3. EPITHELIAL POLARITY AND SORTING

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INTRODUCTION

Epithelia form boundaries between different compartments in the body and allow vectorial flow of molecules and ions from one to the other side (Simons and Fuller, 1985). To serve the transport functions and to protect the cells from external environment, proteins and lipids have to be distributed polarized over the apical and basolateral plasma membrane domains. Generation and maintenance of surface polarity requires sorting and polarized transport of newly synthesized surface molecules (Rodriguez-Boulan and Powell, 1992; Simons, 1995). In recent studies determinants for intracellular sorting into the apical and basolateral pathways have been identified. The two routes appear to use fundamentally different mechanisms. Apical and basolateral determinants are not restricted to proteins in epithelial cells, but appear to function in all cell types to generate polarity. Therefore, epithelial cells provide a good tool to study general mechanisms of membrane trafficking which cannot be easily analysed in cells lacking obvious surface polarity. The aim of this review is to analyse the sorting mechanisms responsible for the polarized distribution of plasma membrane proteins in epithelia.

MODEL SYSTEMS

Sorting has been studied in different cell lines developing polarized monolayers in vitro when grown on a permeable filter support: Madin-Darby canine kidney (MDCK) cells, Caco-2 (human intestinal), Fischer rat thyroid (FRT) cells, LLC-PK1 (pig kidney), retinal pigment epithelial cells (RPE) and hepatocytes. One important outcome from these studies was that the different epithelial cells use different delivery routes for biosynthetic transport (Rodriguez-Boulan and Powell, 1992). While apical and basolateral proteins in MDCK and FRT cells are sorted mainly in the TGN, hepatocytes route plasma membrane proteins first to their basolateral surface and then transcytose the apical components. Caco-2 cells have an intermediate phenotype as apical proteins can use both, a direct apical route and an indirect route via the basolateral membrane (Rodriguez-Boulan and Powell, 1992). Therefore, the same protein can be differently targeted in different cell lines. Analysis of these variations might help to understand the importance and function of individual components of the sorting machinery. Results obtained in culture systems do not always reflect the distribution of the protein in epithelial tissues, because surface distribution can vary for instance in case of the LDL-receptor or the
transferrin-receptor. Proteins can also change polarity under different physiological conditions, a process which has been termed plasticity of epithelial polarity (Schwartz et al., 1985). As most published studies on protein sorting in epithelial cells have been carried out in the MDCK cell line this review will in the following first describe signals and mechanisms identified there and then relate them to observations made in other systems.

SORTING SIGNALS

Secretory Proteins

A large number of exogenous secretory proteins including lysozyme, rat growth hormone (rGH), and alpha-2-microglobulin which were assumed not to contain information for polarized sorting, has been expressed in the MDCK cell line (Table 1). Many of these proteins were secreted in a nonpolarized fashion into the apical and basolateral media, usually somewhat more to the basolateral side, probably following the bulk fluid flow (Gottlieb et al., 1986; Kondor-Koch et al., 1985). This demonstrated that polarized secretion in MDCK cells requires luminal signals. Polarized apical secretion has been detected for two endogenous glycoproteins of unknown function, gp80 and gp20 (Urban et al., 1987; Ulrich et al., 1991), whereas the extracellular matrix components laminin and heparan-sulfate proteoglycan were secreted from the basolateral membrane (Caplan and Jamieson, 1987).

While signals for basolateral secretion are not known, N-glycosylation has been shown to be involved in apical secretion (Scheiffele et al., 1995). The initial observation was that the endogenous glycoprotein gp80 was secreted in a nonpolarized fashion when N-glycosylation was inhibited with the drug tunicamycin (Urban et al., 1987). Rat growth hormone (rGH) is unglycosylated and only to 35% apically secreted. When one or two N-glycosylation sites were introduced, the glycosylated forms of the protein were targeted with increasing efficiency to the apical membrane (65 or 92% apical, respectively; Figure 1) (Scheiffele et al., 1995). Therefore, N-glycans function as a transplantable apical determinant.

Analysis of previously performed studies revealed that N-glycans might be generally employed for apical secretion in epithelial cells. As observed for the singly and doubly glycosylated rGH the sorting efficiency will depend on the number of glycans present and possibly also on their accessibility. However, proteins such as gp20 which are not N- but O-glycosylated and the hepatitis B surface antigen which is not secreted as a conventional soluble protein but as a lipoprotein particle (Gonzalez et al., 1993) must carry other signals (Table 1).

