra sorting requirements in polarized epithelia require further sorting mechanisms, which are reflected both in the different properties of the endocytic organelles and in the different molecular mechanisms that direct the trafficking of individual molecules[10].

SORTING ENDOSOMES
Tubular–vesicular structures that receive direct input from coated-pit-derived endocytosis. They have a key role in sorting material for recycling or degradation.

Understanding endocytic recycling requires a description of the various endocytic compartments, an analysis of the transport pathways that link these compartments, characterization of the molecules that determine the properties of the compartments, and an understanding of how the properties of an individual cargo molecule determine its sorting at various branch points in the trafficking route. At present, we have only a partial understanding of these parameters. Much of what is known at the molecular level about how proteins are transported between membrane compartments is derived from studies of transport through the biosynthetic system and internalization at the plasma membrane. Models that were developed from studies of these pathways provide a framework for understanding endocytic recycling at a molecular level. Discontinuous transport between any two stable membrane compartments involves several common features. These include concentration of the cargo molecules to be transported, formation of a transport intermediate that is enriched in the cargo molecules, and fusion with the proper target membrane compartment. Studies of various transport processes have led to the identification of proteins that control these different steps (BOX 1), and the general molecular mechanisms of vesicle formation, targeting and fusion have been reviewed elsewhere (see REFS 11–16).

Organelles and endocytic recycling pathways

It is important to understand the basis, and limitations of, models such as that shown in FIG. 2. First, the pathways shown are the main routes taken by certain molecules. It is probable that none of the sorting mechanisms is 100% efficient, so molecules will always be found, to some extent, in alternative pathways. Second, all of the compartments are dynamic, and it is probable that no molecule is permanently resident in any compartment. This is either because the individual molecules are transported into or out of the compartment through transport vesicles, or because the compartment itself changes over time (often referred to as 'maturation'). These properties make defining and identifying organelles problematic. For example, many organelle (or compartment) 'resident' proteins are, in fact, transported out of their main sites and are returned there by various recycling (retrieval) pathways. This means that the resident proteins actually move between many organelles, and their average distribution is determined by the net balance of transport into and out of each of these organelles. A second problem is that many organelles are actually not stable over time. For example, as discussed below, SORTING ENDOSOMES mature into late endosomes, and if there are any resident components of sorting endosomes at all, they reside in less than 1% of the volume of the compartment[17]. So, all — or nearly all — of the properties of these endosomes are derived from proteins and lipids that are delivered to it during its brief lifetime.

The lack of truly resident proteins means that there are generally no single, unique markers that can be used to define an organelle. Instead, organelles can be

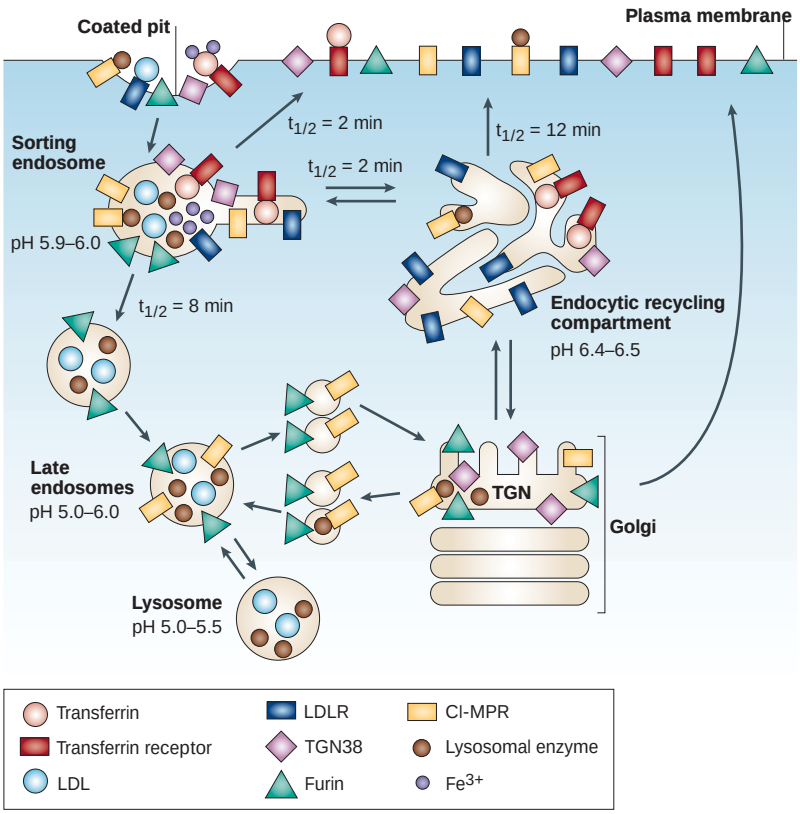


Figure 2 | **Endocytic recycling pathways.** The model shows the post-endocytic itineraries of several molecules. The transferrin receptor binds its ligand, diferric transferrin; the low-density-lipoprotein receptor (LDLR) binds low-density lipoprotein (LDL); and the cation-independent mannose-6-phosphate receptor (CI-MPR) binds lysosomal enzymes. All of these membrane proteins concentrate into clathrin-coated pits, and their initial delivery site is sorting endosomes. The transmembrane proteins furin and *trans*-Golgi network (TGN)38 also enter through clathrin-coated pits. Most membrane proteins rapidly exit sorting endosomes and are either returned directly to the plasma membrane or are transported to the endocytic recycling compartment (ERC). Furin is retained in the sorting endosome as the sorting endosome begins to mature into a late endosome, and furin is delivered to the Golgi from late endosomes. From the ERC, essentially all of the LDLRs and transferrin receptors recycle to the cell surface. Transferrin, unlike most other ligands (for example, LDL), is not released from its receptor in the acidic environment of sorting endosomes. The two irons (Fe^{3+}) are released from diferric transferrin at the acidic pH and transported into the cytoplasm, but iron-free transferrin remains bound to its receptor until it is returned to the cell surface. At the neutral extracellular pH, iron-free transferrin is released from the receptor. About 80% of the internalized TGN38 and CI-MPR also returns to the cell surface, and the rest is delivered to the TGN. The CI-MPR can go from the TGN to late endosomes, where any ligand that is still bound can dissociate as a result of exposure to low pH. From the late endosomes, furin and free CI-MPR can move to the TGN, and molecules in the TGN can be delivered back to the cell surface . It is uncertain whether CI-MPR and furin are transported in the same or different vesicles between the TGN and late endosomes. The $t_{1/2}$ values are approximate and cell-type dependent. They are based on the papers that are cited in the text. Modified with permission from REF. 61 © (2003) the American Society for Cell Biology.

ENDOCYTIC RECYCLING COMPARTMENT (ERC). A component of the endocytic recycling system. A large fraction of recycling membrane components pass through the ERC, which is mainly composed of narrow diameter tubules. In some cells, the ERC is organized around the microtubule-organizing centre.

identified that are relatively enriched in a collection of proteins, even though each of these proteins is also found in other compartments. These proteins might be involved in the functions of the organelle (for example, proteins that control vesicle docking and fusion) or they might be cargo proteins (such as receptors or their ligands). Organelles can also be classified on the basis of their functional properties. For example, sorting endosomes are the first discernible organelles to which internalized transferrin and low-density lipoprotein (LDL) are delivered (FIG. 2). So, an organelle that is filled with these ligands after a one- or two-minute incubation is almost certainly a sorting endosome. It should, however, be noted that it is usually necessary to use more than one characteristic to classify an endocytic organelle experimentally.

The nomenclature for the endocytic system has not been fully standardized — partly because of the difficulties involved in developing simple ways to characterize the organelles experimentally. The commonly used term 'early endosome' actually describes two distinct endosomal organelles — the sorting endosome and the ENDOCYTIC RECYCLING COMPARTMENT (ERC; FIG. 2) . These organelles are distinct, and throughout this review we avoid using the general term early endosome and instead use sorting endosome or ERC. Late endosomes are endocytic organelles that no longer receive a direct input of vesicles that have pinched off from the plasma membrane, and are not on the main endocytic recycling route. They are also a heterogeneous collection of compartments with different properties, and the functional consequences of this heterogeneity are not well understood.

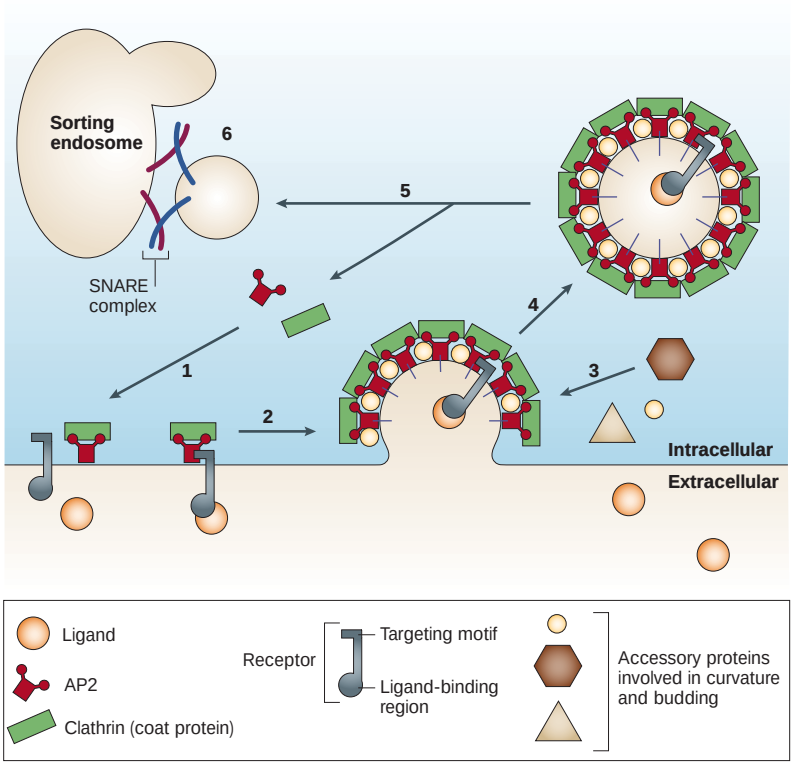
Sorting at sorting endosomes

At the plasma membrane, transmembrane proteins are concentrated in clathrin-coated pits because they have sequence motifs in their cytoplasmic domains that interact with elements of the clathrin-coated pit (BOXES 2,3). Therefore, the targeting of plasma membrane proteins for rapid internalization through clathrin-coated pits is based on protein–protein signal recognition. As discussed below, the predominant sorting mechanism in the sorting endosome is based more on organelle geometry than on the recognition of a specific sorting motif in the cargo proteins.

