Cell. Mol. Life Sci. 63 (2006) 2491–2505
1420-682X/06/212491-15
DOI 10.1007/s00018-006-6210-8

Cellular and Molecular Life Sciences

Review

Apical protein transport

D. Delacour and R. Jacob*

Department of Cell Biology and Cell Pathology, Philipps University of Marburg, Robert-Koch-Str. 6, 35033 Marburg (Germany), Fax: +49 6421 286 6414, e-mail: jacob@staff.uni-marburg.de

Received 5 May 2006; received after revision 12 June 2006; accepted 11 July 2006
Online First 21 August 2006

Abstract. The plasma membrane of epithelial cells and hepatocytes is divided into two separate membrane compartments, the apical and the basolateral domain. This polarity is maintained by intracellular machinery that directs newly synthesized material into the correct target membrane. Apical protein sorting and trafficking require specific signals and different intracellular routes to the cell surface. Some of them depend on the integrity of sphingolipid/cholesterol-enriched membrane microdomains named 'lipid rafts', others use separate transport platforms. Certain characteristics of the heterogeneous population of apical sorting signals are described in this review and cellular factors associated with sorting and transport mechanisms are discussed.

Keywords. Epithelial cells, apical sorting, protein trafficking, cytoskeleton, transport vesicles, membrane fusion.

Introduction

Epithelia represent an interface between the outside world and the internal system of the organism. This is maintained by the characteristic morphology of an epithelial cell integrated into a monolayer of cells that acts as a barrier against water and ion flux. Dependent on the organ or the state of differentiation, epithelial cells specialize in particular functions, such as presentation of hydrolases required for the digestion and absorption of nutrition or ion transporters to pump ions against osmotic gradients. To warrant these functions and to control material flow into one precise direction, epithelial cells maintain a polarized organization. Their plasma membrane is asymmetric and divided into two distinct membrane domains, an apical domain facing the organ lumen or external world, and a basolateral domain facing neighboring cells and the basal lamina. The process of cellular polarization is initiated by an influx of information from the extracellular milieu, provided by cell to cell interactions or contacts with the extracellular matrix (ECM). Based on the outer cellular topology, parts of the cell membrane that are in contact with neighboring cells or the basal lamina develop the basolateral membrane, while areas opposed to lumina form apical membranes [1].

Apical and basolateral membrane domains have distinct morphologies based on their prominent organ-specific function. The apical membrane domain of enterocytes is characterized by a brush border composed of microvilli, which magnify the cell surface and improve absorptive and exchanging properties of the tissue. In hepatocytes the apical pole is concentrated to a membrane area surrounding the *bile canaliculi*, where bile secretion takes place [2]. In general, based on their functional specialization apical and basolateral membrane domains are composed of different proteins and lipids [3]. The apical plasma membrane of intestinal cells is, for example, enriched in intestinal hydrolases, while E-cadherin or integrins are concentrated in the basolateral domain and facilitate the formation of cell/cell or cell/ECM contacts. The composition of membrane lipids is also polarized in epithelial cells: cholesterol and sphingolipids are enriched on the apical part, whereas the basolateral membrane is enriched in phosphatidylcholine [4]. This polarized architecture is stabilized in epithelial cells by the tight junction complex that acts as a barrier against

* Corresponding author.

protein or lipid diffusion from one membrane domain to the other.

Differences in protein and lipid composition of the two membrane domains are maintained by highly specific sorting and transport mechanisms. In this context, the intracellular routing of proteins *per se* is also denoted 'polarized trafficking' [5]. The current knowledge about this sorting machinery and the underlying sorting signals is mainly based on studies with epithelial cells in culture. Following differentiation these cell monolayers reproduce many characteristic features of epithelial cells *in vivo*, providing a useful tool to study the development of polarized cells and basic features of polarized protein transport. Among the most frequently used cell lines are renal Madin-Darby canine kidney (MDCK) cells, the human intestinal cell lines Caco-2 and HT-29 and HepG2 hepatocytes.

Studies on exocytic routes in epithelial cells started by the analysis of virus-infected cells. These studies specified pathways of newly synthesized viral capsids and demonstrated that influenza virus buds from the apical plasma membrane, while vesicular stomatitis virus (VSV) budding is localized to the basolateral membrane. This polarized targeting has also been observed for the corresponding envelope proteins [6], the apical influenza hemagglutinin glycoprotein (HA), and the basolateral VSV glycoprotein G (VSV-G) [7]. HA is synthesized in the endoplasmic reticulum (ER) and travels along the secretory pathway to the cell surface [8]. It is separated from VSV-G in the *trans* Golgi network (TGN) before reaching the cell surface [9–11]. However, viral proteins harbor the potential to manipulate intracellular mechanisms for their own benefit and, therefore, endogenously or exogenously expressed receptors [12, 13] or enzymes [14–16] entered the stage for the characterization of apical transport processes.

Mechanisms ensuring protein transport from the TGN to the plasma membrane can be divided into several steps: protein segregation or sorting, budding and transport of carrier vesicles arising from the TGN, trafficking along intracellular tracks, docking and fusion with the target membrane. This review describes these steps, which certify the specificity of protein transport towards the appropriate membrane domain by fine tuning of membrane and cytosolic factors.

Multiplicity of apical sorting signals and mechanisms

Protein sorting is based on intrinsic motifs that interact with components of the sorting machinery and direct the polypeptide through the distinct transport routes to a correct membrane domain. Early sorting determinants identified are located on basolateral proteins. These initial data demonstrated that short amino acid sequences, containing Tyr, Leu-Leu or Leu-Val motifs are localized in the cytoplasmic region near the transmembrane domain and direct proteins to the basolateral membrane [3, 17–19]. Mostov and coworkers [20] have proposed that theses structures would form a common β-turn signal for basolateral tethering. Nevertheless, a recent study, performed with MDCK and Fischer rat thyroid (FRT) cells, proposed that basolateral sorting signals comprise a multitude of secondary structures like α-helices or β-sheets, and can also be localized in distal parts of the cytoplasmic domain [21]. Basolateral sorting determinants can overlap with endocytosis signals and are decrypted by coat proteins of the clathrin complex [22]. Proteins containing Tyr motifs bind to the γ1 subunit of the AP-1 adaptor complex [23–25], and Leu-Leu motifs interact specifically with the β subunit of AP-1 [26]. Recently, a new adaptor complex, AP-1B, specifically expressed in epithelial cells, has been described for basolateral protein delivery [27].

In contrast to basolateral trafficking, the interaction of apical sorting determinants with cellular adaptors or receptors is still unclear and remains to be identified. Until now a heterogeneous population of apical sorting signals has been described. Among them is the lipid anchoring by glycosylphosphatidylinositol anchorage (GPI), a post-translational modification in the ER, which is the first apical sorting signal that was described [16, 28]. GPI-anchored proteins (GPI-APs) are preferentially localized at the apical plasma membrane of epithelial cells and are resistant to detergent extraction at 4 °C, an indication for the association with sphingolipid/cholesterol-rich membrane microdomains or lipid rafts [16, 29–32]. Nevertheless, although GPI anchoring provides raft association, this is not a general mechanism for protein targeting to the apical membrane. Lipardi et al. [33] have shown that soluble forms of placental alkaline phosphatase (PLAP) are not associated with lipid rafts but are still apically sorted in Fischer rat thyroid cells. Moreover, the addition of a GPI-anchoring motif on rat growth hormone (rGH), a soluble protein that is secreted in a non-polarized manner, is not sufficient for apical targeting [34]. Hence, other determinants, apart from GPI anchoring, ensure proper apical delivery. Those signals could rely on the presence of *N*- or *O*-glycans [34–37].

Early indications for an involvement of *N*-glycosyl chains in apical protein targeting came from studies using *N*-glycosylation inhibitors. Treatment of MDCK cells with tunicamycin, a GlcNAc-analogue that inhibits the first steps of *N*-glycosylation, missorts apical gp80 (clusterin) to both membrane domains of MDCK cells [38]. Glycosylation-deficient cell lines have also been used to demonstrate an involvement of glycans in apical protein sorting. For example, ricin-resistant MDCK cells (MDCK RCAr) are characterized by a defect in galactose incorporation into glycoproteins. The impairment

of glycosylation in this cell line induced mistargeting of the apical glycoprotein gp114 [39]. In addition, recombinantly added *N*-glycosyl chains could be identified as apical sorting determinants. The rGH shows no preferential targeting in epithelial cells, but recombinant addition of two *N*-glycosyl chains results in apical localization of rGH [40]. On the other hand, deletion of one *N*-glycosyl chain on erythropoietin leads to impaired apical secretion in MDCK cells [41]. *N*-glycans also play a role in apical delivery of endolyn [42], the glycine transporter GLYT2 [43] or dipeptidase [44].

Nevertheless, *N*-glycosylation is not a universal apical sorting signal. Indeed, it is important to note that some proteins are apically sorted independent of the presence or absence of *N*-glycosyl chains. Yeaman et al. [37] have shown that for apical targeting of p75NTR, an *N*- and *O*-glycosylated transmembrane protein, *N*-glycans are not required in MDCK cells. Other examples for *N*-glycan-independent apical targeting are provided by the hepatitis B surface antigen (HBAgs) and osteopontin in MDCK cells [45, 46], and ecto-nucleotide pyrophosphatase/phosphodiesterase NPP3 in Caco-2 cells [47]. An intriguing point is that some basolateral proteins are *N*-glycosylated, but nevertheless not delivered to the apical membrane. This could be explained by the simultaneous presence of cytosolic basolateral targeting determinants with a high affinity for adaptor complexes that override apical sorting determinants in the extracytosolic part of the protein [48].

O-linked glycosyl chains may also act as apical targeting signal. P75NTR and the hydrolase sucrase isomaltase (SI) are characterized by the presence of heavily *O*-glycosylated stalk domains in close proximity to the membrane. Deletion of these domains results in a shift in the sorting of these proteins from the apical plasma membrane to both surface domains [36, 37]. Moreover, the position of *O*-glycans in the proximal part of the two stalk domains is crucial for apical sorting [36, 49]. Further evidence comes from the rGH, which is secreted from Caco-2 cells in a non-polarized manner. However, attaching the *O*-glycosylated stalk domain of SI to rGH results in secretion of this hormone from the apical side [50]. In parallel, treatment of cells with GalNAcα-*O*-benzyl, a competitive inhibitor of galactosyltransferases and sialyltransferases, results in the generation of truncated *O*-glycans. Thus, GalNAcα-*O*-benzyl treatment has been shown to inhibit *O*-glycosylation of dipeptidylpeptidase-IV (DPPIV), the carcinoembryonic early antigen (CEA) and the transmembrane mucin MUC1 in HT-29 cells [51, 52] and of SI and DPPIV in Caco-2 or MDCK cells [35, 53, 54]. As a consequence, this treatment results in trafficking defects of apical polypeptides, notably by disturbing the association with lipid rafts [35, 52, 53, 55, 56].

