Apical targeting in polarized epithelial cells: there's more afloat than rafts

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Most metazoan cells are 'polarized'. A crucial aspect of this polarization is that the plasma membrane is divided into two or more domains with different protein and lipid compositions – for example, the apical and basolateral domains of epithelial cells or the axonal and somatodendritic domains of neurons. This polarity is established and maintained by highly specific vesicular membrane transport in the biosynthetic, endocytic and transcytotic pathways. Two important concepts, the 'SNARE' and the 'raft' hypotheses, have been developed that together promise at least a partial understanding of the underlying general mechanisms that ensure the necessary specificity of these pathways.

One particularly well-studied example of polarized cells is epithelial cells, which form a layer lining a surface or cavity. Of the approximately 160 recognized major cell types in humans, around 60% are classified as epithelial. The plasma membrane of epithelial cells is divided by tight junctions into two domains: an apical surface facing a lumen or the outside world, and a basolateral surface facing adjoining cells and underlying connective tissue. These two domains have different protein and lipid compositions, reflecting their very different functions. To appreciate the importance of this polarity, imagine the consequences if the epithelia of the exocrine pancreas or the stomach suddenly secreted digestive enzymes or hydrochloric acid, respectively, to the 'other side'.

Epithelial cells use two pathways to send molecules to the correct surface (Fig. 1). In the 'direct' pathway, new protein molecules are synthesized in the rough endoplasmic reticulum (RER) and transported through the Golgi to the *trans* Golgi network (TGN). In the TGN, proteins are packaged into vesicles that deliver them to the apical or basolateral surface. Alternatively, in the 'indirect' pathway, molecules are sent first from the TGN to the basolateral surface, from which they can then be endocytosed and transported to the apical surface by transcytosis. Transcytosis (at least to the apical surface) involves transit through an apical recycling compartment (ARC), which is a central sorting station in the endosomal system[1]. The steady-state distribution of a protein can depend not only on its vectorial delivery to each location but also on its retention, for example by binding to the membrane cytoskeleton as proposed for the Na^+/K^+-ATPase[2]. We divide polarized transport to the plasma membrane into four steps: segregation, budding, transport and docking (Fig. 1). Here, we concentrate primarily on the first and last steps.

Step 1: segregation

At some point in both the direct and indirect pathways, apical and basolateral proteins must be separated from each other while still in the same membrane. This sorting information must be an intrinsic property of the proteins, although later sorting steps might depend solely on the properties of the vesicle (or the raft: see below) containing the proteins.

Sorting information for basolateral membrane proteins is usually encoded in short (2–10 residue) 'basolateral sorting signals' located in the cytoplasmic domain of a protein[3]. These frequently resemble or even overlap with Tyr-containing or Leu–Leu motifs used for endocytosis from the plasma membrane or for sorting from the TGN to endosomes. However, mutagenesis studies demonstrate distinct sequence requirements for basolateral sorting and endocytosis. A revealing example is the basolateral sorting signal of the polymeric immunoglobulin receptor (pIgR)[4]. This signal lacks a Tyr or Leu–Leu motif, but its secondary structure includes a crucial type I β-turn, like that found in Tyr-containing endocytosis signals. It is possible that the fundamental feature of all of these sorting signals is a type I β-turn.

The best-understood apical signal is the glycosylphosphatidylinositol (GPI) anchor, and its properties have led to the emergence of a general model for sorting of apically targeted proteins and lipids[5]. A central feature of the model is clustering of glycosphingolipids (GSLs) and GPI-anchored proteins (GPIAPs) into distinct membrane subdomains or 'rafts'. In artificial-membrane models, GSLs spontaneously self associate into such rafts, possibly through hydrogen bonding of their head groups and/or packing of their long, saturated acyl chains into a 'liquid ordered' phase[6]. Cholesterol promotes formation of this phase, perhaps by intercalating between the acyl chains of the GSL[5,7]. The outer leaflet of the apical plasma membrane of a typical epithelial cell is enriched in certain glycosphingolipids and depleted of glycerolipids. GPIAPs are found predominantly at the apical surface of most epithelial cells, and the addition of a GPI anchor is sufficient to target a reporter protein to the apical surface. In the 'raft hypothesis', GPIAPs and GSLs meet in the biosynthetic pathway and cluster together spontaneously to form a membrane microdomain, or raft. The association of GPIAPs with rafts may be due to the long acyl chains in the GPI anchor. Raft formation is the sorting process *per se*, and the intact raft is transported in vesicles to the apical surface.

Biophysical techniques have been used to demonstrate the existence of rafts, primarily in artificial membranes[5]. However, for studies in cells, the main