Geometry-based sorting. After shedding their clathrin coats, newly formed endosomes fuse with one another and with pre-existing sorting endosomes. Sorting endosomes are peripherally located, tubular–vesicular structures that have a lumenal pH of ~6.0 (REFS 18,19). Sorting endosomes accept incoming material for only about 5–10 minutes. After this time, they translocate along microtubules, stop fusing with newly endocytosed vesicles and become more acidic. They also start to acquire acid hydrolases and take on the properties of late endosomes. These changes in sorting endosomes are referred to as maturation.

The sorting endosome is the first main branch point in the receptor-mediated endocytosis pathway and, as its name implies, an important function of the sorting endosome is to target molecules to their correct destinations. There are three known destinations after the sorting endosome — the plasma membrane, late endosomes and the ERC (FIG. 2). As a consequence of the low pH in the lumen of the sorting endosome, many ligands are released from their receptors and mix with internalized solute molecules[1]. The uncoupling of receptors from their ligands is the first step in endocytic sorting. As a result

Box 1 | Clathrin-coated-vesicle formation at the plasma membrane



The formation of endosomes exemplifies the general process of vesicle formation. The first step is the recruitment of the clathrin adaptor protein 2 (AP2) and clathrin to the plasma membrane (see step 1 in the figure). The ARF6 GTPase might have a regulatory role in this recruitment[82]. Adaptor proteins bind to coat proteins and to specialized motifs in the cytoplasmic domains of membrane proteins, thereby linking specific cargo to sites of coat assembly. Receptors link soluble extracellular ligands to the site of cytoplasmic coat assembly by interacting with the adaptor proteins (step 2).

The pinching off of the clathrin-coated domain requires the action of accessory proteins (steps 3 and 4). These include, but are not limited to, epsins and endophilin (proteins that are thought to induce the bending of the membrane that is required for vesicle budding)[83–85], dynamin (a protein that is required for the fission of a vesicle from a donor membrane)[86], and amphyphisin, which binds endophilin, clathrin, adaptor proteins and dynamin[87,88]. Epsins and epsin-homology-domain proteins link certain receptors to coat complexes[89]. Many of the proteins that are required for the formation of vesicles bind to phosphoinositides and, consequently, lipid kinases and phosphatases have an important role in vesicle formation. Vesicle formation has been reviewed elsewhere (REFS 11–16).

The coat is removed by an energy-consuming mechanism (step 5), which allows the coat, the adaptor proteins and other proteins that are involved in vesicle formation to be recycled[90,91]. The newly formed endosomes fuse with one another and with pre-existing sorting endosomes in a process that requires SNARE and Rab proteins (step 6)[92].

Vesicle formation and fusion along the endocytic pathway probably requires assemblies of proteins that are similar to those involved in clathrin-coated-vesicle formation. For example, there is evidence that a dominant-inhibitory dynamin mutant inhibits transferrin-receptor cycling, which highlights a requirement for dynamin in the formation of recycling vesicles[93,94]. The SNARE protein cellubrevin (VAMP3) is required for the fusion of transferrin-receptor-containing vesicles with the plasma membrane[95]. Several Rab proteins have also been shown to have a role in specific steps of endocytic trafficking (see BOX 2).

of maturation of the sorting endosome, these soluble molecules, and a subset of membrane proteins and lipids, are 'delivered' to late endosomes without actually leaving the lumen of the organelle. This provides an efficient means of delivering soluble components to the late endosome without requiring a mechanism for concentrating the soluble proteins, as would be the case if the soluble content moved from sorting endosomes to late endosomes using a transport intermediate.

In the period of time before the maturing sorting endosome translocates to the centre of the cell, most recycled molecules are removed rapidly and efficiently. It is believed that most of the membrane is removed by the pinching off of narrow-diameter tubules[17,20]. The surface-area-to-volume ratio of the tubules is greater than that of the vesicular portion of the sorting endosome, therefore the pinching off of tubules preferentially sorts recycled membrane from the soluble molecules. Consequently, in the absence of specific targeting information, a membrane protein that has been internalized into endosomes will be transported from the sorting endosome with the bulk of the membrane. By contrast, as discussed below, the delivery of membrane proteins to late endosomes requires specific targeting information. So, the first step of endocytic sorting is based largely on the geometry of sorting endosomes (FIG. 3), and a membrane protein that is internalized into an endosome will generally be diverted away from late endosomes.

The maturation of sorting endosomes to late endosomes has been extensively studied in Chinese hamster ovary (CHO) cells. In these cells, sorting endosomes have a half-life of about 8 minutes, during which time they continually receive newly endocytosed molecules from the surface (by fusion with newly formed endosomes) and export recycling molecules by the fission of tubules. For transferrin receptors and some fluorescent lipid analogues, it has been shown that exit from the sorting endosomes has a $t_{1/2}$ of 2 minutes or less, and more than 95% of these molecules are removed from the sorting endosomes before they mature[17,20] (the overall recycling efficiency for transferrin receptors is greater than 99%). In this model, the overall efficiency of sorting membrane from the soluble components is determined by the amount of membrane in the tubules and the iteration of the sorting process, with several endosomes fusing with, and tubules pinching off from, the sorting endosome before it matures to form a late endosome.

The molecular mechanism of maturation and the regulation of the timing of maturation are not fully understood. The fusion of coated-pit-derived primary endocytic vesicles with sorting endosomes is regulated in part by Rab5, early endosome antigen 1 (EEA1) and SNAREs[21–23] (BOXES 1,4). Microtubules are required for the 'delivery' of molecules from sorting endosomes to late endosomes[24]. Changes in the fusion compatibility of endosomes (that is, the start of maturation) occur at approximately the same time as the pH drops by about 0.5 units. Bafilomycin A1 — a vacuolar H^+-ATPase inhibitor — slows the progression from sorting endosomes to late

Box 2 | Coat proteins and endocytic recycling

There are three known coat complexes — clathrin, coatomer protein (COP)I and COPII[11,13,96]. Clathrin functions at the plasma membrane and in transport between late endosomes and the Golgi. Clathrin has been observed on endosomes[94,97]. Perturbation of clathrin function using various methods has been shown to slow the internalization of the transferrin receptor without greatly altering the rate or efficiency of its return to the plasma membrane. This indicates that clathrin is not required for recycling to the cell surface[98–100], and is consistent with the observation that deletion of the cytoplasmic domain of the transferrin receptor does not slow its recycling[19,37]. These truncated receptors cannot interact with adaptor proteins and therefore are not concentrated in clathrin-coated pits. Clathrin might have a role in the formation of transport vesicles[94] or in the organization and morphology of endosomes[101], but it is not required to concentrate transferrin receptors for their return to the cell surface in non-polarized cells.

COP complexes function in transport between the endoplasmic reticulum (ER) and the Golgi, and in intra-Golgi transport[102–106]. COPI coat proteins have been localized to endosomes. However, it is still unclear whether the COPI proteins are involved in recycling back to the plasma membrane. The microinjection of antibodies against the COPI protein β-COP did not alter the recycling of transferrin receptors to the cell surface. However, analysis of a Chinese-hamster-ovary cell line that had a temperature-sensitive mutation in the COPI protein ε-COP led to the proposal that COPI has a role in the recycling of transferrin receptors back to the plasma membrane[107]. The fraction of transferrin receptors that were on the cell surface was, however, not altered at the non-permissive temperature, as would be expected if the recycling had been slowed. One difficulty in the attempt to assign a direct role for COP proteins in recycling is that they are required for transport through the Golgi, so alterations in endocytic behaviour might be a secondary effect of altered ER-to-Golgi transport.

endosomes and then to lysosomes[25,26], which indicates that changes in pH might be required for some of these transformations.

Transmembrane-protein transport to late endosomes. The targeting of transmembrane proteins to late endosomes/lysosomes from sorting endosomes has been described for signalling receptors and, in this context, it functions to terminate signalling, as well as to make the cells unresponsive to further signal input until a new complement of receptors has been synthesized[27]. The first step in targeting a membrane protein for downregulation is often the ubiquitylation of its cytoplasmic domain, which ultimately functions as a signal for targeting the protein to invaginated membranes in late endosomes/lysosomes. Ubiquitin is a 76-amino-acid protein that can be covalently linked to lysine residues, and ubiquitylation was first described as a mechanism for targeting cytosolic proteins for degradation by the proteasome. The details of ubiquitylation in membrane-protein targeting are beginning to emerge, with one important finding being that HRS (hepatocyte-growth-factor-regulated tyrosine kinase substrate) links ubiquitylated receptors to flat clathrin lattices in endosomes[28]. This interaction might be important for retaining ubiquitylated receptors in maturing endosomes, which would lead to their delivery to late endosomes. In yeast, in a second sorting step, the ESCRT (endosomal sorting complex required for transport) protein complexes have been shown to recognize and sort ubiquitylated proteins for delivery to the vacuole/lysosome lumen, and they use a mechanism in which the limiting membrane of the endosome invaginates into the late endosome[29,30]. It is probable that a similar mechanism functions in mammalian cells. So, ubiquitylation is a protein modification that can regulate membrane-protein targeting. Furin and some other membrane proteins are targeted to late endosomes by a distinct mechanism, and furin trafficking is discussed in a later section.