Although those findings highlight an involvement of *N*- or *O*-glycosylation in accurate apical delivery, the underlying mechanisms by which glycosylation could control apical trafficking are not known. Current studies tend to define which part of the glycan chain is involved in apical targeting. Weiss and coworkers [57] propose that terminal oligosaccharides constitute apical determinants of endolyn. On the other hand, Vagin et al. [58] postulate that apical sorting signals are located in the core region of glycan chains. According to their model, the function of terminal oligosaccharides is the stabilization of glycoproteins at the apical plasma membrane. Now, it remains to be determined whether glycan motifs are directly involved in apical targeting, or whether they contribute indirectly to apical protein sorting. This could be performed by the stabilization of polypeptides thereby allowing access to a hidden apical sorting signal, or by fixation of a competent conformation for apical sorting. Alternatively, glycosylation could have an impact on the lateral mobility of a protein in the membrane. This could result in the incorporation into membrane microdomains, which are delivered into apical carrier vesicles [40, 48, 59].

Apical sorting signals that mediate the incorporation into membrane microdomains have also been identified in the transmembrane domains of proteins. Deletion mutants of influenza virus neuraminidase (NA) and chimeric constructs containing different domains of NA and the transferrin receptor TfR demonstrated that the NA transmembrane domain can act as a determinant for apical sorting and transport in MDCK cells [60]. This has also been found for the transmembrane domain of HA [61, 62]. Subsequently, Nayak and coworkers [63] reported that several distinct regions of the NA transmembrane domain are involved in apical delivery. Moreover, the apical sorting signal of NA is independent from its raft-association signal, although both motifs are present in the transmembrane domain. In addition to the primary amino acid sequence of the transmembrane domain, its length also seems to be important for raft association and lipid-based sorting [64, 65]. Schuck and Simons [66] propose that the length of the transmembrane domain of a protein determines the affinity for membrane microdomains, and furthermore for apical delivery.

In addition, apical sorting signals of different nature have been located in the cytosolic tails of rhodopsin [67], megalin [68, 69] and receptor guanylyl cyclases [70]. The C terminus of rhodopsin provides a functional link to the cytoskeleton since it interacts directly with Tctex-1, a dynein light chain that mediates apical transport of this eye pigment in epithelial cells [71].

There is still a significant number of apical proteins with unknown apical sorting information [45, 46, 72–75], and therefore other signal variants hidden in primary, secondary, tertiary or quaternary protein structures may also play a role in apical protein delivery.

Apart from those 'structural' apical sorting signals, 'functional' signals for apical transport have been recently re-

ported. In addition to lipid raft association the formation of high molecular weight (HMW) complexes would be required for GPI-AP tethering in MDCK and FRT cells. This GPI-AP oligomerization or association with HMW complexes may stabilize protein-raft association and could cluster small rafts to constitute a functional sorting platform [33, 76, 77].

Lipid rafts as apical sorting platform

An increasing amount of data on apical protein trafficking has highlighted the involvement of membrane microdomains or lipid rafts for protein sorting and transport to the apical plasma membrane. In 1997, Simons and Ikonen [48] postulated that in the TGN apically targeted proteins are segregated into these sphingolipid (SL)- and cholesterol-enriched transport platforms. The concept itself is a classical concept. In 1973, Yu and coworkers [78] first showed that a detergent-resistant membrane (DRM) fraction, rich in SLs, could be isolated from erythrocytes; they suggested that this fraction may contain lipid microdomains, consisting of SLs interacting with each other, and they proposed that those interactions could assist lipids in their association with detergent-resistant proteins. Moreover, some SLs were able to associate and form 'clusters' or 'microdomains' [79], but at that time, these observations had no real biological significance.

A heterogeneous lipid distribution in epithelial cells has been observed by Simons and van Meer [4]. They found a high concentration of sphingomyelin and glycosphingolipids in the apical plasma membrane, while the lipid composition of the basolateral plasma membrane is enriched in phosphatidylcholine and resembles that of non-polarized cells. Moreover, they proposed that the high apical concentration of SLs is a consequence of intracellular lipid sorting in the TGN. Thus, the TGN represents a sorting station that forms apical carrier vesicles composed of lipid clusters, which might recruit apical proteins [4, 80]. Soon afterwards, it was shown that HA becomes detergent resistant in non-ionic Triton X-100 (TX-100) in the late compartments of the Golgi apparatus [81]. The raft concept then emerged from the demonstration that the detergent insolubility of GPI-APs was due to their association with SL-enriched microdomains [29]. Moreover, this insolubility started after they had reached the TGN. Thanks to these studies, membrane microdomains have been defined as insoluble material in non-ionic detergent at low temperature, characterized by light buoyant density on sucrose gradients and a cholesterol- and SL-rich lipid composition [29, 82]. Simons and Ikonen [48] correlated these observations with the biological function of membrane microdomains as transport platform to the apical membrane. According to this model apical proteins are recruited into lipid rafts, thereby excluding basolateral proteins.

Almost 10 years have passed since the raft concept for apical protein transport was postulated, and the concept is still a matter of updates and debates. *In vivo*, membrane microdomains might be small cellular lipid assemblies induced by the specific cellular context or signaling. The coalescence of these small lipid assemblies then generates functional larger platforms or lipid rafts [83, 84]. Recent experiments employing nuclear magnetic resonance spectroscopy (NMR) and calorimetric measurements have shown that the addition of cold TX-100 to artificial lipid mixtures segregates SLs and cholesterol from other lipids. As a consequence, the authors conclude that DRMs might be formed by detergent-lipid interaction and therefore constitute artificially generated, but not pre-existing microdomains [85, 86]. On the other hand, lipid analysis in mass spectrometry showed that the lipid membrane composition of budding HIV virions is comparable to the one of classical DRMs. These experiments, performed in the absence of detergent, suggest that rafts do exist *in vivo* [87].

Several lines of evidence indicated that protein association with lipid rafts could occur before reaching the TGN in the exocytic pathway. GPI-APs are already formed in the ER of yeast cells [88] and prion protein (PrP^c) associates with DRMs in the ER of mammalian cells [89]. Moreover, raft association is not a strict prerequisite for apical protein targeting. Some GPI-APs are found basolaterally in MDCK cells, despite their raft association [76, 89] and several apical transmembrane proteins are not associated with lipid rafts (Table 1). Further studies are required to highlight these additional apical sorting mechanisms in epithelial cells.

Table 1. Apical transmembrane proteins associated or not associated with lipid rafts in epithelial cells.[a]

Associated with lipid rafts	Not associated with lipid rafts
Aminopeptidase A and N [186]	
Aquaporin 5 [187]	Enteropeptidase [188]
Dipeptidyl peptidase IV [186]	gp114 [189]
gp135 [128]	H-K-ATPase [190]
Influenza virus hemagglutinin [191]	Lactase-phlorizin hydrolase [186]
Influenza virus neuraminidase [60]	Neurotrophin receptor p75 [37]
MAL proteolipid [102]	Prominin [192]
Megalin [68]	
Sucrase-isomaltase [186]	

[a] Raft-association was determined by flotation or pelleting following cell lysis in 1% Triton X-100 at 4 °C. Corresponding publications are indicated.

Hierarchy of sorting signals

For a long time, basolateral sorting signals have been considered as being dominant over apical sorting determinants. Indeed, many studies have demonstrated that inactivation of basolateral sorting resulted in protein redirection and their expression at the apical plasma membrane [19, 90–92]. However, this concept was mitigated in the last years, since the removal of basolateral determinants results in a non-polarized protein delivery [93, 94]. For p75NTR, a cytoplasmic basolateral signal is recessive to *O*-glycan-dependent apical sorting signals in MDCK cells [37]. Similar observations indicate that apical sorting signals of endolyn or lactase phlorizine hydrolase (LPH) are superior over cytosolic basolateral determinants [42, 57, 95]. This hierarchy of sorting signals suggests that apical and basolateral sorting information competes in a common compartment along the exocytic route.

One question arises from these observations: why do epithelial cells harbor a variety of apical sorting determinants and mechanisms? We could explain this heterogeneity by the flexible phenotype of epithelial cells. They target proteins to distinct membrane domains based on cell type, cell localization and specialization [96, 97]. Therefore, the final destination of a membrane protein does not mainly depend on the sorting signals themselves, but on the underlying sorting and trafficking machinery provided by the epithelial cell type. This is related to the biological context and physiological functions of an epithelial tissue.

Apical receptors and protein sorting

In analogy to the involvement of adaptor proteins in basolateral protein sorting or the mannose-6 phosphate receptor in lysosomal targeting, apical receptors might identify apical sorting signals to ensure apical protein delivery. Until now, a variety of potential receptors has been discussed. The vesicular integral protein (VIP36) has been isolated by Simons and coworkers [98] from detergent-insoluble fractions. This lectin is present in the Golgi apparatus, at the apical plasma membrane and in endosomal or vesicular structures, accompanied by a putative role in glycoprotein trafficking from the TGN to the plasma membrane. Nevertheless, further studies have demonstrated that VIP36 is in fact involved in early trafficking steps, *e.g.* in trafficking from the ER to the Golgi apparatus, and may not represent an essential sorting factor for apical glycoproteins [99, 100]. Another candidate, the VIP17/MAL protein is present in lipid microdomains, interacts with GPI-APs and has been shown to be required for apical delivery of HA and GPI-APs [101–103].

Recent data have highlighted the involvement of a new lectin family for apical trafficking, the galectins. Galectin-4, a 36-kDa protein, is a major component of lipid rafts in enterocytes of the pig intestine [104] and HT-29 cells [56]. This lectin interacts with high affinity with a specific variant of glycosphingolipids, the sulfatides with long chain-hydroxylated fatty acids, which are specifically enriched in lipid rafts of HT-29 cells. Depletion of galectin-4 by RNA interference impairs raft formation and affects apical trafficking [56]. As a consequence, these data suggest that the interaction between galectin-4 and sulfatides plays a role in the organization of lipid rafts for efficient apical surface delivery. Very recently, we identified galectin-3, a lectin of 29 kDa, in raft-independent apical carrier vesicles from MDCK cells [105]. Galectin-3 interacts directly with the apical proteins LPH, p75NTR and gp114 in a glycan-dependent manner. The observation that those apical glycoproteins are mistargeted to the basolateral membrane in galectin-3-depleted cells suggests a central role to this lectin in the raft-independent apical sorting machinery. As a consequence, galectin-3 could play a role as apical sorting receptor and direct non-raft-associated proteins into apical post-Golgi carriers. The underlying mechanism could be based on the formation of large galectin-3 containing clusters that direct apical glycoproteins into newly formed apical vesicles (Fig. 1). This would pose the question if clustering on the luminal face of a membrane is sufficient or if a cross-talk with cytosolic factors is required to drive the formation of apical vesicles.

