Sorting in the endosomal system in yeast and animal cells

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The endosomal system is a major membrane-sorting apparatus. New evidence reveals that novel coat proteins assist specific sorting steps and docking factors ensure the vectorial nature of trafficking in the endosomal compartment. There is also good evidence for ubiquitin regulating passage of certain proteins into multivesicular late endosomes, which mature by accumulating invaginated membrane. Lipids play a central role in this involution process, as do the class E vacuolar protein-sorting proteins.

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Current Opinion in Cell Biology 2000, **12**:457–466

0955-0674/00/$ – see front matter

Abbreviations

ALP	alkaline phosphatase
CPS	carboxy peptidase S
CPY	carboxy peptidase Y
DPAP A	dipeptidyl aminopeptidase A
HPS	Hermansky–Pudlak syndrome
LBPA	lysobisphosphatidic acid
MPR	mannose 6-phosphate receptor
MVB	multivesicular body
PM	plasma membrane
PTB	phosphotyrosine-binding domain
RF	RING-H finger
SNX1	sorting nexin 1
TGN	*trans*-Golgi network
Vps	vacuolar protein sorting

Introduction

Transport from the *trans*-Golgi network (TGN) to the lysosome or to the yeast equivalent — the vacuole — involves the delivery of cargo in carrier vesicles to an intermediate sorting compartment, the endosome (Figure 1). The endosomal system, which is comprised of multiple vesicular compartments, also serves as collection site for receptors and other plasma-membrane (PM) proteins internalized from the cell surface. Internalized molecules rapidly enter early sorting endosomes, where they are either segregated into tubular membrane extensions or remain within the central portion. The central portion matures into the multivesicular body (MVB)/late endosome by involution of the limiting membrane (Figure 1). From endosomes, proteins may move to the lysosome/vacuole or recycle back to the TGN or cell surface for further rounds of transport. For many years, scientists have been working to define the pathways and the intermediate compartments through which cargo molecules pass, as well as to identify the coat proteins, membrane components and regulatory factors required for sorting and maturation in the endosomal system. New studies in animal cells and model organisms, particularly yeast, have led to enormous advances in our understanding of these processes and this review highlights some of the recent progress.

Trafficking from the biosynthetic pathway: more coats and adaptors

In animal cells, the major pathway for diverting lysosomal enzymes from the flow of proteins to the cell surface involves sorting at the TGN via mannose 6-phosphate receptors (MPRs) [1]. MPR exit from the TGN takes place within clathrin-coated buds containing the AP-1 adaptor, which is recruited by the small GTPase ARF1. AP-1 interacts directly with sorting signals in the MPR cytosolic tail. Although this is a major route for TGN exit of many lysosome-destined proteins in animal cells, additional adaptors and coats that may mediate sorting at the TGN have now been discovered.

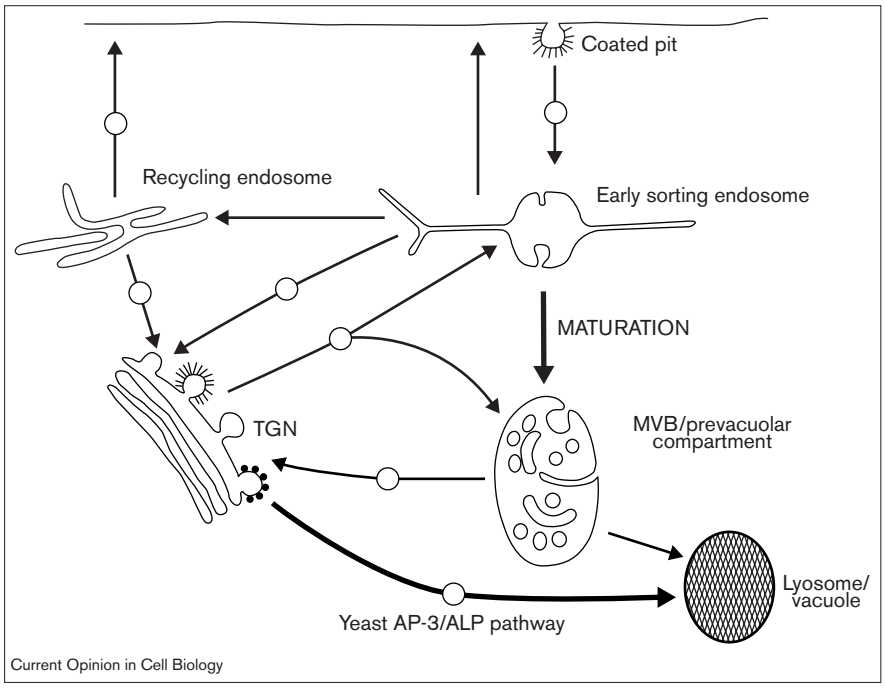
Novel GGA proteins at the TGN

In the yeast *Saccharomyces cerevisiae*, the major pathway to the vacuole is that followed by several soluble vacuolar hydrolases (e.g. carboxypeptidase Y [CPY]) and membrane proteins (e.g. the vacuolar ATPase and carboxypeptidase S [CPS]) [2]. ProCPY transport to the vacuole is mediated by the Vps10p sorting receptor (Table 1), which collects cargo from the TGN for delivery to the prevacuole/endosome, before recycling back to the TGN. In addition, some resident late Golgi membrane proteins (e.g. Kex2p and dipeptidyl aminopeptidase A [DPAP A]) cycle between the TGN and the prevacuole. Clathrin is important for retention of these latter TGN proteins, demonstrated by their appearance at the cell surface in clathrin mutants. However, clathrin and AP-1 are not essential for sorting of CPY via Vps10p (e.g. see [3•,4•]), so another pathway or sorting factor(s) is proposed to be upregulated or substitute for these coats. New candidates are the GGA (Golgi-localizing, gamma-adaptin ear homology domain, ARF-binding) proteins [5•–8•]. In animal cells, GGA proteins are not enriched in clathrin-coated vesicles, but do behave as ARF-dependent coat proteins, localize to the TGN, and cause mislocalization of TGN proteins upon overexpression [5•–8•]. In yeast, *gga1 gga2* knockouts partially missort CPY, and their fragmented vacuolar morphology is reminiscent of a class of Vps mutants (class F) that affect sorting at the TGN [6•,8•].

Clathrin function without APs in yeast

It is unlikely that in yeast AP-1 can be functionally compensated for by one of the other two yeast AP-like complexes (related to AP-2 and AP-3) or AP180-related proteins, which associate with clathrin-coated vesicles in animal cells and bind clathrin in yeast. Yeast with multiple gene deletions removing all of these APs are able to sort CPY efficiently to the vacuole [3•,4•]. In fact, although clathrin mutants have a number of severe sorting and growth defects, multiple AP-mutant strains are essentially normal

Figure 1



Schematic representation of the endocytic system (in yeast and non-polarized animal cells). The major trafficking routes through the endocytic system are indicated by the arrows, with spheres indicating transport steps involving known or surmised vesicular intermediates. The bifurcation in traffic flow from the TGN to both the early sorting endosome and to the MVB reflects the maturation process that underlies MVB formation. Initially, multiple fusion events involving vesicles from both the cell surface and the TGN deliver material to a newly forming early sorting endosome. Recycling components (e.g. transferrin receptors) are sorted into the tubular extensions of the structure, which contain the bulk of the membrane of the early sorting endosome, en route to the tubular recycling endosome or to the cell surface for direct recycling. Over time, the sorting endosome loses the capacity to fuse with PM-derived vesicles, but not with vesicles from the TGN. The radiating tubules are replaced by extensive internal membrane invaginations as the maturing endosome moves toward the center of the cell along microtubules. The available data indicate that in yeast, CPY (not shown) is delivered to a more mature prevacuolar compartment, whereas in animal cells, MPRs may preferentially enter the endocytic pathway at the early sorting endosome. Nevertheless, recent evidence suggests there is a pathway from the TGN to an early endosome in yeast as well and that recycling to the TGN may take place from either the early endosome or prevacuole, similar to animal cells. In yeast, current information supports the direct delivery of ALP to the vacuole in an AP-3-dependent process, although there is presently no evidence for a similar direct route to the lysosome in animal cells.

for all clathrin-related functions. In addition, they still form clathrin-coated vesicles. This surprising finding suggests that heterotetrameric adaptors and AP180-related proteins are not required for clathrin-mediated transport in yeast.