Transport to the plasma membrane. There are two main routes back to the cell surface from sorting endosomes. Some recycling molecules are delivered directly back to the plasma membrane and others are delivered to a long-lived organelle — the ERC (FIG. 2). For some fluorescent lipid analogues, about half of the molecules in the sorting endosomes go back to the cell surface through the direct return pathway with a $t_{1/2}$ of 2 minutes or less, and the rest go through the ERC[31]. However, for technical reasons, it is difficult to analyse the rapid recycling of membrane proteins, and it is not known what fraction returns to the surface using the direct route. In some experiments, the direct return of

Box 3 | Adaptors, other sorting proteins and endocytic recycling

Adaptor proteins bind the cytoplasmic domains of transmembrane proteins and recruit coat proteins to membranes. By providing a link between cargo concentration and vesicle formation (through coat recruitment), they have a crucial role in the sorting of membrane proteins[12,108–111]. One class of adaptors is the adaptor protein (AP) heterotetrameric complexes[14,108]. AP1 is involved in trafficking between the *trans*-Golgi network and endosomes, AP2 functions with clathrin at the plasma membrane, and AP3 is involved in the formation of specialized lysosome-related compartments (for example, melanosomes). The function of AP4 is unknown at present. In addition to the AP family, the GGA (Golgi-localized, γ-ear-containing, ARF-binding protein) family and PACS1 function in membrane-protein trafficking by linking cargo proteins to transport vesicles.

In fibroblast cells, no adaptor protein has been shown to be required for the recycling of proteins that are rapidly and efficiently returned to the cell surface (for example, the transferrin receptor). This finding is not unexpected based on the lack of evidence for a direct role for clathrin in endocytic recycling. However, in polarized cells, the AP1/μ1B ADAPTOR COMPLEX is involved in the efficient endocytic recycling of transferrin receptors and low-density-lipoprotein receptors to the basolateral membrane[112]. These findings indicate that, at least in polarized cells, there are numerous pathways back to the plasma membrane, and that adaptor proteins are involved in targeting proteins to a specific pathway. In addition, a family of phosphoinositide-binding proteins — the sorting nexins — that are involved in intracellular membrane transport have been described[113–116], although the molecular basis for their action is not well-characterized at present (for a review, see REF. 117).

SNAREs
(soluble *N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein (SNAP) receptors). SNARE proteins function in cognate pairs, with one set of the pair being localized to the vesicle and the other to the target membrane. The resultant SNARE pair has a role in the fusion of the bilayer. Assembly of the proper SNARE pair is also involved in establishing the specificity of fusion.

AP1/μ1B ADAPTOR COMPLEX
A form of adaptor protein 1 (AP1) that is expressed in polarized cells and that has a role in polarized endocytic recycling.

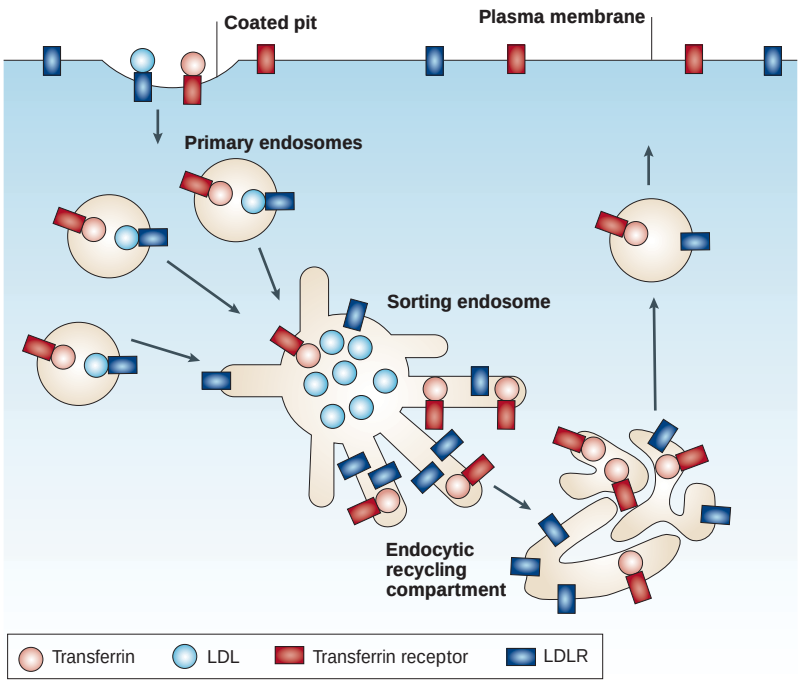


Figure 3 | **The iterative, geometric sorting endosome.** Sorting endosomes use a strikingly simple mechanism to recycle most membrane components and to retain a large fraction of solubilized ligands. Many primary endocytic vesicles (that is, coated-pit-derived vesicles) and endosomes fuse with a sorting endosome. At the same time, narrow-diameter tubules bud out from the sorting endosome, and carry away a large fraction of membrane components with relatively little lumenal volume. Up to 80% of the membrane surface area of the sorting endosome is in its tubules[135,136]. This geometrical mechanism is not sufficient to account for the high efficiency of recycling in a single sorting operation. However, as tubule budding is repeated many times during the lifetime of the sorting endosome, highly efficient recycling can be achieved using this simple physical mechanism[17]. LDL, low-density lipoprotein; LDLR, low-density-lipoprotein receptor. Modified with permission from REF. 17 © (1989) The Rockefeller University Press.

proteins or lipids to the cell surface has been reported to have a $t_{1/2}$ of about 6 minutes (REF. 32), but these rates were determined in experiments that involved low-temperature steps, which might slow membrane trafficking even after re-warming. The molecular differences that determine whether the sorting of components back to the plasma membrane occurs using the direct or indirect recycling pathway are not known. Furthermore, what determines the amount of membrane that flows through these two pathways is also unclear.

The endocytic recycling compartment

The ERC is mainly a collection of tubular organelles with diameters of about 60 nm that are associated with microtubules[33–35]. The distribution of the ERC varies among different cell types. In some cell types (for example, CHO cells), the ERC tubules are mostly condensed around the microtubule-organizing centre, but, in other cells, ERC tubules are distributed more widely throughout the cytoplasm[36]. The kinetics of recycling from the ERC do not seem to be affected significantly by the differences in distribution. As discussed below, the ERC can sort molecules to several different destinations, but most molecules in the ERC return to the plasma membrane. Transferrin receptors and a fluorescent lipid analogue (C6-NBD-sphingomyelin) return to the cell surface from the ERC with identical kinetics ($t_{1/2}$ about 10 minutes)[20]. Transferrin receptors with only a three-residue cytoplasmic domain show a significantly reduced endocytic rate at the plasma membrane, but their intracellular itinerary after endocytosis and the rate constant for their return to the plasma membrane from the ERC are not measurably different from wild-type receptors[19,37]. This indicates that, like protein transport from the sorting endosome to the ERC, protein-sorting determinants are not absolutely required for the export of transmembrane proteins from the ERC to the plasma membrane in non-polarized cells (BOXES 2,3). Of course, in some cases, protein determinants might be involved in regulating the recycling of membrane proteins and, as is discussed in a later section, molecules can be sorted within the ERC using either protein- or lipid-based sorting mechanisms. In polarized cells, protein-determinants (sorting motifs) are required for the targeting of proteins to the appropriate plasma membrane domain (basolateral or apical).

The ERC and related organelles rival the Golgi in the complexity of their sorting processes. The ERC is a long-lived compartment, and therefore transport from the ERC requires the formation of transport intermediates — either vesicles or tubules. Regardless of whether or not cargo is specifically concentrated in these intermediates, proteins are required to form the transport intermediates. This machinery has not been completely elucidated, although it is probable that similar mechanisms to those that are used to form other transport intermediates are used to form the recycling vesicles (BOXES 2,3). For example, clathrin and its associated adaptor proteins have been observed on endosomes, and perturbation of clathrin function can alter recycling (BOXES 2,3). These results are, however, not inconsistent with the proposal that the recycling of membrane proteins is independent of a protein-sorting determinant in non-polarized cells, because, in endosomes, clathrin and its associated proteins might be required for vesicle formation and not required for the selection of most recycling cargo. Two examples of proteins that specifically regulate transport from the ERC are Rab11 (BOX 4) and the Eps15-homology-domain protein EHD1/Rme1 (REFS 38,39). Interestingly, transport to both the *trans*-Golgi network (TGN) and the plasma membrane are affected when the activities of these proteins are altered[38,40]. It should be noted, however, that there might be several routes from the endosomal system to the TGN[41], and that Rab6a′ (an isoform of Rab6) might have a more essential role than Rab11 in transport from endosomes to the TGN[42].

Sorting in the endocytic recycling compartment

One type of sorting in the ERC is exemplified by the endocytic trafficking of TGN38, a protein that has a predominantly TGN distribution (FIG. 2). About 10% of TGN38 is on the cell surface at steady state, and the surface pool is internalized and returned to the TGN.