In the past, the TGN has been the presumed sorting site for newly synthesized apical and basolateral proteins [80, 106, 107], and live cell imaging monitored the formation and trafficking of apical and basolateral transport carriers [106, 108–111] (Fig. 2). However, these data could not exclude the possibility that at least some sorting occurred in an endosomal compartment beyond the TGN. Evidence for this comes from recent studies indicating that recycling endosomes play a decisive role in the sorting of basolateral proteins [112]. Here, the VSV-G was found to enter transferrin-positive recycling endosomes in the exocytic pathway within a few minutes after exit from the TGN. Interestingly, a newly synthesized variant of VSV-G that is sorted to the apical cell surface has also been detected in this endocytic compartment. Since recycling endosomes in polarized epithelial cells must be able to sort apical from basolateral proteins, returning each to its appropriate plasma membrane domain [113], it is very attractive to suggest that recycling endosomes are the primary site of signal-dependent polarized sorting. However, lipid rafts as one platform for apical protein sorting are present in the TGN, but also in endosomal compartments such as recycling endosomes, late endosomes and internal membranes of multivesicular bodies (MVBs) [114–116]. In living cells, the separation of two distinct apical vesicle populations has been monitored in a compartment beyond the TGN [108]. This compartment cre-

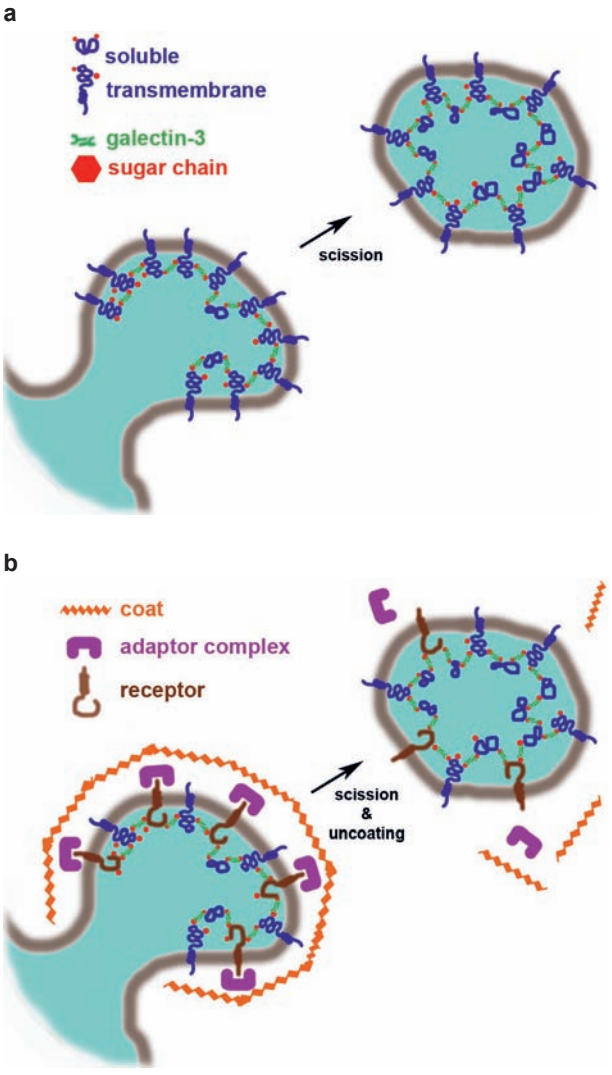

Figure 1. Two proposed models for non-raft dependent apical sorting in epithelial cells. Both models describe oligomerized galectin-3 as a lectin-receptor that clusters membrane bound and soluble apical glycoproteins. In (*a*) this cluster formation is sufficient to induce membrane curvature, which results in the scission of apical vesicles. (*b*) Alternatively, transmembrane receptors are co-clustered by galectin-3 and drive the formation of a vesicle coat. This coat bends the membrane to a globular structure and facilitates vesicle formation.

ates vesicles that carry raft-associated SI, SI-associated vesicles (SAVs) or non-associated LPH, LPH-associated vesicles (LAVs). Both vesicle populations are transported to the apical membrane along distinct cytoskeletal tracks and harbor characteristic protein components [117]. Here, in contrast to the classical view, a major sorting step in direct apical trafficking proceeds in a compartment beyond the TGN (Fig. 2). As suggested previously, this might be the common endosome [118] or the subapical compartment [119], which is defined as large accumulations of short tubules localized just beneath the apical recycling endosome.

It has also been suggested that in MDCK cells raft-dependent trafficking of GPI-APs encompasses a transcytotic route across the basolateral membrane [120], but new data based on biochemical analysis combined with fluorescence microscopy demonstrate that these proteins are directly sorted to the apical cell surface [111, 121].
These observations indicate that a number of unsolved questions still exist concerning the exact trafficking of apical cargo after release from the TGN.

Budding of post-Golgi carriers

Unlike the formation of basolateral vesicles, the budding process for apical protein carriers is not well characterized. In 1988, Simons and van Meer [4] hypothesized that an adaptor or 'bridge-protein' binds to raft-associated ligands and generates a curvature in the vesicular membrane. This phenomenon may favor vesicle budding [4, 83]. Recent data present novel coat proteins for apical carrier formation. PtdIns(4)P, a product of phosphatidylinositol 4-kinase [PI(4)K] activity, is localized in the Golgi apparatus and regulates constitutive transport from the Golgi complex to the plasma membrane [122]. Moreover, FAPP1 and FAPP2 have been identified as effectors of PtdIns (4)P at the TGN level. Hence, they might control carrier vesicle formation, budding and fission [123]. Also Simons and coworkers [124] have shown that FAPP2 was required for apical carrier formation in MDCK cells. Consequently, FAPP proteins could be considered as new coat proteins involved in apical carrier formation [125].

Cytoskeletal tracks in apical trafficking

Polarization of epithelial cells is not restricted to the separation of two plasma membrane domains, but also includes the orientation of cytosolic organelles and the cytoskeleton. Highly organized actin microfilaments stabilize microvilli in the apical brush border of epithelial cells [126]. These microfilaments are anchored in the terminal web, a dense meshwork of F-actin filaments and actin binding proteins close to the apical cell pole that is fixed at the adherens junctional complex. The integrity of actin microfilaments is a prerequisite for an efficient delivery of various polypeptides to the apical membrane. This has been demonstrated by utilizing the fungal inhibitor cytochalasin D to specifically disrupt the subcellular architecture of actin microfilaments [117, 127–129]. The necessity of the terminal web in the transport of a variety of apical proteins is further reflected by the familial enteropathy microvillus inclusion disease (MID), which is characterized by microvillus atrophy and microvillar inclusions on the subcellular level. Here, EM studies reveal thin layers of apical F-actin and reduced amounts of

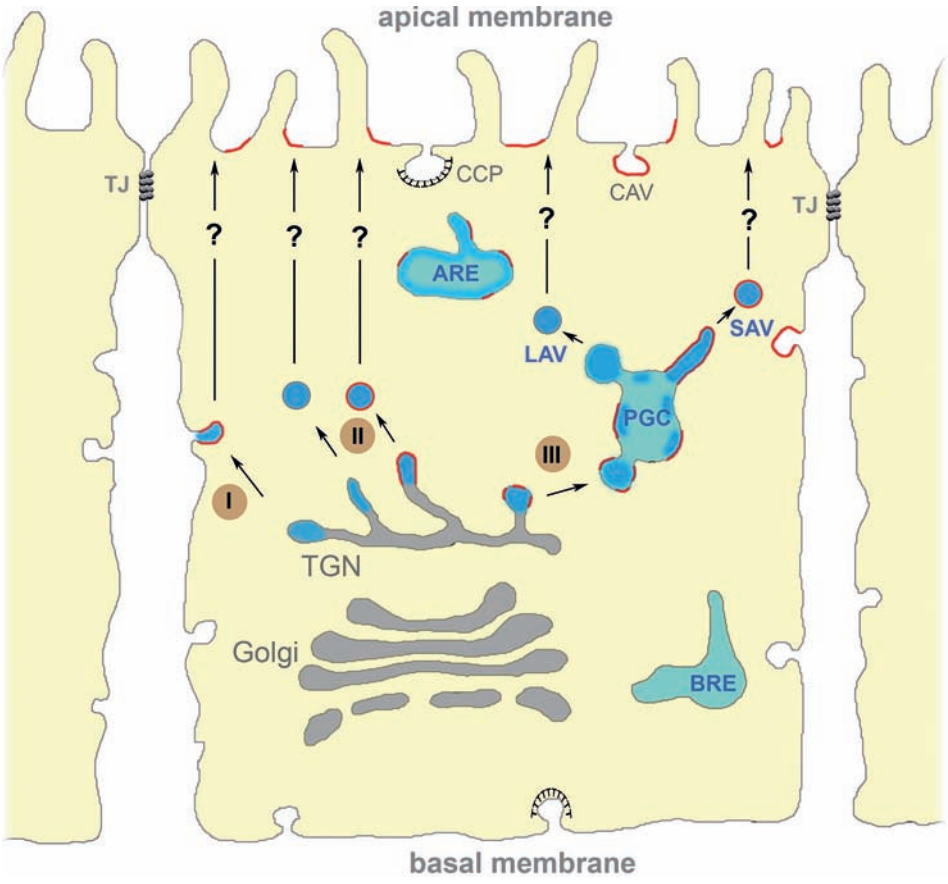

Figure 2. Alternative models for apical protein sorting. Three scenarios for apical protein sorting are represented in this figure. This may involve transcytosis across the basolateral membrane (scenario I) or direct trafficking routes (scenarios II and III). A classical view of direct transport to the apical membrane describes the sorting of raft-associated (red membrane) and non-associated material in the TGN into apical vesicle populations (II). In the new alternative (III) raft-associated and non-associated cargo is transported jointly to a post Golgi compartment [108, 117]. This compartment segregates apical material into distinct vesicle populations (LAVs and SAVs) [117]. ARE, apical recycling endosome; BRE, basolateral recycling endosome; CAV; caveolae; CCP, clathrin-coated pits; PGC, post Golgi compartment; TJ, tight junctions.

marker molecules in the apical membrane [130]. However, not all transport pathways of apical polypeptides require an intact F-actin network. In MDCK cells apical delivery of endogenous gp80 or exogenously expressed LPH is not perturbed by actin-depolarization [117, 131], which suggests that actin microfilaments modulate the trafficking of a subset of apical carriers. Many studies refer to a central role of actin in endocytic trafficking or recycling to the apical cell pole. Cytochalasin D treatment of MDCK cells prevented the apical uptake of receptor bound ferritin or fluid phase markers like Lucifer yellow [132] and similar results were observed in Caco-2 cells [133, 134], in opossum kidney cells [135] and in small intestinal tissue [136]. Moreover, basolateral to apical transcytosis and apical recycling were significantly inhibited by cytochalasin D [127], thus suggesting that postendoycytic apical traffic requires an intact actin cytoskeleton. What is the function of these filaments in apical transport processes? It is tempting to propose that actin stress fibers could function as tracks to direct the motor-driven movement of transport vesicles or carriers towards the endosomal compartment or to the cell membrane.