Treatment of cells with inhibitors of terminal glycosylation (deoxy-mannojirimycin or swainsonine) does not influence polarity of the apical protein gp80, therefore no complex type glycans are required, but rather the GlcNAc-Man-core (Parczyk and Koch-Brandt, 1991; Wagner et al., 1995). So far the role of N-glycans in the secretory pathway was thought to be restricted to folding and quality control in the ER (Helenius, 1994), the mannose-6-phosphate signal for lysosomal transport being the only exception. Now it appears that glycosylation is also used as a general determinant for apical transport (Fiedler and Simons, 1995). An open
### Table 1 Polarized secretion from MDCK cells.

<table>
<thead>
<tr>
<th>Apical secretion</th>
<th>N-glycosyl</th>
<th>Reference</th>
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<tbody>
<tr>
<td>gp80</td>
<td>required</td>
<td>(Urban et al., 1987)</td>
</tr>
<tr>
<td>erythropoietin</td>
<td>required</td>
<td>(Kitagawa et al., 1994)</td>
</tr>
<tr>
<td>hCBG</td>
<td>required</td>
<td>(Musco, 1993)</td>
</tr>
<tr>
<td>gp20</td>
<td>–</td>
<td>(Ulrich et al., 1991)</td>
</tr>
<tr>
<td>HBsAg</td>
<td>not required</td>
<td>(Gonzalez et al., 1993)</td>
</tr>
<tr>
<td>endoglucanase E</td>
<td>+</td>
<td>(Soole et al., 1992)</td>
</tr>
<tr>
<td>NEP (sec)</td>
<td>+</td>
<td>(Corbeil et al., 1992)</td>
</tr>
<tr>
<td>APN (sec)</td>
<td>+</td>
<td>(Vogel et al., 1992)</td>
</tr>
<tr>
<td>plgAR (sec)</td>
<td>+</td>
<td>(Mostov et al., 1987)</td>
</tr>
<tr>
<td>Thy-1 (sec)</td>
<td>+</td>
<td>(Powell et al., 1991)</td>
</tr>
<tr>
<td>PLAP (sec)</td>
<td>+</td>
<td>(Brown et al., 1989)</td>
</tr>
<tr>
<td>DAF (sec)</td>
<td>+</td>
<td>(Lisanti et al., 1989)</td>
</tr>
<tr>
<td>NA (sec)</td>
<td>+</td>
<td>(Kundu and Nayak, 1994)</td>
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<thead>
<tr>
<th>Basolateral secretion</th>
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<tbody>
<tr>
<td>Laminin</td>
<td>+</td>
<td>(Caplan and Jamieson, 1987)</td>
</tr>
<tr>
<td>HSPG</td>
<td>+</td>
<td>(Caplan and Jamieson, 1987)</td>
</tr>
<tr>
<td>gD-1 sec</td>
<td>+</td>
<td>(Lisanti et al., 1989)</td>
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<table>
<thead>
<tr>
<th>Nonpolar secretion</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>growth hormone, lysozyme,</td>
<td>–</td>
<td>(Goetzl et al., 1986)</td>
</tr>
<tr>
<td>α-2-microglobulin,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ig K-chain, prochymosin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lysozyme</td>
<td>–</td>
<td>(Kondor-Koch et al., 1985)</td>
</tr>
<tr>
<td>urothoglobin</td>
<td>–</td>
<td>(Vogel et al., 1993)</td>
</tr>
<tr>
<td>cystatin C</td>
<td>–</td>
<td>(Vogel et al., 1992)</td>
</tr>
<tr>
<td>HA (sec), VSV G (sec)</td>
<td>+</td>
<td>(Gonzalez et al., 1987)</td>
</tr>
<tr>
<td>uPA</td>
<td>–</td>
<td>(Ragno et al., 1992)</td>
</tr>
<tr>
<td>bGH</td>
<td>–</td>
<td>(Lisanti et al., 1989)</td>
</tr>
</tbody>
</table>

'Sec' indicates anchor-minus forms of transmembrane proteins.

hCBP, human corticosteroid binding globulin; HBsAg, hepatitis B surface antigen; NEP, neutral endopeptidase; APN, aminopeptidase N; plgAR, plasminogen activator; PLAP, placental alkaline phosphatase; DAF, decay accelerating factor; NA, influenza virus neuraminidase; HSPG, heparan sulfate proteoglycan; gD-1, herpes simplex virus glycoprotein D; HA, influenza virus hemagglutinin; VSV G, vesicular stomatitis virus glycoprotein; uPA, mouse urokinase-type plasminogen activator; bGH, human growth hormone.

