Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane

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Summary

Polarized cells establish and maintain functionally distinct surface domains by an elaborate sorting process, which ensures accurate delivery of biosynthetic cargo to different parts of the plasma membrane. This is particularly evident in polarized epithelial cells, which have been used as a model system for studies of sorting mechanisms. The clustering of lipid rafts through the oligomerization of raft components could be utilized for segregating apical from basolateral cargo and for the generation of intracellular transport carriers. Besides functioning in polarized sorting in differentiated cells, raft clustering might also play an important role in the biogenesis of apical membrane domains during development.

Key words: Protein sorting, Epithelial cells, Rafts, Apical membrane

Introduction

Most cell types generated by multicellular organisms are polarized. A characteristic feature of polarized cells is the division of their surface into functionally distinct membrane domains. This requires an intricate sorting machinery that delivers proteins and lipids to the right membrane domains. Hence, the sorting machinery is fundamental both to the generation of polarity and its maintenance in the face of continuous plasma membrane turnover by endocytosis.

Epithelial cells provide a paradigm of cell polarization. They constitute a protective barrier against the external environment, but also serve as exchange interfaces with the outside world. To fulfil these functions, epithelial cells have evolved characteristic apical and basolateral membrane domains. The basolateral membrane contacts neighbouring cells and the underlying tissue, whereas the apical membrane faces the lumen of an internal organ. The two membrane domains are separated by tight junctions, which help to prevent mixing of apical and basolateral membrane components and seal the epithelium (Tsukita et al., 2001).

Here, we discuss the mechanisms of polarized sorting in epithelial cells, focusing on the biogenesis of the apical membrane and specifically the role of lipid rafts in this process. Compared with basolateral sorting, current knowledge about apical sorting has been difficult to integrate into a coherent picture. However, the apical pathway plays a major role in the plasticity of epithelial tissues during development (Lubarsky and Krasnow, 2003). Therefore, insight into apical sorting will be vital to understanding cell polarization and its role in tissue generation.

Properties of the apical membrane

The apical membrane mediates many of the functions specific to epithelial cells. Exposed to hostile environments marked by, for example, high osmotic pressure or the presence of digestive enzymes, it needs to be particularly sturdy. The unusual robustness of the apical membrane is largely due to its special lipid composition. It is strongly enriched in sphingolipids (Simons and van Meer, 1988), which, together with cholesterol, have the propensity to form tightly packed membrane microdomains called lipid rafts. Rafts probably exist in the plasma membranes of almost all cells as small, highly dynamic liquid-ordered assemblies embedded in the surrounding liquid-disordered membrane (Kusumi et al., 2004; Simons and Vaz, 2004; Munro, 2003). Rafts have been estimated to cover 30-40% of the plasma membrane of non-polarized cells (Simons and Toomre, 2000; Prior et al., 2003). However, the high proportion of sphingolipids and cholesterol in the apical membrane could make it a continuous raft membrane in which non-raft domains are embedded (Verkade et al., 2000; Crane and Tamm, 2004).

The apical membrane is by no means only a rigid shield. Epithelial cells in the intestine and the kidney have a large capacity for absorption and secretion, and the apical membrane can be internalized and replaced entirely within hours. Given this combination of protective and exchange functions, the composition of the apical membrane needs to be tightly controlled. Insertion of the wrong transport proteins into the apical membrane of kidney cells, for example, could destroy the steep ion gradients across the epithelium, or lead to influx of toxic compounds such as uric acid. Similarly, delivery of unsaturated glycerophospholipids to the apical membrane of hepatocytes might disturb its resistance to bile acid detergents.

Given this need for strict exclusion of basolateral membrane components from the apical membrane, it is interesting that basolateral targeting is usually more accurate than apical targeting (Pfeiffer et al., 1985). Thus, the physiological context of epithelial cells may place constraints on how much mis-sorting can be tolerated and, therefore, on how sorting to the plasma membrane can be organized.

Mechanisms of polarized sorting

A polarized surface distribution of proteins and lipids can be achieved by targeted delivery or selective retention (Yeaman et al., 1999; Matter, 2000). Targeted delivery relies on the segregation of cargo molecules destined for different plasma membrane domains before they reach the cell surface. Selective retention works by trapping them once they arrive – for instance, by anchoring to a domain-specific cytoskeletal scaffold. Proteins delivered to the wrong surface domain are removed by endocytosis and are then degraded or undergo another round of delivery. Targeted delivery and selective retention can be combined such that selective retention enhances the accuracy of intracellular sorting (Mays et al., 1995).

The basolateral as well as the apical sorting pathway has been suspected to involve bulk flow, but it is now clear that neither is a general default pathway for the surface delivery of proteins. Instead, apical and basolateral sorting are both governed by sorting determinants embedded in cargo proteins.

Sorting determinants

Sorting determinants in membrane proteins can reside in the cytoplasmic domain, membrane anchor or extracellular domain (Table 1). Cytoplasmic domain determinants include the basolateral-targeting, tyrosine-based and dileucine motifs (Matter and Mellman, 1994), PDZ-domain-binding motifs and a growing list of unrelated sequences (Altschuler et al., 2003; Muth and Caplan, 2003). Determinants in membrane anchors include the transmembrane domains of some apically targeted viral proteins (Kundu et al., 1996; Lin et al., 1998) and glycosylphosphatidylinositol (GPI) anchors. The latter usually confer localization to the apical membrane (Brown et al., 1989; Lisanti et al., 1989), but alone are not always sufficient (Brown and London, 1998; Benting et al., 1999a). Both *N*- and *O*-glycosylation of the extracellular domain have been implicated in apical targeting (Scheiffele et al., 1995; Yeaman et al., 1997; Gut et al., 1998; Spodsberg et al., 2001). Finally, oligomerization of membrane proteins may be an important sorting determinant, particularly for apical transport. Evidence in epithelial cells is lacking so far, but sorting of the voltage-gated potassium channel Kv1 into the axonal pathway of neurons, which is related to the apical pathway of epithelial cells (Horton and Ehlers, 2003), requires oligomerization (Gu et al., 2003).

Overall, the known apical sorting determinants are more diverse and not as strict as prototypical basolateral determinants. Basolateral determinants seem to dominate over apical ones, because addition of a tyrosine-based or dileucine motif usually redirects apical proteins to the basolateral membrane. Such a hierarchy of sorting determinants could help to ensure stringent sorting of basolateral proteins away from the apical membrane. However, there are also examples of recessive basolateral determinants (Monlauzeur et al., 1995; Jacob et al., 1999; Ihrke et al., 2001), indicating that the hierarchy of sorting determinants is more complicated or that their relative strength depends on the protein in which they are present.

Sorting by receptor-mediated cargo capture

Certain cytoplasmic domain determinants are recognized by specific sorting receptors. Best characterized are the adaptor protein (AP) complexes, which sort proteins along many intracellular trafficking routes (Robinson, 2004). Several tyrosine-based motifs interact with the epithelia-specific μ1B subunit of the AP-1 complex, and this interaction is crucial for the basolateral sorting of the low-density lipoprotein (LDL) receptor and the transferrin receptor (Folsch et al., 1999; Sugimoto et al., 2002). Other adaptors implicated in the recognition of basolateral determinants are the AP-3 and AP-4 complexes (Nishimura et al., 2002; Simmen et al., 2002). How the binding of adaptor complexes to cargo proteins is coupled to the machinery responsible for the formation and movement of transport carriers is not understood. The ability of the AP-1 complex to bind to clathrin makes an involvement of clathrin-coated vesicles in basolateral transport plausible, but direct evidence has been difficult to obtain. Adaptor complexes can also bind to motor proteins, which then move cargo containers towards the cell periphery (Nakagawa et al., 2000; Setou et al., 2000). Alternatively, motor proteins themselves may serve as receptors for cytoplasmic domain determinants, as is the case for the dynein-mediated apical transport of rhodopsin (Tai et al., 2001). In any event, receptor-mediated cargo capture by specific protein-protein interactions provides a stringent mechanism for the inclusion of proteins into transport carriers.