Likely candidates to catalyze this movement are myosin motors. Class I myosins were found on apical Golgi-derived vesicles from intestinal or MDCK cells [117, 137, 138]. These monomeric, non-processive motors are characterized by a large ATP- and actin-binding head domain and a smaller tail domain with calmodulin-binding motifs and a positively charged region that facilitates binding to phospholipids vesicles [139]. It has been suggested that myosin Ia associated with SI in lipid rafts is implicated in the retention of this enzyme within the apical brush border of enterocytes [140]. A mislocalization of SI and galectin-4 to the basolateral surface in intestinal epithelia of myosin Ia knockout mice can be explained by the destabilization of lipid rafts in the absence of the actin motor [141]. However, biochemical and microscopic studies localized myosin Ia on tubulo-vesicular structures and indicate that this motor is involved in endosomal membrane trafficking [117, 142, 143]. The link between the two observations is

that SI associates with lipid rafts already in the TGN, and is transported with these lipid platforms to the cell surface. An absence of myosin Ia in knockout mice could therefore alter the trafficking of SI containing carriers and sort this enzyme to the basolateral membrane. Evidence for an impact of myosin Ia on sorting endosomes comes from exogenous expression of truncated mutants with a dominant negative effect on this actin motor in Caco-2 cells [144]. In living mouse hepatoma cells, the expression of non-functional myosin Ia impaired the directionality of long-range lysosomal movements, thus suggesting a general function of this motor in organelle transport [145]. Nevertheless, the concrete mechanism of myosin Ia activity on vesicular or endosomal carriers still remains to be solved. Many myosin motors are regulated by phosphorylation and the recently identified SAV-associated protein kinase, alpha kinase I (ALPK1), is capable of phosphorylating myosin Ia *in vitro* [142]. Moreover, siRNA-mediated depletion of this kinase in MDCK cells results in a significant decrease in delivery of SI to the apical membrane. The observation that ALPK1 knockdown does not result in redirection of SI to the basolateral cell surface indicates that this alpha kinase plays a role in the apical transport process and not in polarized sorting of SI.

Some polypeptides of the annexin family also participate in apical transport of raft-associated membrane proteins. Annexin-13b has been identified in post-Golgi vesicles from MDCK cells and associates with lipid rafts [146, 147]. This annexin recruits the ubiquitin protein ligase Nedd4, which might modulate the dynamics of membrane microdomains [148]. Antibodies directed against annexin-13b inhibited the transport of the HA glycoprotein from the TGN to the apical plasma membrane [146], and a similar effect on apical trafficking of SI could be observed after siRNA-mediated depletion of annexin-2 in MDCK cells [149]. Annexin-2 associates with lipid raft containing apical vesicular or tubulo-vesicular carrier populations, but is not involved in raft-independent apical trafficking [149–151]. Neither the HA glycoprotein nor SI are shifted to the basolateral surface following annexin-13b or annexin-2 depletion [146, 149]. Hence, the two annexins participate in lipid raft-dependent apical transport pathways, while the underlying mechanisms of action appear to be diverse. For annexin-2, it is well known that it interacts in a heterotetrameric complex with a smaller S100A10 subunit with lipid rafts in a cholesterol-dependent manner [152], and it has been published that the S100A10 subunit is also implicated in membrane trafficking of a variety of channel proteins [153–155]. Based on its binding capacity to lipid rafts and to F-actin [151, 156], a suggested role for the annexin-2/S100A10 complex is to form a link between lipid raft carrying vesicles and cytoskeletal microfilaments.

In fact, the microtubular network of polarized epithelial cells has a crucial function in surface delivery of membrane proteins. In contrast to the classical organization of microtubules in fibroblastic cells, most microtubules of epithelial cells are noncentrosomal and align along the apico-basal polarity axis of the cell [157, 158]. Moreover, MDCK and Caco-2 cells also harbor a horizontal meshwork of microtubules with mixed orientations underlying the cell apex and at the cell base [157–159]. The perturbation of this microtubular architecture has dramatic effects on apical protein transport, as has been demonstrated using microtubule-acting drugs [131, 160–163], even though the dependence of apical traffic on the integrity of microtubules varies for distinct proteins analyzed [164, 165]. In fact, an involvement of microtubules in apical transport has been confirmed by live cell imaging of apical proteins in non-polarized and fully polarized MDCK cells [106, 109, 117, 166], which depicts vesicular or tubulo-vesicular structures leaving the TGN along microtubular tracks. Hence, microtubular motors exposed on the cytosolic surface of these transport carriers catalyze the observed movements.

Evidence for a substantial role of kinesin motors comes from studies based on microinjection of anti-kinesin antibodies. Here, surface delivery of the apical marker p75NTR to the apical membrane was blocked following kinesin inhibition [166]. For lipid raft-dependent apical transport routes, an involvement of the minus end directed kinesin KIFC3 in surface delivery of influenza HA has been demonstrated [167]. This motor interacts with annexin 13b in TX-100-insoluble membrane organelles. Moreover, a variant of the dynein family is also implicated in apical trafficking, since cytoplasmic dynein binds to the cytosolic tail of rhodopsin and mediates translocation of this exogenously expressed photo-pigment to the apical membrane domain of MDCK cells [168]. Interestingly, this dynein-dependent apical transport can be modulated by the equilibrium between dynein light chain Tctex-1, which directly interacts with rhodopsin's cytosolic tail, and its homologue RP3. Altogether, microtubular motors so far identified in apical transport move to the minus end of microtubules, presumably along microtubules oriented from basal to apical along the apico-basal polarity axis.

Docking and fusion of carrier vesicles at the plasma membrane

Docking and fusion of carrier vesicles is based on protein tethering at the plasma membrane, and results in secretion of the transported material from the cell. The specificity of this process is ensured by interactions of SNARE proteins (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor), which have been identified initially in rat neuronal cells [169]. They can be divided into two groups, the vesicle-SNAREs or v-SNAREs, related to synaptobrevin or vesicle associated membrane

protein (VAMP), a protein localized on synaptic vesicles, and target-SNAREs or t-SNAREs, related to syntaxin-1 and synaptosomal-associated protein of 25 kDa (SNAP-25). V- and t-SNAREs define associated membranes for fusion. Nevertheless, they are not sufficient to ensure the fusion process itself and require accessory proteins for exocytosis [170, 171]. A polarized membrane distribution of t-SNAREs can be found in several cell types. Syntaxin-4 is localized to the basolateral plasma membrane of MDCK cells, hepatocytes, pancreatic cells [172–174], and syntaxin-3 accumulates at the apical plasma membrane of MDCK cells [174], enterocytes [175] and hepatocytes [172]; it is also present on zymogen granules, which fuse with the apical plasma membrane in pancreatic cells [173]. In MDCK cells, this apical localization of syntaxin-3 depends on intact microtubules [109]. Overexpression of syntaxin-3 or injection of anti-syntaxin-3 antibodies results in an accumulation of transport vesicles beneath the apical membrane and perturbs protein integration into the apical membrane [176, 177]. In addition, two isoforms of the SNARE machinery, SNAP-23 and α-SNAP, contribute to syntaxin-3-mediated apical fusion events [177]. Recently, Mostov and coworkers [178] have shown that syntaxin-3 plays an essential role for apical membrane localization of gp80 and p75NTR, and so for specificity of membrane fusion at the plasma membrane, using chimeric proteins between syntaxin-3 and syntaxin-4.

In view of the fact that lipid rafts represent one platform for apical transport, further studies indicated that syntaxin-3 and the corresponding v-SNARE Ti-VAMP together with SNAP-23 could be also detected in lipid rafts [176]. It had been demonstrated before, that Ti-VAMP (VAMP-7) is part of a SNARE complex at the apical plasma membrane of Caco-2 cells [179]. The accessibility of SNAREs is also regulated. For example, in yeast, Sec1p plays a role as t-SNARE 'protector' [180]. A similar phenomenon has been observed for the homologous variants nSec-1 or Munc18 in epithelial cells [181]. Munc18-2 is localized in association with syntaxin-3 on the apical plasma membrane of epithelial cells [182]. Furthermore, Munc18-2 displaced syntaxin-3 from SNAP-23 and overexpression of Munc18-2 inhibited apical delivery of HA [183], thus suggesting that this factor controls apical vesicle fusion in epithelial cells.

Conclusions

The past three decades have witnessed a notable increase in our knowledge about signals for apical protein sorting and cellular factors that escort and direct them to the apical membrane compartment. This knowledge raises new questions about their interplay in protein trafficking, the compartments traversed in this intracellular journey and how distinct trafficking pathways are coordinated. Based on an increase in information about protein transport routes, we are now confronted with a remarkable number of trafficking connections between distinct intracellular compartments and domains of the plasma membrane. Therefore, it will be helpful to define main stream routes to the apical or the basolateral cell surface of epithelial cells and separate them from bypassing ones. The whole scenario becomes even more complicated by the observation that similar mechanisms for cell surface transport are used in polarized and non-polarized cells, *e.g.* fibroblasts [184, 185]. As a consequence, key elements of the sorting and transport machinery are available in both cell types. Undoubtedly, it will be complex to solve these unanswered questions. We will depend on carefully chosen model systems, powerful imaging techniques and highly sensitive protein interaction assays to understand the molecular details underlying apical protein trafficking.

Acknowledgements. We are indebted to the countless scientists whose research provided the basis for this review. Work from our laboratory was supported by the Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany grants JA 1033, the Sonderforschungsbereich 593, and by the International relationship INSERM (Institut National de la Sante et de la Recherche Medicale)-DFG.

1 Yeaman, C., Grindstaff, K. K. and Nelson, W. J. (1999) New perspectives on mechanisms involved in generating epithelial cell polarity. Physiol. Rev. 79, 73–98.
2 Aroeti, B., Okhrimenko, H., Reich, V. and Orzech, E. (1998) Polarized trafficking of plasma membrane proteins: emerging roles for coats, SNAREs, GTPases and their link to the cytoskeleton. Biochim. Biophys. Acta 1376, 57–90.
3 Rodriguez-Boulan, E. and Powell, S. K. (1992) Polarity of epithelial and neuronal cells. Annu. Rev. Cell Biol. 8, 395–427.
4 Simons, K. and van Meer, G. (1988) Lipid sorting in epithelial cells. Biochemistry 27, 6197–6202.
5 Caplan, M. J. (1997) Membrane polarity in epithelial cells: protein sorting and establishment of polarized domains. Am. J. Physiol. 272, F425–429.
6 Boulan, E. R. and Sabatini, D. D. (1978) Asymmetric budding of viruses in epithelial monlayers: a model system for study of epithelial polarity. Proc. Natl. Acad. Sci. USA 75, 5071–5075.
7 Boulan, E. R. and Pendergast, M. (1980) Polarized distribution of viral envelope proteins in the plasma membrane of infected epithelial cells. Cell 20, 45–54.
8 Rodriguez-Boulan, E., Paskiet, K. T., Salas, P. J. and Bard, E. (1984) Intracellular transport of influenza virus hemagglutinin to the apical surface of Madin-Darby canine kidney cells. J. Cell Biol. 98, 308–319.
9 Griffiths, G. and Simons, K. (1986) The trans Golgi network: sorting at the exit site of the Golgi complex. Science 234, 438–443.
10 Matlin, K. S. and Simons, K. (1984) Sorting of an apical plasma membrane glycoprotein occurs before it reaches the cell surface in cultured epithelial cells. J. Cell Biol. 99, 2131–2139.
11 Misek, D. E., Bard, E. and Rodriguez-Boulan, E. (1984) Biogenesis of epithelial cell polarity: intracellular sorting and vectorial exocytosis of an apical plasma membrane glycoprotein. Cell 39, 537–546.
12 Le Bivic, A., Sambuy, Y., Patzak, A., Patil, N., Chao. M, and Rodriguez-Boulan, E. (1991) An internal deletion in the cy-