The question is how the glycosylation signal functions. Glycans might be used to change physical properties of proteins for sorting. Another possibility is that the carbohydrate moiety is directly recognized by a lectin-like sorting receptor which concentrates the protein in apical carrier vesicles (see below). Since also basolaterally secreted proteins are N-glycosylated, they must carry dominant sorting determinants which would allow their segregation from the apical glycoproteins, either earlier in the secretory pathway or by binding to basolateral receptors with higher affinity than the putative apical lectin.

The ectodomains of many membrane proteins have been expressed as anchorless secreted forms to analyse their targeting information. For viral proteins the
results were often inconsistent and even contradictory. However, for cellular proteins the ectodomain was often found to be secreted apically, independently of the polarity of the membrane protein (Table 1). The fact that all the analysed proteins were N-glycosylated could explain the presence of a apical sorting signal in their ectodomain (Simons, 1995).

**Basolateral Membrane Proteins**

Sorting signals of basolateral transmembrane proteins are located to their cytoplasmic tails (Hunziker *et al.*, 1991). In many cases truncation of the cytoplasmic tail or mutation of crucial cytoplasmic amino acids results in apical sorting, probably facilitated by the carbohydrate signal in the ectodomain (Table 2, Figure 2). On the other hand the basolateral signal when transplanted onto an apical protein will always dominate over the apical signal present and result in basolateral sorting. Sorting signals therefore function hierarchically, the basolateral information being dominant (Matter and Mellman, 1994; Simons, 1995).

Basolateral signals have been extensively characterized by mutagenesis. Two classes of signals have been defined, one related to clathrin-coated pit localization motifs and another unrelated motif (Matter and Mellman, 1994). Many of the clathrin-coated pit related signals depend on tyrosine residues, but the requirements for amino acids surrounding the critical tyrosine are different for basolateral sorting.
Table 2 Sorting of tail-minus forms of basolateral transmembrane proteins.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Apical</td>
<td>(Casanova et al., 1991)</td>
</tr>
<tr>
<td>plgAR</td>
<td>(Hunziker et al., 1991)</td>
</tr>
<tr>
<td>LDL-receptor</td>
<td>(Prill et al., 1993)</td>
</tr>
<tr>
<td>LAP</td>
<td>(Hunziker et al., 1991)</td>
</tr>
<tr>
<td>Fc-receptor</td>
<td>(Hunziker et al., 1991)</td>
</tr>
<tr>
<td>IgG120</td>
<td>(Hobert and Carlin, 1995)</td>
</tr>
<tr>
<td>Nonpolarized</td>
<td></td>
</tr>
<tr>
<td>transferrin-receptor</td>
<td>(Kundu and Nayak, 1994)</td>
</tr>
<tr>
<td>VSV G</td>
<td>(Thomas et al., 1993)</td>
</tr>
<tr>
<td>ASGP-receptor</td>
<td>(Geffen et al., 1993)</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>(Koller and Ranacht, 1996)</td>
</tr>
</tbody>
</table>

*tail-minus N-cadherin is basolaterally stabilized. plgAR, polymeric immunoglobulin A-receptor; LDL, low-density-lipoprotein; LAP, lysosomal acid phosphatase; EGF, epidermal growth factor; ASGP, asialoglycoprotein.

and endocytosis. Interestingly, replacement of the tyrosine in the cytoplasmic tail of lysosomal acid phosphatase with phenylalanine blocks endocytosis while basolateral sorting is not significantly affected (Prill et al., 1993). In other proteins signals for endocytosis and basolateral sorting are physically separated. The cytoplasmic tail of low density lipoprotein (LDL)-receptor contains two targeting determinants. The proximal one overlaps with the clathrin-coated pit signal and can be inactivated by replacement of the critical tyrosine by alanine (mutant A18; Figure 3). The resulting receptor is not endocytosed efficiently through clathrin-coated pits, but