Whether cytoplasmic PDZ-domain-binding motifs are recognized by sorting receptors or mediate selective retention at the target membrane is not clear. In the case of the cystic fibrosis transmembrane conductance regulator (CFTR), a

Table 1. Determinants for the polarized sorting of membrane proteins

Site	Determinant	Polarity	Examples*
Cytoplasmic domain	Tyrosine-based motif	Basolateral	LDL receptor, transferrin receptor, vesicular stomatitis virus glycoprotein
	Dileucine motif	Basolateral	IgG Fc receptor, E-cadherin
	PDZ-binding motif	Apical/basolateral	CFTR (a), BGT-1 GABA transporter (bl), ERbB-2 receptor tyrosine kinase (bl)
	Others	Apical/basolateral	H,K-ATPase α-subunit (a), megalin (a), GAT-2 GABA transporter (bl)
Membrane anchor	Transmembrane domain	Apical	Influenza virus hemagglutinin and neuraminidase
	GPI-anchor	Apical	Placental alkaline phosphatase, decay-accelerating factor, Thy-1
Extracellular domain	*N*-glycosylation	Apical	Occludin (truncated version), FcLR (Fc receptor-LDL receptor chimera), GLYT2 glycine transporter
	O-glycosylation	Apical	Neurotrophin receptor, sucrase isomaltase
	Oligomerization domain?	Apical?	Kv1 potassium channel (neurons)

*a, apical; bl, basolateral.

PDZ-domain-binding motif seems to be responsible for its selective apical retention after initial transport to both membrane domains (Swiatecka-Urban et al., 2002). The PDZ protein NHERF links CFTR to the actin network beneath the apical membrane (Short et al., 1998), and NHERF probably plays this scaffolding role for various apical proteins (Shenolikar and Weinman, 2001; Altschuler et al., 2003). Similarly, the epithelial gamma aminobutyric acid (GABA) transporter BGT-1 and the receptor tyrosine kinase ErbB-2 have PDZ-domain-binding motifs that mediate basolateral retention by interacting with the PDZ protein LIN-7 (Perego et al., 1999; Shelly et al., 2003). However, there are reports arguing that the PDZ-domain-binding motif is dispensable for the apical targeting of CFTR (Benharouga et al., 2003; Ostedgaard et al., 2003), and basolateral targeting of BGT-1 and ErbB-2 still occurs when the motifs are deleted. Localization of these proteins therefore appears to involve a combination of targeting and retention mechanisms.

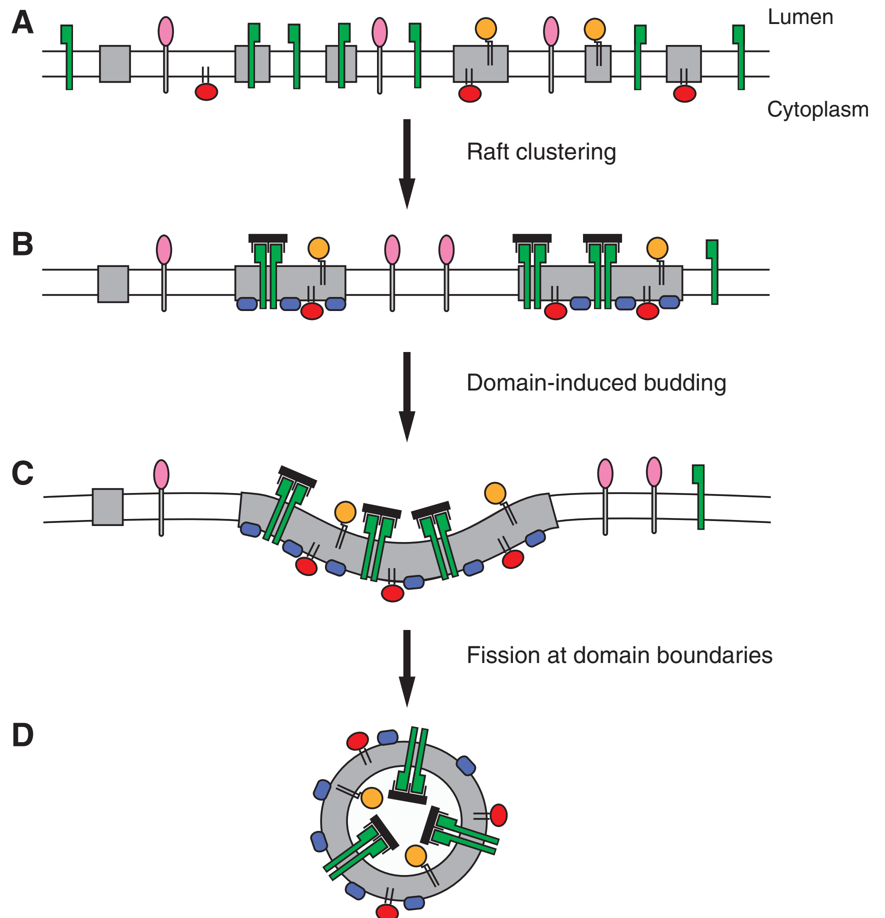

Fig. 1. Raft clustering and domain-induced budding. Before clustering, proteins associate with rafts (grey) to various extents. A GPI-anchored protein (gold) resides exclusively in rafts, a doubly acylated protein (red) is mainly in rafts, a transmembrane protein (green) is mainly outside rafts, and another transmembrane protein (pink) is excluded from rafts (A). Clustering is induced, for example, by the binding of a multimeric protein of the annexin type (blue) to the cytoplasmic face of rafts. The strongly raft-associated GPI-anchored and doubly acylated proteins partition into clustered rafts. The weakly raft-associated transmembrane protein is driven into clustered rafts by crosslinking with a divalent interaction partner, e.g. a lectin (black). Note that the recruitment of the weakly raft-associated transmembrane protein is not complete, nor are all rafts clustered. For simplicity, only one type of raft cluster is shown, i.e. differential clustering of rafts into separate domains with different constituents is not depicted (B). Growth of the clustered raft domain beyond a critical size induces budding (C). Finally, a transport container consisting of raft components pinches off from the parent membrane by fission at the domain boundaries (D).

Lipid rafts as apical sorting platforms

Sorting receptors that engage in direct protein-protein interactions with apical sorting determinants in membrane anchors or extracellular domains have not been identified. Instead, the sorting of many apical proteins may be governed by lipid-lipid and lipid-protein interactions. The enrichment of sphingolipids in the apical membrane, together with their propensity to associate with cholesterol to form lipid rafts, has led to the concept that rafts preferentially traffic to the apical membrane after intracellular assembly. Since certain proteins associate with rafts during apical transport, rafts could act as apical sorting platforms (Simons and Ikonen, 1997).