AP-3 sorting

Two other heterotetrameric adaptors, AP-3 and AP-4, associate with the TGN in an ARF-dependent fashion. Although little is known about the function of AP-4 [9,10], AP-3 is known to be important in lysosomal trafficking. This was illuminated by studies of mutants in yeast, as well as animals manifesting pigmentation defects [11,12]. Each AP-3 subunit is represented in the *Drosophila* 'granule group' of eye-color mutations, which affect the delivery of proteins to lysosomes and pigment granules (see Table 1) [12–14]. *mocha* and *pearl* mice have mutations in their AP-3 δ and β3A (a non-neuronal β3 isoform) subunits, respectively [15,16] and are among several mutations that cause coat- and eye-color dilution and platelet storage pool deficiency. These mice are also models for Hermansky–Pudlak syndrome (HPS), a related lysosomal disorder in humans affecting the lysosome-related organelles, melanosomes and platelet dense granules. Recently two HPS patients with mutations in β3A were identified [17••].

AP-3 is also involved in the sorting of lysosome-associated membrane proteins (e.g. LAMP-1 and LIMP-II). AP-3 deficient fibroblasts show increased surface expression of LAMPs but not of MPR [18,19]. However, the location at which AP-3 sorts these proteins is still not completely resolved. There is evidence for TGN and endosomal distributions of AP-3 and for both TGN to endosome or endosome to lysosome sorting by this adaptor (e.g. see [17••,18,20]).

In yeast, AP-3 directs the vacuolar membrane proteins alkaline phosphatase (ALP) and Vam3p, a vacuolar t-SNARE, to the vacuole via an alternative pathway from the well studied CPY pathway [11]. In animal cells, AP-3 binds to clathrin and may have both clathrin-dependent and -independent sorting functions [19,21], whereas in yeast, sorting via the AP-3 pathway is clathrin independent [22]. Clathrin-independent AP-3 sorting could still require an outer coat component to generate a vesicular intermediate. One candidate is Vps41p, which binds directly to Apl5p, the yeast AP-3 δ subunit [23••]. Also, formation of AP-3 vesicles is blocked at the TGN in *vps41-ts* mutants [23••]. Vps41p might recruit the additional factors needed for budding from the TGN, but a coat-like function is appealing as Vps41p has a clathrin heavy chain homology region that may be involved in oligomerization. However, compared to AP-3 mutants, a *vps41-Δ* mutation causes pleiotropic phenotypes including defective sorting of

Table 1

Proteins involved in sorting to the yeast vacuole.

Yeast name*	Animal counterparts/related-proteins†	Function/features/reference
Apl5p	AP-3 δ, Dm Garnet, Mm Mocha	AP-3 complex subunit [11,12,15,23••]
Apl6p	AP-3 β, Dm Ruby, Mm Pearl, Hs β3A	AP-3 complex subunit [11,14,16,17••]
Apm3p	AP-3 μ, Dm Carmine	AP-3 complex subunit [11,13]
Aps3p	AP-3 σ, Dm Orange	AP-3 complex subunit [11,14]
Fab1p	Mm p235/PIKfyve, type III PIP 5-kinase	PtdIns(3)P 5-kinase, FYVE domain [26,28,43••,44,45]
Gga1p/Gga2p	Hs GGA1, 2, 3	VHS and γ-adaptin ear homology domains, ARF binding, TGN sorting (?) [5•,6•,7•,8•]
Grd19p	Hs SNX3	Retrograde transport from prevacuole [65]
Mvp1p	SNX-related	Vesicle budding at TGN [64]
Pep12p	Syntaxin-related	Prevacuole t-SNARE (Class D *vps*) [2,26,28,31••,36,39]
Tlg1p	Syntaxin-related	Golgi t-SNARE, retrograde transport via early endosome [31••,39]
Tlg2p	Syntaxin-related	Early endosome retrograde t-SNARE, cytosol to vacuole transport [31••,39]
Vac1p/Pep7p	Hs EEA1	Prevacuole/endosomal FYVE tethering component (class D *vps*) [2,26–28,35]
Vam3p	Syntaxin-related	Vacuolar t-SNARE [2,11,23,39]
Vps4p	Mm SKD1, Hs VPS4	AAA ATPase, endosomal maturation/exit (class E *vps*) [2,49,50••,51••]
Vps5p	Hs SNX1, SNX2	Retromer subunit, retrograde vesicle formation from prevacuole [2,60,62,63,66]
Vps10p	Sortilin-related	Vacuolar sorting receptor [2,84,87,91]
Vps17p		Retromer subunit, retrograde vesicle formation from prevacuole [2,60]
Vps21p/Ypt51p	Rab5	Prevacuole or early endosomal Rab (class D *vps*) [2,26-28,29•,30•]
Vps23p/Stp22p	Mm/Hs TSG101	Endosomal maturation/exit (class E *vps*), UBC-related [83••,84]
Vps24p		Endosomal maturation/exit (class E *vps*) [49]
Vps26p	Hs Vps26, Mm Vps26	Retromer subunit, retrograde vesicle formation from prevacuole [60,66]
Vps29p	Hs Vps29	Retromer subunit, retrograde vesicle formation from prevacuole [2,60,66]
Vps32p		Endosomal maturation/exit (class E *vps*) [49]
Vps34p	Hs Vps34p, class III PI 3-kinase	PtdIns 3-kinase (class D *vps*) [2,26,28,42•]
Vps35p	Hs Vps35, Mm Mem3	Retromer subunit, retrograde vesicle formation from prevacuole [2,60,61•,66]
Vps39p/Vam6p		Binds Vps41p, localizes to vacuole membrane region [2,25]
Vps41p/Vam2p	Dm light	Binds Apl5p and Vps39p. AP-3 pathway sorting, other? [2,23••,24,25]
Vps45p	Hs Vps45	Sec1-related, multiple endosomal steps (class D *vps*) [2,26–28,31••,34]
Vps52p		Retrograde transport from prevacuole, docking at the TGN [67••]
Vps53p		Retrograde transport from prevacuole, docking at the TGN [67••]
Vps54p		Retrograde transport from prevacuole, docking at the TGN [67••]

*Many of the genes encoding these proteins have multiple names, but for simplification we have indicated the more widely used terms. We refer you to the Proteome Database (http://www.proteome.com) for further information, including alternative names, functional details, and other identifications. †Known or surmised functional counterparts of the yeast protein, or information on the related protein family in animals is listed. Much of this information can be accessed through the Proteome Database. Note that there are many sequences encoding proteins related to these yeast products in the EST and other databases. Species designations are: Dm, *Drosophila*; Mm, Murine; Hs, Human. Other pertinent references can be found in the Proteome Database.

both ALP and CPY pathway cargo and fragmented vacuoles [24,25]. Furthermore, Vps41p (also called Vam2p) complexes with Vam6p/Vps39p, which localizes in structures associated with the vacuole [25]. Therefore Vps41p may be a more general coat protein that functions in several endosomal-sorting pathways, whereas AP-3 is more specialized for sorting select cargo that follow the ALP pathway.

Delivery to the endosome

In the past few years, many details of vesicle docking/fusion with the endosome have been elucidated,

and questions about specificity of delivery and convergence of TGN and endocytic traffic are beginning to be addressed. In yeast, delivery of TGN vesicles to the prevacuole has been defined primarily by the class D Vps proteins, which includes Ypt51p/Vps21p Rab-like GTPase, Vac1p/Pep7p, a FYVE-domain protein that binds phosphatidylinositol 3-phosphate (PtdIns(3)P), Vps45p, an endosomal Sec1p homologue, and the t-SNARE Pep12p [2,26–28]. These yeast proteins are analogous to components of the well studied animal cell Rab5 GTPase/EEA1-FYVE protein effector complex [26–28]. This animal complex is required for fusion at the early endosome, where both TGN and endocytic traffic can converge and recycle. In contrast, the prevacuole is generally thought to be a later endosome, yet the convergence point for traffic from the cell surface (e.g. see [29•,30•]). However, recent evidence indicates that endocytic and TGN traffic can meet in an early endosome in yeast as well [31••], similar to its mammalian counterparts [32,22].