![Figure 2 Sorting of truncated basolateral transmembrane proteins (BL) in the trans-Golgi-network (TGN). As in the case of LDL-receptor and other proteins (table 2) deletion of the cytoplasmic signal results in apical (AP) sorting and removal of the membrane anchor results in apical secretion of the ectodomain.](image-url)
Figure 3  Signals in the cytoplasmic tail of LDL-receptor and pIgA-receptor. Crucial amino acid residues are shown in bold.
(A) The wild-type (WT) LDL-receptor contains two basolateral determinants. In the mutant A18 the tyrosine residue of the clathrin-coated pit internalisation signal (underlined) is mutated to alanine. CT22 is a truncation mutant containing only the membrane proximal 22 amino acids of the cytoplasmic tail (Matter and Mellman, 1994).
(B) Minimal cytoplasmic signal (truncation mutant stop670) and secondary structure of the polymeric Immunoglobulin A-receptor (pIgA-R). The indicated mutation of the serine residue to aspartate (mimicking phosphorylation) results in apical sorting of the receptor (Aroeti et al., 1993).

the distal determinant is still sufficient for basolateral targeting. On the other hand truncation of the cytoplasmic tail leaving only 22 cytoplasmic residues (mutant CT22; Figure 3) results in an apical receptor which can be efficiently endocytosed via clathrin coated pits (Matter et al., 1992; Matter et al., 1994).

The fact that basolateral sorting information can overlap with endocytic signals suggests that the machinery mediating sorting and clathrin-dependent endocytosis might be functionally similar. Secondary structure analysis of some basolateral signals reveals additional similarities as they adopt a β-turn conformation which can also be found for endocytosis signals (Figure 3) (Aroeti et al., 1993; Vaux, 1992). Surface plasmon resonance studies have shown that the in vitro binding of the cytoplasmic adaptor complexes AP-1 and AP-2 to sorting signals correlates with sorting efficiency and endocytosis (Heilker et al., 1996). A more detailed analysis of signals carrying information for sorting but not for endocytosis might reveal differences in binding of the Golgi adaptors versus the plasma membrane adaptor AP-2. Also the lipid environment will influence adaptor binding to cytoplasmic signals (Rapoport et al., 1997). However, so far basolateral markers have not been
detected in exocytic clathrin-coated vesicles, and the coat components employed remain to be discovered.

Apical Membrane Proteins

GPI-anchored proteins

The glycosylphosphatidylinositol (GPI)-anchor was the first apical signal to be identified. Addition of a GPI-anchor is sufficient to target proteins to the apical plasma membrane (Brown et al., 1989; Lisanti et al., 1989). When the cytoplasmic tail and transmembrane domain of basolateral proteins like vesicular stomatitis virus glycoprotein (VSV G) or herpes simplex virus glycoprotein D was replaced by a GPI-anchor, the chimeric proteins were transported apically. The apical polarity is not simply due to deletion of the cytoplasmic basolateral signal because also a nonpolarized secreted protein, human growth hormone, is directed apically when fused to a GPI-moiety (Lisanti et al., 1989). As the human growth hormone is an unglycosylated protein this also demonstrates that GPI-anchored proteins do not need N-glycosylation for apical polarity. Therefore, the GPI-anchor is sufficient for apical targeting and must somehow be recognized by the sorting machinery.

GPI-anchored proteins enter a detergent-insoluble glycolipid-rich complex (DIG) during transport to the apical cell surface which contains a specific subset of lipids and transmembrane proteins (Brown and Rose, 1992; Melkonian et al., 1995). The inclusion into these complexes could reflect the recognition of the GPI-anchor by the sorting machinery and will be discussed later.