Support for this hypothesis has come from two types of experiment. First, apical sorting is particularly sensitive to depletion of cholesterol and sphingolipids (Mays et al., 1995; Keller and Simons, 1998; Hansen et al., 2000; Lipardi et al., 2000). However, this is only indirect evidence, because depletion of these raft lipids could also affect apical trafficking in a more general way. Second, certain apically targeted proteins such as the GPI-anchored placental alkaline phosphatase (PLAP) and influenza virus hemagglutinin, but not typical basolateral protein, enter rafts before they reach the cell surface (Skibbens et al., 1989; Brown and Rose, 1992). Apical sorting of these proteins thus correlates with a dramatic change in their lipid environment during surface transport, but whether this change is required for accurate targeting is unclear. In addition, in these experiments, rafts were defined as detergent-resistant membranes (DRMs), i.e. membranes that resist solubilization with mild detergents such as Triton X-100. Although useful, detergent resistance is a rather crude criterion to determine raft association (London and Brown, 2000; Schuck et al., 2003). Indeed, several endogenous apical proteins in the well-characterized Madin-Darby canine kidney (MDCK) cell line are fully detergent soluble, and some DRM-associated proteins still reach the apical membrane when rendered detergent soluble by mutation or cholesterol depletion (Lin et al., 1998; Lipardi et al., 2000). Detergent extraction probably disrupts weak interactions of proteins with raft domains, but these interactions might nonetheless be important for sorting (Shvartsman et al., 2003). Thus, a lack of suitable methods has

hampered the quest for conclusive evidence for or against sorting by raft association.

Sorting by cargo recruitment into clustered rafts

Recent work has revealed the ability of individual rafts to cluster selectively into large domains, and this allows us to propose a more comprehensive model for the role of rafts in polarized sorting (Fig. 1). To introduce this model, we first examine the clustering of rafts at the cell surface. We then discuss how raft clustering might be utilized for sorting, how known apical sorting determinants fit into this picture and what kind of protein machinery could mediate raft clustering. Last, we argue that different raft clusters can be produced.

The size and stability of rafts in the unperturbed state is controversial, but there is a growing consensus that individual rafts can be induced to form large, stable clusters (Kusumi et al., 2004; Simons and Vaz, 2004). Raft clustering can be initiated by the oligomerization of raft components. At the cell surface, clustering can be brought about artificially by antibody crosslinking, but it also occurs naturally, for example when interactions between the T-cell receptor and peptides bound to major histocompatibility complexes (MHC) on antigen-presenting cells trigger the clustering of rafts during formation of the immunological synapse (Harder and Engelhardt, 2004). As a consequence of raft clustering, raft association of proteins and lipids that have an affinity for liquid-ordered domains is stabilized, whereas non-raft components are excluded. For example, antibody crosslinking of the GPI-anchored PLAP or the apical transmembrane protein gp114 at the plasma membrane of MDCK cells strengthens their raft association, as judged by increased detergent resistance (Harder et al., 1998; Verkade et al., 2000). This happens because oligomerization by crosslinking multiplies the tendency of these proteins to associate with raft domains. In thermodynamic terms, the partitioning coefficient of oligomers between the raft and the non-raft phase is given by the product of the partitioning coefficients of the monomers they are composed of. This should lead to an exponential increase in raft affinity as oligomer size increases (Simons and Vaz, 2004).

Importantly, antibody crosslinking of PLAP leads to co-clustering of the doubly acylated raft protein Fyn, a peripheral membrane protein that resides on the opposite side of the membrane. Thus, the crosslinking of one raft protein moves whole lipid microdomains together in both membrane leaflets (Harder et al., 1998; Prior et al., 2003). The same is true for raft lipids, as shown by crosslinking of the glycosphingolipid GM1 with the pentameric cholera toxin, which leads to co-clustering with various raft proteins (Janes et al., 1999). This presumably occurs because lipids and proteins within raft microdomains associate tightly enough to allow these domains to behave as stable entities (Pralle et al., 2000). In contrast to raft components, non-raft proteins, such as the transferrin receptor, are excluded from clustered rafts, even when crosslinked (Harder et al., 1998; Janes et al., 1999). These examples show that the clustering of individual rafts reinforces the segregation of the raft and the non-raft phase, and sharpens the distribution of membrane components between the two phases. Thus, raft clustering is a mechanism for the selective recruitment of proteins and lipids that have an affinity for liquid-ordered domains, as well as the efficient exclusion of non-raft proteins.

We envisage that the clustering of lipid rafts is used as a cellular sorting mechanism to recruit certain cargo proteins yet exclude others. Stabilization of rafts should attract proteins and lipids that have a strong affinity for ordered domains. Indeed, clustered rafts might exhibit an even greater degree of lipid ordering than individual rafts, thus providing a preferable environment for these molecules. Recruitment into clustered rafts could be assisted by additional interactions in the case of weakly raft-associated proteins. Crosslinking by a multivalent interaction partner outside rafts could augment their raft affinity. Also, they could bind to other proteins that drag them into clustered rafts. There, they could become entrapped by further modifications that strengthen their raft association, such as palmitoylation, oligomerization or a conformational change (Bagnat et al., 2001; Cherukuri et al., 2004). Note that certain proteins and lipids that have raft affinity might still partially localize to non-raft membranes or non-clustered rafts. Nevertheless, clustering should create membrane patches consisting of a select group of molecules whose trafficking fates are connected.

Certain apical sorting determinants are able to facilitate raft association, especially when rafts are clustered. First, GPI-anchored proteins generally associate with lipid rafts on the basis of the favourable packing of the GPI anchor into liquid-ordered domains. However, GPI anchors are structurally diverse, and so this might not be true for all GPI-linked proteins (Benting et al., 1999b; Mayor and Riezman, 2004). Interestingly, raft association of GPI-anchored proteins might need to be stabilized by oligomerization for them to be sorted apically (Paladino et al., in press). Second, transmembrane domains known to act as apical sorting determinants also mediate raft association (Scheiffele et al., 1997; Barman and Nayak, 2000). The principles underlying the association of transmembrane proteins with rafts are poorly understood. One element is binding to raft lipids and, possibly, conformational changes induced by specific protein-lipid interactions (Simons and Vaz, 2004). Another factor might be the length of the transmembrane domain. It has been proposed that the targeting of transmembrane proteins to different cell membranes is based on the length of the membrane-spanning region (Bretscher and Munro, 1993). Long transmembrane domains could have a preference for raft membranes, whereas proteins that have short transmembrane domains might be excluded. Indeed, owing to the ordering of lipid side chains, raft membranes are probably thicker than non-raft membranes. In addition, they have different elastic properties, e.g. they are more difficult to bend or compress. Recently, a theoretical study showed that these elastic properties, rather than membrane thickness, provide the primary driving force for the exclusion of proteins that have short transmembrane domains from cholesterol-enriched membranes (Lundbaek et al., 2003). If clustering were to increase the lipid order of rafts and make them even less elastic, raft clustering would potentiate the effect of transmembrane domain length on the raft association of transmembrane proteins. Third, oligomerization should amplify the affinity of proteins for rafts by the same mechanism as antibody crosslinking does. This could explain why oligomerization might be an important determinant for raft-mediated apical sorting.

How might raft clustering be induced? From what is known about the clustering process, any oligomerization of raft components could be sufficient. Several proteins might promote raft clustering, although the available evidence is circumstantial. One type of 'clustering agent' might be represented by VIP17/MAL and the closely related MAL2. Both are tightly raft-associated integral membrane proteins and play a role in apical targeting (Puertollano et al., 1999; Cheong et al., 1999; de Marco et al., 2002). Moreover, VIP17/MAL can form oligomers, which might function to cluster rafts at the sites at which sorting takes place. Similarly, caveolins, flotillins and stomatin are raft-associated membrane proteins that form oligomers (Monier et al., 1995; Snyers et al., 1999; Neumann-Giesen et al., 2004). Caveolins clearly have the ability to cluster rafts, acting as scaffolds for caveolae (van Deurs et al., 2003). Sotgia et al. have proposed that caveolin-1 is required for transport of GPI-anchored proteins to the plasma membrane (Sotgia et al., 2002), but evidence from our laboratory argues against a major role of caveolin-1 in polarized sorting in MDCK cells (A. Manninen, J. Füllekrug and K.S., unpublished).