Whether docking/fusion factors are unique or shared for PM- and TGN-derived vesicles converging on the endosome is still being worked out. In yeast, Vps45p does not seem to be required for fusion of endocytic intermediates with the prevacuole [34], whereas Vac1p, Vps21p, and Pep12p are required for transport from both pathways [30•,35,36]. Similarly, in animal cells, Rab5/EEA1 may function with syntaxin 13 in endocytic early endosome fusion [37] and may act with syntaxin 6 in TGN to early endosome transport [38]. The role of specific v-SNAREs and t-SNAREs in these events is complicated by their ability to function in multiple transport steps [39]. SNAREs must also be recycled after fusion in order to be reused, so localization may not be a reliable indicator of function. Therefore, further dissection of the functions of the docking/fusion components will be crucial for understanding how directionality of membrane transport in the endosomal system is achieved.

Biogenesis of and sorting within multivesicular bodies

Morphologically identifiable MVBs are present in both yeast and animals and contain substantial amounts of internal membrane. This compartment is downstream of the sorting endosome and carries material destined for the lysosome/vacuole. Certain proteins (MPRs, Vps10p, furin) recycle to the TGN from MVBs rather than early endosomes, indicating that sorting is still ongoing as the MVB matures. Recent advances have been the appreciation of the role of lipids in maintaining the morphology and function of the MVB and the identification of the class E Vps proteins as central regulators of MVB biogenesis.

The role of lipids

Long saturated acyl-chain-containing synthetic lipids traffic preferentially to the late endocytic compartment when added to mammalian cells in culture, whereas short-chain unsaturated lipids are sorted to the recycling endosome and returned to the cell surface [40•]. Different combinations of lipids are thus likely to be found in the MVB and early-endocytic compartment membranes. In addition, polyphosphoinositides are certainly involved in MVB function. In yeast, Vps34p, a PtdIns 3-kinase, is required for proper sorting to the vacuole and wortmannin, an inositide kinase inhibitor, perturbs trafficking to the lysosome in mammalian cells [28,41•]. Curiously, turnover of PtdIns(3)P requires vacuolar hydrolases [42•], so it has been proposed that this lipid plays an important role in the formation of intralumenal vesicles discharged into the vacuolar lumen on fusion of the prevacuolar compartment (MVB) with the vacuole [42•]. Fab1p, a PtdIns(3)P 5-kinase is also involved [28,43••]. This enzyme might consume PtdIns(3)P generated by Vps34p, thereby producing a spatially/temporally-distinct second messenger [28]. *fab1-ts* mutants show rapid and massive expansion of the vacuole at the restrictive temperature. The expanded vacuole is poorly acidified and the transmembrane segment of CPS, which is usually degraded within the vacuole lumen, remains associated with the limiting vacuolar membrane [43••]. Intravacuolar vesicles are also absent in *fab1* mutants that are unable to synthesize PtdIns(3,5)P_2, so this lipid also seems important in the process of involution in the MVB [28]. Homologues of Fab1p, which complement a *fab1* strain, have recently been identified in many organisms [44,45], so we anticipate rapid clarification of the precise role of this lipid kinase in sorting.

Lysobisphosphatidic acid (LBPA), a relatively rare phospholipid, is enriched in mammalian MVBs [46]. Ingested anti-LBPA antibodies accumulate within MVBs, altering both the morphology and function of the organelle [46,47••]. MPRs and unesterified cholesterol become entrapped within these aberrant, LAMP-positive endosomes. The flow of LDL-derived unesterified cholesterol from the late endosome/lysosome is regulated by NPC1, a large polytopic transmembrane protein with a sterol-sensing domain [48•] that is mutated in the majority of patients with Niemann-Pick type-C (NPC) disease. In NPC1-mutant fibroblasts, LBPA abnormally codistributes with accumulated cholesterol in MVBs positive for both MPRs and LAMPs [47••]. Thus, abnormalities in the lipid composition of the MVB prevent certain recycling proteins from segregating away from lysosome-destined material, demonstrating that lipid composition, membrane involution and protein sorting in the late endosome are tightly coupled.

Protein regulators

Mutation of any of the 13 class E Vps proteins in yeast causes accumulation of CPY, Vps10p, endocytosed proteins, and late-Golgi proteins (such as Kex2p and DPAP A, which usually recycle back from the prevacuole) within aberrant perivacuolar endosomes. The overall phenotype is consistent with a generalized failure in late endosomal sorting, traffic stalling in an acidified compartment of stacked tubular structures — the E compartment [2,43••]. Mammalian homologues of a few

class E proteins are now also being functionally characterized (Table 1); the results confirm that class E proteins are central regulators of late endosome function and MVB involution.

One class E regulatory protein, Vps4p, is a member of the AAA ATPase family. It cycles onto membranes in an ATP-dependent manner [49]. An ATPase-defective form of Vps4p collects on the class E compartment and is associated with a large, detergent-insoluble structure [49]. Vps4p might disassemble a class E–protein complex required for invagination of MVB membranes, much like N-ethylmaleimide-sensitive factor disassembles SNARE complexes. Evidence for this comes from the demonstration that two other class E proteins (Vps24p and Vps32p), which are also found in higher organisms, accumulate at the same sites in *vps4* ATPase mutants [49]. Studies on mouse (SKD1) and human (hVPS4) Vps4p homologues support this idea. An ATP-restricted point mutant causes severe endosomal abnormalities and sorting defects in transfected cells [50••,51••]. Dilated vacuoles positive for LAMPs, MPRs, unesterified cholesterol, transferrin and EEA1 develop, which, by EM, appear as a profusion of tangled tubular/vesicular structures closely opposed, and occasionally connected, to a large central electron-lucent vacuole [50••,51••]. The phenotype induced by the mutant Vps4p homologues is strikingly similar to that induced by wortmannin [41•], arguing that the E compartment might be equivalent to the dilated vacuoles that arise in wortmannin-treated mammalian cells. Thus, it is probable that Vps4p functions along the pathway that requires PtdIns(3)P formation.

Retrograde traffic to the TGN and the recycling machinery

Although the pathways followed by membrane proteins returning to the TGN from endosomes are becoming clearer, the machinery involved is only beginning to emerge. In both yeast and animals, proteins can return to the Golgi from early or late endosomes. Shiga toxin B fragment or a chimeric protein containing the cytoplasmic tail of the TGN protein, TGN38, are transported directly via early/recycling endosomes to the TGN [52,53], whereas furin and MPRs are transported back to the Golgi from the late endosome [32,54•]. This distinction is not merely due to the latter proteins being delivered directly to the late endosome from the TGN. Furin and MPRs can return to the TGN from the cell surface following a pathway that takes them through early sorting and then late endosomes [54•]. Also, the bulk of newly synthesized MPRs enter the endosomal system at the sorting endosome, where transferrin receptors are being segregated for return to the cell surface (see update). MPRs also require Rab7 to progress from early to late endosomes prior to recycling the TGN [32,33]. In yeast, the secretory v-SNARE Snc1p recycles to the TGN from the early endosome after internalization, whereas Vps10p returns via the late endosome/prevacuole [2,31••].

Information on the machinery that mediates retrieval from the endosome to the Golgi in animal cells is still relatively limited. Retrograde transport from the late endosome appears to be vesicle mediated and requires the small GTPase Rab9 [55]. Dynamin associates with late endosomes and seems to play a role in recycling of MPR to the TGN [56]. Microtubules and microtubule-associated proteins improve the efficiency of MPR return [57]. In a few cases, cargo-selective components have been identified, such as TIP47 for retrieval of MPR [58] and PACS-1, which interacts with the furin cytosolic tail [59].

Clues to other components involved in endosomal retrieval have emerged from investigation of *vps* and Golgi retention defective (*grd*) mutants in yeast. Some of these mutants secrete proCPY and display rapid mislocalization of Vps10p, Kex2p and A-ALP, a chimeric model TGN protein, to the vacuole [2]. This retrieval phenotype is distinct from that of many other *vps* mutants, where transport is blocked at an intermediate step along the forward pathway to the vacuole. A set of these retrieval mutants define the components of a complex that has properties of a vesicle coat, termed the retromer [60]. The retromer assembles from two subcomplexes: one includes Vps35p, Vps29p and Vps26p/Pep8p, and a second includes Vps5p and Vps17p. The Vps35p component may play a direct role in cargo selection, as cargo-specific alleles of *VPS35* that affect retrieval of Vps10p but not A-ALP (and vice versa) have been isolated [61•]. Vps5p localizes to the budding rims of the prevacuole and it has self-assembly properties, suggesting that it may play a more general structural role in vesicle formation [60].