Apical transmembrane proteins

Two different signals seem to mediate apical transport of transmembrane proteins. One, most likely involving glycosylation, is located in the ectodomain (Matter and Mellman, 1994; Simons, 1995). As mentioned above this could explain why secretory forms of apical and basolateral transmembrane proteins are secreted apically and deletion of the basolateral signal in a transmembrane protein mostly results in apical delivery (Figure 2). A second signal was proposed to be located in the transmembrane domain (TMD) of the type II transmembrane protein influenza virus neuraminidase (Kundu et al., 1996). A number of chimeric proteins assembled from the basolateral transferrin receptor and the apical neuraminidase were analysed. Truncation of the cytoplasmic signal in transferrin receptor yielded a nonpolarized transported molecule. When the TMD of the neuraminidase was introduced into this truncated receptor the protein was transported apically. It is not clear why the cytoplasmic deletion mutant of the transferrin receptor is not targeted apically as observed for many other basolateral transmembrane proteins (Table 2). This could suggest that N-glycosylation is not sufficient for apical targeting of some transmembrane proteins although it can mediate apical secretion of their ectodomains. Apical targeting of transmembrane proteins could generally need TMD determinants. According to this, proteins like the LDL-receptor would contain such a determinant which must be recessive versus the cytoplasmic
basolateral signal. Another explanation would be that the carbohydrate moieties in the transferrin receptor are not sufficiently exposed to be recognized by the apical sorting machinery. Only analysis of unglycosylated transmembrane proteins will clarify this question and the involvement of other proteinaceous signals for apical sorting cannot be excluded.

Like GPI-anchored proteins also several apical transmembrane proteins are found in DIGs, whereas basolateral transmembrane proteins are excluded from these complexes (Kurzchalia et al., 1992; Danielsen, 1995). In the following we will discuss the nature of these microdomains and then propose a model for sorting into the apical pathway of MDCK cells.

SPHINGOLIPID-CHOLESTEROL RAFTS AND APICAL TRANSPORT

The raft-hypothesis is based on the observation that not only apical proteins but also glycosphingolipids are sorted to the apical membrane (van Meer et al., 1987) (see below). Transport of the lipids and proteins was proposed to be coupled because GPI-anchored proteins and some apical transmembrane proteins could be recovered together with glycosphingolipids and cholesterol in a detergent-insoluble glycolipid-enriched complex (DIG) after extraction with Triton X-100 at 4°C. This was further supported by the fact that also apical transport vesicles contain a detergent-insoluble subcomplex (Brown and Rose, 1992; Kurzchalia et al., 1992; Skibbens et al., 1989).

Evidence and a model for the structure of sphingolipid-cholesterol rafts has been recently summarized (Simons and Ikonen, 1997). Briefly, glycosphingolipids and cholesterol would form microdomains and thereby create a lateral organization of cellular membranes. Inclusion of lipids into the microdomain would be favoured by long saturated fatty acid chains (Schroeder et al., 1994). As glycosphingolipids have large headgroups, their lateral association would tend to create voids in the exoplasmic leaflet of the membrane which could be filled with cholesterol molecules (Simons and Ikonen, 1997) (Figure 4). These sphingolipid-cholesterol assemblies would be small and dynamic, and float like rafts in the surrounding membrane consisting of glycerophospholipids and ("non-raft") cholesterol (Harder and Simons, 1997; Simons and Ikonen, 1997).

GPI-anchored proteins like placental alkaline phosphatase (PLAP) can directly associate with the rafts by their two mostly saturated fatty acid chains in the phosphatidylinositol (C_{16:0} and C_{18:0}) (Schroeder et al., 1994; Redman et al., 1994). For the apical transmembrane protein influenza virus hemagglutinin (HA) bulky hydrophobic amino acids in the transmembrane domain (TMD) were shown to be crucial for association with the rafts. In both cases cellular proteins were not required for binding to raft lipids, therefore this interaction is completely encoded in the protein (Schroeder et al., 1994; Scheiffele et al., 1997). Whether the TMD determinant of HA also functions in apical sorting remains to be shown. However, the apical TMD signal in the influenza virus neuraminidase (NA) also seems to mediate raft association (Kundu et al., 1996). Therefore, TMD determinants might be generally used for apical sorting by intercalating proteins directly into the lipid environment which recruits the apical sorting machinery.
Figure 4 Model for the assembly of raft lipids. Sphingomyelin and glycosphingolipids are enriched in the exoplasmic leaflet and glycerolipids in the cytoplasmic leaflet. Cholesterol fills space under the large headgroups of sphingolipids or extends interdigitating fatty acyl chains in the opposite leaflet (Simons and Ikonen, 1997).