toplasmic tail reverses the apical localization of human NGF receptor in transfected MDCK cells. J. Cell Biol. 115, 607–618.
13 Mostov, K. E. and Deitcher, D. L. (1986) Polymeric immunoglobulin receptor expressed in MDCK cells transcytoses IgA. Cell 46, 613–621.
14 Danielsen, E. M., Sjostrom, H. and Noren, O. (1983) Biosynthesis of intestinal microvillar proteins. Pulse-chase labelling studies on maltase-glucoamylase, aminopeptidase A and dipeptidyl peptidase IV. Biochem. J. 210, 389–393.
15 Hauri, H. P., Sterchi, E. E., Bienz, D., Fransen, J. A. and Marxer, A. (1985) Expression and intracellular transport of microvillus membrane hydrolases in human intestinal epithelial cells. J. Cell Biol. 101, 838–851.
16 Lisanti, M. P., Sargiacomo, M., Graeve, L., Saltiel, A. R. and Rodriguez-Boulan, E. (1988) Polarized apical distribution of glycosyl-phosphatidylinositol-anchored proteins in a renal epithelial cell line. Proc. Natl. Acad. Sci. USA 85, 9557–9561.
17 Casanova, J. E., Apodaca, G. and Mostov, K. E. (1991) An autonomous signal for basolateral sorting in the cytoplasmic domain of the polymeric immunoglobulin receptor. Cell 66, 65–75.
18 Matter, K., Hunziker, W. and Mellman, I. (1992) Basolateral sorting of LDL receptor in MDCK cells: the cytoplasmic domain contains two tyrosine-dependent targeting determinants. Cell 71, 741–753.
19 Mostov, K. E., de Bruyn Kops, A. and Deitcher, D. L. (1986) Deletion of the cytoplasmic domain of the polymeric immunoglobulin receptor prevents basolateral localization and endocytosis. Cell 47, 359–364.
20 Aroeti, B., Kosen, P. A., Kuntz, I. D., Cohen, F. E. and Mostov, K. E. (1993) Mutational and secondary structural analysis of the basolateral sorting signal of the polymeric immunoglobulin receptor. J. Cell Biol. 123, 1149–1160.
21 Beau, I., Groyer-Picard, M. T., Desroches, A., Condamine, E., Leprince, J., Tome, J. P., Dessen, P., Vaudry, H. and Misrahi, M. (2004) The basolateral sorting signals of the thyrotropin and luteinizing hormone receptors: an unusual family of signals sharing an unusual distal intracellular localization, but unrelated in their structures. Mol. Endocrinol. 18, 733–746.
22 Lin, S., Naim, H. Y. and Roth, M. G. (1997) Tyrosine-dependent basolateral sorting signals are distinct from tyrosine-dependent internalization signals. J. Biol. Chem. 272, 26300–26305.
23 Odorizzi, G. and Trowbridge, I. S. (1997) Structural requirements for basolateral sorting of the human transferrin receptor in the biosynthetic and endocytic pathways of Madin-Darby canine kidney cells. J. Cell Biol. 137, 1255–1264.
24 Ohno, H., Stewart, J., Fournier, M. C., Bosshart, H., Rhee, I., Miyatake, S., Saito, T., Gallusser, A., Kirchhausen, T. and Bonifacino, J. S. (1995) Interaction of tyrosine-based sorting signals with clathrin-associated proteins. Science 269, 1872–1875.
25 Bonifacino, J. S. and Dell'Angelica, E. C. (1999) Molecular bases for the recognition of tyrosine-based sorting signals. J. Cell Biol. 145, 923–926.
26 Rapoport, I., Chen, Y. C., Cupers, P., Shoelson, S. E. and Kirchhausen, T. (1998) Dileucine-based sorting signals bind to the beta chain of AP-1 at a site distinct and regulated differently from the tyrosine-based motif-binding site. EMBO J. 17, 2148–2155.
27 Folsch, H., Ohno, H., Bonifacino, J. S. and Mellman, I. (1999) A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. Cell 99, 189–198.
28 Brown, D. A., Crise, B. and Rose, J. K. (1989) Mechanism of membrane anchoring affects polarized expression of two proteins in MDCK cells. Science 245, 1499–1501.
29 Brown, D. A. and Rose, J. K. (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. Cell 68, 533–544.
30 Garcia, M., Mirre, C., Quaroni, A., Reggio, H. and Le Bivic, A. (1993) GPI-anchored proteins associate to form microdomains during their intracellular transport in Caco-2 cells. J. Cell Sci. 104, 1281–1290.
31 Wilson, J. M., Fasel, N. and Kraehenbuhl, J. P. (1990) Polarity of endogenous and exogenous glycosyl-phosphatidylinositol-anchored membrane proteins in Madin-Darby canine kidney cells. J. Cell Sci. 96, 143–149.
32 Powell, S. K., Cunningham, B. A., Edelman, G. M. and Rodriguez-Boulan, E. (1991) Targeting of transmembrane and GPI-anchored forms of N-CAM to opposite domains of a polarized epithelial cell. Nature 353, 76–77.
33 Lipardi, C., Nitsch, L. and Zurzolo, C. (2000) Detergent-insoluble GPI-anchored proteins are apically sorted in fischer rat thyroid cells, but interference with cholesterol or sphingolipids differentially affects detergent insolubility and apical sorting. Mol. Biol. Cell 11, 531–542.
34 Benting, J. H, Rietveld, A. G. and Simons, K. (1999) *N*-Glycans mediate the apical sorting of a GPI-anchored, raft- associated protein in Madin-Darby canine kidney cells. J. Cell Biol. 146, 313–320.
35 Alfalah, M., Jacob, R., Preuss, U., Zimmer, K. P., Naim, H. and Naim, H. Y. (1999) O-linked glycans mediate apical sorting of human intestinal sucrase-isomaltase through association with lipid rafts. Curr. Biol. 9, 593–596.
36 Jacob, R., Alfalah, M., Grunberg, J., Obendorf, M. and Naim, H. Y. (2000) Structural determinants required for apical sorting of an intestinal brush-border membrane protein. J. Biol. Chem. 275, 6566–6572.
37 Yeaman, C., Le Gall, A. H., Baldwin, A. N., Monlauzeur, L., Le Bivic, A. and Rodriguez-Boulan, E. (1997) The *O*-glycosylated stalk domain is required for apical sorting of neurotrophin receptors in polarized MDCK cells. J. Cell Biol. 139, 929–940.
38 Urban, J., Parczyk, K., Leutz, A., Kayne, M. and Kondor-Koch, C. (1987) Constitutive apical secretion of an 80-kD sulfated glycoprotein complex in the polarized epithelial Madin-Darby canine kidney cell line. J. Cell Biol. 105, 2735–2743.
39 Le Bivic, A., Garcia, M. and Rodriguez-Boulan, E. (1993) Ricin-resistant Madin-Darby canine kidney cells missort a major endogenous apical sialoglycoprotein. J. Biol. Chem. 268, 6909–6916.
40 Scheiffele, P., Peranen, J. and Simons, K. (1995) *N*-glycans as apical sorting signals in epithelial cells. Nature 378, 96–98.
41 Kitagawa, Y., Sano, Y., Ueda, M., Higashio, K., Narita, H., Okano, M., Matsumoto, S. and Sasaki, R. (1994) *N*-glycosylation of erythropoietin is critical for apical secretion by Madin-Darby canine kidney cells. Exp. Cell Res. 213, 449–457.
42 Ihrke, G., Bruns, J. R., Luzio, J. P. and Weisz, O. A. (2001) Competing sorting signals guide endolyn along a novel route to lysosomes in MDCK cells. EMBO J. 20, 6256–6264.
43 Martinez-Maza, R., Poyatos, I., Lopez-Corcuera, B., Núñez, E., Gimenez, C., Zafra, F. and Aragon, C. (2001) The role of *N*-glycosylation in transport to the plasma membrane and sorting of the neuronal glycine transporter GLYT2. J. Biol. Chem. 276, 2168–2173.
44 Pang, S., Urquhart, P. and Hooper, N. M. (2004) N-glycans, not the GPI anchor, mediate the apical targeting of a naturally glycosylated, GPI-anchored protein in polarised epithelial cells. J. Cell Sci. 117, 5079–5086.
45 Marzolo, M. P., Bull, P. and Gonzalez, A. (1997) Apical sorting of hepatitis B surface antigen (HBsAg) is independent of *N*-glycosylation and glycosylphosphatidylinositol-anchored protein segregation. Proc. Natl. Acad. Sci. USA 94, 1834–1839.
46 Trischler, M., Koch-Brandt, C. and Ullrich, O. (2001) Apical transport of osteopontin is independent of *N*-glycosylation and sialylation. Mol. Membr. Biol. 18, 275–281.

47 Meerson, N. R., Bello, V., Delaunay, J. L., Slimane, T. A., Delautier, D., Lenoir, C., Trugnan, G. and Maurice, M. (2000) Intracellular traffic of the ecto-nucleotide pyrophosphatase/phosphodiesterase NPP3 to the apical plasma membrane of MDCK and Caco-2 cells: apical targeting occurs in the absence of N-glycosylation. J. Cell Sci. 113, 4193–4202.

48 Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. Nature 387, 569–572.

49 Breuza, L., Garcia, M., Delgrossi, M. H. and Le Bivic, A. (2002) Role of the membrane-proximal O-glycosylation site in sorting of the human receptor for neurotrophins to the apical membrane of MDCK cells. Exp. Cell Res. 273, 178–186.

50 Spodsberg, N., Alfalah, M. and Naim, H. Y. (2001) Characteristics and structural requirements of apical sorting of the rat growth hormone through the O-glycosylated stalk region of intestinal sucrase-isomaltase. J. Biol. Chem. 276, 46597–46604.

51 Huet, G., Hennebicq-Reig, S., de Bolos, C., Ulloa, F., Lesuffleur, T., Barbat, A., Carriere, V., Kim, I., Real, F. X., Delannoy, P. and Zweibaum, A. (1998) GalNAc-alpha-O-benzyl inhibits NeuAcalpha2-3 glycosylation and blocks the intracellular transport of apical glycoproteins and mucus in differentiated HT-29 cells. J. Cell Biol. 141, 1311–1322.

52 Gouyer, V., Leteurtre, E., Delmotte, P., Steelant, W. F., Krzewinski-Recchi, M. A., Zanetta, J. P., Lesuffleur, T., Trugnan, G., Delannoy, P. and Huet, G. (2001) Differential effect of GalNAcalpha-O-bn on intracellular trafficking in enterocytic HT-29 and Caco-2 cells: correlation with the glycosyltransferase expression pattern. J. Cell Sci. 114, 1455–1471.

53 Naim, H. Y., Joberty, G., Alfalah, M. and Jacob, R. (1999) Temporal association of the N- and O-linked glycosylation events and their implication in the polarized sorting of intestinal brush border sucrase-isomaltase, aminopeptidase N, and dipeptidyl peptidase IV. J. Biol. Chem. 274, 17961–17967.