Interestingly, Vps5p is related to mammalian sorting nexins 1 and 2 (SNX1, SNX2) and other proteins with a phox homology (PX) domain originally identified in the 40 and 47 kDa subunits of NADPH oxidase [62,63]. In yeast, this includes Mvp1p, which may function in vesicle budding at the TGN [64] and Grd19p [65], which is related to SNX3 [63]. Grd19p has also been implicated in endosomal retrieval of DPAP A and Kex2p, but not Vps10p, via direct association with DPAP A/Kex2p sorting signals [65]. SNX1 and SNX2 bind to the cytosolic domains of a number of receptor tyrosine kinases (RTKs) and other internalized receptors and localize to perinuclear vesicles [62,63]. Recently, human orthologs of Vps26p, Vps29p and Vps35p were characterized and shown to associate with SNX1/2 in a multimeric complex [66]. Thus, although we still do not understand the exact function of the SNX1/2 retromer-like complex, it is likely to play a role in endosomal trafficking.

Three new *vps* mutants — *vps52*, *vps53* and *vps54* — have similar sorting phenotypes to those described for retromer mutants [67••]. However, these proteins form a peripheral membrane complex that localizes to the TGN, rather than the prevacuole, suggesting that this complex may be involved in docking or fusion of retrograde vesicles [67••]. Retrograde transport from the early endosome involves

Vps45p and the t-SNAREs Tlg1p and Tlg2p [31••], which may be required for docking of return vesicles at the TGN. However, class E Vps proteins or retromer subunits, which are required for retrieval from the prevacuolar endosome, are not required for retrieval from the early endosome. So far it is not known whether Vps45p, Tlg1p and Tlg2p are also required for retrograde transport from the late endosome/prevacuole, or whether Vps52p, Vps53p and Vps54p are used in early endosome retrieval. Now that cargo proteins are available to follow both retrieval pathways in yeast [31••] and animal Vps52, Vps53, Vps54 homologues can be examined [67••] it should be possible to address these questions.

Regulated sorting of proteins in the endosomal system: role of ubiquitin

In response to alterations in nutrient status or ligand binding, PM proteins, such as signal-transducing receptors and small-molecule permeases, can be rapidly removed or downregulated by endocytosis and degraded in the lysosome/vacuole. This process is an important mechanism for cellular regulation, as exemplified by the loss of cell growth control when growth factor receptors cannot be internalized. Ubiquitination is a key mechanism for targeting surface proteins for internalization and downregulation [68] and new evidence suggests that this may also regulate sorting at the endosome and TGN (see also update).

Ligand-stimulated RTKs, such as the EGF receptor (EGFR/ErbB-1), are internalized and sorted into internal compartments of MVBs for transport to the lysosome, whereas unstimulated receptors are more efficiently recycled to the cell surface. A factor regulating this is the tyrosine kinase adaptor Cbl, which was originally identified as a viral oncogene in mice [69]. Overexpression of c-Cbl increases ligand-induced ubiquitination and downregulation of EGFR and a number of other RTKs [70••,71–73,74•]. Cbl does not accelerate internalization of EGFR but may function at the endosome to facilitate sorting into MVBs, thereby attenuating kinase signaling [70••]. Still, there may be other factors that determine sorting of EGFR into MVBs, as a dileucine signal in the cytosolic portion of EGFR seems to be required for diversion of the receptor from the recycling pathway [75].

c-Cbl contains an amino-terminal phosphotyrosine-binding (PTB) domain and a central C_3HC_4 RING-H finger (RF) domain [69]. The amino-terminal PTB is required for receptor binding. The RF domain stimulates receptor ubiquitination [73,76,77••,78,79••,80], similar to other RF proteins that serve as E3 ligases for E2 ubiquitin-conjugating enzymes [81]. A question that remains is whether the ubiquitinated receptors are degraded in lysosomes or by the proteasome. Studies using lysosomal protease and proteasome inhibitors have implicated both pathways in degradation (e.g. [74•,79••]). However, inhibition of proteasomal degradation of ubiquitinated proteins can deplete intracellular ubiquitin pools [82•], which might lead to reduced ubiquitination of the target receptor and resistance to downregulation.

Another protein that may regulate sorting into MBVs is the class E Vps protein, Vps23p (or Stp22p), which is related to TSG101, the tumor susceptibility gene product in animals [83••,84]. In *tsg101* mutant cells, much of the cellular pool of MPR spills out to the cell surface, and processing of the lysosomal enzyme cathepsin D is impaired. Although internalization and recycling of transferrin receptors is normal, downregulation of activated EGFR is markedly attenuated because in the *tsg101* mutant the receptor is efficiently recycled, along with MPRs, back to the surface [83••]. Mutant Vps23p also permits recycling of damaged Ste2p to the cell surface in yeast (see below) [84]. Interestingly, TSG101 and Vps23p contain a domain related to E2 ubiquitin-conjugating enzymes [83••,84]. These are unlikely to catalyze ubiquitination because they lack the active-site cysteine, but perhaps these proteins are involved in ubiquitin recognition or modification. TSG101/Vps23p could bind to ubiquitinated membrane proteins to direct them into forming multivesicular late endosomes, or they might act as E3 accessory proteins regulating ubiquitination of cargo. Alternatively, the sorting machinery itself could be regulated by ubiquitination (see also update).

Ubiquitin modification may also be involved in regulated sorting of amino-acid permeases from the TGN in yeast. The tryptophan permease, Tat2p, is expressed at the surface in nitrogen-rich conditions, but it is diverted from the Golgi to the vacuole during nitrogen starvation [85••]. Inverse regulation is observed for the general amino-acid permease, Gap1p [86]. In both cases, there is a large pool of internal permease that is turned over, along with surface permease, under downregulating conditions. Mutation of the sites of Tat2p ubiquitin modification stabilizes both internal and surface Tat2p, suggesting that ubiquitination is required for diverting intracellular Tat2p directly to the vacuole [85••]. A similar mechanism for re-routing of Gap1p from the Golgi to the vacuole in nitrogen-rich conditions is likely, although in both cases, the location of internal ubiquitination and the ubiquitination factors responsible are not known.

Another type of regulated sorting that can take place in the TGN/endosomal system is a quality control system that mediates turnover of misfolded soluble or membrane proteins [87–89]. In yeast, certain misfolded Ste2p and plasma-membrane ATPase (Pma1p) mutants escape ER quality control, but are directed to the vacuole for degradation at the level of the TGN without reaching the cell surface [88,89]. The phenotypic consequences of loss of localization at the cell surface can be suppressed by a number of *vps* mutants, including *vps23* (TSG101) [84,90]. How these proteins are recognized as misfolded in the first place is not entirely clear. Vps10p, which has distinct domains for recognition of vacuolar hydrolases and unfolded ligands, may function in quality control [84,87,91]. Also,

misfolded Ste2p accumulates in higher mw species, suggesting a role for post-translational modification, possibly ubiquitination, in this process [89].

Conclusions

Membrane flow through the endosomal compartment is both enormously complex and precisely regulated. The past few years have clarified some of the many different sorting itineraries, and important new tools, including mutants and chimeric and tagged proteins, are available for following the precise trajectory of cargo proteins. Until recently, the factors required for MVB involution and sorting were largely unknown. The finding that yeast class E *vps* mutations affect late endosome maturation, and the realization that lipids and possibly ubiquitination play a role in sorting at the endosome, will allow us to characterize this process in detail. There are still many questions to address regarding vesicle formation and docking in the endosomal system. In a few cases, cargo-selective components, such as the retromer and AP-3, are known, and a number of factors involved in fusion with the endosome or TGN have been identified. However, what other factors are involved in regulating recruitment of coats for budding or attachment of the vesicle to the target membrane? Also, many components seem to be used at multiple transport steps, so a major challenge is to identify the specialized factors that provide directionality to sorting within these compartments. The high degree of conservation though metazoan evolution will continue to facilitate studies in this field and enable us to advance to the next step of deciphering the mechanistic details of sorting in the endosomal system.

Update

A recent study, following transferrin uptake in cells stably transfected with GFP-tagged Rab proteins, reveals that early endosomes contain morphologically discernible subdomains, demarcated by discrete Rab proteins [92••]. As transferrin recycles along the endocytic pathway, it overlaps sequentially with Rab5, then Rab4 and then Rab11. These GTPases can be found on continuous membrane structures (a single endosome) but do not appear to mix significantly.