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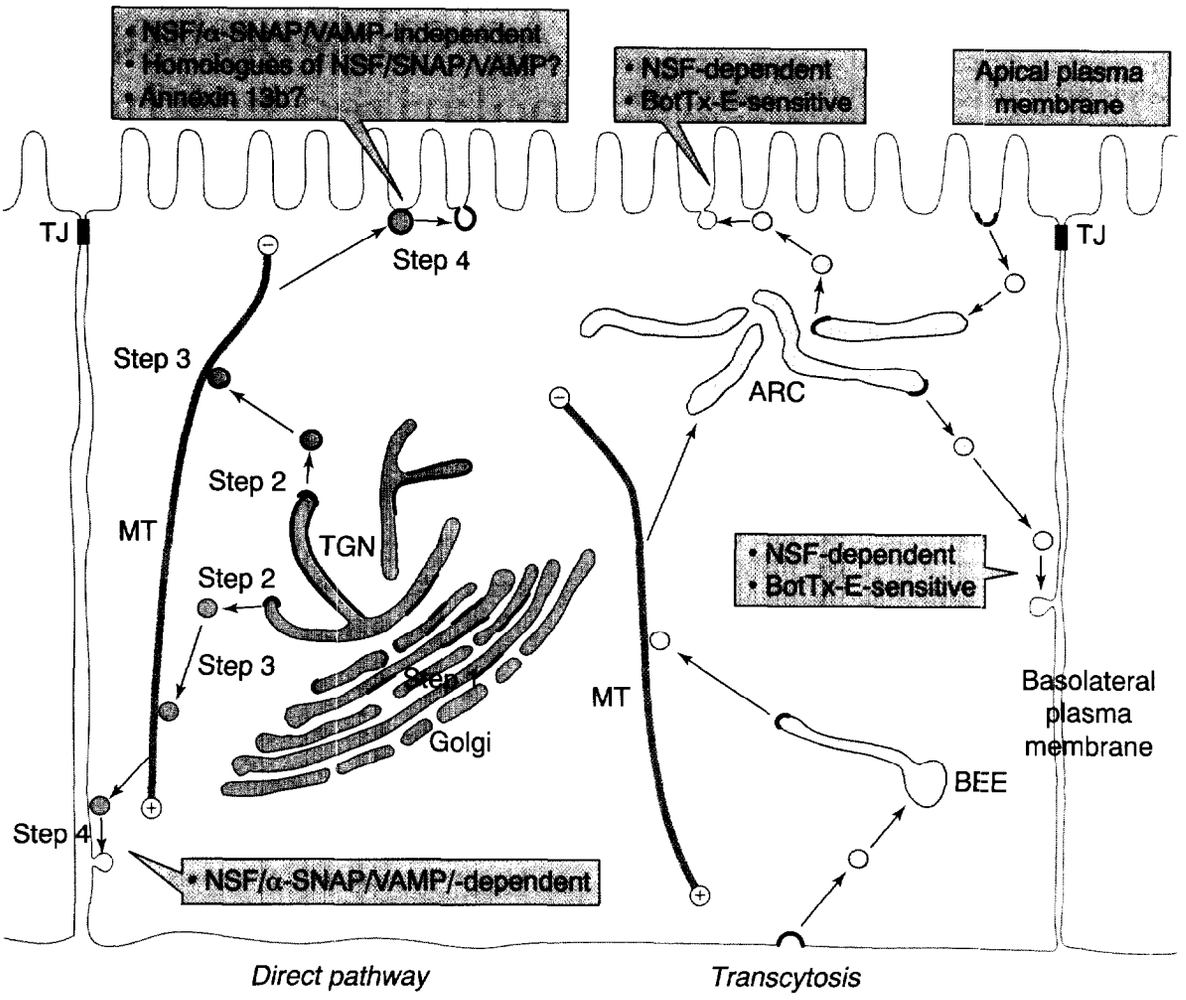

FIGURE 1

Summary of the vesicular membrane-trafficking pathways in a typical epithelial cell (e.g. MDCK). Epithelial cells possess two plasma membrane domains, apical and basolateral, separated by tight junctions (TJs). Two principal pathways exist for the targeting of plasma membrane proteins: in the 'direct' pathway, proteins are sorted in the Golgi apparatus, possibly by clustering into or exclusion from glycosphingolipid-rich membrane microdomains (rafts, step 1). Transport vesicles destined for the apical and basolateral membranes bud from the *trans* Golgi network (TGN), in a process probably mediated by coat proteins (step 2). Vesicles are transported directionally along microtubules (MTs) or other cytoskeletal elements using vesicle-associated motors (step 3). After reaching the plasma membrane, vesicles dock and fuse utilizing the SNARE machinery at the basolateral and possibly also at the apical surface (step 4, see text for explanations). In the 'indirect' pathway, newly synthesized membrane proteins are first transported from the TGN to the basolateral surface and are then endocytosed into basolateral early endosomes (BEE). From here, apical proteins are transported along microtubules to the tubovesicular 'apical recycling compartment' (ARC), which also receives proteins internalized from the apical surface. The final transport step to the apical plasma membrane involves the SNARE machinery since it is NSF-dependent and sensitive to botulinum toxin E (BotTx-E), which cleaves certain t-SNAREs[58].

experimental tool used to determine whether a molecule partitions into rafts, and indeed part of the underpinnings of the raft hypothesis, is to solubilize the cells in non-ionic detergents (e.g. Triton X-100) at 4°C. Under these conditions, GSLs and GPIAPs are found in low-density insoluble membrane structures that can be isolated on sucrose gradients. Although copurification with such floating material is often taken as evidence that a molecule is part of a raft, this, *per se*, is a poor indication that such a molecule is in rafts or even that the rafts themselves exist in intact cells[8,9]. For instance, sphingomyelin is almost entirely detergent insoluble[10], yet it, as well as other sphingolipids such as galactosylceramide and sulfatide, is preferentially transported basolaterally in Madin–Darby canine kidney (MDCK) epithelial cells[11,12]. Similarly, several non-apical proteins are found in the detergent-insoluble material. The choice of detergent also has a strong influence on the protein and lipid composition of the isolated final material[13].