Raft clustering might be promoted in a slightly different way by annexin 13b and annexin 2, which are cytosolic proteins that facilitate apical transport in MDCK cells (Lafont et al., 1998; Jacob et al., 2004). Both are unusual annexins because they preferentially associate with cholesterol-rich membranes (Lafont et al., 1998; Rescher and Gerke, 2004). Again, they could act as membrane organizers through their ability to oligomerize, since some annexins, including annexin 2, form large, two-dimensional ordered arrays (Oling et al., 2001). Little is known about the cytoplasmic side of rafts, but it might contain enough cholesterol to serve as a preferred docking site for annexin 13b and annexin 2. Although rafts are primarily thought of as assemblies in the exoplasmic membrane leaflet, there clearly is leaflet coupling such that raft domains on the exoplasmic side are matched by raft domains on the cytoplasmic side (Harder et al., 1998; Korlach et al., 1999; Prior et al., 2003). Thus, clustering of raft lipids on the cytoplasmic side by annexin oligomers should cluster raft components in the exoplasmic leaflet.

Lectins of the galectin family might be involved in driving glycolipids and glycoproteins into clustered rafts. Galectins are usually multivalent owing to self-association and can organize glycoproteins into large regular arrays (Brewer et al., 2002). Galectin 4 tightly associates with rafts on the outside of the brush border membrane of intestinal cells and stabilizes raft domains (Braccia et al., 2003). Recent studies implicate galectin 4 in apical sorting in enterocytes (D. Delacour, V. Gouyer, A. Manninen, K.S. and G. Huet, unpublished). Galectins are secreted from cells by a non-classical mechanism that bypasses the Golgi complex. How they could enter the secretory pathway is therefore unclear. However, they might be re-internalized by endocytosis and thus gain access to intracellular sorting sites.

Finally, it is important to realize that rafts can be clustered differentially. For example, migrating T lymphocytes are able to set up separate raft domains that have distinct compositions at the leading edge and the uropod (Gomez-Mouton et al., 2001). Likewise, although the mating projection in yeast is a specialized membrane domain that might arise by raft clustering, raft proteins are also found elsewhere in the plasma membrane (Bagnat and Simons, 2002). The immunological synapse formed by activated T cells is viewed as a raft cluster but nevertheless excludes raft proteins that have no function in immune signalling (Harder and Kuhn, 2000; Bunnell et al., 2002). In all these processes, the actin cytoskeleton plays a key role. However, even when raft components are clustered artificially, selective co-clustering of raft components is still observed (Wilson et al., 2004). Hence, differential raft clustering appears to be a general principle. In the context of intracellular sorting, the differential clustering of lipid rafts might be used to segregate raft components that have different destinations.

Generation of transport carriers by raft clustering

Raft clustering could also provide a mechanism for the generation of transport carriers. If phases with different properties coexist in the same membrane, as in the case of liquid-ordered microdomains in a membrane mainly in the liquid-disordered phase, there is line tension at the phase boundaries. Line tension is the two-dimensional equivalent of surface tension and arises from the immiscibility of membrane components that prefer different phases. The energetic cost of line tension can be reduced by decreasing the contact between phases, i.e. by decreasing the boundary length of domains that constitute the minority phase. This is achieved most effectively when domains that are part of the minority phase bud out of the majority phase and eventually detach from the parent membrane. Line-tension-driven budding is opposed by the energy required to bend the membrane of the incipient bud. Importantly, the balance between line tension and bending energy depends on the size of the membrane domain in the minority phase. The line tension increases with increasing domain size, whereas the bending energy is independent of the domain size. Small domains give rise to buds that have higher degrees of curvature than large buds, but the energy to deform the membrane into a sphere is the same. As a result, a growing domain within a membrane will reach a critical size beyond which budding becomes energetically favourable (Lipowsky, 1993; Lipowsky, 2002). This mechanism, termed domain-induced budding, was originally postulated on theoretical grounds but has recently received experimental support from studies of model membranes (Baumgart et al., 2003). As predicted, domains in the minority phase were observed to bud out of the surrounding membrane, with fission occurring at the phase boundaries.

In cell membranes, individual rafts usually form a dispersed minority phase within a continuous non-raft phase (Simons and Toomre, 2000; Prior et al., 2003). Therefore, clustering of small rafts into large domains should induce budding, followed by fission at the domain boundaries and the generation of vesicles strongly enriched in raft components (Fig. 1C,D). However, the factors governing the budding size of clustered raft domains are likely to be more complex in cell membranes. The line tension between raft and non-raft membranes could be modulated by surfactancy effects of lipids and proteins at the domain interfaces (Simons and Vaz, 2004). The spontaneous curvature of raft domains that results from lipid asymmetry between the two membrane leaflets could play a part in controlling the budding process (Huttner and Zimmerberg, 2001). Moreover, budding must be regulated in

cells by proteins and probably also be supported by traction forces generated by molecular motors, and scission of the bud neck could be assisted by proteins such as dynamin (Kreitzer et al., 2000).

Post-Golgi transport routes to the plasma membrane

Apical and basolateral proteins traverse the Golgi together until they reach the trans-Golgi network (TGN). There, they are segregated from Golgi proteins, from proteins trafficking to endosomes, and sometimes from each other, giving rise to a complicated intracellular trafficking map (Fig. 2). Cargo sorting may not be confined to a single station along a given pathway, but may take place sequentially. It is therefore difficult to assign sorting events to specific cellular locations. The enormous morphological and molecular complexity of the post-Golgi membrane system, together with the rapid and extensive exchange of molecules within this system by means of membrane trafficking, makes the definition of distinct compartments problematic. Therefore, we restrict our 'compartment vocabulary' to the terms TGN, early endosomes, recycling endosomes and late endosomes. Moreover, these simplifying terms are not meant to describe independent entities, but only the more identifiable elements of a highly interconnected system. This interconnectivity makes it unlikely that cargo molecules that have different destinations are segregated from each other in a single step. Despite these difficulties, a few basic mechanisms of cargo delivery can be distinguished.

In some epithelial cells, such as MDCK cells, the TGN appears to be a major site for sorting apical from basolateral biosynthetic cargo. Raft association of apical proteins, as well as the packaging of sphingolipids and cholesterol into transport carriers destined for the plasma membrane, also takes place in the TGN. The TGN therefore probably is a compartment in which sorting based on raft clustering operates. In the TGN of endocrine and exocrine cells, raft clustering may mediate not only constitutive sorting to the plasma membrane, but also formation of immature secretory granules (Thiele and Huttner, 1998; Schmidt et al., 2001). This again underscores the idea that the clustering of rafts does not lead to a random aggregation of raft components.

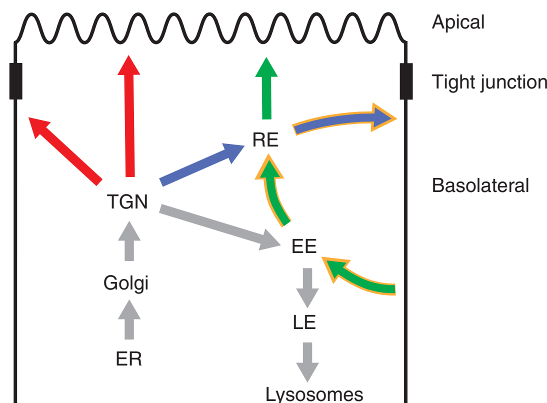

Fig. 2. Trafficking pathways in polarized epithelial cells. From the TGN, cargo can be transported directly to the apical or basolateral membrane (red). Basolateral cargo can also reach the plasma membrane through endosomes (blue). Following initial transport to the basolateral membrane, apical cargo can be sorted to the apical membrane via the transcytotic route (green). The arrows marked with a gold outline together constitute the basolateral recycling pathway. The apical recycling pathway and the apical-to-basolateral transcytotic pathway are omitted for clarity. EE, early endosomes; ER, endoplasmic reticulum; LE, late endosomes; RE, recycling endosomes; TGN, trans-Golgi network.