The proposed model suggests a crucial role for cholesterol in rafts. Recent experiments demonstrated that cholesterol is required for association of HA and GPI-anchored proteins to rafts (Scheiffele et al., 1997; Hanada et al., 1995). Cholesterol depletion by a steroid binding cyclodextrin inhibits apical transport of HA and causes missorting of HA to the basolateral membrane domain whereas basolateral transport of VSV G is not significantly affected (Keller and Simons, 1998). This establishes the importance of sphingolipid-cholesterol rafts for the apical pathway in epithelial cells.

One problem is that not all apical proteins are found in DIGs (Danielsen, 1995; Arreaza and Brown, 1995). Therefore, direct and tight raft association by a TMD determinant cannot be the only mechanism for apical sorting. It seems most likely that similarly as for apical secretion also the N-glycosylation in the ectodomain contributes to apical targeting (Scheiffele et al., 1995). Some proteins might exhibit a low or no affinity to rafts, but they would associate to the apical sorting machinery by their carbohydrates. Other proteins (glycosylated or unglycosylated) could bind with high affinity to rafts by their TMDs. Therefore both signals would not be strictly required but could act cooperatively. However, another possibility is that also a raft independent pathway to the apical surface exists.
VIP36, a leguminous lectin homologue, has been identified in TGN-derived vesicle preparations (Fiedler et al., 1994). The protein belongs to a new family of intracellular lectins including ERGIC53 (human), Emp47p and YK24 (yeast) which share about 20% sequence identity with each other and with members of the leguminous lectin family (Fiedler and Simons, 1994) (Schröder et al., 1995). While ERGIC53 is localized to the ER-Golgi-intermediate compartment (Schweizer et al., 1988), VIP36 resides at steady state in the Golgi-complex and might recycle between the Golgi-complex and the plasma membrane (Fiedler et al., 1994). VIP36 can also be recovered in detergent-insoluble complexes, but in contrast to other raft associated proteins its interaction is strongly pH-dependent as it is weak at neutral pH and much stronger at pH 6.2 (Scheiffele and Simons, unpublished) — approximately the pH of the TGN (Anderson and Orci, 1988). We propose that VIP36 functions as a clustering factor which binds to carbohydrate residues of glycoproteins in the TGN, promoted by the high local concentration and the chemical conditions. The binding would increase the affinity of VIP36 and the cargo to the sphingolipid-cholesterol rafts. VIP36 is an oligomer and could form larger assemblies of cargo molecules in rafts which could then bud off as apical vesicles. As the raft association of VIP36 is strongly pH dependent, the whole complex will disassemble after arrival at the plasma membrane to release cargo proteins and allow recycling of VIP36 and other components of the apical targeting machinery back to the Golgi-complex. The hypothesised function of VIP36 would be to cluster glycoproteins in the TGN. Interestingly, antibody clustering of raft proteins on the cell surface has been demonstrated to concentrate them in a raft lipid environment and also to increase their detergent insolubility (Harder et al., 1998). Similarly, VIP36 clustered on the cell surface by antibodies is partially recruited to caveolae which can be conceived as large immobilized raft domains (Fiedler et al., 1994). This antibody clustering effect might mimic the recruitment of VIP36 to caveolin-1-containing patches of the TGN which will form the apical transport vesicles. The observations made for clustered proteins also suggest that oligomerisation and multivalency are important parameters for raft association in vivo (Harder and Simons, 1997).

TRANSCYTOSIS

Besides the direct route from the TGN to the apical surface also an indirect transcytotic route exists which has been well characterised for the polymeric IgA receptor (pIgAR) (Mostov et al., 1992). The protein is first targeted from the TGN to the basolateral plasma membrane by its cytoplasmic sorting signal. The receptor then is endocytosed and delivered to basolateral endosomes where a serine residue in the cytoplasmic tail of the receptor is phosphorylated (Casanova et al., 1990). Subsequently, the protein is directed to the apical recycling compartment, a tubular structure underlying the apical plasma membrane, and finally reaches the apical surface (Apodaca et al., 1994; Barroso and Szul, 1994). The function of the phosphorylation event is most likely to inactivate the basolateral signal which would allow the recessive apical signal in the ectodomain to come into play and target the protein apically. This is supported by the fact that a serine to aspartate mutant (with the aspartate mimicking the phosphorylation, see Figure 3) is already in the
TGN directed to the apical membrane (Aroeti and Mostov, 1994). Therefore, transcytosis does not appear to require specific signals, but rather the inactivation of the basolateral signal in endosomes to reveal the recessive apical sorting information in the ectodomain. This scenario implies that the sorting signals functioning in the TGN can be also decoded in endosomes (see below).