Besides GPI-anchored proteins, some apical transmembrane proteins are also raft associated as judged by the detergent-insolubility criterion; these include influenza haemagglutinin (HA) and neuraminidase (NA)[14,15]. The transmembrane domain of NA was shown to be responsible for apical targeting as well as for detergent insolubility[15], but this appears not to be the case for HA[16]. These findings led to an extension of the raft hypothesis as a general mechanism of protein sorting in the biosynthetic pathway. Raft formation and hence sorting may take place as early as in the *cis* or medial Golgi[10]. Although this appears to contradict earlier studies in which no segregation of apically and basolaterally targeted membrane proteins

was observed in the Golgi[17], the possibility that the sorting power of the entire Golgi apparatus could be used for this process is intriguing.

N-linked oligosaccharides can act as an apical signal for secretory proteins, for example gp80, in MDCK cells[18–20]. Some basolateral membrane proteins appear to possess a recessive apical signal in their lumenal domain because removal of their cytoplasmic basolateral targeting signal often results in apical targeting[21]. It has been suggested that this signal is the N-glycans[21]. Moreover, it has been hypothesized that N-glycans might interact with a raft-bound lectin, such as VIP36, in the TGN, thereby accomplishing sorting[21]. This latter conjecture is, however, incompatible with the finding that certain apical proteins, including gp80, can be transported independently of GPIAPs and GSLs (Refs 22 and 23 and see below).

Although the raft model is extremely appealing, it is useful to examine how well it explains sorting in various epithelial cell types (Table 1). The most commonly used epithelial cell line is MDCK. The original line was heterogeneous, and different clones with distinctive properties have been isolated by several groups. In general, type I clones have high transmonolayer electrical resistance (~1000 ohm cm^{-2}), whereas type II clones have lower resistance (~100 ohm cm^{-2}). One of the most frequently used MDCK type II clones was isolated at the EMBL in Heidelberg ('Heidelberg clone'). Remarkably, another type II clone ('J clone') sorts GSLs and GPIAPs equally to both surfaces, whereas both a transmembrane apical protein (gp135) and a secretory apical protein (gp80) are still sorted to the apical surface[23]. This cell line can therefore sort certain proteins to the apical surface independently of GSLs and GPIAPs. Another MDCK type II strain, isolated by selection for concanavalin A resistance, sorts GSLs correctly to the apical surface, but GPIAPs are sorted to both surfaces[24].

Fischer rat thyroid (FRT) cells sort GSLs and GPIAPs entirely to the basolateral surface[24,25]. Several other non-GPI-anchored proteins that are apical in MDCK cells are also apical in these cells, indicating that FRT cells can still sort proteins to the apical surface in the absence of apical GSL- and GPIAP-traffic. HT-29 cells can be grown under conditions where the cells are non-polarized and the 'apical membrane' forms an intracellular compartment. It was reported that GPIAPs are transported to this 'apical' compartment, whereas some normally apical transmembrane proteins are still transported to the surface[26].

There are several other shortcomings in the experimental support for the raft model. First, much of the work on sphingolipid sorting has been based on using soluble, short chain, fluorescent sphingolipids, such as NBD ceramide and its metabolites. Although these analogues can be a useful tool for analysing lipid transport, they have a bulky artificial fluorescent group and lack the long acyl chain that may be involved in packaging into rafts. Second, it has been realized recently that these short-chain lipid analogues can be transported independently of vesicular traffic owing to their high water-solubility[27], requiring a revision of the interpretation of many previously published data. Third, little attention has been paid to variations in the length and saturation of the acyl groups in various lipids. Such variations could account for at least some of the differences in sorting seen in different cell types. Similarly, the structure of the GPI anchors may vary in different proteins and cell types, which might also account for some of the differences described above.

We therefore suggest that, although the raft model is appealing, it is far from well established and should

TABLE 1 – SUMMARY OF SORTING PATTERNS FOUND IN A VARIETY OF EPITHELIAL CELLS[a]

Cell type	Glycolipids	GPI-proteins	Other apical proteins	Triton-insoluble	Basolateral proteins	Refs
MDCK II strains						
Heidelberg strain	Apical	Apical	Apical	GSL, GPI, HA	Basolateral	3,5
'J' strain	Mixed	Mixed	Apical (secreted gp80, membrane gp135)	Not reported	Mixed (Na^+/K^+-ATPase) Basolateral (E-cadherin)	2,23
$ConA^r$ mutant	Apical	Mixed	Apical	GSL, GPI	Basolateral	24
Fischer rat thyroid (FRT) cells	Basolateral	Basolateral	Apical	GSL, not GPI	Basolateral	22,24, 25
Caco2 intestinal cells	Apical	Apical	Apical (some predominantly basolateral, then transcytosed apical)	GSL, GPI	Basolateral	31,32
Hepatocytes	Mixed	Basolateral (followed by transcytosis in both cases)	Basolateral	Not reported	Basolateral	33,34

[a]These data indicate that there is tremendous plasticity in the sorting patterns used by various epithelial cells. In particular, the notion that glycolipid and GPI-anchored proteins are sorted in Triton-insoluble rafts to the apical surface was established in the Heidelberg strain of MDCK cells. Clearly, this pattern does not extend even to other strains of MDCK cells, much less to other epithelial cell types.