A novel F-box protein, Rcy1p, is required for transport out of the early endosome in yeast [93•]. While internalization is normal, mutant Rcy1p leads to accumulation of endocytic markers in an early endosomal compartment, preventing both recycling to the cell surface and transport on to the prevacuole. Interestingly, several members of the F-box family of proteins mediate ubiquitination of substrates as components of SKP1/cullin/F-box ubiquitin ligase complexes. Therefore, Rcy1p may function in a ubiquitination pathway that regulates transport in the endosomal system.

Acknowledgements

We would like to thank our many colleagues for willingly sending us manuscripts prior to publication and apologize to those whose work or references have not been cited due to space limitations. We also thank Linda Hicke for helpful discussions and Stuart Kornfeld for critical comments on our manuscript. Our research is supported by National Institutes of Health Grants GM55796 (SKL) and DK53249 (LMT).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Traub LM, Kornfeld S: **The trans-Golgi network: a late secretory sorting station.** *Curr Opin Cell Biol* 1997, **9**:527-533.

2. Conibear E, Stevens TH: **Multiple sorting pathways between the late Golgi and the vacuole in yeast.** *Biochim Biophys Acta* 1998, **1404**:211-230.

3. • Huang KM, D'Hondt K, Riezman H, Lemmon SK: **Clathrin functions in the absence of heterotetrameric adaptors and AP180-related proteins in yeast.** *EMBO J* 1999, **18**:3897-3908.
Yeast strains carrying multiple gene deletions eliminating all AP complexes and/or AP180 homologues have normal clathrin function. Further, clathrin-coated vesicles can form in the absence of adaptors and AP180 proteins. This paper and [4•] question the long-held view that APs are required for clathrin recruitment during vesicle budding.

4. • Yeung BG, Phan HL, Payne GS: **Adaptor complex-independent clathrin function in yeast.** *Mol Biol Cell* 1999, **10**:3643-3659.
Multiple AP-β chain disruption strains are not impaired in clathrin function or coated vesicle formation, indicating that AP-complexes are not required for clathrin recruitment during vesicle budding.

5. • Boman AL, Zhang C-J, Zhu A, Kahn RA: **A family of ARF effectors that can alter membrane transport through the trans-Golgi.** *Mol Biol Cell* 2000, **11**:1241-1255.
GGA1, 2, 3 (Golgi-localizing, Gamma-adaptin ear homology domain, ARF-binding proteins) were isolated through their interaction with the GTP-bound conformation of ARF3. GGA association with the TGN is brefeldin A sensitive, and overexpression of GGA causes redistribution of MPR and TGN38, suggesting a role in sorting at the TGN.

6. • Hirst J, Lui W, Bright N, Totty N, Seaman M, Robinson M: **A family of proteins with γ-adaptin and VHS domains that facilitate trafficking between the TGN and the vacuole/lysosome.** *J Cell Biol* 2000, **149**:67-79.
Human GGA1–3 are identified by their homology to the γ-adaptin ear domain and found to localize to the TGN. Double deletion mutations in the yeast homologues, *GGA1* and *GGA2*, cause missorting of CPY and a fragmented vacuolar morphology.

7. • Poussu A, Lohi O, Lehto VP: **Vear, a novel Golgi-associated protein with VHS and gamma-adaptin 'Ear' Domains.** *J Biol Chem* 2000, **275**:7176-7183.
Vear (GGA-2) is characterized as a ubiquitous Golgi-associated protein with an amino-terminal VHS domain and a carboxy-terminal γ-adaptin ear domain. Vear dissociates from the TGN in the presence of brefeldin A. The central portion of the protein (encompassing the GAT region) facilitates the specific association with the Golgi.

8. • Dell'Angelica EC, Puertollano R, Mullins C, Aguilar RC, Vargas JD, Hartnell LM, Bonifacino JS: **GGAs: a family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex.** *J Cell Biol* 2000, **149**:81-93.
GGA2 and 3 are characterized and shown to localize to coated regions of the TGN, as well as to peripheral structures. Membrane association is brefeldin sensitive, and requires the central GAT region, which binds to GTP-activated ARF. Expression of the GAT domain causes AP-1, AP-3 and COPI redistribution into the cytosol, validating the role of ARF in the recruitment of these coat proteins. In yeast, a *gga1 gga2* double-deletion strain secretes proCPY.

9. Hirst J, Bright NA, Rous B, Robinson MS: **Characterization of a fourth adaptor-related protein complex.** *Mol Biol Cell* 1999, **10**:2787-802.

10. Dell'Angelica EC, Mullins C, Bonifacino JS: **AP-4, a novel protein complex related to clathrin adaptors.** *J Biol Chem* 1999, **274**:7278-7285.

11. Odorizzi G, Cowles CR, Emr SD: **The AP-3 complex: a coat of many colours.** *Trends Cell Biol* 1998, **8**:282-288.

12. Lloyd V, Ramaswami M, Kramer H: **Not just pretty eyes: *Drosophila* eye-colour mutations and lysosomal delivery.** *Trends Cell Biol* 1998, **8**:257-259.

13. Mullins C, Hartnell LM, Wassarman DA, Bonifacino JS: **Defective expression of the mu3 subunit of the AP-3 adaptor complex in**

the *Drosophila* pigmentation mutant *carmine*. *Mol Gen Genet* 1999, **262**:401-412.

14. Mullins C, Hartnell LM, Wassarman DA, Bonifacino J: **Mutations in subunits of the AP-3 adaptor complex result in defective pigment granule biogenesis in *Drosophila melanogaster*.** *Mol Biol Cell* 1999, **10**:223a.

15. Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, Carskadon SL, Kapfhamer D, Sufalko D, Robinson MS, Noebels JL, Burmeister M: **Mutation in AP-3 delta in the *mocha* mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles.** *Neuron* 1998, **21**:111-122.

16. Feng L, Seymour AB, Jiang S, To A, Peden AA, Novak EK, Zhen L, Rusiniak ME, Eicher EM, Robinson MS *et al.*: **The beta3A subunit gene (AP3β1) of the AP-3 adaptor complex is altered in the mouse hypopigmentation mutant *pearl*, a model for Hermansky–Pudlak syndrome and night blindness.** *Hum Mol Genet* 1999, **8**:323-330.

17. •• Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS: **Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor.** *Mol Cell* 1999, **3**:11-21.

This paper provides the first evidence for human mutations in the AP-3 complex in patients with HPS. Patients' fibroblasts display increased surface expression of lysosomal membrane proteins but not MPR.

18. Le Borgne R, Alconada A, Bauer U, Hoflack B: **The mammalian AP-3 adaptor-like complex mediates the intracellular transport of lysosomal membrane glycoproteins.** *J Biol Chem* 1998, **273**:29451-29461.

19. Dell'Angelica EC, Klumperman J, Stoorvogel W, Bonifacino JS: **Association of the AP-3 adaptor complex with clathrin.** *Science* 1998, **280**:431-434.

20. Gough NR, Zweifel ME, Martinez-Augustin O, Aguilar RC, Bonifacino JS, Fambrough DM: **Utilization of the indirect lysosome targeting pathway by lysosome-associated membrane proteins (LAMPs) is influenced largely by the C-terminal residue of their GYXXphi targeting signals.** *J Cell Sci* 1999, **112**:4257-4269.

21. Faundez V, Horng JT, Kelly RB: **A function for the AP-3 coat complex in synaptic vesicle formation from endosomes.** *Cell* 1998, **93**:423-432.

22. Vowels JJ, Payne GS: **A dileucine-like sorting signal directs transport into an AP-3-dependent, clathrin-independent pathway to the yeast vacuole.** *EMBO J* 1998, **17**:2482-2493.

23. •• Rehling P, Darsow T, Katzmann DJ, Emr SD: **Formation of AP-3 transport intermediates requires Vps41 function.** *Nat Cell Biol* 1999, **1**:346-353.

A *vam3-ts* mutant, which blocks fusion with the vacuole, was used to isolate AP-3 vesicle intermediates of 50-130 nm that are enriched in AP-3 pathway cargo. Formation of the vesicles is blocked in *vps41-ts* mutants and Vps41p binds AP-3 δ-adaptin, suggesting that Vps41p functions as a coat or effector in formation of the AP-3 vesicle intermediates.

24. Radisky D, Snyder WB, Emr SD, Kaplan J: **Characterization of *VPS41*, a gene required for vacuolar trafficking and high-affinity iron transport in yeast.** *Proc Natl Acad Sci USA* 1997, **94**:5662-5666.