Box 4 | Rab proteins and endocytic recycling

Rab proteins are a family of more than 60 small GTPase regulatory proteins, and members of this family have key regulatory roles in most membrane-transport steps. A great deal is known about Rab function in the endosomal system. Rab5, one of the most well-characterized Rabs, is involved in the formation and function of the sorting endosome. Rab5 together with early endosome antigen 1 (EEA1)[118–120] — a phosphoinositide-binding protein — regulate fusion between primary endocytic vesicles and sorting endosomes. More recently, it has been appreciated that the effects of Rab5 are not limited to regulating fusion, but that it also has a role in controlling endosome dynamics by recruiting microtubule motors to endosomes. In fact, one of the main roles of Rab5 might be to recruit various protein components and thereby establish specific membrane domains (regions) in endosomes[121–123].

Rab4, Rab5 and Rab11 have roles in regulating recycling. These Rabs have been localized to single endosomes, in which case they segregate to distinct regions of the membrane and probably define specialized functional membrane domains[122]. Rab4 and Rab5 are localized to sorting endosomes and some Rab4 is also associated with the endocytic recycling compartment (ERC). Rab4 seems to have a role in recycling membrane from the sorting endosomes, which perhaps includes direct recycling back to the plasma membrane, although the exact site(s) of its action has not been described[124]. Rab11, which is localized to the ERC and *trans*-Golgi-network (TGN) membranes, has a role in recycling back to the plasma membrane[125,126]. In conditions of perturbed Rab11 function, the transferrin receptor remains concentrated in the ERC, which indicates that transport from the ERC is blocked. The transport of Shiga toxin from the ERC to the TGN and the transport of the glucose transporter GLUT4 from the ERC to the insulin-regulated compartment both require Rab11 function, which indicates that Rab11 is required by numerous sorting pathways that begin at the ERC[40,48]. The mechanism(s) by which Rab11 regulates transport from the ERC is, however, not known.

Using a hybrid construct that consisted of an extracellular interleukin-receptor-α-chain domain (Tac) and the cytoplasmic domain of TGN38, and by labelling surface receptors with high affinity anti-Tac monoclonal antibodies, it was shown that Tac–TGN38 passes through the ERC *en route* to the TGN[43]. The sorting of TGN38 from the ERC to the TGN is an example of a process that is not very efficient in a single step, but that nevertheless achieves a high overall efficiency. Approximately 80% of the Tac–TGN38 in the ERC returns to the cell surface with kinetics that are very similar to those for the recycling of transferrin receptors. So, on average, a TGN38 molecule at the plasma membrane will recycle several times before it is delivered to the TGN. A predominantly Golgi steady-state distribution is achieved because the endocytic recycling is fast compared with the exit rate from the Golgi back to the cell surface.

Shiga toxin, which binds to a glycolipid (globotriaosylceramide), is also transported from the ERC to the TGN by a pathway that is very similar to that taken by TGN38, which indicates that lipid-based sorting occurs in the ERC[44]. Further evidence for this comes from an examination of the endocytosis and recycling of glycosylphosphatidylinositol (GPI)-anchored proteins, such as the folate receptor or decay-accelerating factor. In CHO cells, both of these GPI-anchored proteins enter the ERC. However, whereas transferrin receptors or C6-NBD-sphingomyelin return to the surface with a $t_{1/2}$ of about 10 minutes, the GPI-anchored proteins return with a $t_{1/2}$ of about 30 minutes[45]. When cellular cholesterol levels are reduced by about 30%, the recycling rate of the GPI-anchored proteins is increased threefold and matches the rate constant for the return of transferrin receptors. These data provide evidence for a role for LIPID MICRODOMAINS in sorting in the ERC. It should be noted that, in CHO cells, the ERC membrane is the main intracellular cholesterol repository[46].

One of the most significant sorting events in the ERC/endosomes involves the trafficking of the insulin-regulated glucose transporter GLUT4 in fat and muscle cells[47]. Controlling the fraction of GLUT4 transporters that are expressed on the cell surface regulates glucose uptake into these cells. Insulin causes a rapid net translocation of GLUT4 from the internal stores to the plasma membrane. In fat cells, GLUT4 follows an endocytic itinerary that is initially similar to the transferrin-receptor pathway shown in FIG. 2 and, in the presence of insulin, most of the GLUT4 can be found in compartments that also contain transferrin receptors. However, in the absence of insulin, GLUT4 is transported from the ERC to a distinct compartment — the INSULIN-REGULATED COMPARTMENT (IRC) — which does not contain transferrin receptors[48,49]. The GLUT4 in the IRC continues to cycle through the other compartments, which include the plasma membrane under low insulin conditions. This indicates that the accumulation of GLUT4 in the internal pool is maintained by a dynamic process, rather than by sequestering the transporters into a regulated secretory compartment, from which they can be released only after insulin stimulation. The kinetics of GLUT4 trafficking are such that low insulin concentrations result in GLUT4 being equally distributed between the ERC and the IRC. So, the ERC/endosomes are not only involved in the sorting of GLUT4 to the specialized insulin-regulated pathway, they are also a reservoir for insulin-recruited GLUT4.

Two kinetically distinct routes that lead from the ERC directly back to the cell surface have been characterized in CHO cells, and this shows that specialized recycling pathways are not limited to differentiated cell types. Transferrin receptors, C6-NBD-sphingomyelin and other constitutively recycled molecules are rapidly returned to the cell surface with a $t_{1/2}$ of about 10 minutes. One cargo protein of the slow pathway ($t_{1/2}$ ~30 minutes) is the transmembrane

LIPID MICRODOMAINS
Localized membrane regions that differ from surrounding regions in their lipid composition and order. There are probably many types of lipid microdomain that coexist within the same membrane bilayer. One type of microdomain is a lipid raft.

INSULIN-REGULATED COMPARTMENT
A specialized endosomal compartment found in fat and muscle cells. This poorly described compartment is the site of storage of the glucose transporter GLUT4. Insulin recruits GLUT4 to the plasma membrane from this compartment.

Box 5 | Mechanisms for sorting lipids

Lipids can be sorted very efficiently whenever a vesicle or tubule buds from a parent organelle, but the general principles for lipid sorting are not fully understood. Three properties are key to lipid sorting — headgroup interactions, lipid shape and MEMBRANE-ORDER PARAMETERS (that is, the fluidity and packing density of the hydrocarbon core).

Lipid headgroups can interact with other lipids or with proteins. One possible mechanism for sorting glycolipids is based on headgroup interactions with lectins[127], but it has been difficult to provide direct evidence for this. Headgroup interactions between lipids could facilitate the formation of microdomains, which might be preferentially included or excluded from vesicles or tubules as they form. Lipid interactions with a protein might cause the lipid to be sorted on the basis of the characteristics of the protein, and such mechanisms are important for the trafficking of some glycosylphosphatidylinositol-anchored proteins[128].

The general shapes of lipids, with their long axis orientated perpendicular to the bilayer, can be characterized as cylinders, cones or inverted cones. Lysophospholipids — which have a larger cross-sectional area at the headgroup than the tail — are examples of inverted cones. Lipids such as phosphatidic acid or phosphatidylserine, which have a smaller cross-sectional area at the headgroup than the tail, are approximately cone shaped. Based on their geometry, a cylinder (for example, phosphatidylcholine with a single *cis*-unsaturated bond) would fit well in a planar bilayer, whereas cone-shaped and inverted-cone-shaped lipids would fit best in membranes of opposing curvatures. It is noteworthy that, in many cases, the formation of vesicles or tubules is accompanied by sharp local changes in membrane curvature. Lipids with different shapes are preferentially included or excluded from these curved regions.

Finally, different types of lipids have varying propensities for forming microdomains, for example, LIPID RAFTS. Well-ordered, densely packed lipids are resistant to the bending that is required for the formation of highly curved membranes. So, the preference of lipids for highly ordered microdomains can also affect their inclusion in transport intermediates.

insulin-regulated amino peptidase (IRAP)[50,51]. IRAP is transported in the same way as GLUT4 in adipocytes, but it is naturally expressed in many other cell types. The recycling of IRAP in fibroblasts is increased by insulin. In CHO cells, IRAP is sorted from the transferrin receptor in the ERC and is returned to the plasma membrane in distinct transport vesicles, which shows that there are at least two distinct pathways to the plasma membrane from the ERC[49]. The targeting of IRAP to the slow recycling pathway is dependent on a cytoplasmic acidic-cluster–dileucine motif, which also functions as an internalization motif. The adaptor that binds the acidic-cluster–dileucine motif of IRAP and targets it to the slow recycling pathway has not been identified.

Complex recycling itineraries

Most proteins that cycle between the cell interior and the cell surface accumulate in the ERC, because this is the slow step in their return to the plasma membrane. TGN38 and furin are examples of proteins that continually cycle to the cell surface, but that are found predominantly in the TGN. The recycling route for TGN38 has already been described. Similar to TGN38, furin is cleared rapidly from the cell surface through clathrin-coated pits and is delivered to sorting endosomes (FIG. 2). However, furin is retained in these endosomes as they begin to mature into late endosomes[52]. Furin is then transferred to the TGN by a process that seems to occur abruptly — organelles that contain furin do not lose it gradually[53]. After delivery to the TGN, furin can cycle between the Golgi and late endosomes. The net rate of export back to the cell surface is low, in part because much of the furin is involved in this internal recycling.