Abbreviations: $ConA^r$, concanavalin A resistant; GPI; glycosylphosphatidylinositol; GSL, glycosphingolipid; HA, haemagglutinin; MDCK, Madin–Darby canine kidney.

not be taken as dogma. Rafts clearly can exist in artificial membranes, and they probably also exist in cells. At present, we have no firm idea of the size, localization or dynamic properties of rafts in living cells. The enormous complexity of lipids and proteins in real cells may substantially alter the properties of cellular rafts, compared with those formed in artificial membranes. For instance, caveolin binds to cholesterol and is found in cholesterol-rich caveolae, which may be a specialized type of stabilized, morphologically discernible raft[28]. In another example, annexin II associates peripherally with the cytoplasmic surface of cholesterol-rich membranes, and may therefore interact with rafts, and perhaps link them to the cytoskeleton[29]. If rafts do exist in cells, much work is required to establish their physiological relevance in protein sorting and other processes.

Some epithelial cells rely on transcytosis for delivery of most of their apical surface components. As FRT cells develop polarity, transcytosis is initially used for apical delivery, whereas, later in development, the direct TGN-to-apical route predominates[30]. Thus, transcytosis may be more fundamental in ontogeny and even in evolution. In intestinal cells, proteins use a mixture of the direct TGN and transcytotic pathways to the apical surface, with the exact percentage for each pathway depending on the individual protein[31,32]. Hepatocytes use transcytosis nearly exclusively for apical delivery of proteins, even GPIAPs[33], but they can deliver GSLs directly from the TGN to the apical surface[34].

Is the raft mechanism used for sorting during transcytosis? Earlier evidence did not detect GSL sorting and presumably rafting during transcytosis in MDCK cells[35]. Recently, however, GSL sorting was observed during transcytosis in hepatocytes[36]. How non-GPI-anchored proteins that are not incorporated into rafts transcytose to the apical surface remains an important question. Perhaps the oligosaccharides on transcytosing proteins interact with a lectin that is targeted to the apical surface by rafts or another mechanism. This model might explain how binding of heavily glycosylated IgA to the pIgR stimulates apical transcytosis of the pIgR[1].

Even 'non-polarized fibroblasts', such as baby hamster kidney (BHK) and Chinese hamster ovary (CHO) cells, produce two types of TGN-derived vesicles, corresponding to the apical and basolateral vesicles leaving the TGN in polarized cells[37,38]. These cells therefore have separate TGN-to-apical and TGN-to-basolateral pathways, including rafts for the apical pathway, but they do not provide separate apical and basolateral plasma membrane targets as these are mixed in one undifferentiated plasma membrane. However, many fibroblastic cell lines, including the BHK and CHO cells used in these studies, are derived from epithelia and may have only partially lost epithelial polarity. More surprising is that osteoclasts, which are of non-epithelial, haematopoietic lineage, also have apical and basolateral surfaces as well as transcytosis[39]. The principle of 'apical' and 'basolateral' pathways may even hold for *Saccharomyces cerevisiae*, which has two pathways from the TGN to the cell surface[40]. Since yeast have GPIAPs, one of these pathways may be the equivalent of the raft pathway. By extension of this idea, the division into apical (GSL raft) and basolateral (cytoplasmic signal) circuits has been proposed to exist throughout the exocytic and endocytic pathways in both polarized and non-polarized cells[5]. In this model, the apical and basolateral pathways would meet in several intracellular compartments, and each compartment would resegregate components into apical and basolateral vesicles.

Step 2: vesicle formation

Once apical and basolateral proteins have been sorted from each other, they must be packaged into vesicles that transport them to the respective surfaces. The process of recruitment into a vesicle may in fact also contribute to sorting of certain membrane or soluble proteins (in which case, steps 1 and 2 would overlap). This would be analogous to the recruitment of receptors and ligands into clathrin-coated pits at the plasma membrane. Although the compartment from which the final transport vesicles bud off is generally assumed to be the TGN, some newly synthesized plasma membrane proteins might first travel from the TGN to endosomes before reaching the plasma membrane[41,42]. Also, TGN-to-surface transport might involve an intermediate(s) that is larger than a classical carrier vesicle, such as a tubule.

How vesicles destined for the apical membrane (including rafts) bud off the TGN is unknown; even the involvement of a coat protein is conjectural. For basolateral proteins, sorting in the membrane (step 1) and recruitment into budding vesicles (step 2) may both involve a coat protein; the similarity of basolateral and endocytosis signals suggests that this coat might be a member of the clathrin adaptor and COP family of coat proteins[43,44]. A novel adaptor-like complex, termed AP3, has recently been described[45,46]. Like the μ1 and μ2 subunits of the AP1 and AP2 adaptor complexes, the μ3 subunit of the AP3 adaptor complex can bind to tyrosine-based sorting signals. It is possible therefore that this AP3 adaptor could be involved in recognition of signals involved in polarized sorting, although similar roles for AP1, as well as other novel adaptors, remain to be investigated. Myosin II appears to be involved in budding of basolateral vesicles (Ref. 47, but see also Ref. 48). It might provide the force for budding or otherwise act on the Golgi spectrin cytoskeleton[49]. Regardless of how sorting and budding occur, a new vesicle must contain information that specifies its translocation, docking and fusion properties.