Transport carriers often arise from the TGN as large tubules (Hirschberg et al., 1998), which then undergo fission and fusion (Toomre et al., 1999). Segregation of apical and basolateral cargo might continue after transport containers have left the TGN, so that mixed carriers would mature into apical carriers through removal of basolateral proteins (Musch, 2004). This would be analogous to the maturation of immature secretory granules into dense core granules. TGN-derived apical and basolateral transport carriers can then directly fuse with their respective target plasma membrane domain (Keller et al., 2001).

TGN-derived carriers can also intersect with other trafficking routes before their cargo is delivered to the surface. Basolateral proteins such as the transferrin receptor, the asialoglycoprotein receptor and vesicular stomatitis glycoprotein (VSV-G) can pass through endosomes before they reach the cell surface (Futter et al., 1995; Leitinger et al., 1995; Ang et al., 2004). Given the similarity between certain basolateral sorting determinants and endocytosis signals, basolateral cargo and molecules endocytosed from the plasma membrane might meet in an endosomal compartment, probably in recycling endosomes. From there, basolateral cargo could move to the basolateral membrane together with molecules that are being recycled (Matter and Mellman, 1994; Traub and Apodaca, 2003). A similar endosomal stop-over has not been observed for apical cargo, except for mutants of the normally basolateral polymeric immunoglobulin receptor and VSV-G (Orzech et al., 2000; Ang et al., 2004). However, this might simply reflect a sorting defect along the basolateral route. These mutants might first travel from the TGN to recycling endosomes like their wild-type counterparts, but then fail to find their way to the basolateral membrane. They could subsequently leak into the pathway from recycling endosomes to the apical membrane that is normally used for apical recycling and transcytosis.

In some epithelial cells, such as hepatocytes, most apical cargo is first delivered to the basolateral membrane, followed by transcytosis (Bastaki et al., 2002). This could be achieved by selective endocytosis of apical proteins or, more likely, by sorting after endocytosis so that basolateral proteins are recycled whereas apical proteins are transcytosed. It has been suggested that the transcytotic route involves another transport intermediate, the apical recycling endosome (Brown et al., 2000; Mostov et al., 2000). However, it is not clear whether this intermediate really is a distinct compartment or a part of recycling endosomes (Sheff et al., 1999; Hoekstra et al., 2004).

These three modes of apical sorting (direct sorting from the TGN, sorting via endosomes and indirect sorting via the basolateral membrane) probably operate simultaneously in all polarized cell types but are used to different extents. Hepatocytes favour the transcytotic route, but also sort apical proteins directly (Kipp and Arias, 2000). Similarly, direct delivery is preferred in MDCK cells (Matlin and Simons, 1984;

Misek et al., 1984), but transcytotic sorting may nevertheless occur. Polishchuk et al. have recently claimed that GPI-anchored proteins are sorted to the apical surface of MDCK cells along the transcytotic pathway (Polishchuk et al., 2004). However, their observations contradict several previous studies (Brown et al., 1989; Lisanti et al., 1989; Keller et al., 2001). Thus, it remains to be seen whether they can be substantiated.

The underlying sorting mechanisms in the TGN and in endosomes are probably similar. Basolateral cytoplasmic domain determinants are likely to be recognized by similar sorting receptors (Folsch et al., 2003) and raft clustering might be reused to govern the trafficking of endocytosed molecules. The sorting machinery might also be reused. For instance, the closely related proteins VIP17/MAL and MAL2 have been proposed to participate in apical sorting at the TGN and in endosomes, respectively (Puertollano et al., 1999; Cheong et al., 1999; de Marco et al., 2002). Similarly, Rab11, in addition to functioning in recycling endosomes, has been suggested to regulate exit of basolateral cargo from the TGN (Chen et al., 1998).

Implications of apical membrane biogenesis by raft clustering

The hypothesis that basolateral sorting is mainly based on specific cargo capture by sorting receptors, whereas apical sorting relies on cargo recruitment into raft domains, could explain why basolateral proteins are sorted more accurately. Receptor-mediated cargo capture is a stringent mechanism for the inclusion of a particular set of proteins into transport carriers, but may not exclude other proteins very effectively. Hence, apical proteins could become part of basolateral transport containers simply by accident. Recruitment of apical cargo into clustered rafts may not be complete, because it ultimately relies on differential affinities for different lipid environments. However, raft clustering followed by domain-induced budding is a mechanism well suited to stringently exclude non-raft proteins.

A more fundamental reason for this mechanistic organization of epithelial exocytosis is that apical sorting must ensure the special lipid composition of the apical membrane responsible for its robustness. A raft-based sorting mechanism, in which selective lipid-lipid associations provide the driving force for sorting and the formation of transport containers, may be best suited for this purpose. By contrast, sorting based on specific protein-protein interactions may not exert much control over which lipids are included into the transport containers. The basolateral membrane contains a sizeable fraction of raft lipids that could simply have been delivered by default.

Another feature of sorting by recruitment into clustered rafts is that cargo can be transported by substoichiometric interactions with the machinery responsible for moving transport containers. When molecular motors bind to adaptors that directly interact with cargo proteins, a single motor protein controls the transport only of those proteins it captures using the adaptor. By contrast, a motor protein that associates with a raft domain could potentially move all lipids and proteins present in that domain. Apical transport involves kinesin motors (Kreitzer et al., 2000). So far, the only kinesin known to bind membranes by protein-lipid recognition is KIF1A (Klopfenstein and Vale, 2004), but there may be more. An interesting candidate is KIFC3, which has been proposed to associate with rafts and mediate the apical transport of raft proteins (Noda et al., 2001). Movement of transport containers is followed by fusion with the target membrane. Again, raft association of only a few proteins that mediate fusion may be sufficient to govern the targeting of whole raft domains. Indeed, syntaxin 3 and TI-VAMP, two components of the apical fusion machinery (Galli et al., 1998; Low et al., 1998), associate with rafts (Lafont et al., 1999). Hence, KIFC3, syntaxin 3 and TI-VAMP could act as molecular guideposts to ensure the economical, coordinated targeting of apical cargo.

Apical membrane biogenesis during tube formation by epithelial cells could be another process in which raft clustering plays a key role. Tube formation occurs in a number of ways, all of which involve the generation of a new apical membrane (Lubarsky and Krasnow, 2003). For instance, MDCK cells, as well as epithelial cells of the *Caenorhabditis elegans* gut and *Drosophila* heart, can depolarize and form a cord consisting of two layers of cells. In this unpolarized state, apical membrane components are deposited in an intracellular vacuolar apical compartment (VAC), which may be a specialized endosome (Vega-Salas et al., 1987; Low et al., 2000). Once the cord is established, the cells repolarize and form a new apical membrane at the site where the two cell layers face each other, thus generating a tube lumen. They achieve this by exocytosing the entire VAC (Vega-Salas et al., 1988). Similarly, newly formed *Drosophila* tracheal tubes are expanded in a two-step process that involves the cytoplasmic accumulation of vesicles containing apical membrane components and their subsequent fusion with the apical membrane, thereby increasing its area (Beitel and Krasnow, 2000; Lubarsky and Krasnow, 2003). Raft clustering clearly is a mechanism suited to bring about the collective transport of large sets of molecules. Because only a few ‘guidepost’ proteins may be necessary to make whole patches of membrane moveable, raft clustering could be used to deliver blocks of prospective apical membrane to a new destination.