A cytoplasmic-domain bipartite motif determines furin trafficking[52]. First, a typical tyrosine-based motif (YKGL) and a leucine–isoleucine motif direct furin internalization through clathrin-coated pits[54,55]. These sequences might also be involved in the transport of furin from the TGN to endosomes, although not much is known about this pathway at the molecular level. The second element of the motif — a casein-kinase-II-phosphorylated acidic-cluster motif (SDSEEDE, in which the serines can be phosphorylated) — regulates furin trafficking from endosomes to the TGN[54,56,57]. The phosphorylated motif binds the PACS1 adaptor protein[58,59]. PACS1 also binds the adaptor protein 1 (AP1)–clathrin complex, and thereby links phosphorylated furin to AP1 transport vesicles[58,59]. PACS1 function is not limited to furin trafficking, as it can function in the transport of several proteins from endosomes to the TGN[52]. The binding of furin by PACS1 seems to have a role in determining whether furin that is internalized from the plasma membrane is transported from endosomes back to the cell surface or is transferred to the TGN (the latter is dependent on phosphorylation and PACS1 binding). The distribution of furin between the TGN and endosomes can therefore be regulated rapidly by modulating the phosphorylation state of the serine residues in the acidic-cluster motif, which adds an extra level of complexity to furin trafficking.

The cation-independent mannose-6-phosphate receptor (CI-MPR) further illustrates the complexity and individuality of membrane-trafficking pathways[60]. Like furin, this molecule also passes through the TGN, late endosomes and the plasma membrane (FIG. 2). The Golgi-to-late-endosome traffic delivers newly made lysosomal enzymes to late endosomes, and the plasma-membrane receptors can capture and internalize enzymes that have been secreted. As CI-MPR moves among the same organelles as furin in CHO cells, it might seem probable that it would follow a similar trafficking itinerary. However, the CI-MPR follows an initial itinerary from the plasma membrane that is nearly indistinguishable from TGN38 (REF. 61). After the sorting endosome, it goes to the ERC and, from there, a high fraction of CI-MPR returns to the cell surface. However, during each cycle of internalization, a fraction also goes to the TGN and late endosomes. As with furin, the net rate of CI-MPR return to the cell surface is low, because much of it is involved in the intracellular Golgi-to-late-endosome recycling loop. CI-MPR trafficking is determined by an acidic-cluster–dileucine motif and aromatic-residue-based motifs[60,62–66]. Transport of CI-MPR from the TGN to endosomes is mediated by the interaction of the acidic-cluster–dileucine motif with GGA2 (Golgi-localized, γ-ear-containing, ARF-binding protein 2)[67–70]. The GGAs are a recently described family of adaptor proteins that regulate traffic between endosomes and the TGN[71–73]. It is uncertain

MEMBRANE-ORDER PARAMETERS
These parameters describe the organization and dynamics of lipids in a bilayer. Highly ordered lipids have tight packing and restricted motion of the hydrocarbon core.

LIPID RAFTS
One type of lipid microdomain that is characterized by a relatively high content of cholesterol, sphingomyelin and glycosphingolipids. These microdomains are resistant to solubilization by non-ionic detergent because of their tight packing. They resemble liquid-ordered domains that are found in model membranes, which are characterized by tight packing and the high lateral mobility of lipids within the bilayer.

precisely where the endocytosed enzymes dissociate from CI-MPRs. This might occur in the sorting endosomes, or it might occur only after the receptor–enzyme complexes enter the late endosomes after passing through the ERC and the TGN.

Endocytic recycling of lipids

In addition to the protein components of the membrane, lipids are also internalized and then delivered to various intracellular destinations. As mentioned above, a high fraction of internalized C6-NBD-sphingomyelin is recycled to the cell surface through an itinerary that is morphologically and kinetically indistinguishable from recycling transferrin receptors[20,74]. This is consistent with the hypothesis that most internalized molecules recycle back to the cell surface in the absence of a positive sorting signal, because the tubules that bud out from the sorting endosome have a high surface-area-to-volume ratio. However, many internalized lipids are delivered to other branches of the endocytic pathways, and this sorting helps to maintain the distinct lipid compositions of various organelles. The mechanisms for the endocytic sorting of lipids are only partially understood[75,76], and they depend on the properties of both the head groups and the hydrophobic portions of the lipids (BOX 5). It seems that lipid sorting occurs at every step in which a vesicle or tubule buds from a parent organelle, and that lipid composition and sorting can influence protein sorting. It will therefore be important to develop a deeper understanding of the mechanisms involved in lipid sorting.

Cholesterol is, of course, a very important lipid constituent of membranes. The cholesterol content of membranes in mammalian cells can range from being just a few percent of the total lipids to being nearly stoichiometrically equivalent to phospholipids. Because cholesterol can desorb rapidly from membranes in the presence of appropriate acceptors[77], it can move between compartments as part of a lipid bilayer or by diffusion through the cytoplasm in association with carrier proteins[78]. Both vesicular and non-vesicular mechanisms are used to transport cholesterol in cells, and this makes the analysis of cholesterol trafficking particularly difficult. Some other lipids are also transported by non-vesicular transport in association with carriers. This type of pathway is essential for the delivery of lipids to organelles such as mitochondria, which do not engage in vesicle-mediated transport with other organelles. Interestingly, in fibroblasts and non-polarized HepG2 hepatoma cells, the ERC is the main intracellular site of cholesterol accumulation, and sterol can be delivered from the plasma membrane to the ERC by an ATP-independent, non-vesicular process[79,80]. In polarized epithelia, cholesterol is enriched in the apical membranes, and transport to this membrane domain can also occur by ATP-independent processes[80].

Reversible membrane sequestration

In a recent study of the cell–cell adhesion molecule PECAM (platelet–endothelial-cell adhesion molecule), it was observed that there was a sequestered pool of PECAM in endothelial cells that becomes available for binding to PECAM on monocytes that are crossing the endothelial monolayer[81] (FIG. 4). It was also observed that molecules of PECAM on the endothelial cells could recycle between the sequestered pool and the accessible pool. Although this process seemed much like an endocytic recycling process, the sequestered PECAM molecules did not colocalize with transferrin or with other endocytic tracers. In fact, it could be shown that all of the sequestered PECAM was actually in compartments that were open to the extracellular space. Using electron microscopy, these compartments could be seen to be morphologically similar to clusters or chains of caveolae, although the compartments could not be stained with anti-caveolin antibodies. This new recycling process shows that there are many ways to regulate the expression of molecules on the cell surface (FIG. 4).

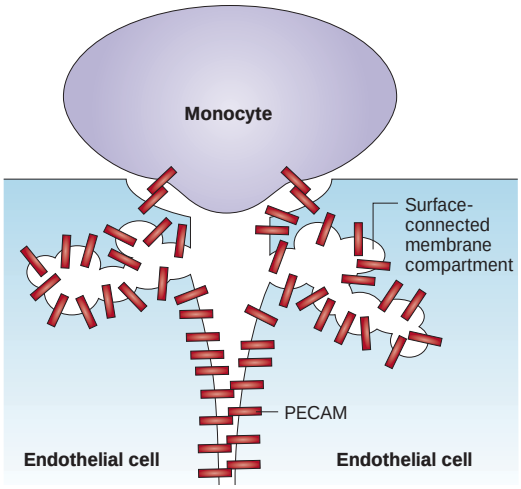


Figure 4 | **Functional recycling without removal from the plasma membrane.** Endothelial cells express surface adhesion molecules that facilitate the transmigration of white blood cells at sites of inflammation. One of these adhesion molecules, PECAM (platelet–endothelial-cell adhesion molecule), has a crucial role in supporting monocyte transmigration, and it also functions as a homotypic adhesion molecule that links adjacent endothelial cells to one another. Endothelial cells maintain a reservoir of PECAM that is functionally sequestered away from the cell surface, but that can be made available at the plasma membrane very rapidly when a monocyte is squeezing between adjacent endothelial cells. The pool of sequestered PECAM molecules can exchange slowly with the surface pool. In the resting state, this reservoir of PECAM is inaccessible to antibody binding, and it does not participate in endothelial cell–cell adhesion. Although this movement of PECAM between a sequestered pool and a surface pool shares many functional properties with endocytic recycling, sequestered PECAM is never fully removed from the cell surface. Instead, the PECAM reservoir is in a reticulum of small, surface-connected invaginations that resemble caveolae, but that lack the caveolin protein[81]. This system allows a pool of the PECAM molecules to be available only when it is needed to support monocyte transmigration. Modified with permission from REF. 137 © (2003) Elsevier.

Conclusion and perspective

The analysis of endocytic recycling pathways is a rich arena for continued studies of the molecular mechanisms of membrane transport. Our understanding of endocytic recycling has lagged behind that of biosynthetic pathways, in part because of the complexity that is inherent in the endosomal system, which is composed of many compartments that are connected by numerous pathways. However, most of the pathways have now been mapped, and this knowledge — together with technical advances in quantitative real-time imaging, the availability of reporters for these pathways that can be labelled with green fluorescent protein, and new methods for disrupting the functions of proteins (for example, small molecular inhibitors and small interfering RNAs) — indicates that in the near future we will have a more complete understanding of the molecular mechanisms of endocytic recycling.