54 Slimane, T. A., Lenoir, C., Sapin, C., Maurice, M. and Trugnan, G. (2000) Apical secretion and sialylation of soluble dipeptidyl peptidase IV are two related events. Exp. Cell Res. 258, 184–194.

55 Delacour, D., Gouyer, V., Leteurtre, E., Ait-Slimane, T., Drobecq, H., Lenoir, C., Moreau-Hannedouche, O., Trugnan, G. and Huet, G. (2003) 1-Benzyl-2-acetamido-2-deoxy-alpha-D-galactopyranoside blocks the apical biosynthetic pathway in polarized HT-29 cells. J. Biol. Chem. 278, 37799–37809.

56 Delacour, D., Gouyer, V., Zanetta, J. P., Drobecq, H., Leteurtre, E., Grard, G., Moreau-Hannedouche, O., Maes, E., Pons, A., Andre, S., Le Bivic, A., Gabius, H. J., Manninen, A., Simons, K. and Huet, G. (2005) Galectin-4 and sulfatides in apical membrane trafficking in enterocyte-like cells. J. Cell Biol. 169, 491–501.

57 Potter, B. A., Ihrke, G., Bruns, J. R., Weixel, K. M. and Weisz, O. A. (2004) Specific N-glycans direct apical delivery of transmembrane, but not soluble or glycosylphosphatidylinositol-anchored forms of endolyn in Madin-Darby canine kidney cells. Mol. Biol. Cell 15, 1407–1416.

58 Vagin, O., Turdikulova, S. and Sachs, G. (2004) The H,K-ATPase beta subunit as a model to study the role of N-glycosylation in membrane trafficking and apical sorting. J. Biol. Chem. 279, 39026–39034.

59 Rodriguez-Boulan E and Gonzalez, A. (1999) Glycans in post-Golgi apical targeting: sorting signals or structural props? Trends Cell Biol. 9, 291–294.

60 Kundu, A., Avalos, R. T., Sanderson, C. M. and Nayak, D. P. (1996) Transmembrane domain of influenza virus neuraminidase, a type II protein, possesses an apical sorting signal in polarized MDCK cells. J. Virol. 70, 6508–6515.

61 Lin, S., Naim, H. Y., Rodriguez, A. C. and Roth, M. G. (1998) Mutations in the middle of the transmembrane domain reverse the polarity of transport of the influenza virus hemagglutinin in MDCK epithelial cells. J. Cell Biol. 142, 51–57.

62 Scheiffele, P., Roth, M. G. and Simons, K. (1997) Interaction of influenza virus haemagglutinin with sphingolipid- cholesterol membrane domains via its transmembrane domain. EMBO J. 16, 5501–5508.

63 Barman S and Nayak, D. P. (2000) Analysis of the transmembrane domain of influenza virus neuraminidase, a type II transmembrane glycoprotein, for apical sorting and raft association. J. Virol. 74, 6538–6545.

64 Munro, S. (1995) An investigation of the role of transmembrane domains in Golgi protein retention. EMBO J. 14, 4695–4704.

65 Nezil, F. A. and Bloom, M. (1992) Combined influence of cholesterol and synthetic amphiphillic peptides upon bilayer thickness in model membranes. Biophys. J. 61, 1176–1183.

66 Schuck, S. and Simons, K. (2004) Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. J. Cell Sci. 117, 5955–5964.

67 Chuang, J. Z. and Sung, C. H. (1998) The cytoplasmic tail of rhodopsin acts as a novel apical sorting signal in polarized MDCK cells. J. Cell Biol. 142, 1245–1256.

68 Marzolo, M. P., Yuseff, M. I., Retamal, C., Donoso, M., Ezquer, F., Farfan, P., Li, Y. and Bu, G. (2003) Differential distribution of low-density lipoprotein-receptor-related protein (LRP) and megalin in polarized epithelial cells is determined by their cytoplasmic domains. Traffic 4, 273–288.

69 Takeda, T., Yamazaki, H. and Farquhar, M. G. (2003) Identification of an apical sorting determinant in the cytoplasmic tail of megalin. Am. J. Physiol. Cell Physiol. 284, C1105–C1113.

70 Hodson, C. A., Ambrogi, I. G., Scott, R. O., Mohler, P. J. and Milgram, S. L. (2006) Polarized apical sorting of guanylyl cyclase C is specified by a cytosolic signal. Traffic 7, 456–464.

71 Tai, A. W., Chuang, J. Z., Bode, C., Wolfrum, U. and Sung, C. H. (1999) Rhodopsin's carboxy-terminal cytoplasmic tail acts as a membrane receptor for cytoplasmic dynein by binding to the dynein light chain Tctex-1. Cell 97, 877–887.

72 Alonso, M. A., Fan, L. and Alarcon, B. (1997) Multiple sorting signals determine apical localization of a nonglycosylated integral membrane protein. J. Biol. Chem. 272, 30748–30752.

73 Kadowaki, T., Tsukuba, T., Bertenshaw, G. P. and Bond, J. S. (2000) N-Linked oligosaccharides on the meprin A metalloprotease are important for secretion and enzymatic activity, but not for apical targeting. J. Biol. Chem. 275, 25577–25584.

74 Kuhn, U., Cohn, D. V. and Gorr, S. U. (2000) Polarized secretion of the regulated secretory protein chromogranin A. Biochem. Biophys. Res. Commun. 270, 631–636.

75 Larsen, J. E., Avvakumov, G. V., Hammond, G. L. and Vogel, L. K. (1999) N-glycans are not the signal for apical sorting of corticosteroid binding globulin in MDCK cells. FEBS Lett. 451, 19–22.

76 Paladino, S., Sarnataro, D., Pillich, R., Tivodar, S., Nitsch, L. and Zurzolo, C. (2004) Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins. J. Cell Biol. 167, 699–709.

77 Helms, J. B and Zurzolo, C. (2004) Lipids as targeting signals: lipid rafts and intracellular trafficking. Traffic 5, 247–254.

78 Yu, J., Fischman, D. A. and Steck, T. L. (1973) Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. J. Supramol. Struct. 1, 233–248.

79 Thompson, T. E and Tillack, T. W. (1985) Organization of glycosphingolipids in bilayers and plasma membranes of mammalian cells. Annu. Rev. Biophys. Biophys. Chem. 14, 361–386.

80 Wandinger-Ness, A., Bennett, M. K., Antony, C. and Simons, K. (1990) Distinct transport vesicles mediate the delivery of plasma membrane proteins to the apical and basolateral domains of MDCK cells. J. Cell Biol. 111, 987–1000.

81 Skibbens, J. E., Roth, M. G. and Matlin, K. S. (1989) Differential extractability of influenza virus hemagglutinin during

intracellular transport in polarized epithelial cells and nonpolar fibroblasts. J. Cell Biol. 108, 821–832.
82 Hooper, N. M. (1999) Detergent-insoluble glycosphingolipid/cholesterol-rich membrane domains, lipid rafts and caveolae. Mol. Membr. Biol. 16, 145–156.
83 Simons K and Vaz, W. L. (2004) Model systems, lipid rafts, and cell membranes. Annu. Rev. Biophys. Biomol. Struct. 33, 269–295.
84 Zurzolo, C., van Meer, G. and Mayor, S. (2003) The order of rafts. Conference on microdomains, lipid rafts and caveolae. EMBO Rep. 4, 1117–1121.
85 Heerklotz, H. (2002) Triton promotes domain formation in lipid raft mixtures. Biophys. J. 83, 2693–2701.
86 Edidin, M. (2003) The state of lipid rafts: from model membranes to cells. Annu. Rev. Biophys. Biomol. Struct. 32, 257–283.
87 Brugger, B., Glass, B., Haberkant, P., Leibrecht, I., Wieland, F. T. and Krausslich, H. G. (2006) The HIV lipidome: A raft with an unusual composition. Proc. Natl. Acad. Sci. USA 103, 2641–2646.
88 Bagnat, M., Keranen, S., Shevchenko, A. and Simons, K. (2000) Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. Proc. Natl. Acad. Sci. USA 97, 3254–3259.
89 Sarnataro, D., Campana, V., Paladino, S., Stornaiuolo, M., Nitsch, L. and Zurzolo, C. (2004) PrP(C) association with lipid rafts in the early secretory pathway stabilizes its cellular conformation. Mol. Biol. Cell 15, 4031–4042.
90 Gut, A., Kappeler, F., Hyka, N., Balda, M. S., Hauri, H. P. and Matter, K. (1998) Carbohydrate-mediated Golgi to cell surface transport and apical targeting of membrane proteins. EMBO J. 17, 1919–1929.
91 Hunziker, W., Harter, C., Matter, K. and Mellman, I. (1991) Basolateral sorting in MDCK cells requires a distinct cytoplasmic domain determinant. Cell 66, 907–920.
92 Prill, V., Lehmann, L., von Figura, K. and Peters, C. (1993) The cytoplasmic tail of lysosomal acid phosphatase contains overlapping but distinct signals for basolateral sorting and rapid internalization in polarized MDCK cells. EMBO J. 12, 2181–2193.
93 Matter, K., Yamamoto, E. M. and Mellman, I. (1994) Structural requirements and sequence motifs for polarized sorting and endocytosis of LDL and Fc receptors in MDCK cells. J. Cell Biol. 126, 991–1004.
94 Thomas, D. C. and Roth, M. G. (1994) The basolateral targeting signal in the cytoplasmic domain of glycoprotein G from vesicular stomatitis virus resembles a variety of intracellular targeting motifs related by primary sequence but having diverse targeting activities. J. Biol. Chem. 269, 15732–15739.
95 Jacob, R., Preuss, U., Panzer, P., Alfalah, M., Quack, S., Roth, M. G., Naim, H. and Naim, H. Y. (1999) Hierarchy of sorting signals in chimeras of intestinal lactase-phlorizin hydrolase and the influenza virus hemagglutinin. J. Biol. Chem. 274, 8061–8067.
96 Rodriguez-Boulan, E., Kreitze, G. and Musch, A. (2005) Organization of vesicular trafficking in epithelia. Nat. Rev. Mol. Cell Biol. 6, 233–247.
97 Muth, T. R. and Caplan, M. J. (2003) Transport protein trafficking in polarized cells. Annu. Rev. Cell Dev. Biol. 19, 333–366.
98 Fiedler, K., Parton, R. G., Kellner, R., Etzold, T. and Simons, K. (1994) VIP36, a novel component of glycolipid rafts and exocytic carrier vesicles in epithelial cells. EMBO J. 13, 1729–1740.
99 Fullekrug, J., Scheiffele, P. and Simons, K. (1999) VIP36 localisation to the early secretory pathway. J. Cell Sci. 112, 2813–2821.
100 Hara-Kuge, S., Ohkura, T., Ideo, H., Shimada, O., Atsumi, S. and Yamashita, K. (2002) Involvement of VIP36 in intracellular transport and secretion of glycoproteins in polarized Madin-Darby canine kidney (MDCK) cells. J. Biol. Chem. 277, 16332–16339.
101 Cheong, K. H., Zacchetti, D., Schneeberger, E. E. and Simons, K. (1999) VIP17/MAL, a lipid raft-associated protein, is involved in apical transport in MDCK cells. Proc. Natl. Acad. Sci. USA 96, 6241–6248.
102 Martin-Belmonte, F., Puertollano, R., Millan, J. and Alonso, M. A. (2000) The MAL proteolipid is necessary for the overall apical delivery of membrane proteins in the polarized epithelial madin-darby canine kidney and fischer rat thyroid cell lines. Mol. Biol. Cell 11, 2033–2045.
103 Puertollano, R., Martin-Belmonte, F., Millan, J., de Marco MC., Albar, J. P., Kremer, L. and Alonso, M. A. (1999) The MAL proteolipid is necessary for normal apical transport and accurate sorting of the influenza virus hemagglutinin in Madin- Darby canine kidney cells. J. Cell Biol. 145, 141–151.
104 Danielsen, E. M and van Deurs, B. (1997) Galectin-4 and small intestinal brush border enzymes form clusters. Mol. Biol. Cell 8, 2241–2251.
105 Delacour, D., Cramm-Behrens, C. I., Drobecq, H., Le Bivic, A., Naim, H. Y. and Jacob, R. (2006) Requirement for galectin-3 in apical protein sorting. Curr. Biol. 16, 408–414.
106 Keller, P., Toomre, D., Diaz, E., White, J. and Simons, K. (2001) Multicolour imaging of post-Golgi sorting and trafficking in live cells. Nat. Cell Biol. 3, 140–149.
107 Rindler, M. J., Ivanov, I. E., Plesken, H. and Sabatini, D. D. (1985) Polarized delivery of viral glycoproteins to the apical and basolateral plasma membranes of Madin-Darby canine kidney cells infected with temperature-sensitive viruses. J. Cell Biol. 100, 136–151.
108 Jacob, R. and Naim, H. Y. (2001) Apical membrane proteins are transported in distinct vesicular carriers. Curr. Biol. 11, 1444–1450.
109 Kreitzer, G., Schmoranzer, J., Low, S. H., Li, X., Gan, Y., Weimbs, T., Simon, S. M. and Rodriguez-Boulan, E. (2003) Three-dimensional analysis of post-Golgi carrier exocytosis in epithelial cells. Nat. Cell Biol. 5, 126–136.
110 Toomre, D., Keller, P., White, J., Olivo, J. C. and Simons, K. (1999) Dual-color visualization of trans-Golgi network to plasma membrane traffic along microtubules in living cells. J. Cell Sci. 112, 21–33.
111 Hua, W., Sheff, D., Toomre, D. and Mellman, I. (2006) Vectorial insertion of apical and basolateral membrane proteins in polarized epithelial cells revealed by quantitative 3D live cell imaging. J. Cell Biol. 172, 1035–1044.
112 Ang AL., Taguchi, T., Francis, S., Folsch, H., Murrells, L. J., Pypaert, M., Warren, G. and Mellman, I. (2004) Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. J. Cell Biol. 167, 531–543.
113 Sheff, D. R., Daro, E. A., Hull, M. and Mellman, I. (1999) The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions. J. Cell Biol. 145, 123–139.
114 Gagescu, R., Demaurex, N., Parton, R. G., Hunziker, W., Huber, L. A. and Gruenberg, J. (2000) The recycling endosome of Madin-Darby canine kidney cells is a mildly acidic compartment rich in raft components. Mol. Biol. Cell 11, 2775–2791.
115 Fivaz, M., Vilbois, F., Thurnheer, S., Pasquali, C., Abrami, L., Bickel, P. E., Parton, R. G. and van der Goot, F. G. (2002) Differential sorting and fate of endocytosed GPI-anchored proteins. EMBO J. 21, 3989–4000.
116 Mobius, W., van Donselaar, E., Ohno-Iwashita, Y., Shimada, Y., Heijnen, H. F., Slot, J. W. and Geuze, H. J. (2003) Recycling compartments and the internal vesicles of multivesicular bodies harbor most of the cholesterol found in the endocytic pathway. Traffic 4, 222–231.