25. Nakamura N, Hirata A, Ohsumi Y, Wada Y: **Vam2/Vps41p and Vam6/Vps39p are components of a protein complex on the vacuolar membranes and involved in the vacuolar assembly in the yeast *Saccharomyces cerevisiae*.** *J Biol Chem* 1997, **272**:11344-11349.

26. Corvera S, D'Arrigo A, Stenmark H: **Phosphoinositides in membrane traffic.** *Curr Opin Cell Biol* 1999, **11**:460-465.

27. Waters MG, Pfeffer SR: **Membrane tethering in intracellular transport.** *Curr Opin Cell Biol* 1999, **11**:453-459.

28. Wurmser AE, Gary JD, Emr SD: **Phosphoinositide 3-kinases and their FYVE domain-containing effectors as regulators of vacuolar/lysosomal membrane trafficking pathways.** *J Biol Chem* 1999, **274**:9129-9132.

29. • Mulholland J, Konopka J, Singer-Kruger B, Zerial M, Botstein D: **Visualization of receptor-mediated endocytosis in yeast.** *Mol Biol Cell* 1999, **10**:799-817.

This immunoEM study found substantial colocalization of internalized Ste2p pheromone receptors with CPY, the vacuolar ATPase and Vps21p in late perivacuolar MVBs. This supports the idea that endocytic and TGN traffic converge at late endosomes.

30. • Gerrard SR, Bryant NJ, Stevens TH: **VPS21 controls entry of endocytosed and biosynthetic proteins into the yeast prevacuolar compartment.** *Mol Biol Cell* 2000, **11**:613-626.

In *vps21* mutants, TGN- and endocytically-derived vesicles accumulate in distinct intermediates blocked before prevacuole fusion, indicating that Vps21 is involved in delivery of vesicle intermediates from both pathways. This also suggests that TGN and endocytic vesicles are normally delivered to the prevacuole/late endosome, although it cannot be ruled out that *vps21* blocks fusion with both early and late endosomes.

31. •• Lewis MJ, Nichols BJ, Prescianotto-Baschong C, Riezman H, Pelham HR: **Specific retrieval of the exocytic SNARE Snc1p from early yeast endosomes.** *Mol Biol Cell* 2000, **11**:23-38.

After fusion with the plasma membrane, the exocytic v-SNARE, Snc1p, is re-internalized and recycled from early endosomes to the TGN. Interestingly, it co-mingles with Kex2p in the endosomal recycling structures. As the recycling of Snc1p is not blocked by mutations that prevent retrieval from the prevacuolar endosome, Lewis *et al.* argue that this recycling compartment is an early endosome, distinct from the prevacuole. Moreover, these results suggest that TGN-resident proteins can follow a pathway from the TGN to the early endosome.

32. Hirst J, Futter CE, Hopkins CR: **The kinetics of mannose 6-phosphate receptor trafficking in the endocytic pathway in HEp-2 cells: the receptor enters and rapidly leaves multivesicular endosomes without accumulating in a prelysosomal compartment.** *Mol Biol Cell* 1998, **9**:809-816.

33. Press B, Feng Y, Hoflack B, Wandinger-Ness A: **Mutant Rab7 causes the accumulation of cathepsin D and cation- independent mannose 6-phosphate receptor in an early endocytic compartment.** *J Cell Biol* 1998, **140**:1075-1089.

34. Bryant NJ, Piper RC, Gerrard SR, Stevens TH: **Traffic into the prevacuolar/endosomal compartment of *Saccharomyces cerevisiae*: a VPS45-dependent intracellular route and a VPS45-independent, endocytic route.** *Eur J Cell Biol* 1998, **76**:43-52.

35. Webb GC, Zhang ZJ, Garlow SJ, Wesp A, Riezman H, Jones EW: **Pep7p provides a novel protein that functions in vesicle-mediated transport between the yeast Golgi and endososme.** *Mol Biol Cell* 1997, **8**:871-895.

36. Gerrard SR, Levi BP, Stevens TH: **Pep12p is a multifunctional yeast syntaxin that controls entry of biosynthetic, endocytic and retrograde traffic into the prevacuolar compartment.** *Traffic* 2000, **1**:259-279.

37. McBride HM, Rybin V, Murphy C, Giner A, Teasdale R, Zerial M: **Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13.** *Cell* 1999, **98**:377-386.

38. Simonsen A, Gaullier JM, D'Arrigo A, Stenmark H: **The Rab5 effector EEA1 interacts directly with syntaxin-6.** *J Biol Chem* 1999, **274**:28857-28860.

39. Pelham HR: **SNAREs and the secretory pathway-lessons from yeast.** *Exp Cell Res* 1999, **247**:1-8.

40. • Mukherjee S, Soe TT, Maxfield FR: **Endocytic sorting of lipid analogues differing solely in the chemistry of their hydrophobic tails.** *J Cell Biol* 1999, **144**:1271-1284.

Fluorescent lipid analogues added to culture medium label the surface of cells and are endocytosed, together with transferrin receptors, into common early sorting endosomes. Although short unsaturated acyl-chain probes then enter the recycling endosome with the transferrin receptor, long saturated acyl-chain probes segregate from this path and enter morphologically distinct late endosomes. This indicates that early and late endosomes can be distinguished by different bilayer properties that probably also affect protein sorting events

41. • Fernandez-Borja M, Wubbolts R, Calafat J, Janssen H, Divecha N, Dusseljee S, Neefjes J: **Multivesicular body morphogenesis requires phosphatidylinositol 3-kinase activity.** *Curr Biol* 1999, **9**:55-58.

In antigen-presenting Mel JuSo cells, wortmannin causes vacuolation of endocytic compartments. However, MPR and MHC class II are found in separate swollen vacuoles. These enlarged vacuoles contain few internal vesicles, so PtdIns 3-kinase activity appears necessary for the involution required to form MVBs.

42. • Wurmser AE, Emr SD: **Phosphoinositide signaling and turnover: PtdIns(3)P, a regulator of membrane traffic, is transported to the vacuole and degraded by a process that requires lumenal vacuolar hydrolase activities.** *EMBO J* 1998, **17**:4930-4942.

Ablation of fusion between the prevacuolar compartment and the vacuole causes an ~five-fold increase in cellular PtdIns(3)P levels. Similar results are obtained if vacuolar hydrolase activity is inhibited. In these cells, where MVB-vacuole

fusion is intact, the vacuole interior is filled with many small vesicles. Turnover of PtdIns(3)P seems, therefore, to require membrane delivery to the vacuole.

43. Odorizzi G, Babst M, Emr SD: **Fab1p PtdIns(3)P 5-kinase function**
•• **essential for protein sorting in the multivesicular body.** *Cell* 1998, **95**:847-858.
On sorting CPS to the vacuole, the protease is processed, releasing the catalytic portion from the membrane. Compromising vacuolar function by deleting a vacuolar ATPase subunit gene allows CPS to be visualized within intravacuolar vesicles. Inactivation of Fab1p, a PtdIns(3)P 5-kinase causes the cytosolic and transmembrane portions of CPS to remain associated with the limiting vacuolar membrane. This indicates that PtdIns(3,5)P_2 is important in getting CPS into the lumen of the vacuole, presumably by facilitating involution into the MVB. Internalized Ste2p also enters the vacuole via this pathway. Further, Class E Vps proteins seem to be required at the stage of MVB formation and/or sorting.

44. Sbrissa D, Ikonomov OC, Shisheva A: **PIKfyve, a mammalian ortholog of yeast Fab1p lipid kinase, synthesizes 5-phosphoinositides. Effect of insulin.** *J Biol Chem* 1999, **274**:21589-21597.

45. McEwen RK, Dove SK, Cooke FT, Painter GF, Holmes AB, Shisheva A, Ohya Y, Parker PJ, Michell RH: **Complementation analysis in PtdInsP kinase-deficient yeast mutants demonstrates that *Schizosaccharomyces pombe* and murine Fab1p homologues are phosphatidylinositol 3-phosphate 5-kinases.** *J Biol Chem* 1999, **274**:33905-33912.

46. Kobayashi T, Stang E, Fang KS, de Moerloose P, Parton RG, Gruenberg J: **A lipid associated with the antiphospholipid syndrome regulates endosome structure and function.** *Nature* 1998, **392**:193-197.