Step 3: vesicle transport to the plasma membrane

The polarized organization of microtubules (MTs) in epithelia[50] suggests that dynein- and kinesin-like motors could be used for delivery of TGN-derived vesicles to the apical and basolateral domains, respectively. Indeed, differential requirements for these motors have been demonstrated in polarized delivery in MDCK cells[51]. Actin-based motors are also likely to play a role, at least in apical delivery in both the direct[52] and transcytotic pathways[53]. Disruption of MTs by nocodazole has a kinetic effect on delivery

of proteins to both surfaces. From the handful of proteins examined, it appears that direct apical and transcytotic delivery are particularly affected, with many proteins being missorted to some degree to the basolateral surface. There are two interpretations for this disparity. First, transport of apical vesicles from the TGN or basolateral endosomes may rely more on MTs than basolaterally directed pathways; and, in the absence of MTs, these vesicles are relatively free to fuse with either plasma membrane. Such random fusion properties of vesicles does not agree well with either the annexin or SNARE mechanisms of fusion (see below). Second, TGN/endosome sorting of apical proteins could rely more on intact MTs than does that of basolateral proteins, and, in the absence of MTs, apical proteins are incorporated into basolateral vesicles. Sorting in the TGN may be closely coupled to tubulation[54]. The role of MTs, if any, in this process is unknown, but there is evidence for MT motor involvement in tubulation of many organelles, including the TGN and endosomes[50]. Thus, while MTs are clearly involved in cellular organization and vesicle transport in polarized epithelia, their role in targeting specificity is not understood.

Step 4: docking and fusion of transport vesicles with the plasma membrane

Once the transport vesicles reach their destination, they must dock to and fuse with the plasma membrane. The SNARE hypothesis provides a unified model for intracellular membrane fusion[55]. This hypothesis postulates that 'addressing' proteins, called SNAREs (see Box 1), determine the specificity of membrane fusion by requiring the correct pairing of a v-SNARE on the vesicle membrane with its cognate t-SNARE on the target membrane. When a vesicle carrying digestive enzymes happens to be mistargeted by a failure of prior specificity mechanisms, the pancreas cell has one last chance to prevent the secretion of digestive enzymes into the interstitium: it can prohibit docking and fusion. The SNARE mechanism might provide such a final proofreading mechanism.

Recently, however, Ikonen *et al.* reported that, while targeting from the TGN to the basolateral membrane involved SNAREs, apical targeting from the TGN was not inhibited by antibodies to the general SNARE-dependent fusion factor, NSF, and was insensitive to tetanus toxin, which cleaves several v-SNAREs[56]. It was suggested therefore that TGN-to-apical fusion uses a novel, non-SNARE-dependent pathway and that the apical surface might even utilize this novel mechanism exclusively. Hence, mistargeted vesicles could never fuse with the 'wrong' membrane because of a complete incompatibility of the machineries. This group found that apically targeted vesicles contain the epithelium-specific annexin 13b, and that bivalent antibodies to annexin 13b blocked TGN-to-apical delivery, which was interpreted to suggest that annexin 13b is involved in apical membrane fusion[57].

Recently, a similar experimental system was used to test the role of SNAREs in transcytosis[58]. The results showed that both receptor-mediated transcytosis of IgA to the apical surface as well as recycling to the basolateral surface required NSF and were inhibited by botulinum E toxin, which cleaves the neuron-specific t-SNARE SNAP-25. Although it is not clear what the target of this toxin is in MDCK cells, new homologues of SNAP-25 have been discovered recently[59,60] that are good candidates. Thus it appears that the apical plasma membrane domain can utilize the SNAREs, although possibly only for a subset of vesicles.

If SNAREs control the specificity of apical and basolateral docking/fusion, these domains should contain different t-SNARE isoforms. This was shown recently for the t-SNARE subunits of the syntaxin family in MDCK[61], pancreatic acinar[62] and gastric parietal cells[63] (Fig. 2). Syntaxins 2, 3 and 4 are expressed in MDCK cells, but have strikingly different localizations. Syntaxin 2 was found on both the apical and basolateral surfaces, whereas syntaxins 3 and 4 localize non-overlappingly to the apical and

BOX 1 – GLOSSARY

Miscellaneous

GPIAP: glycosylphosphatidylinositol (GPI)-anchored protein.
GSL: glycosphingolipid.
Raft: a membrane microdomain that forms by clustering of GSLs and GPIAPs.
TGN: *trans* Golgi network, a proposed major cellular sorting organelle.

Cell lines (all are of epithelial origin)

Caco2 and HT-29 cells: human cell lines derived from colon carcinoma.
FRT cells: Fischer rat thyroid cells.
MDCK: Madin–Darby canine kidney cells. Different subclones have been isolated:
MDCK I: high transmonolayer electrical resistance.
MDCK II: low transmonolayer electrical resistance. Two subclones of MDCK II cells have been characterized extensively: 'Heidelberg clone' and 'J clone'.