Perspective

The challenge now is to gain more insight into the mechanisms of polarized trafficking by defining the protein machinery involved. The interactions of this machinery with lipid microdomains should provide further clues about the role of rafts in sorting. In addition, the localization of the machinery should help to clarify at which cellular sites segregation of different cargo molecules occurs. Together with more information on the natural cargo proteins, this will lead to a more complete understanding of how different sorting mechanisms and trafficking routes are employed according to the physiological functions of epithelial cells.

This will, however, yield only a static picture of how membrane trafficking works. During differentiation, various epithelial cells switch between the modes of surface transport they utilize (Zurzolo et al., 1992; Tuma et al., 2002; Kreitzer et al., 2003), but we know very little about how this is brought about. The signalling events that regulate such a reorganization of trafficking during differentiation are also largely unknown. Insight here will come from applying what has been learnt about cell polarity in other model organisms (Baas et al., 2004;

Cohen et al., 2004a; Cohen et al., 2004b). Bringing together what is known about polarized membrane trafficking and the many developmental processes that involve the biogenesis of new membrane domains will deepen our understanding of how polarized sorting works at the cellular level, and how it is put into action when cells collaborate to create tissues.

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References

Altschuler, Y., Hodson, C. and Milgram, S. L. (2003). The apical compartment: trafficking pathways, regulators and scaffolding proteins. *Curr. Opin. Cell Biol.* **15**, 423-429.

Ang, L. A., Taguchi, T., Francis, S., Folsch, H., Murrells, L., 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.

Baas, A. F., Kuipers, J., van der Wel, N. N., Batlle, E., Koerten, H. K., Peters, P. J. and Clevers, H. C. (2004). Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* **116**, 457-466.

Bagnat, M. and Simons, K. (2002). Cell surface polarization during yeast mating. *Proc. Natl. Acad. Sci. USA* **99**, 14183-14188.

Bagnat, M., Chang, A. and Simons, K. (2001). Plasma membrane proton ATPase Pma1p requires raft association for surface delivery in yeast. *Mol. Biol. Cell* **12**, 4129-4138.

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.

Bastaki, M., Braiterman, L. T., Johns, D. C., Chen, Y. H. and Hubbard, A. L. (2002). Absence of direct delivery for single transmembrane apical proteins or their 'Secretory' forms in polarized hepatic cells. *Mol. Biol. Cell* **13**, 225-237.

Baumgart, T., Hess, S. T. and Webb, W. W. (2003). Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. *Nature* **425**, 821-824.

Beitel, G. J. and Krasnow, M. A. (2000). Genetic control of epithelial tube size in the *Drosophila* tracheal system. *Development* **127**, 3271-3282.

Benharouga, M., Sharma, M., So, J., Haardt, M., Drzymala, L., Popov, M., Schwapach, B., Grinstein, S., Du, K. and Lukacs, G. L. (2003). The role of the C terminus and Na^+/H^+ exchanger regulatory factor in the functional expression of cystic fibrosis transmembrane conductance regulator in nonpolarized cells and epithelia. *J. Biol. Chem.* **278**, 22079-22089.

Benting, J. H., Rietveld, A. G. and Simons, K. (1999a). 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.

Benting, J. H., Rietveld, A. G., Ansorge, I. and Simons, K. (1999b). Acyl and alkyl chain length of GPI-anchors is critical for raft association in vitro. *FEBS Lett.* **462**, 47-50.

Braccia, A., Villani, M., Immerdal, L., Niels-Christiansen, L. L., Nystrom, B. T., Hansen, G. H. and Danielsen, E. M. (2003). Microvillar membrane microdomains exist at physiological temperature. Role of galectin-4 as lipid raft stabilizer revealed by 'superrafts'. *J. Biol. Chem.* **278**, 15679-15684.

Bretscher, M. S. and Munro, S. (1993). Cholesterol and the Golgi apparatus. *Science* **261**, 1280-1281.

Brewer, C. F., Miceli, M. C. and Baum, L. G. (2002). Clusters, bundles, arrays and lattices: novel mechanisms for lectin-saccharide-mediated cellular interactions. *Curr. Opin. Struct. Biol.* **12**, 616-623.

Brown, D. A. and London, E. (1998). Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* **14**, 111-136.

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.

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.

Brown, P. S., Wang, E., Aroeti, B., Chapin, S. J., Mostov, K. E. and Dunn, K. W. (2000). Definition of distinct compartments in polarized Madin-Darby canine kidney (MDCK) cells for membrane-volume sorting, polarized sorting and apical recycling. *Traffic* **1**, 124-140.

Bunnell, S. C., Hong, D. I., Kardon, J. R., Yamazaki, T., McGlade, C. J., Barr, V. A. and Samelson, L. E. (2002). T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. *J. Cell Biol.* **158**, 1263-1275.

Chen, W., Feng, Y., Chen, D. and Wandinger-Ness, A. (1998). Rab11 is required for trans-golgi network-to-plasma membrane transport and a preferential target for GDP dissociation inhibitor. *Mol. Biol. Cell* **9**, 3241-3257.

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.

Cherukuri, A., Carter, R. H., Brooks, S., Bornmann, W., Finn, R., Dowd, C. S. and Pierce, S. K. (2004). B cell signaling is regulated by induced palmitoylation of CD81. *J. Biol. Chem.* **279**, 31973-31982.

Cohen, D., Brennwald, P. J., Rodriguez-Boulan, E. and Musch, A. (2004a). Mammalian PAR-1 determines epithelial lumen polarity by organizing the microtubule cytoskeleton. *J. Cell Biol.* **164**, 717-727.

Cohen, D., Rodriguez-Boulan, E. and Musch, A. (2004b). Par-1 promotes a hepatic mode of apical protein trafficking in MDCK cells. *Proc. Natl. Acad. Sci. USA* **101**, 13792-13797.

Crane, J. M. and Tamm, L. K. (2004). Role of cholesterol in the formation and nature of lipid rafts in planar and spherical model membranes. *Biophys. J.* **86**, 2965-2979.

de Marco, M. C., Martin-Belmonte, F., Kremer, L., Albar, J. P., Correas, I., Vaerman, J. P., Marazuela, M., Byrne, J. A. and Alonso, M. A. (2002). MAL2, a novel raft protein of the MAL family, is an essential component of the machinery for transcytosis in hepatoma HepG2 cells. *J. Cell Biol.* **159**, 37-44.

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.

Folsch, H., Pypaert, M., Maday, S., Pelletier, L. and Mellman, I. (2003). The AP-1A and AP-1B clathrin adaptor complexes define biochemically and functionally distinct membrane domains. *J. Cell Biol.* **163**, 351-362.

Futter, C. E., Connolly, C. N., Cutler, D. F. and Hopkins, C. R. (1995). Newly synthesized transferrin receptors can be detected in the endosome before they appear on the cell surface. *J. Biol. Chem.* **270**, 10999-11003.

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.

Gomez-Mouton, C., Abad, J. L., Mira, E., Lacalle, R. A., Gallardo, E., Jimenez-Baranda, S., Illa, I., Bernad, A., Manes, S. and Martinez, A. C. (2001). Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. *Proc. Natl. Acad. Sci. USA* **98**, 9642-9647.

Gu, C., Jan, Y. N. and Jan, L. Y. (2003). A conserved domain in axonal targeting of Kv1 (Shaker) voltage-gated potassium channels. *Science* **301**, 646-649.

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.

Hansen, G. H., Niels-Christiansen, L. L., Thorsen, E., Immerdal, L. and Danielsen, E. M. (2000). Cholesterol depletion of enterocytes. Effect on the Golgi complex and apical membrane trafficking. *J. Biol. Chem.* **275**, 5136-5142.