1. Mukherjee, S., Ghosh, R. N. & Maxfield, F. R. Endocytosis. *Physiol. Rev.* **77**, 759–803 (1997).
2. Sandvig, K. & van Deurs, B. Transport of protein toxins into cells: pathways used by ricin, cholera toxin and Shiga toxin. *FEBS Lett.* **529**, 49–53 (2002).
3. Sabharanjak, S., Sharma, P., Parton, R. G. & Mayor, S. GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. *Dev. Cell* **2**, 411–423 (2002).
4. Sharma, D. K. *et al.* Glycosphingolipids internalized via caveolar-related endocytosis rapidly merge with the clathrin pathway in early endosomes and form microdomains for recycling. *J. Biol. Chem.* **278**, 7564–7572 (2003).
5. Naslavsky, N., Weigert, R. & Donaldson, J. G. Convergence of non-clathrin- and clathrin-derived endosomes involves Arf6 inactivation and changes in phosphoinositides. *Mol. Biol. Cell* **14**, 417–431 (2003).
6. Wang, E. *et al.* Apical and basolateral endocytic pathways of MDCK cells meet in acidic common endosomes distinct from a nearly-neutral apical recycling endosome. *Traffic* **1**, 480–493 (2000).
7. Schell, M. J., Maurice, M., Stieger, B. & Hubbard, A. L. 5′ nucleotidase is sorted to the apical domain of hepatocytes via an indirect route. *J. Cell Biol.* **119**, 1173–1182 (1992).
8. Kipp, H. & Arias, I. M. Newly synthesized canalicular ABC transporters are directly targeted from the Golgi to the hepatocyte apical domain in rat liver. *J. Biol. Chem.* **275**, 15917–15925 (2000).
9. Zegers, M. M. & Hoekstra, D. Mechanisms and functional features of polarized membrane traffic in epithelial and hepatic cells. *Biochem. J.* **336**, 257–269 (1998).
10. Mostov, K., Su, T. & ter Beest, M. Polarized epithelial membrane traffic: conservation and plasticity. *Nature Cell Biol.* **5**, 287–293 (2003).
11. Kirchhausen, T. Three ways to make a vesicle. *Nature Rev. Mol. Cell Biol.* **1**, 187–198 (2000).
12. Kirchhausen, T. Clathrin adaptors really adapt. *Cell* **109**, 413–416 (2002).
13. Bonifacino, J. S. & Lippincott-Schwartz, J. Coat proteins: shaping membrane transport. *Nature Rev. Mol. Cell Biol.* **4**, 409–414 (2003).
14. Boehm, M. & Bonifacino, J. S. Adaptins: the final recount. *Mol. Biol. Cell* **12**, 2907–2920 (2001).
15. Waters, M. G. & Hughson, F. M. Membrane tethering and fusion in the secretory and endocytic pathways. *Traffic* **1**, 588–597 (2000).
16. Kavalali, E. T. SNARE interactions in membrane trafficking: a perspective from mammalian central synapses. *Bioessays* **24**, 926–936 (2002).
17. Dunn, K. W., McGraw, T. E. & Maxfield, F. R. Iterative fractionation of recycling receptors from lysosomally destined ligands in an early sorting endosome. *J. Cell Biol.* **109**, 3303–3314 (1989).
Provides experimental evidence that supports the maturation model for delivery from sorting endosomes to late endosomes.
18. Presley, J. F., Mayor, S., McGraw, T. E., Dunn, K. W. & Maxfield, F. R. Bafilomycin A1 treatment retards transferrin receptor recycling more than bulk membrane recycling. *J. Biol. Chem.* **272**, 13929–13936 (1997).
19. Johnson, L. S., Dunn, K. W., Pytowski, B. & McGraw, T. E. Endosome acidification and receptor trafficking: bafilomycin A1 slows receptor externalization by a mechanism involving the receptor's internalization motif. *Mol. Biol. Cell* **4**, 1251–1266 (1993).
20. Mayor, S., Presley, J. F. & Maxfield, F. R. Sorting of membrane components from endosomes and subsequent recycling to the cell surface occurs by a bulk flow process. *J. Cell Biol.* **121**, 1257–1269 (1993).
Used a detailed kinetic analysis of the trafficking of a lipid probe to show that efficient sorting can occur in the absence of a protein-sorting motif.
21. Mills, I. G., Jones, A. T. & Clague, M. J. Regulation of endosome fusion. *Mol. Membr. Biol.* **16**, 73–79 (1999).
22. Clague, M. J. Membrane transport: take your fusion partners. *Curr. Biol.* **9**, R258–R260 (1999).
23. Woodman, P. G. Biogenesis of the sorting endosome: the role of Rab5. *Traffic* **1**, 695–701 (2000).
24. Gruenberg, J., Griffiths, G. & Howell, K. E. Characterization of the early endosome and putative endocytic carrier vesicles *in vivo* and with an assay of vesicle fusion *in vitro*. *J. Cell Biol.* **108**, 1301–1316 (1989).
This paper describes an *in vitro* endosome-fusion assay, which has been of great value in establishing the molecular mechanisms of specific trafficking events in the endocytic pathway.
25. van Weert, A. V. M., Dunn, K. W., Geuze, H. J., Maxfield, F. R. & Stoorvogel, W. Transport from late endosomes to lysosomes, but not sorting of integral membrane proteins in endosomes, depends on the vacuolar proton pump. *J. Cell Biol.* **130**, 821–834 (1995).
26. Aniento, F., Gu, F., Parton, R. G. & Gruenberg, J. An endosomal β COP is involved in the pH-dependent formation of transport vesicles destined for late endosomes. *J. Cell Biol.* **133**, 29–41 (1996).
27. Katzmann, D. J., Odorizzi, G. & Emr, S. D. Receptor downregulation and multivesicular-body sorting. *Nature Rev. Mol. Cell Biol.* **3**, 893–905 (2002).
28. Raiborg, C. *et al.* Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nature Cell Biol.* **4**, 394–398 (2002).
29. Babst, M., Katzmann, D. J., Estepa-Sabal, E. J., Meerloo, T. & Emr, S. D. Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. *Dev. Cell* **3**, 271–282 (2002).
30. Babst, M., Katzmann, D. J., Snyder, W. B., Wendland, B. & Emr, S. D. Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. *Dev. Cell* **3**, 283–289 (2002).
References 28–30 provide an initial molecular description of the molecular machinery that is involved in the development of multivesicular bodies and the targeting of receptors for downregulation.
31. Hao, M. & Maxfield, F. R. Characterization of rapid membrane internalization and recycling. *J. Biol. Chem.* **275**, 15279–15286 (2000).
32. Sheff, D. R., Daro, E. A., Hull, M. & Mellman, I. The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions. *J. Cell Biol.* **145**, 123–139 (1999).
33. Hopkins, C. R. Intracellular routing of transferrin and transferrin receptors in epidermoid carcinoma A431 cells. *Cell* **35**, 321–330 (1983).
34. Yamashiro, D. J., Tycko, B., Fluss, S. R. & Maxfield, F. R. Segregation of transferrin to a mildly acidic (pH 6.5) para-Golgi compartment in the recycling pathway. *Cell* **37**, 789–800 (1984).
References 33 and 34 provide the first description of the endosomal recycling compartments traversed by the transferrin receptor, the most widely used reporter of endocytic recycling.
35. McGraw, T. E., Dunn, K. W. & Maxfield, F. R. Isolation of a temperature-sensitive variant Chinese hamster ovary cell line with a morphologically altered endocytic recycling compartment. *J. Cell. Physiol.* **155**, 579–594 (1993).
36. Lin, S. X., Gundersen, G. G. & Maxfield, F. R. Export from pericentriolar endocytic recycling compartment to cell surface depends on stable, detyrosinated (glu) microtubules and kinesin. *Mol. Biol. Cell* **13**, 96–109 (2002).
37. Jing, S. Q., Spencer, T., Miller, K., Hopkins, C. & Trowbridge, I. S. Role of the human transferrin receptor cytoplasmic domain in endocytosis: localization of a specific signal sequence for internalization. *J. Cell Biol.* **110**, 283–294 (1990).
38. Lin, S. X., Grant, B., Hirsh, D. & Maxfield, F. R. Rme-1 regulates the distribution and function of the endocytic recycling compartment in mammalian cells. *Nature Cell Biol.* **3**, 567–572 (2001).
39. Caplan, S., Dell'Angelica, E. C., Gahl, W. A. & Bonifacino, J. S. Trafficking of major histocompatibility complex class II molecules in human B-lymphoblasts deficient in the AP-3 adaptor complex. *Immunol. Lett.* **72**, 113–117 (2000).
40. Wilcke, M. *et al.* Rab11 regulates the compartmentalization of early endosomes required for efficient transport from early endosomes to the *trans*-golgi network. *J. Cell Biol.* **151**, 1207–1220 (2000).
41. Iversen, T. G. *et al.* Endosome to Golgi transport of ricin is independent of clathrin and of the Rab9- and Rab11-GTPases. *Mol. Biol. Cell* **12**, 2099–2107 (2001).
42. Mallard, F. *et al.* Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a Rab6 isoform. *J. Cell Biol.* **156**, 653–664 (2002).
43. Ghosh, R. N., Mallet, W. G., Soe, T. T., McGraw, T. E. & Maxfield, F. R. An endocytosed TGN38 chimeric protein is delivered to the TGN after trafficking through the endocytic recycling compartment in CHO cells. *J. Cell Biol.* **142**, 923–936 (1998).
44. Mallard, F. *et al.* Direct pathway from early/recycling endosomes to the Golgi apparatus revealed through the study of shiga toxin B-fragment transport. *J. Cell Biol.* **143**, 973–990 (1998).
45. Mayor, S., Sabharanjak, S. & Maxfield, F. R. Cholesterol-dependent retention of GPI-anchored proteins in endosomes. *EMBO J.* **17**, 4626–4638 (1998).
46. Hao, M. *et al.* Vesicular and non-vesicular sterol transport in living cells. The endocytic recycling compartment is a major sterol storage organelle. *J. Biol. Chem.* **277**, 609–617 (2002).
47. Bryant, N. J., Govers, R. & James, D. E. Regulated transport of the glucose transporter GLUT4. *Nature Rev. Mol. Cell Biol.* **3**, 267–277 (2002).
48. Zeigerer, A. *et al.* GLUT4 retention in adipocytes requires two intracellular insulin-regulated transport steps. *Mol. Biol. Cell* **13**, 2421–2435 (2002).
49. Lampson, M. A., Schmoranzer, J., Zeigerer, A., Simon, S. M. & McGraw, T. E. Insulin-regulated release from the endosomal recycling compartment is regulated by budding of specialized vesicles. *Mol. Biol. Cell* **12**, 3489–4501 (2001).
This work establishes that distinct sets of vesicles formed from the ERC transport cargo to the plasma membrane in non-polarized cells.
50. Johnson, A. O., Lampson, M. A. & McGraw, T. E. A di-leucine sequence and a cluster of acidic amino acids are required for dynamic retention in the endosomal recycling compartment of fibroblasts. *Mol. Biol. Cell* **12**, 367–381 (2001).
51. Johnson, A. O. *et al.* Identification of an insulin-responsive, slow endocytic recycling mechanism in Chinese hamster ovary cells. *J. Biol. Chem.* **273**, 17968–17977 (1998).
52. Thomas, G. Furin at the cutting edge: from protein traffic to embryogenesis and disease. *Nature Rev. Mol. Cell Biol.* **3**, 753–766 (2002).