47. Kobayashi T, Beuchat MH, Lindsay M, Frias S, Palmiter RD,
•• Sakuraba H, Parton RG, Gruenberg J: **Late endosomal membranes rich in lysobisphosphatidic acid regulate cholesterol transport.** *Nat Cell Biol* 1999, **1**:113-118.
Accumulated, unesterified cholesterol in NPC-patient fibroblasts colocalizes with Rab7, LBPA and MPRs in MVBs, indicating endosomal involvement in cholesterol mobilization. Antibodies against LBPA from anti-phospholipid syndrome patients cause cholesterol to accumulate in LBPA-positive elements, thus demonstrating the relationship between lipid flow and MVB sorting functions.

48. Neufeld EB, Wastney M, Patel S, Suresh S, Cooney AM, Dwyer NK,
• Roff CF, Ohno K, Morris JA, Carstea ED *et al.*: **The Niemann-Pick C1 protein resides in a vesicular compartment linked to retrograde transport of multiple lysosomal cargo.** *J Biol Chem* 1999, **274**:9627-9635.
This provides the first thorough characterization of the trafficking of NPC1. In normal fibroblasts, NPC1 colocalizes with a subset of LAMP-1-positive structures but not with cholesterol or MPRs. U18666A treatment, which mimics the NPC phenotype by causing cholesterol stagnation in late endosomes/lysosomes, results in NPC1 redistribution into cholesterol-positive vacuoles. NPC appears to traffic between the lysosome and a late endocytic subcompartment, facilitating LDL-derived cholesterol mobilization to the cell surface.

49. Babst M, Wendland B, Estepa EJ, Emr SD: **The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function.** *EMBO J* 1998, **17**:2982-2993.

50. Bishop N, Woodman P: **ATPase-defective mammalian VPS4**
•• **localizes to aberrant endosomes and impairs cholesterol trafficking.** *Mol Biol Cell* 2000, **11**:227-239.
The human homologue of Vps4p, hVPS4, is predominantly soluble when expressed exogenously, but an ATP-restricted form of the ATPase associates with aberrantly enlarged intracellular vesicles. The accumulated swollen membranes are from the late endosome and contain MPR, LAMP-1, and accumulated cholesterol. Expression of the mutant hVPS4 has minimal effect on early endocytic compartments.

51. Yoshimori T, Yamagata F, Yamamoto A, Mizushima N, Kabeya Y,
•• Nara A, Miwako I, Ohashi M, Ohsumi M, Ohsumi Y: **The mouse SKD1, a homologue of yeast Vps4p, is required for normal endosomal trafficking and morphology in mammalian cells.** *Mol Biol Cell* 2000, **11**:747-763.
This is a similar study to [50••] using the mouse VPS4 homologue, SKD1. Although the late endocytic defects using an ATPase-defective mutant are similar to those observed in [50••], this study shows there are severe early endosomal defects as well. The reason for this discrepancy remains to be determined.

52. Ghosh RN, Mallet WG, Soe TT, McGraw TE, Maxfield FR: **An endocytosed TGN38 chimeric protein is delivered to the TGN after trafficking through the endocytic recycling compartment in CHO cells.** *J Cell Biol* 1998, **142**:923-936.

53. Mallard F, Antony C, Tenza D, Salamero J, Goud B, Johannes L: **Direct pathway from early/recycling endosomes to the Golgi apparatus revealed through the study of shiga toxin B-fragment transport.** *J Cell Biol* 1998, **143**:973-990.

54. Mallet WG, Maxfield FR: **Chimeric forms of furin and TGN38 are**
• **transported with the plasma membrane in the trans-Golgi network via distinct endosomal pathways.** *J Cell Biol* 1999, **146**:345-359.
Chimeras membrane proteins containing the cytoplasmic domains of TGN38 or furin are delivered back to the TGN, after endocytosis, from the endocytic recycling pathway or from late endosomes, respectively. The retrieval from late endosomes is efficient and requires one pass, whereas the proteins retrieved from recycling endosomes may undergo multiple passes through the plasma membrane/early endosome before reaching the TGN.

55. Riederer MA, Soldati T, Shapiro AD, Lin J, Pfeffer SR: **Lysosome biogenesis requires Rab9 function and receptor recycling from endosomes to the trans-Golgi network.** *J Cell Biol* 1994, **125**:573-582.

56. Nicoziani P, Vilhardt F, Llorente A, Hilout L, Courtoy J, Sandvig K, van Deurs B: **Role for dynamin in late endosome dynamics and trafficking of the cation-independent mannose 6-phosphate receptor.** *Mol Biol Cell* 2000, **11**:481-495.

57. Itin C, Ulitzur N, Muhlbauer B, Pfeffer SR: **Mapmodulin, cytoplasmic dynein, and microtubules enhance the transport of mannose 6-phosphate receptors from endosomes to the trans-Golgi network.** *Mol Biol Cell* 1999, **10**:2191-2197.

58. Diaz E, Pfeffer SR: **TIP47: a cargo selection device for mannose 6-phosphate receptor trafficking.** *Cell* 1998, **93**:433-443.

59. Wan L, Molloy SS, Thomas L, Liu G, Xiang Y, Rybak SL, Thomas G: **PACS-1 defines a novel gene family of cytosolic sorting proteins required for trans-Golgi network localization.** *Cell* 1998, **94**:205-216.

60. Seaman MN, McCaffery JM, Emr SD: **A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast.** *J Cell Biol* 1998, **142**:665-681.

61. Nothwehr SF, Bruinsma P, Strawn LA: **Distinct domains within**
• **Vps35p mediate the retrieval of two different cargo proteins from the yeast prevacuolar/endosomal compartment.** *Mol Biol Cell* 1999, **10**:875-890.
Different Vps35p retromer mutants are selectively defective in retrieval of A-ALP or Vps10p. This suggests that Vps35p is a coat protein involved in cargo selection and has distinct domains for recognition of the cytosolic domains of different proteins recycling from the prevacuole to the TGN.

62. Kurten RC, Cadena DL, Gill GN: **Enhanced degradation of EGF receptors by a sorting nexin, SNX1.** *Science* 1996, **272**:1008-1010.

63. Haft CR, Luz Sierra M, Barr V, Haft DH, Taylor SI: **Identification of a family of sortin nexin molecules and characterization of their association with receptors.** *Mol Cell Biol* 1998, **18**:7278-7287.

64. Ekena K, Stevens TH: **The *Saccharomyces cerevisiae MVP1* gene interacts with *VPS1* and is required for vacuolar protein sorting.** *Mol Cell Biol* 1995, **15**:1671-1678.

65. Voos W, Stevens TH: **Retrieval of resident late-Golgi membrane proteins from the prevacuolar compartment of *Saccharomyces cerevisiae* is dependent on the function of Grd19p.** *J Cell Biol* 1998, **140**:577-590.

66. Haft CR, Sierra L, Barr VA, Bafford R, Taylor SI: **Sorting nexins (SNX) 1 and 2: Interaction domains involved in self association and associations with human retromer proteins.** *Mol Biol Cell* 1999, **10**:114a.

67. Conibear E, Stevens TH: **Vps52p, Vps53p, and Vps54p form a novel**
•• **multisubunit complex required for protein sorting at the yeast late Golgi.** *Mol Biol Cell* 2000, **11**:305-323.
Three new Vps proteins, Vps52p, Vps53p and Vps54p form a complex and localize to the late Golgi. TGN-membrane proteins are mislocalized to the vacuole when the genes are mutated, suggesting a defect in retrograde transport from the prevacuole blocking docking/fusion with the TGN.

68. Hicke L: **Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels.** *Trends Cell Biol* 1999, **9**:107-112.

69. Lupher ML Jr, Rao N, Eck MJ, Band H: **The Cbl protooncoprotein: a negative regulator of immune receptor signal transduction.** *Immunol Today* 1999, **20**:375-382.

70. Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY,
•• Beguinot L, Geiger B, Yarden Y: **c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor.** *Genes Dev* 1998, **12**:3663-3674.

This study provides evidence that c-Cbl mediates ligand induced downregulation of the EGF receptor (ErbB-1) in the early endosome, possibly by promoting sorting of the receptor into the MVB pathway. This downregulation requires receptor kinase activity and involves c-Cbl-dependent covalent attachment of ubiquitin to EGF receptor. Oncogenic Cbl inhibits downregulation by allowing recycling to the cell surface.