Harder, T. and Engelhardt, K. R. (2004). Membrane domains in lymphocytes – from lipid rafts to protein scaffolds. *Traffic* **5**, 265-275.

Harder, T. and Kuhn, M. (2000). Selective accumulation of raft-associated membrane protein LAT in T cell receptor signaling assemblies. *J. Cell Biol.* **151**, 199-208.

Harder, T., Scheiffele, P., Verkade, P. and Simons, K. (1998). Lipid domain structure of the plasma membrane revealed by patching of membrane components. *J. Cell Biol.* **141**, 929-942.

Hirschberg, K., Miller, C. M., Ellenberg, J., Presley, J. F., Siggia, E. D., Phair, R. D. and Lippincott-Schwartz, J. (1998). Kinetic analysis of secretory protein traffic and characterization of golgi to plasma membrane transport intermediates in living cells. *J. Cell Biol.* **143**, 1485-1503.

Hoekstra, D., Tyteca, D. and van IJzendoorn, S. C. (2004). The subapical compartment: a traffic center in membrane polarity development. *J. Cell Sci.* **117**, 2183-2192.

Horton, A. C. and Ehlers, M. D. (2003). Neuronal polarity and trafficking. *Neuron* **40**, 277-295.

Huttner, W. B. and Zimmerberg, J. (2001). Implications of lipid microdomains for membrane curvature, budding and fission. *Curr. Opin. Cell Biol.* **13**, 478-484.

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.

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.

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.

Janes, P. W., Ley, S. C. and Magee, A. I. (1999). Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. *J. Cell Biol.* **147**, 447-461.

Keller, P. and Simons, K. (1998). Cholesterol is required for surface transport of influenza virus hemagglutinin. *J. Cell Biol.* **140**, 1357-1367.

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.

Kipp, H. and Arias, I. M. (2000). Newly synthesized canalicular ABC transporters are directly targeted from the Golgi to the hepatocyte apical domain in rat liver. *J. Biol. Chem.* **275**, 15917-15925.

Klopfenstein, D. R. and Vale, R. D. (2004). The lipid binding pleckstrin homology domain in UNC-104 kinesin is necessary for synaptic vesicle transport in *Caenorhabditis elegans*. *Mol. Biol. Cell* **15**, 3729-3739.

Korlach, J., Schwille, P., Webb, W. W. and Feigenson, G. W. (1999). Characterization of lipid bilayer phases by confocal microscopy and fluorescence correlation spectroscopy. *Proc. Natl. Acad. Sci. USA* **96**, 8461-8466.

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.

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.

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.

Kusumi, A., Koyama-Honda, I. and Suzuki, K. (2004). Molecular dynamics and interactions for creation of stimulation-induced stabilized rafts from small unstable steady-state rafts. *Traffic* **5**, 213-230.

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.

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.

Leitinger, B., Hille-Rehfeld, A. and Spiess, M. (1995). Biosynthetic transport of the asialoglycoprotein receptor H1 to the cell surface occurs via endosomes. *Proc. Natl. Acad. Sci. USA* **92**, 10109-10113.

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.

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.

Lipowsky, R. (1993). Domain-induced budding of fluid membranes. *Biophys. J.* **64**, 1133-1138.

Lipowsky, R. (2002). Domains and rafts in membranes – hidden dimensions of self-organization. *J. Biol. Phys.* **28**, 195-210.

Lisanti, M. P., Caras, I. W., Davitz, M. A. and Rodriguez-Boulan, E. (1989). A glycophospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. *J. Cell Biol.* **109**, 2145-2156.

London, E. and Brown, D. A. (2000). Insolubility of lipids in triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domains (rafts). *Biochim. Biophys. Acta* **1508**, 182-195.

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.

Low, S. H., Miura, M., Roche, P. A., Valdez, A. C., Mostov, K. E. and Weimbs, T. (2000). Intracellular redirection of plasma membrane trafficking after loss of epithelial cell polarity. *Mol. Biol. Cell* **11**, 3045-3060.

Lubarsky, B. and Krasnow, M. A. (2003). Tube morphogenesis: making and shaping biological tubes. *Cell* **112**, 19-28.

Lundbaek, J. A., Andersen, O. S., Werge, T. and Nielsen, C. (2003). Cholesterol-induced protein sorting: an analysis of energetic feasibility. *Biophys. J.* **84**, 2080-2089.

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.

Matter, K. (2000). Epithelial polarity: sorting out the sorters. *Curr. Biol.* **10**, R39-R42.

Matter, K. and Mellman, I. (1994). Mechanisms of cell polarity: sorting and transport in epithelial cells. *Curr. Opin. Cell Biol.* **6**, 545-554.

Mayor, S. and Riezman, H. (2004). Sorting GPI-anchored proteins. *Nat. Rev. Mol. Cell Biol.* **5**, 110-120.

Mays, R. W., Siemers, K. A., Fritz, B. A., Lowe, A. W., van Meer, G. and Nelson, W. J. (1995). Hierarchy of mechanisms involved in generating Na/K-ATPase polarity in MDCK epithelial cells. *J. Cell Biol.* **130**, 1105-1115.

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.

Monier, S., Parton, R. G., Vogel, F., Behlke, J., Henske, A. and Kurzchalia, T. V. (1995). VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. *Mol. Biol. Cell* **6**, 911-927.

Monlauzeur, L., Rajasekaran, A., Chao, M., Rodriguez-Boulan, E. and le Bivic, A. (1995). A cytoplasmic tyrosine is essential for the basolateral localization of mutants of the human nerve growth factor receptor in Madin-Darby canine kidney cells. *J. Biol. Chem.* **270**, 12219-12225.

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

Munro, S. (2003). Lipid rafts: elusive or illusive? *Cell* **115**, 377-388.

Musch, A. (2004). Microtubule organization and function in epithelial cells. *Traffic* **5**, 1-9.

Muth, T. R. and Caplan, M. J. (2003). Transport protein trafficking in polarized cells. *Annu. Rev. Cell Dev. Biol.* **19**, 333-366.

Nakagawa, T., Setou, M., Seog, D., Ogasawara, K., Dohmae, N., Takio, K. and Hirokawa, N. (2000). A novel motor, KIF13A, transports mannose-6-phosphate receptor to plasma membrane through direct interaction with AP-1 complex. *Cell* **103**, 569-581.

Neumann-Giesen, C., Falkenbach, B., Beicht, P., Claasen, S., Luers, G., Stuermer, C. A., Herzog, V. and Tikkanen, R. (2004). Membrane and raft association of reggie-1/flotillin-2: role of myristoylation, palmitoylation and oligomerization and induction of filopodia by overexpression. *Biochem. J.* **378**, 509-518.

Nishimura, N., Plutner, H., Hahn, K. and Balch, W. E. (2002). The delta subunit of AP-3 is required for efficient transport of VSV-G from the trans-Golgi network to the cell surface. *Proc. Natl. Acad. Sci. USA* **99**, 6755-6760.

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.

Oling, F., Bergsma-Schutter, W. and Brisson, A. (2001). Trimers, dimers of trimers, and trimers of trimers are common building blocks of annexin a5 two-dimensional crystals. *J. Struct. Biol.* **133**, 55-63.

Orzech, E., Cohen, S., Weiss, A. and Aroeti, B. (2000). Interactions between the exocytic and endocytic pathways in polarized Madin-Darby canine kidney cells. *J. Biol. Chem.* **275**, 15207-15219.

Ostedgaard, L. S., Randak, C., Rokhlina, T., Karp, P., Vermeer, D., Ashbourne Excoffon, K. J. and Welsh, M. J. (2003). Effects of C-terminal deletions on cystic fibrosis transmembrane conductance regulator function in cystic fibrosis airway epithelia. *Proc. Natl. Acad. Sci. USA* **100**, 1937-1942.