53. Mallet, W. G. & Maxfield, F. R. Chimeric forms of furin and TGN38 are transported from the plasma membrane to the *trans*-Golgi network via distinct endosomal pathways. *J. Cell Biol.* **146**, 345–359 (1999).
54. Voorhees, P. *et al.* An acidic sequence within the cytoplasmic domain of furin functions as a determinant of *trans*-Golgi network localization and internalization from the cell surface. *EMBO J.* **14**, 4961–4975 (1995).
55. Schafer, W. *et al.* Two independent targeting signals in the cytoplasmic domain determine *trans*-Golgi network localization and endosomal trafficking of the proprotein convertase furin. *EMBO J.* **14**, 2424–2435 (1995).
56. Molloy, S. S., Thomas, L., Van Slyke, J. K., Stenberg, P. E. & Thomas, G. Intracellular trafficking and activation of the furin proprotein convertase: localization to the TGN and recycling from the cell surface. *EMBO J.* **13**, 18–33 (1994).
57. Jones, B. G. *et al.* Intracellular trafficking of furin is modulated by the phosphorylation state of a casein kinase II site in its cytoplasmic tail. *EMBO J.* **14**, 5869–5883 (1995).
Provides an initial description of the trafficking pathway of furin.
58. Wan, L. *et al.* PACS-1 defines a novel gene family of cytosolic sorting proteins required for *trans*-Golgi network localization. *Cell* **94**, 205–216 (1998).
59. Crump, C. M. *et al.* PACS-1 binding to adaptors is required for acidic cluster motif-mediated protein traffic. *EMBO J.* **20**, 2191–2201 (2001).
60. Ghosh, P., Dahms, N. M. & Kornfeld, S. Mannose 6-phosphate receptors: new twists in the tale. *Nature Rev. Mol. Cell Biol.* **4**, 202–212 (2003).
61. Lin, S. X., Mallet, W. G., Huang, A. Y. & Maxfield, F. R. Endocytosed cation-independent mannose 6-phosphate receptor traffics via the endocytic recycling compartment *en route* to the *trans*-Golgi network and a sub-population of late endosomes. *Mol. Biol. Cell* 31 Oct 2003 (doi:10.1091/mbc.E03-07-0497).
62. Chen, H. J., Remmler, J., Delaney, J. C., Messner, D. J. & Lobel, P. Mutational analysis of the cation-independent mannose 6-phosphate/insulin-like growth factor II receptor. A consensus casein kinase II site followed by 2 leucines near the carboxyl terminus is important for intracellular targeting of lysosomal enzymes. *J. Biol. Chem.* **268**, 22338–22346 (1993).
63. Chen, H. J., Yuan, J. & Lobel, P. Systematic mutational analysis of the cation-independent mannose 6-phosphate/insulin-like growth factor II receptor cytoplasmic domain. An acidic cluster containing a key aspartate is important for function in lysosomal enzyme sorting. *J. Biol. Chem.* **272**, 7003–7012 (1997).
64. Schweizer, A., Kornfeld, S. & Rohrer, J. Proper sorting of the cation-dependent mannose 6-phosphate receptor in endosomes depends on a pair of aromatic amino acids in its cytoplasmic tail. *Proc. Natl Acad. Sci. USA* **94**, 14471–14476 (1997).
65. Rohrer, J., Schweizer, A., Johnson, K. F. & Kornfeld, S. A determinant in the cytoplasmic tail of the cation-dependent mannose 6-phosphate receptor prevents trafficking to lysosomes. *J. Cell Biol.* **130**, 1297–1306 (1995).
66. Johnson, K. F. & Kornfeld, S. The cytoplasmic tail of the mannose 6-phosphate/insulin-like growth factor-II receptor has two signals for lysosomal enzyme sorting in the Golgi. *J. Cell Biol.* **119**, 249–257 (1992).
67. Doray, B., Bruns, K., Ghosh, P. & Kornfeld, S. Interaction of the cation-dependent mannose 6-phosphate receptor with GGA proteins. *J. Biol. Chem.* **277**, 18477–18482 (2002).
68. Doray, B., Ghosh, P., Griffith, J., Geuze, H. J. & Kornfeld, S. Cooperation of GGAs and AP-1 in packaging MPRs at the *trans*-Golgi network. *Science* **297**, 1700–1703 (2002).
69. Zhu, Y., Doray, B., Poussu, A., Lehto, V. P. & Kornfeld, S. Binding of GGA2 to the lysosomal enzyme sorting motif of the mannose 6-phosphate receptor. *Science* **292**, 1716–1718 (2001).
70. Puertollano, R., Aguilar, R. C., Gorshkova, I., Crouch, R. J. & Bonifacino, J. S. Sorting of mannose 6-phosphate receptors mediated by the GGAs. *Science* **292**, 1712–1716 (2001).
References 69 and 70 provide insights into the possible functions of GGAs in a process that was previously ascribed to AP1.
71. Costaguta, G., Stefan, C. J., Bensen, E. S., Emr, S. D. & Payne, G. S. Yeast Gga coat proteins function with clathrin in Golgi to endosome transport. *Mol. Biol. Cell* **12**, 1885–1896 (2001).
72. Boman, A. L. GGA proteins: new players in the sorting game. *J. Cell Sci.* **114**, 3413–3418 (2001).
73. Puertollano, R., Randazzo, P. A., Presley, J. F., Hartnell, L. M. & Bonifacino, J. S. The GGAs promote ARF-dependent recruitment of clathrin to the TGN. *Cell* **105**, 93–102 (2001).
74. Koval, M. & Pagano, R. E. Lipid recycling between the plasma membrane and intracellular compartments: transport and metabolism of fluorescent sphingomyelin analogues in cultured fibroblasts. *J. Cell Biol.* **108**, 2169–2181 (1989).
75. Mukherjee, S. & Maxfield, F. R. Cholesterol: stuck in traffic. *Nature Cell Biol.* **1**, E37–E38 (1999).
76. Mukherjee, S. & Maxfield, F. R. Role of membrane organization and membrane domains in endocytic lipid trafficking. *Traffic* **1**, 203–211 (2000).
77. Steck, T. L., Ye, J. & Lange, Y. Probing red cell membrane cholesterol movement with cyclodextrin. *Biophys. J.* **83**, 2118–2125 (2002).
78. Maxfield, F. R. & Wustner, D. Intracellular cholesterol transport. *J. Clin. Invest.* **110**, 891–898 (2002).
79. Hao, M. *et al.* Vesicular and non-vesicular sterol transport in living cells. The endocytic recycling compartment is a major sterol storage organelle. *J. Biol. Chem.* **277**, 609–617 (2002).
80. Wustner, D., Herrmann, A., Hao, M. & Maxfield, F. R. Rapid nonvesicular transport of sterol between the plasma membrane domains of polarized hepatic cells. *J. Biol. Chem.* **277**, 30325–30336 (2002).
81. Mamdouh, Z., Chen, X., Pierini, L. M., Maxfield, F. R. & Muller, W. A. Targeted recycling of PECAM from endothelial surface-connected compartments during diapedesis. *Nature* **421**, 748–753 (2003).
82. Krauss, M. *et al.* ARF6 stimulates clathrin/AP-2 recruitment to synaptic membranes by activating phosphatidylinositol phosphate kinase type Iγ. *J. Cell Biol.* **162**, 113–124 (2003).
83. Farsad, K. *et al.* Generation of high curvature membranes mediated by direct endophilin bilayer interactions. *J. Cell Biol.* **155**, 193–200 (2001).
84. Schmidt, A. *et al.* Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. *Nature* **401**, 133–141 (1999).
85. Ford, M. G. *et al.* Curvature of clathrin-coated pits driven by epsin. *Nature* **419**, 361–366 (2002).
86. Hinshaw, J. E. Dynamin and its role in membrane fission. *Annu. Rev. Cell Dev. Biol.* **16**, 483–519 (2000).
87. Wigge, P. & McMahon, H. T. The amphiphysin family of proteins and their role in endocytosis at the synapse. *Trends Neurosci.* **21**, 339–344 (1998).
88. Slepnev, V. I. & De Camilli, P. Accessory factors in clathrin-dependent synaptic vesicle endocytosis. *Nature Rev. Neurosci.* **1**, 161–172 (2000).
89. Wendland, B. Epsins: adaptors in endocytosis? *Nature Rev. Mol. Cell Biol.* **3**, 971–977 (2002).
90. Lemmon, S. K. Clathrin uncoating: auxilin comes to life. *Curr. Biol.* **11**, R49–R52 (2001).
91. Ma, Y. *et al.* Identification of domain required for catalytic activity of auxilin in supporting clathrin uncoating by Hsc70. *J. Biol. Chem.* **277**, 49267–49274 (2002).
92. Weber, T. *et al.* SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759–772 (1998).
93. van Dam, E. M., Ten, B. T., Jansen, K., Spijkers, P. & Stoorvogel, W. Endocytosed transferrin receptors recycle via distinct dynamin and phosphatidylinositol 3-kinase-dependent pathways. *J. Biol. Chem.* **277**, 48876–48883 (2002).
94. van Dam, E. M. & Stoorvogel, W. Dynamin-dependent transferrin receptor recycling by endosome-derived clathrin-coated vesicles. *Mol. Biol. Cell* **13**, 169–182 (2002).
95. Galli, T. *et al.* Tetanus toxin-mediated cleavage of cellubrevin impairs exocytosis of transferrin receptor-containing vesicles in CHO cells. *J. Cell Biol.* **125**, 1015–1024 (1994).
96. Jackson, T. Transport vesicles: coats of many colours. *Curr. Biol.* **8**, R609–R612 (1998).
97. Stoorvogel, W., Oorschot, V. & Geuze, H. J. A novel class of clathrin-coated vesicles budding from endosomes. *J. Cell Biol.* **132**, 21–33 (1996).
This paper identifies clathrin-coated structures on endosomes.
98. Wettey, F. R. *et al.* Controlled elimination of clathrin heavy-chain expression in DT40 lymphocytes. *Science* **297**, 1521–1525 (2002).
99. Iversen, T. G., Skretting, G., van Deurs, B. & Sandvig, K. Clathrin-coated pits with long, dynamin-wrapped necks upon expression of a clathrin antisense RNA. *Proc. Natl Acad. Sci. USA* **100**, 5175–5180 (2003).
100. Moskowitz, H. S., Heuser, J., McGraw, T. E. & Ryan, T. A. Targeted chemical disruption of clathrin function in living cells. *Mol. Biol. Cell* **14**, 4437–4447 (2003).
101. Bennett, E., Lin, S., Towler, M., Maxfield, F. & Brodsky, F. Clathrin hub expression affects early endosome distribution with minimal impact on receptor sorting and recycling. *Mol. Biol. Cell* **12**, 2790–2799 (2001).
102. Pelham, H. R. Traffic through the Golgi apparatus. *J. Cell Biol.* **155**, 1099–1101 (2001).
103. Barlowe, C. Traffic COPs of the early secretory pathway. *Traffic* **1**, 371–377 (2000).
104. Antonny, B. & Schekman, R. ER export: public transportation by the COPII coach. *Curr. Opin. Cell. Biol.* **13**, 438–443 (2001).
105. Barlowe, C. COPII-dependent transport from the endoplasmic reticulum. *Curr. Opin. Cell. Biol.* **14**, 417–422 (2002).
106. Wieland, F. & Harter, C. Mechanisms of vesicle formation: insights from the COP system. *Curr. Opin. Cell Biol.* **11**, 440–446 (1999).
107. Daro, E., Sheff, D., Gomez, M., Kreis, T. & Mellman, I. Inhibition of endosome function in CHO cells bearing a temperature-sensitive defect in the coatomer (COPI) component epsilon-COP. *J. Cell Biol.* **139**, 1747–1759 (1997).
108. Boehm, M. & Bonifacino, J. S. Genetic analyses of adaptin function from yeast to mammals. *Gene* **286**, 175–186 (2002).
109. Kirchhausen, T., Bonifacino, J. S. & Riezman, H. Linking cargo to vesicle formation: receptor tail interactions with coat proteins. *Curr. Opin. Cell Biol.* **9**, 488–495 (1997).
110. Kirchhausen, T. Adaptors for clathrin-mediated traffic. *Annu. Rev. Cell Dev. Biol.* **15**, 705–732 (1999).
111. Le Borgne, R. & Hoflack, B. Mechanisms of protein sorting and coat assembly: insights from the clathrin-coated vesicle pathway. *Curr. Opin. Cell Biol.* **10**, 499–503 (1998).
112. Gan, Y., McGraw, T. E. & Rodriguez-Boulan, E. The epithelial-specific adaptor AP1B mediates post-endocytic recycling to the basolateral membrane. *Nature Cell Biol.* **4**, 605–609 (2002).
113. Phillips, S. A., Barr, V. A., Haft, D. H., Taylor, S. I. & Haft, C. R. Identification and characterization of SNX15, a novel sorting nexin involved in protein trafficking. *J. Biol. Chem.* **276**, 5074–5084 (2001).
114. Barr, V. A., Phillips, S. A., Taylor, S. I. & Haft, C. R. Overexpression of a novel sorting nexin, SNX15, affects endosome morphology and protein trafficking. *Traffic* **1**, 904–916 (2000).
115. Haft, C. R., de la Luz Sierra, M., Barr, V. A., Haft, D. H. & Taylor, S. I. Identification of a family of sorting nexin molecules and characterization of their association with receptors. *Mol. Cell. Biol.* **18**, 7278–7287 (1998).
116. Horazdovsky, B. F. *et al.* A sorting nexin-1 homologue, Vps5p, forms a complex with Vps17p and is required for recycling the vacuolar protein-sorting receptor. *Mol. Biol. Cell* **8**, 1529–1541 (1997).
117. Worby, C. A. & Dixon, J. E. Sorting out the cellular functions of sorting nexins. *Nature Rev. Mol. Cell Biol.* **3**, 919–931 (2002).
118. Dumas, J. J. *et al.* Multivalent endosome targeting by homodimeric EEA1. *Mol. Cell* **8**, 947–958 (2001).
119. Lawe, D. C. *et al.* Sequential roles for phosphatidylinositol 3-phosphate and Rab5 in tethering and fusion of early endosomes via their interaction with EEA1. *J. Biol. Chem.* **277**, 8611–8617 (2002).
120. McBride, H. M. *et al.* Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. *Cell* **98**, 377–386 (1999).
121. Zerial, M. & McBride, H. Rab proteins as membrane organizers. *Nature Rev. Mol. Cell Biol.* **2**, 107–117 (2001).
122. Sonnichsen, B., De, R. S., Nielsen, E., Rietdorf, J. & Zerial, M. Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. *J. Cell Biol.* **149**, 901–914 (2000).
123. Murray, J. T., Panaretou, C., Stenmark, H., Miaczynska, M. & Backer, J. M. Role of Rab5 in the recruitment of hVps34/p150 to the early endosome. *Traffic* **3**, 416–427 (2002).
124. van der Sluijs, P. *et al.* The small GTP-binding protein rab4 controls an early sorting event on the endocytic pathway. *Cell* **70**, 729–740 (1992).