SNARE machinery

NSF: *N*-ethylmaleimide sensitive factor; a soluble cytoplasmic ATPase.
SNAP: soluble NSF attachment protein; recruits NSF to membranes after SNAP binds to a SNARE.
SNAP-25: Synaptosomal-associated protein of 25 kDa: neuron-specific founding member of the second protein family acting as t-SNAREs when bound to a member of the syntaxin family. Unrelated to SNAP.
SNAP-23: ubiquitously expressed homologue of SNAP-25.
SNARE: SNAP receptor; membrane proteins on the target membrane (t-SNARE) or on the vesicle membrane (v-SNARE).
Syntaxins: a family of membrane proteins acting as t-SNAREs. Some syntaxins bind to a member of the SNAP-25 family to form a heterodimeric t-SNARE.
VAMP ('vesicle associated membrane protein')/synaptobrevin: a family of membrane proteins acting as v-SNAREs, discovered independently by two groups.

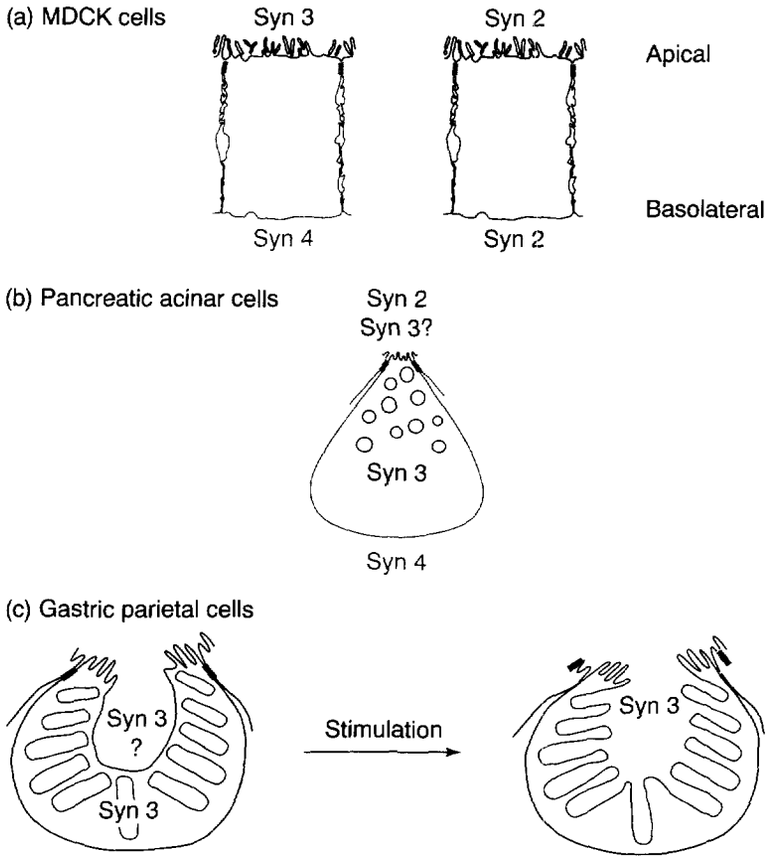

FIGURE 2

Differential localization of t-SNAREs in epithelial cells. According to the SNARE hypothesis, every membrane compartment that utilizes this fusion machinery should contain a specific target-SNARE (t-SNARE), which allows docking and fusion only of transport vesicles possessing a matching v-SNARE. Plasma membrane t-SNAREs appear to consist of two subunits: one is a member of the syntaxin family and the other a member of the SNAP-25 family. Recently, the distribution of some syntaxin isoforms has been studied in MDCK[61], pancreatic acinar[62] and gastric parietal cells[63], which revealed their differential distribution at the apical and basolateral plasma membrane domains. Syntaxin 4 is restricted to the basolateral domain in both MDCK (a) and acinar cells (b). Syntaxin 2 was found at both domains in MDCK cells but appeared to be only apical in acinar cells. Syntaxin 3 was studied in all three cell types and was found at the apical domain in MDCK (with some additional lysosomal localization) and possibly also in acinar cells. Interestingly, syntaxin 3 could also be detected on the large secretory granules that ultimately fuse with the (small) apical plasma membrane of acinar cells. Moreover, in parietal cells (c), at least some syntaxin 3 was localized to the H^+/K^+-ATPase-containing tubovesicles that fuse with the apical membrane after gastric stimulation. It is not clear whether this intracellular pool of syntaxin 3 arises from insufficient retention during membrane retrieval from the apical surface or whether it has a specific function.

basolateral surfaces, respectively[61]. These disparate localizations tantalizingly suggest that the syntaxins serve different polarized targeting pathways and perhaps contribute to the specificity of polarized targeting. Moreover, the presence of t-SNAREs at the apical plasma membrane domain implies that this domain does utilize the SNARE machinery and makes it unlikely that fusion to it depends entirely on an unrelated mechanism.

The involvement of t-SNAREs in TGN-to-apical delivery was not tested by Ikonen *et al.* and it is possible that syntaxins 2 and/or 3 are involved in TGN-to-apical transport and transcytosis. Transport from the TGN to the apical surface might involve not NSF and the v-SNARE VAMP/synaptobrevin themselves but possibly homologues of these proteins (homologues of NSF have been reported recently; see Ref. 64). It is even likely that apical fusion does not involve the tetanus-toxin-sensitive VAMP/synaptobrevin isoforms I and II, which do not bind to syntaxins 2 and 3 (Ref. 65). An involvement of SNAREs in apical fusion is compatible with all reported data. Therefore, we suggest that SNAREs are involved in all vesicle-fusion events with both the basolateral and the apical plasma membrane and that the specificity of fusion depends on the utilization of different isoforms of the constituents of the SNARE machineries.