Regulation of membrane trafficking has been analysed using different inhibitors and activators of protein kinases and trimeric G proteins. For example, activation of Gs by aluminium tetafluoride causes stimulation of apical but inhibition of basolateral transport (Pimplikar and Simons, 1993). Also stimulation of the expected downstream molecules adenylate cyclase and protein kinase A increased apical TGN-surface transport and at the same time stimulated apical transcytosis (Hansen and Casanova, 1994; Pimplikar and Simons, 1994; Mostov and Cardone, 1995). As both pathways respond in the same way to pharmacological treatments they might be similarly regulated. One the other hand apical transport does not appear to pass through the apical recycling compartment, but is thought to occur direct. Also the machinery for fusion of transcytotic vesicles with the apical plasma membrane seems to be different than that of TGN-derived vesicles and does not appear to involve raft formation (see below). Future work will have to establish the similarities and differences between the two (direct and indirect) apical pathways.

LIPID SORTING

The apical and basolateral plasma membrane domains have different lipid compositions which are achieved by vectorial transport of lipids. Intermixing of the lipids in the outer leaflet of the lipid bilayer is prevented by the tight junctions (van Meer et al., 1986). Analysis of lipid sorting has received considerable attention, also because according to the raft hypothesis it should be prerequisite for protein sorting. Initially, the lipid contents of viruses budding in a polarized fashion from MDCK cells was determined. When the phospholipid compositions of the basolateral vesicular stomatitis viruses (VSV) and the apical fowl plague viruses (FPV) produced in polarized MDCK cell monolayers were compared, VSV showed an enrichment in sphingomyelin, phosphatidylinositol and phosphatidylethanolamine whereas FPV was enriched in phosphatidylserine and phosphatidylethanolamine. As the phospholipid compositions of the viruses produced in unpolarized MDCK cells were very similar to each other, the detected differences do reflect the composition of the apical and basolateral plasma membrane domain rather than preferences of lipid incorporation by the different viruses (van Meer and Simons, 1982).

Sorting of sphingomyelin and glycosphingolipids in the exocytic pathway has been analysed with the fluorescently labelled short-chain ceramide analog N-6[7-nitro-2,1,3-benzoxadiazol-4-yl]aminocapryl sphingosine (C6-NBD-ceramide) (Lipsky and Pagano, 1983). C6-NBD-ceramide can be introduced into cells at low temperature and is concentrated in the Golgi complex. Here it will be converted to C6-NBD-sphingomyelin and C6-NBD-glucosylceramide which then move to the plasma membrane (Lipsky and Pagano, 1983). Recently, it was demonstrated that a multidrug resistance protein localized to the apical surface of polarized epithelial cells can translocate these short-chain lipids from the cytosol through the plasma.
membrane (van Helvoort et al., 1996). Therefore, part of the apical NBD-signal measured, can be independent of vesicular transport. This makes the interpretation of previous studies employing C6-NBD-ceramide difficult. However, also in permeabilized cells C6-NBD-glycosylceramide was preferentially transported to the apical membrane when cytosolic short-chain lipids were captured with BSA (Kobayashi et al., 1992). The other problem with C6-NBD-ceramide is that it is lacking the long and saturated fatty acid chains, but instead carries the NBD-group after a C6-chain. Thereby, most of the raft character of the molecule is removed and C6-NBD-sphingomyelin indeed rather behaves like a bulk-membrane marker than like a raft lipid (Simons and Ikonen, 1997). C6-NBD-glycosylceramide might retain some raft lipid properties by its bulky headgroup, capable of interacting with other glycolipid headgroups. However, lipid polarity and sorting of raft lipids to the apical membrane of MDCK II cells is confirmed by the fact that also endogenous glycolipids such as the Forsman antigen are enriched in the apical plasma membrane (van Genderen et al., 1991).