117 Jacob, R., Heine, M., Alfalah, M. and Naim, H. Y. (2003) Distinct cytoskeletal tracks direct individual vesicle populations to the apical membrane of epithelial cells. Curr. Biol. 13, 607–612.

118 Mostov, K. E., Verges, M. and Altschuler, Y. (2000) Membrane traffic in polarized epithelial cells. Curr. Opin. Cell Biol. 12, 483–490.

119 Van IJzendoorn, S. C and Hoekstra, D. (1999) The subapical compartment: a novel sorting centre? Trends Cell Biol. 9, 144–149.

120 Polishchuk, R., Di Pentima, A. and Lippincott-Schwartz, J. (2004) Delivery of raft-associated, GPI-anchored proteins to the apical surface of polarized MDCK cells by a transcytotic pathway. Nat. Cell Biol. 6, 297–307.

121 Paladino, S., Pocard, T., Catino, M. A. and Zurzolo, C. (2006) GPI-anchored proteins are directly targeted to the apical surface in fully polarized MDCK cells. J. Cell Biol. 172, 1023–1034.

122 De Matteis, M. A. and Godi, A. (2004) PI-loting membrane traffic. Nat. Cell Biol. 6, 487–492.

123 Godi, A., Di Campli, A., Konstantakopoulos, A., Di Tullio, G., Alessi, D. R., Kular, G. S., Daniele, T., Marra, P., Lucocq, J. M. and De Matteis, M. A. (2004) FAPPs control Golgi-to-cell-surface membrane traffic by binding to ARF and PtdIns(4)P. Nat. Cell Biol. 6, 393–404.

124 Vieira, O. V., Verkade, P., Manninen, A. and Simons, K. (2005) FAPP2 is involved in the transport of apical cargo in polarized MDCK cells. J. Cell Biol. 170, 521–526.

125 Roth, M. (2004) New candidates for vesicle coat proteins. Nat. Cell Biol. 6, 384–385.

126 Tilney, L. G., Hatano, S., Ishikawa, H. and Mooseker, M. S. (1973) The polymerization of actin: its role in the generation of the acrosomal process of certain echinoderm sperm. J. Cell Biol. 59, 109–126.

127 Maples, C. J., Ruiz, W. G. and Apodaca, G. (1997) Both microtubules and actin filaments are required for efficient postendocytotic traffic of the polymeric immunoglobulin receptor in polarized Madin-Darby canine kidney cells. J. Biol. Chem. 272, 6741–6751.

128 Ojakian, G. K and Schwimmer, R. (1988) The polarized distribution of an apical cell surface glycoprotein is maintained by interactions with the cytoskeleton of Madin-Darby canine kidney cells. J. Cell Biol. 107, 2377–2387.

129 Valentijn, K. M., Gumkowski, F. D. and Jamieson, J. D. (1999) The subapical actin cytoskeleton regulates secretion and membrane retrieval in pancreatic acinar cells. J. Cell Sci. 112, 81–96.

130 Ameen, N. A. and Salas, P. J. (2000) Microvillus inclusion disease: a genetic defect affecting apical membrane protein traffic in intestinal epithelium. Traffic 1, 76–83.

131 Parczyk, K., Haase, W. and Kondor-Koch, C. (1989) Microtubules are involved in the secretion of proteins at the apical cell surface of the polarized epithelial cell, Madin-Darby canine kidney. J. Biol. Chem. 264, 16837–16846.

132 Gottlieb, T. A., Ivanov, I. E., Adesnik, M. and Sabatini, D. D. (1993) Actin microfilaments play a critical role in endocytosis at the apical but not the basolateral surface of polarized epithelial cells. J. Cell Biol. 120, 695–710.

133 Jackman, M. R., Shurety, W., Ellis, J. A. and Luzio, J. P. (1994) Inhibition of apical but not basolateral endocytosis of ricin and folate in Caco-2 cells by cytochalasin D. J. Cell Sci. 107, 2547–2556.

134 Shurety, W., Bright, N. A. and Luzio, J. P. (1996) The effects of cytochalasin D and phorbol myristate acetate on the apical endocytosis of ricin in polarised Caco-2 cells. J. Cell Sci. 109, 2927–2935.

135 Gekle, M., Mildenberger, S., Freudinger, R., Schwerdt, G. and Silbernagl, S. (1997) Albumin endocytosis in OK cells: dependence on actin and microtubules and regulation by protein kinases. Am. J. Physiol. 272, F668–F677.

136 Blok, J., Scheven, B. A., Mulder-Stapel, A. A., Ginsel, L. A. and Daems, W. T. (1982) Endocytosis in absorptive cells of cultured human small-intestinal tissue: effect of cytochalasin B and D. Cell Tissue Res. 222, 113–126.

137 Fath, K. R and Burgess, D. R. (1993) Golgi-derived vesicles from developing epithelial cells bind actin filaments and possess myosin-I as a cytoplasmically oriented peripheral membrane protein. J. Cell Biol. 120, 117–127.

138 Montes, D. O., Lezama, R. A., Mondragon, R., Castillo, A. M. and Meza, I. (1997) Myosin I interactions with actin filaments and trans-Golgi-derived vesicles in MDCK cell monolayers. Arch. Med. Res. 28, 321–328.

139 Hayden, S. M., Wolenski, J. S. and Mooseker, M. S. (1990) Binding of brush border myosin I to phospholipid vesicles. J. Cell Biol. 111, 443–451.

140 Tyska, M. J and Mooseker, M. S. (2004) A role for myosin-1A in the localization of a brush border disaccharidase. J. Cell Biol. 165, 395–405.

141 Tyska, M. J., Mackey, A. T., Huang, J. D., Copeland, N. G., Jenkins, N. A. and Mooseker, M. S. (2005) Myosin-1a is critical for normal brush border structure and composition. Mol. Biol. Cell 16, 2443–2457.

142 Heine, M., Cramm-Behrens, C. I., Ansari, A., Chu, H. P., Ryazanov, A. G., Naim, H. Y. and Jacob, R. (2005) α-Kinase 1, a new component in apical protein transport. J. Biol. Chem. 280, 25637–25643.

143 Raposo, G., Cordonnier, M. N., Tenza, D., Menichi, B., Durrbach, A., Louvard, D. and Coudrier, E. (1999) Association of myosin I alpha with endosomes and lysosomes in mammalian cells. Mol. Biol. Cell 10, 1477–1494.

144 Durrbach, A., Raposo, G., Tenza, D., Louvard, D. and Coudrier, E. (2000) Truncated brush border myosin I affects membrane traffic in polarized epithelial cells. Traffic 1, 411–424.

145 Cordonnier, M. N., Dauzonne, D., Louvard, D. and Coudrier, E. (2001) Actin filaments and myosin I alpha cooperate with microtubules for the movement of lysosomes. Mol. Biol. Cell 12, 4013–4029.

146 Fiedler, K., Lafont, F., Parton, R. G. and Simons, K. (1995) Annexin XIIIb: a novel epithelial specific annexin is implicated in vesicular traffic to the apical plasma membrane. J. Cell Biol. 128, 1043–1053.

147 Lafont, F., Lecat, S., Verkade, P. and Simons, K. (1998) Annexin XIIIb associates with lipid microdomains to function in apical delivery. J. Cell Biol. 142, 1413–1427.