71. Miyake S, Lupher ML Jr, Druker B, Band H: **The tyrosine kinase regulator Cbl enhances the ubiquitination and degradation of the platelet-derived growth factor receptor alpha.** *Proc Natl Acad Sci USA* 1998, **95**:7927-7932.

72. Wang Y, Yeung YG, Stanley ER: **CSF-1 stimulated multiubiquitination of the CSF-1 receptor and of Cbl follows their tyrosine phosphorylation and association with other signaling proteins.** *J Cell Biochem* 1999, **72**:119-134.

73. Miyake S, Mullane-Robinson KP, Lill NL, Douillard P, Band H: **Cbl-mediated negative regulation of platelet-derived growth factor receptor-dependent cell proliferation. A critical role for Cbl tyrosine kinase-binding domain.** *J Biol Chem* 1999, **274**:16619-16628.

74. Lee PS, Wang Y, Dominguez MG, Yeung YG, Murphy MA, Bowtell DD,
• Stanley ER: **The Cbl protooncoprotein stimulates CSF-1 receptor multiubiquitination and endocytosis, and attenuates macrophage proliferation.** *EMBO J* 1999, **18**:3616-3628.

Ligand mediated downregulation of endogenous CSF-1 receptor by c-Cbl was studied in primary macrophages. Lysosomal, but not proteasomal inhibitors, blocked turnover of the receptor, suggesting that ubiquitinated receptors are turned over in the lysosome.

75. Kil SJ, Hobert M, Carlin C: **A leucine-based determinant in the epidermal growth factor receptor juxtamembrane domain is required for the efficient transport of ligand- receptor complexes to lysosomes.** *J Biol Chem* 1999, **274**:3141-3150.

76. Lill NL, Douillard P, Awwad RA, Ota S, Lupher ML, Jr., Miyake S, Meissner-Lula N, Hsu VW, Band H: **The evolutionarily conserved N-terminal region of Cbl is sufficient to enhance down-regulation of the epidermal growth factor receptor.** *J Biol Chem* 2000, **275**:367-377.

77. Joazeiro CA, Wing SS, Huang H, Leverson JD, Hunter T, Liu YC: **The**
•• **tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase.** *Science* 1999, **286**:309-312.

This is one of several papers showing that c-Cbl is recruited to activated RTKs via its PTB domain. c-Cbl regulates ubiquitin ligation of the receptor via its RING-H finger domain (See also [73,76,78,79••,80]).

78. Waterman H, Levkowitz G, Alroy I, Yarden Y: **The RING finger of c-Cbl mediates desensitization of the epidermal growth factor receptor.** *J Biol Chem* 1999, **274**:22151-22154.

79. Levkowitz G, Waterman H, Ettenberg SA, Katz M, Tsygankov AY,
•• Alroy I, Lavi S, Iwai K, Reiss Y, Ciechanover A, Lipkowitz S, Yarden Y: **Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1.** *Mol Cell* 1999, **4**:1029-1040.

Ligand-bound EGF receptor undergoes tyrosine autophosphorylation at a lysosomal-targeting motif, which serves as a docking site for c-Cbl via its PTB domain. This binding is followed by tyrosine phosphorylation of c-Cbl at a site flanking the RING-H finger, enabling activation of c-Cbl E3 ubiquitin ligase activity.

80. Yokouchi M, Kondo T, Houghton A, Bartkiewicz M, Horne WC, Zhang H, Yoshimura A, Baron R: **Ligand-induced ubiquitination of the epidermal growth factor receptor involves the interaction of the c-Cbl RING finger and UbcH7.** *J Biol Chem* 1999, **274**:31707-31712.

81. Tyers M, Willems AR: **One ring to rule a superfamily of E3 ubiquitin ligases.** *Science* 1999, **284**:601-604.

82. Swaminathan S, Amerik AY, Hochstrasser M: **The Doa4**
• **deubiquitinating enzyme is required for ubiquitin homeostasis in yeast.** *Mol Biol Cell* 1999, **10**:2583-2594.

This paper shows that inhibition of recycling of conjugated ubiquitin in *doa4* yeast depletes ubiquitin pools and thereby impairs ubiquitin-dependent processes, including receptor downregulation. Interestingly, the evidence from this paper suggests that endocytosed plasma membrane proteins may be deubiquitinated by cytosolic deubiquitinating enzymes before their delivery and degradation in the vacuole.

83. Babst M, Odorizzi G, Estepa EJ, Emr SD: **Mammalian TSG101 and**
•• **the yeast homologue, Vps23p, both function in late endosomal trafficking.** *Traffic* 2000, **1**:248-258.

TSG101 and Vps23p (a class E *vps*) are involved in MVB formation and late endosome maturation. Deletion of *VPS23* causes CPS to accumulate in the perivacuolar class E compartment with abnormal proteolytic processing. Mutation of tumor susceptibility gene 101 (*TSG101*), the mouse homologue of *VPS23*, causes an analogous defect in cathepsin D delivery to the lysosome. Endocytosis and recycling are normal in *tsg101* mutants, but trafficking of both MPRs and activated EGF receptors into MVBs is disrupted, which causes them to recycle back to the surface efficiently. Impaired downregulation of EGF-receptor signaling leads to chronic MAPK activation that probably explains the tumor-susceptibility phenotype.

84. Li Y, Kane T, Tipper C, Spatrick P, Jenness DD: **Yeast mutants affecting possible quality control of plasma membrane proteins.** *Mol Cell Biol* 1999, **19**:3588-3599.

85. Beck T, Schmidt A, Hall MN: **Starvation induces vacuolar targeting**
• **and degradation of the tryptophan permease in yeast.** *J Cell Biol* 1999, **146**:1227-1238.

Tat2p tryptophan permease undergoes regulated turnover during nutrient deprivation by diversion of the permease from the late secretory pathway to the vacuolar pathway. This paper suggests that this regulated sorting involves ubiquitination of Tat2p.

86. Roberg KJ, Rowley N, Kaiser CA: **Physiological regulation of membrane protein sorting late in the secretory pathway of *Saccharomyces cerevisiae*.** *J Cell Biol* 1997, **137**:1469-1482.

87. Hong E, Davidson AR, Kaiser CA: **A pathway for targeting soluble misfolded proteins to the yeast vacuole.** *J Cell Biol* 1996, **135**:623-633.

88. Luo WJ, Chang A: **Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant.** *J Cell Biol* 1997, **138**:731-746.

89. Jenness DD, Li Y, Tipper C, Spatrick P: **Elimination of defective alpha-factor pheromone receptors.** *Mol Cell Biol* 1997, **17**:6236-6245.

90. Luo W, Chang A: **An endosome-to-plasma membrane pathway involved in trafficking of a mutant plasma membrane ATPase in yeast.** *Mol Biol Cell* 2000, **11**:579-592.

91. Jorgensen MU, Emr SD, Winther JR: **Ligand recognition and domain structure of Vps10p, a vacuolar protein sorting receptor in *Saccharomyces cerevisiae*.** *Eur J Biochem* 1999, **260**:461-469.

92. Sonnichsen B, De Renzis S, Nielsen E, Rietdorf J, Zerial M: **Distinct**
•• **membrane domains on endosomes in the recycling pathways visualized by multicolor imaging of rab4, rab5, and rab11.** *J Cell Biol* 2000, **149**:901-904.

This study, following transferrin uptake in cells stably transfected with GFP-tagged Rab proteins, reveals that early endosomes contain morphologically discernible subdomains, demarcated by discrete Rab proteins [92••]. As transferrin recycles along the endocytic pathway, it overlaps sequentially with Rab5, then Rab4 and then Rab11. These GTPases can be found on continuous membrane structures (a single endosome) but do not appear to mix significantly.

93. Wiederkehr A, Avaro S, Prescianotto-Baschong C,
• Haguenauer-Tsapis R, Riezman H: **The F-box protein Rcy1p is involved in endocytic membrane traffic and recycling out of an early endosome in *Saccharomyces cerevisiae*.** *J Cell Biol* 2000, **149**:397-410.

A novel F-box protein, Rcy1p, is required for transport out of the early endosome in yeast [93•]. While internalization is normal, mutant Rcy1p leads to accumulation of endocytic markers in an early endosomal compartment, preventing both recycling to the cell surface and transport on to the prevacuole. Interestingly, several members of the F-box family of proteins mediate ubiquitination of substrates as components of SKP1/cullin/F-box ubiquitin ligase complexes. Therefore, Rcy1p may function in a ubiquitination pathway that regulates transport in the endosomal system.