Conclusions

Recent studies have uncovered several possible mechanisms that may provide cells with the tools necessary for the polarized targeting of membrane proteins and lipids. Association with lipid rafts would be an elegant sorting mechanism, but the physiological significance of 'rafting' still requires further experimental confirmation. It is becoming increasingly clear that the SNARE machinery, besides being a membrane-fusion machinery, may play a role in ensuring the specificity of vesicle fusion as a final proofreading mechanism. Careful regulation of these mechanisms – e.g. by changes in lipid metabolism, redistribution of v- and/or t-SNAREs, utilizing different motors and/or different coat proteins – may yield the plasticity needed to generate the 160 different major human cell types. Considerable challenges for the future will be to determine whether our current hypotheses are true and, if so, how each of these mechanisms functions in molecular terms and how they are regulated.

References

1 MOSTOV, K. E. *et al.* (1995) *Cold Spring Harbor Symp. Quant. Biol.* 60, 775–781
2 HAMMERTON, R. W., KRZEMINSKI, K. A., MAYS, R. W., RYAN, T. A., WOLLNER, D. A. and NELSON, W. J. (1991) *Science* 254, 847–850
3 MATTER, K. and MELLMAN, I. (1994) *Curr. Opin.Cell Biol.* 6, 545–554
4 AROETI, B., KOSEN, P. A., KUNTZ, I. D., COHEN, F. E. and MOSTOV, K. E. (1993) *J. Cell Biol.* 123, 1149–1160
5 SIMONS, K. and IKONEN, E. (1997) *Nature* 387, 569–572
6 SCHROEDER, R., LONDON, E. and BROWN, D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 12130–12134
7 AHMED, S. N., BROWN, D. A. and LONDON, E. *Biochemistry* (in press)
8 KURZCHALIA, T. V., HARTMANN, E. and DUPREE, P. (1995) *Trends Cell Biol.* 5, 187–191
9 MAYOR, S. and MAXFIELD, F. R. (1995) *Mol. Biol. Cell* 6, 929–944
10 BROWN, D. A. and ROSE, J. K. (1992) *Cell* 68, 533–544
11 van MEER, G. (1993) *Curr. Opin. Cell Biol.* 5, 661–673
12 van der BIJL, P., LOPES-CARDOZO, M. and van MEER, G. (1996) *J. Cell Biol.* 132, 813–821
13 FIEDLER, K., KOBAYASHI, T., KURZCHALIA, T. V. and SIMONS, K. (1993) *Biochemistry* 32, 6365–6373
14 SKIBBENS, J. E., ROTH, M. G. and MATLIN, K. S. (1989) *J. Cell Biol.* 108, 821–832
15 KUNDU, A., AVALOS, R. T., SANDERSON, C. M. and NAYAK, D. P. (1996) *J. Virol.* 70, 6508–6515