148 Plant, P. J., Lafont, F., Lecat, S., Verkade, P., Simons, K. and Rotin, D. (2000) Apical membrane targeting of Nedd4 is mediated by an association of its C2 domain with annexin XIIIb. J. Cell Biol. 149, 1473–1484.

149 Jacob, R., Heine, M., Eikemeyer, J., Frerker, N., Zimmer, K. P., Rescher, U., Gerke, V. and Naim, H. Y. (2004) Annexin II is required for apical transport in polarized epithelial cells. J. Biol. Chem. 279, 3680–3684.

150 Danielsen, E. M., van Deurs, B. and Hansen, G. H. (2003) 'Nonclassical' secretion of annexin A2 to the lumenal side of the enterocyte brush border membrane. Biochemistry 42, 14670–14676.

151 Harder, T. and Gerke, V. (1994) The annexin II2p11(2) complex is the major protein component of the Triton X-100-insoluble low-density fraction prepared from MDCK cells in the presence of Ca^{2+}. Biochim. Biophys. Acta 1223, 375–382.

152 Oliferenko, S., Paiha, K., Harder, T., Gerke, V., Schwarzler, C., Schwarz, H., Beug, H., Gunthert, U. and Huber, L. A. (1999) Analysis of CD44-containing lipid rafts: Recruitment of annexin II and stabilization by the actin cytoskeleton. J. Cell Biol. 146, 843–854.

153 Girard, C., Tinel, N., Terrenoire, C., Romey, G., Lazdunski, M. and Borsotto, M. (2002) p11, an annexin II subunit, an auxiliary protein associated with the background K^+ channel, TASK-1. EMBO J. 21, 4439–4448.

154 Okuse, K., Malik-Hall, M., Baker, M. D., Poon, W. Y., Kong, H., Chao, M. V. and Wood, J. N. (2002) Annexin II light chain regulates sensory neuron-specific sodium channel expression. Nature 417, 653–656.
155 van de Graaf, S. F., Hoenderop, J. G., Gkika, D., Lamers, D., Prenen, J., Rescher, U., Gerke, V., Staub, O., Nilius, B. and Bindels, R. J. (2003) Functional expression of the epithelial Ca(2+) channels (TRPV5 and TRPV6) requires association of the S100A10-annexin 2 complex. EMBO J. 22, 1478–1487.
156 Filipenko, N. R and Waisman, D. M. (2001) The C terminus of annexin II mediates binding to F-actin. J. Biol. Chem. 276, 5310–5315.
157 Bacallao, R., Antony, C., Dotti, C., Karsenti, E., Stelzer, E. H. and Simons, K. (1989) The subcellular organization of Madin-Darby canine kidney cells during the formation of a polarized epithelium. J. Cell Biol. 109, 2817–2832.
158 Bre, M. H., Pepperkok, R., Hill, A. M., Levilliers, N., Ansorge, W., Stelzer, E. H. and Karsenti, E. (1990) Regulation of microtubule dynamics and nucleation during polarization in MDCK II cells. J. Cell Biol. 111, 3013–3021.
159 Meads, T. and Schroer, T. A. (1995) Polarity and nucleation of microtubules in polarized epithelial cells. Cell Motil. Cytoskeleton 32, 273–288.
160 Breitfeld, P. P., McKinnon, W. C. and Mostov, K. E. (1990) Effect of nocodazole on vesicular traffic to the apical and basolateral surfaces of polarized MDCK cells. J. Cell Biol. 111, 2365–2373.
161 Ojakian, G. K. and Schwimmer, R. (1992) Antimicrotubule drugs inhibit the polarized insertion of an intracellular glycoprotein pool into the apical membrane of Madin-Darby canine kidney (MDCK) cells. J. Cell Sci. 103, 677–687.
162 Rindler, M. J., Ivanov, I. E. and Sabatini, D. D. (1987) Microtubule-acting drugs lead to the nonpolarized delivery of the influenza hemagglutinin to the cell surface of polarized Madin-Darby canine kidney cells. J. Cell Biol. 104, 231–241.
163 van Zeijl, M. J. and Matlin, K. S. (1990) Microtubule perturbation inhibits intracellular transport of an apical membrane glycoprotein in a substrate-dependent manner in polarized Madin-Darby canine kidney epithelial cells. Cell Regul. 1, 921–936.
164 Grindstaff, K. K., Bacallao, R. L. and Nelson, W. J. (1998) Apiconuclear organization of microtubules does not specify protein delivery from the trans-Golgi network to different membrane domains in polarized epithelial cells. Mol. Biol. Cell 9, 685–699.
165 Saunders, C. and Limbird, L. E. (1997) Disruption of microtubules reveals two independent apical targeting mechanisms for G-protein-coupled receptors in polarized renal epithelial cells. J. Biol. Chem. 272, 19035–19045.
166 Kreitzer, G., Marmorstein, A., Okamoto, P., Vallee, R. and Rodriguez-Boulan, E. (2000) Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. Nat. Cell Biol. 2, 125–127.
167 Noda, Y., Okada, Y., Saito, N., Setou, M., Xu, Y., Zhang, Z. and Hirokawa, N. (2001) KIFC3, a microtubule minus end-directed motor for the apical transport of annexin XIIIb-associated Triton-insoluble membranes. J. Cell Biol. 155, 77–88.
168 Tai, A. W., Chuang, J. Z. and Sung, C. H. (2001) Cytoplasmic dynein regulation by subunit heterogeneity and its role in apical transport. J. Cell Biol. 153, 1499–1509.
169 Sollner, T., Whiteheart, S. W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J. E. (1993) SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318–324.
170 Chen, Y. A and Scheller, R. H. (2001) SNARE-mediated membrane fusion. Nat. Rev. Mol. Cell Biol. 2, 98–106.
171 Pfeffer, S. R. (1999) Transport-vesicle targeting: tethers before SNAREs. Nat. Cell Biol. 1, E17–E22.
172 Fujita, H., Tuma, P. L., Finnegan, C. M., Locco, L. and Hubbard, A. L. (1998) Endogenous syntaxins 2, 3 and 4 exhibit distinct but overlapping patterns of expression at the hepatocyte plasma membrane. Biochem. J. 329, 527–538.
173 Gaisano, H. Y., Ghai, M., Malkus, P. N., Sheu, L., Bouquillon, A., Bennett, M. K. and Trimble, W. S. (1996) Distinct cellular locations of the syntaxin family of proteins in rat pancreatic acinar cells. Mol. Biol. Cell 7, 2019–2027.
174 Low, S. H., Chapin, S. J., Weimbs, T., Komuves, L. G., Bennett, M. K. and Mostov, K. E. (1996) Differential localization of syntaxin isoforms in polarized Madin-Darby canine kidney cells. Mol. Biol. Cell 7, 2007–2018.
175 Delgrossi, M. H., Breuza, L., Mirre, C., Chavrier, P. and Le Bivic, A. (1997) Human syntaxin 3 is localized apically in human intestinal cells. J. Cell Sci. 110, 2207–2214.
176 Lafont, F., Verkade, P., Galli, T., Wimmer, C., Louvard, D. and Simons, K. (1999) Raft association of SNAP receptors acting in apical trafficking in Madin-Darby canine kidney cells. Proc. Natl. Acad. Sci. USA 96, 3734–3738.
177 Low, S. H., Chapin, S. J., Wimmer, C., Whiteheart, S. W., Komuves, L. G., Mostov, K. E. and Weimbs, T. (1998) The SNARE machinery is involved in apical plasma membrane trafficking in MDCK cells. J. Cell Biol. 141, 1503–1513.
178 ter Beest, M. B., Chapin, S. J., Avrahami, D. and Mostov, K. E. (2005) The role of syntaxins in the specificity of vesicle targeting in polarized epithelial cells. Mol. Biol. Cell 16, 5784–5792.
179 Galli, T., Zahraoui, A., Vaidyanathan, V. V., Raposo, G., Tian, J. M., Karin, M., Niemann, H. and Louvard, D. (1998) A novel tetanus neurotoxin-insensitive vesicle-associated membrane protein in SNARE complexes of the apical plasma membrane of epithelial cells. Mol. Biol. Cell 9, 1437–1448.
180 Carr, C. M., Grote, E., Munson, M., Hughson, F. M. and Novick, P. J. (1999) Sec1p binds to SNARE complexes and concentrates at sites of secretion. J. Cell Biol. 146, 333–344.
181 Rizo, J. and Sudhof, T. C. (2002) Snares and Munc18 in synaptic vesicle fusion. Nat. Rev. Neurosci. 3, 641–653.
182 Riento, K., Galli, T., Jansson, S., Ehnholm, C., Lehtonen, E. and Olkkonen, V. M. (1998) Interaction of Munc-18-2 with syntaxin 3 controls the association of apical SNAREs in epithelial cells. J. Cell Sci. 111, 2681–2688.
183 Riento, K., Kauppi, M., Keranen, S. and Olkkonen, V. M. (2000) Munc18-2, a functional partner of syntaxin 3, controls apical membrane trafficking in epithelial cells. J. Biol. Chem. 275, 13476–13483.
184 Musch, A., Xu, H., Shields, D. and Rodriguez-Boulan, E. (1996) Transport of vesicular stomatitis virus G protein to the cell surface is signal mediated in polarized and nonpolarized cells. J. Cell Biol. 133, 543–558.
185 Yoshimori, T., Keller, P., Roth, M. G. and Simons, K. (1996) Different biosynthetic transport routes to the plasma membrane in BHK and CHO cells. J. Cell Biol. 133, 247–256.
186 Danielsen, E. M. (1995) Involvement of detergent-insoluble complexes in the intracellular transport of intestinal brush border enzymes. Biochemistry 34, 1596–1605.
187 Ishikawa, Y., Yuan, Z., Inoue, N., Skowronski, M. T., Nakae, Y., Shono, M., Cho, G., Yasui, M., Agre, P. and Nielsen, S. (2005) Identification of AQP5 in lipid rafts and its translocation to apical membranes by activation of M3 mAChRs in interlobular ducts of rat parotid gland. Am. J. Physiol Cell Physiol. 289, C1303–C1311.
188 Zheng, X., Lu, D. and Sadler, J. E. (1999) Apical sorting of bovine enteropeptidase does not involve detergent-resistant association with sphingolipid-cholesterol rafts. J. Biol. Chem. 274, 1596–1605.
189 Verkade, P., Harder, T., Lafont, F. and Simons, K. (2000) Induction of caveolae in the apical plasma membrane of Madin-Darby canine kidney cells. J. Cell Biol. 148, 727–739.

190 Dunbar, L. A., Aronson, P. and Caplan, M. J. (2000) A transmembrane segment determines the steady-state localization of an ion-transporting adenosine triphosphatase. J. Cell Biol. 148, 769–778.
191 Fiedler, K., Kobayashi, T., Kurzchalia, T. V. and Simons, K. (1993) Glycosphingolipid-enriched, detergent-insoluble complexes in protein sorting in epithelial cells. Biochemistry 32, 6365–6373.
192 Roper, K., Corbeil, D. and Huttner, W. B. (2000) Retention of prominin in microvilli reveals distinct cholesterol- based lipid micro-domains in the apical plasma membrane. Nat. Cell Biol. 2, 582–592.