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*. (in press).

Perego, C., Vanoni, C., Villa, A., Longhi, R., Kaech, S. M., Frohli, E., Hajnal, A., Kim, S. K. and Pietrini, G. (1999). PDZ-mediated interactions retain the epithelial GABA transporter on the basolateral surface of polarized epithelial cells. *EMBO J.* **18**, 2384-2393.

Pfeiffer, S., Fuller, S. D. and Simons, K. (1985). Intracellular sorting and basolateral appearance of the G protein of vesicular stomatitis virus in Madin-Darby canine kidney cells. *J. Cell Biol.* **101**, 470-476.

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.

Pralle, A., Keller, P., Florin, E. L., Simons, K. and Horber, J. K. (2000). Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. *J. Cell Biol.* **148**, 997-1008.

Prior, I. A., Muncke, C., Parton, R. G. and Hancock, J. F. (2003). Direct visualization of Ras proteins in spatially distinct cell surface microdomains. *J. Cell Biol.* **160**, 165-170.

Puertollano, R., Martin-Belmonte, F., Millan, J., de Marco, M. C., 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.

Rescher, U. and Gerke, V. (2004). Annexins – unique membrane binding proteins with diverse functions. *J. Cell Sci.* **117**, 2631-2639.

Robinson, M. S. (2004). Adaptable adaptors for coated vesicles. *Trends Cell Biol.* **14**, 167-174.

Scheiffele, P., Peranen, J. and Simons, K. (1995). N-glycans as apical sorting signals in epithelial cells. *Nature* **378**, 96-98.

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.

Schmidt, K., Schrader, M., Kern, H. F. and Kleene, R. (2001). Regulated apical secretion of zymogens in rat pancreas. Involvement of the glycosylphosphatidylinositol-anchored glycoprotein GP-2, the lectin ZG16p, and cholesterol-glycosphingolipid-enriched microdomains. *J. Biol. Chem.* **276**, 14315-14323.

Schuck, S., Honsho, M., Ekroos, K., Shevchenko, A. and Simons, K. (2003). Resistance of cell membranes to different detergents. *Proc. Natl. Acad. Sci. USA* **100**, 5795-5800.

Setou, M., Nakagawa, T., Seog, D. H. and Hirokawa, N. (2000). Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* **288**, 1796-1802.

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.

Shelly, M., Mosesson, Y., Citri, A., Lavi, S., Zwang, Y., Melamed-Book, N., Aroeti, B. and Yarden, Y. (2003). Polar expression of ErbB-2/HER2 in epithelia. Bimodal regulation by Lin-7. *Dev. Cell* **5**, 475-486.

Shenolikar, S. and Weinman, E. J. (2001). NHERF: targeting and trafficking membrane proteins. *Am. J. Physiol. Renal Physiol.* **280**, F389-F395.

Short, D. B., Trotter, K. W., Reczek, D., Kreda, S. M., Bretscher, A., Boucher, R. C., Stutts, M. J. and Milgram, S. L. (1998). An apical PDZ protein anchors the cystic fibrosis transmembrane conductance regulator to the cytoskeleton. *J. Biol. Chem.* **273**, 19797-19801.

Shvartsman, D. E., Kotler, M., Tall, R. D., Roth, M. G. and Henis, Y. I. (2003). Differently anchored influenza hemagglutinin mutants display distinct interaction dynamics with mutual rafts. *J. Cell Biol.* **163**, 879-888.

Simmen, T., Honing, S., Icking, A., Tikkanen, R. and Hunziker, W. (2002). AP-4 binds basolateral signals and participates in basolateral sorting in epithelial MDCK cells. *Nat. Cell Biol.* **4**, 154-159.

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

Simons, K. and Toomre, D. (2000). Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1**, 31-39.

Simons, K. and van Meer, G. (1988). Lipid sorting in epithelial cells. *Biochemistry* **27**, 6197-6202.

Simons, K. and Vaz, W. L. (2004). Model systems, lipid rafts, and cell membranes. *Annu. Rev. Biophys. Biomol. Struct.* **33**, 269-295.

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.

Snyers, L., Umlauf, E. and Prohaska, R. (1999). Association of stomatin with lipid-protein complexes in the plasma membrane and the endocytic compartment. *Eur. J. Cell Biol.* **78**, 802-812.

Sotgia, F., Razani, B., Bonuccelli, G., Schubert, W., Battista, M., Lee, H., Capozza, F., Schubert, A. L., Minetti, C., Buckley, J. T. et al. (2002). Intracellular retention of glycosylphosphatidyl inositol-linked proteins in caveolin-deficient cells. *Mol. Cell. Biol.* **22**, 3905-3926.

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.

Sugimoto, H., Sugahara, M., Folsch, H., Koide, Y., Nakatsu, F., Tanaka, N., Nishimura, T., Furukawa, M., Mullins, C., Nakamura, N. et al. (2002). Differential recognition of tyrosine-based basolateral signals by AP-1B subunit mu1B in polarized epithelial cells. *Mol. Biol. Cell* **13**, 2374-2382.

Swiatecka-Urban, A., Duhaime, M., Coutermarsh, B., Karlson, K. H., Collawn, J., Milewski, M., Cutting, G. R., Guggino, W. B., Langford, G. and Stanton, B. A. (2002). PDZ domain interaction controls the endocytic recycling of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* **277**, 40099-40105.

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.

Thiele, C. and Huttner, W. B. (1998). Protein and lipid sorting from the trans-Golgi network to secretory granules – recent developments. *Semin. Cell Dev. Biol.* **9**, 511-516.

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.

Traub, L. M. and Apodaca, G. (2003). AP-1B: polarized sorting at the endosome. *Nat. Cell Biol.* **5**, 1045-1047.

Tsukita, S., Furuse, M. and Itoh, M. (2001). Multifunctional strands in tight junctions. *Nat. Rev. Mol. Cell Biol.* **2**, 285-293.

Tuma, P. L., Nyasae, L. K. and Hubbard, A. L. (2002). Nonpolarized cells selectively sort apical proteins from cell surface to a novel compartment, but lack apical retention mechanisms. *Mol. Biol. Cell* **13**, 3400-3415.

van Deurs, B., Roepstorff, K., Hommelgaard, A. M. and Sandvig, K. (2003). Caveolae: anchored, multifunctional platforms in the lipid ocean. *Trends Cell Biol.* **13**, 92-100.

Vega-Salas, D. E., Salas, P. J. and Rodriguez-Boulan, E. (1987). Modulation of the expression of an apical plasma membrane protein of Madin-Darby canine kidney epithelial cells: cell-cell interactions control the appearance of a novel intracellular storage compartment. *J. Cell Biol.* **104**, 1249-1259.

Vega-Salas, D. E., Salas, P. J. and Rodriguez-Boulan, E. (1988). Exocytosis of vacuolar apical compartment (VAC): a cell-cell contact controlled mechanism for the establishment of the apical plasma membrane domain in epithelial cells. *J. Cell Biol.* **107**, 1717-1728.

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.

Wilson, B. S., Steinberg, S. L., Liederman, K., Pfeiffer, J. R., Surviladze, Z., Zhang, J., Samelson, L. E., Yang, L. H., Kotula, P. G. and Oliver, J. M. (2004). Markers for detergent-resistant lipid rafts occupy distinct and dynamic domains in native membranes. *Mol. Biol. Cell* **15**, 2580-2592.

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.

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.

Zurzolo, C., le Bivic, A., Quaroni, A., Nitsch, L. and Rodriguez-Boulan, E. (1992). Modulation of transcytotic and direct targeting pathways in a polarized thyroid cell line. *EMBO J.* **11**, 2337-2344.