125. Chen, W., Feng, Y., Chen, D. & Wandinger-Ness, A. Rab11 is required for *trans*-golgi network-to-plasma membrane transport and a preferential target for GDP dissociation inhibitor. *Mol. Biol. Cell* **9**, 3241–3257 (1998).
126. Ren, M. *et al.* Hydrolysis of GTP on rab11 is required for the direct delivery of transferrin from the pericentriolar recycling compartment to the cell surface but not from sorting endosomes. *Proc. Natl Acad. Sci. USA* **95**, 6187–6192 (1998).
127. Fiedler, K., Parton, R. G., Kellner, R., Etzold, T. & Simons, K. VIP36, a novel component of glycolipid rafts and exocytic carrier vesicles in epithelial cells. *EMBO J.* **13**, 1729–1740 (1994).
128. Kjoller, L., Simonsen, A. C., Ellgaard, L. & Andreasen, P. A. Differential regulation of urokinase-type-1 inhibitor complex endocytosis by phorbol esters in different cell lines is associated with differential regulation of α_2-macroglobulin receptor and urokinase receptor expression. *Mol. Cell. Endocrinol.* **109**, 209–217 (1995).
129. Araki, N., Johnson, M. T. & Swanson, J. A. A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages. *J. Cell Biol.* **135**, 1249–1260 (1996).
130. Parton, R. G. Caveolae — from ultrastructure to molecular mechanisms. *Nature Rev. Mol. Cell Biol.* **4**, 162–167 (2003).
131. Thomsen, P., Roepstorff, K., Stahlhut, M. & van Deurs, B. Caveolae are highly immobile plasma membrane microdomains, which are not involved in constitutive endocytic trafficking. *Mol. Biol. Cell* **13**, 238–250 (2002).
132. Morrissette, N., Gold, E. & Aderem, A. The macrophage — a cell for all seasons. *Trends Cell Biol.* **9**, 199–201 (1999).
133. Myers, J. N., Tabas, I., Jones, N. L. & Maxfield, F. R. β-very low density lipoprotein is sequestered in surface-connected tubules in mouse peritoneal macrophages. *J. Cell Biol.* **123**, 1389–1402 (1993).
134. Sandvig, K. & van Deurs, B. Membrane traffic exploited by protein toxins. *Annu. Rev. Cell Dev. Biol.* **18**, 1–24 (2002).
135. Griffiths, G., Back, R. & Marsh, M. A quantitative analysis of the endocytic pathway in baby hamster kidney cells. *J. Cell Biol.* **109**, 2703–2720 (1989).
136. Marsh, M., Griffiths, G., Dean, G. E., Mellman, I. & Helenius, A. Three-dimensional structure of endosomes in BHK-21 cells. *Proc. Natl Acad. of Sci. USA* **83**, 2899–2903 (1986).
137. Muller, W. A. Leukocyte–endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunol.* **24**, 327–334 (2003).

Acknowledgement
The work in the authors' laboratories is supported by grants from the National Institutes of Health.

Competing interests statement
The authors declare that they have no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
Interpro: http://www.ebi.ac.uk/interpro/
amphyphysin | dynamin
Swiss-Prot: http://us.expasy.org/sprot/
ARF6 | CI-MPR | β-COP | ε-COP | EEA1 | EHD1 | endophilin | Furin | GGA2 | HRS | PECAM | Rab5 | Rab6a′ | Rab11 | TGN38 | Ubiquitin | VAMP3
Access to this interactive links box is free online.