16 ARREAZA, G. and BROWN, D. A. (1995) *J. Biol. Chem.* 270, 23641–23647
17 GRIFFITHS, G. and SIMONS, K. (1986) *Science* 234, 438–443
18 KITAGAWA, Y. *et al.* (1994) *Exp. Cell Res.* 213, 449–457
19 URBAN, J., PARCZYK, K., LEUTZ, A., KAYNE, M. and KONDOR-KOCH, C. (1987) *J. Cell Biol.* 105, 2735–2743
20 SCHEIFFELE, P., PERÄNEN, J. and SIMONS, K. (1995) *Nature* 378, 96–98
21 FIEDLER, K. and SIMONS, K. (1995) *Cell* 81, 309–312
22 GRAICHEN, R., LOSCH, A., APPEL, D. and KOCH-BRANDT, C. (1996) *J. Biol. Chem.* 271, 15854–15857
23 MAYS, R. W., SIEMERS, K. A., FRITZ, B. A., LOWE, A. W., van MEER, G. and NELSON, W. J. (1995) *J. Cell Biol.* 130, 1105–1115
24 ZURZOLO, C., van't HOF, W., van MEER, G. and RODRIGUEZ-BOULAN, E. (1994) *EMBO J.* 13, 42–53
25 ZURZOLO, C., LISANTI, M. P., CARAS, I. W., NITSCH, L. and RODRIGUEZ-BOULAN, E. (1993) *J. Cell Biol.* 121, 1031–1039
26 NICOLAS, F., TIVERON, M. C., DAVOUST, J. and REGGIO, H. (1994) *J. Cell Sci.* 107, 2679–2689
27 van HELVOORT, A. *et al.* (1996) *Cell* 87, 507–517
28 HARDER, T. and SIMONS, K. (1997) *Curr. Opin. Cell Biol.* 9, 534–542
29 HARDER, T., KELLNER, R., PARTON, R. G. and GRUENBERG, J. (1997) *Mol. Biol. Cell* 8, 533–545
30 ZURZOLO, C., le BIVIC, A., QUARONI, A., NITSCH, L. and RODRIGUEZ-BOULAN, E. (1992) *EMBO J.* 11, 2337–2344
31 MATTER, K., BRAUCHBAR, M., BUCHER, K. and HAURI, H-P. (1990) *Cell* 60, 429–437
32 le BIVIC, A., QUARONI, A., NICHOLS, B. and RODRIGUEZ-BOULAN, E. (1990) *J. Cell Biol.* 111, 1351–1361
33 BARTLES, J. R. and HUBBARD, A. L. (1988) *Trends Biochem. Sci.* 13, 181–184
34 ZAAL, K. J., KOK, J. W., SORMUNEN, R., ESKELINEN, S. and HOEKSTRA, D. (1994) *Eur. J. Cell Biol.* 63, 10–19
35 van GENDEREN, I. and van MEER, G. (1995) *J. Cell Biol.* 131, 645–654
36 van IJZENDOORN, S. C., ZEGERS, M. M., KOK, J. W. and HOEKSTRA, D. (1997) *J. Cell Biol.* 137, 347–357
37 MÜSCH, A., XU, H., SHIELDS, D. and RODRIGUEZ-BOULAN, E. (1996) *J. Cell Biol.* 133, 543–558
38 YOSHIMORI, T., KELLER, P., ROTH, M. G. and SIMONS, K. (1996) *J. Cell Biol.* 133, 247–256
39 MOSTOV, K. E. and WERB, Z. (1997) *Science* 276, 219–220
40 HARSAY, E. and BRETSCHER, A. (1995) *J. Cell Biol.* 131, 297–310
41 LEITINGER, B., HILLE-REHFELD, A. and SPIESS, M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 10109–10113
42 FUTTER, C. E., CONNOLLY, C. N., CUTLER, D. F. and HOPKINS, C. R. (1995) *J. Biol. Chem.* 270, 10999–11003
43 HEILKER, R., MANNING-KRIEG, U., ZUBER, J. F. and SPIESS, M. (1996) *EMBO J.* 15, 2893–2899
44 ROBINSON, M. S. (1997) *Trends Cell Biol.* 7, 99–102
45 SIMPSON, F., PEDEN, A. A., CHRISTOPOULOU, L. and ROBINSON, M. S. (1997) *J. Cell Biol.* 137, 835–845
46 DELL'ANGELICA, E. C., OHNO, H., OOI, C. E., RABINOVICH, E., ROCHE, K. W. and BONIFACINO, J. S. (1997) *EMBO J.* 16, 917–928
47 MÜSCH, A., COHEN, D. and RODRIGUEZ-BOULAN, E. (1997) *J. Cell Biol.* 138, 291–306
48 IKONEN, E., PARTON, R. G., LAFONT, F. and SIMONS, K. (1996) *Mol. Biol. Cell* 7, 961–974
49 BECK, K. A. and NELSON, W. J. (1996) *Am. J. Physiol.* 270, C1263–C1270
50 COLE, N. B. and LIPPINCOTT-SCHWARTZ, J. (1995) *Curr. Opin. Cell Biol.* 7, 55–64
51 LAFONT, F., BURKHARDT, J. K. and SIMONS, K. (1994) *Nature* 372, 801–803
52 FATH, K. R., TRIMBUR, G. M. and BURGESS, D. R. (1994) *J. Cell Biol.* 126, 661–675
53 MAPLES, C. J., RUIZ, W. G. and APODACA, G. (1997) *J. Biol. Chem.* 272, 6741–6751
54 LADINSKY, M. S., KREMER, J. R., FURCINITTI, P. S., McINTOSH, J. R. and HOWELL, K. E. (1994) *J. Cell Biol.* 127, 29–38
55 ROTHMAN, J. E. and WARREN, G. (1994) *Curr. Biol.* 4, 220–233
56 IKONEN, E., TAGAYA, M., ULLRICH, O., MONTECUCCO, C. and SIMONS, K. (1995) *Cell* 81, 571–580
57 FIEDLER, K., LAFONT, F., PARTON, R. G. and SIMONS, K. (1995) *J. Cell Biol.* 128, 1043–1053
58 APODACA, G., CARDONE, M. H., WHITEHEART, S. W., DASGUPTA, B. R. and MOSTOV, K. E. (1996) *EMBO J.* 15, 1471–1481
59 RAVICHANDRAN, V., CHAWLA, A. and ROCHE, P. A. (1996) *J. Biol. Chem.* 271, 13300–13303
60 WEIMBS, T., LOW, S. H., CHAPIN, S. J., MOSTOV, K. E., BUCHER, P. and HOFMANN, K. (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 3046–3051
61 LOW, S. H., CHAPIN, S. J., WEIMBS, T., KÖMÜVES, L. G., BENNETT, M. K. and MOSTOV, K. E. (1996) *Mol. Biol. Cell* 7, 2007–2018
62 GAISANO, H. Y. *et al.* (1996) *Mol. Biol. Cell* 7, 2019–2027
63 PENG, X-R., YAO, X., CHOW, D-C., FORTE, J. G. and BENNETT, M. K. (1997) *Mol. Biol. Cell* 8, 399–407
64 MELLMAN, I. (1995) *Cell* 82, 869–872
65 CALAKOS, N., BENNETT, M. K., PETERSON, K. E. and SCHELLER, R. H. (1994) *Science* 263, 1146–1149

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