SORTING IN OTHER MODEL SYSTEMS

Variant and Mutant MDCK Strains

Two lectin-resistant cell lines derived from the parental MDCK cells have been obtained which retained the characteristic structural and functional epithelial polarity (Meiss et al., 1982). A ricin-resistant line (MDCK-RCA²) carries a defect in an UDP-galactose transporter in the Golgi complex and therefore is unable to incorporate galactose and sialic acid into glycoproteins. Another concanavalin A-resistant line (MDCK-ConA⁸), with a so far undefined defect, transfers unusual core oligosaccharides on glycoproteins. Despite the defects in N-glycosylation both cell lines target gp80 apically, underlining that the apical N-glycan signal apparently does not require specific terminal sugar structures (see above) (Parczyk and Koch-Brandt, 1991; Wagner et al., 1995). Basolateral missorting in MDCK-RCA² cells was observed for the apical glycoprotein gp114. However, treatment with glycosylation inhibitors revealed no requirement for terminal sugar modifications in sorting (Le Bivic et al., 1993). All other apical proteins analysed so far are correctly sorted in MDCK-RCA² cells and in contrast to wild-type MDCK cells gp114 in MDCK-RCA² cells is efficiently endocytosed. This suggested that the observed missorting might be due to a mutation in gp114 creating a dominant basolateral signal rather than being a consequence of the aberrant N-glycosylation (Le Bivic et al., 1993).

MDCK-ConA² cells transport some GPI-anchored proteins in an unpolarized fashion, but sort four out of five detectable endogenous GPI-anchored proteins, gp80, influenza virus hemagglutinin, and C6-NBD-glycosylceramide normally to the apical membrane (Lisanti et al., 1990). Also the partially missorted GPI-anchored proteins can associate with rafts (Zurzolo et al., 1994). It is not known whether GPI-anchored proteins, transmembrane proteins and secretory proteins generally use the same vesicles for surface delivery. One explanation for missorting of GPI-anchored proteins could be that their carrier vesicles are partially mistargeted or that some of the molecules can be accommodated in basolateral vesicles.
Unpolarized delivery of a GPI-anchored protein is also observed in a special clone of MDCK cells, termed MDCK/J (Mays et al., 1995). In MDCK/J cells also C6-NBD-glycosylceramide and the Na+,K+-ATPase are equally transported to the apical and basolateral membrane, but at steady state Na+,K+-ATPase is found in the basolateral membrane where it is stabilised by interaction with the membrane skeleton (Hammerton et al., 1991). This is in contrast to another clone termed MDCK/G which sorts GPI-anchored proteins and C6-NBD-glycosylceramide apically and Na+,K+-ATPase exclusively to the basolateral membrane. Treatment of MDCK/G cells with Fumonisin B1, an inhibitor of sphingolipid synthesis, converts them to the same sorting phenotype as MDCK/J cells (Mays et al., 1993). This clearly demonstrates a requirement for lipids in polarized sorting. Na+,K+-ATPase appears to be excluded from sphingolipid-cholesterol rafts which transport GPI-anchored proteins and C6-NBD-glycosylceramide. This example also illustrates the complexity of the sorting processes as in MDCK/J cells transport of GPI-anchored proteins and C6-NBD-glycosylceramide is coupled, whereas some GPI-anchored proteins appear independently sorted in MDCK-ConA cells. However, as mentioned above, C6-NBD-glycosylceramide might not always reflect sorting of endogenous glycosphingolipids. Influenza virus haemagglutinin and gp80 are directly transported to the apical membrane, also in the clone MDCK/J cells (Mays et al., 1995). As suggested above this could mean that parallel pathways exist to the apical surface.

FRT Cells

The rat thyroid cell line FRT shows a very interesting sorting phenotype. In fully polarized cells proteins mainly use the direct route from the TGN to the cell surface as in MDCK cells (Zurzolo et al., 1992). One striking difference is, however, that FRT cells sort some GPI-anchored proteins preferentially to the basolateral plasma membrane domain (Zurzolo et al., 1993). Also C6-NBD-glycosylceramide is sorted basolaterally (Zurzolo et al., 1994). According to their detergent-solubility the GPI-anchored protein GDI-DAF is not found associated with raft lipids, in contrast to MDCK and MDCK-ConA cells where even basolaterally missedorted GPI-anchored proteins are detergent-insoluble (Zurzolo et al., 1994). On the other hand other apical proteins as influenza virus HA, dipeptidyl peptidase IV and gp80 are transported with the same polarity in FRT as in MDCK cells (Zurzolo et al., 1992; Graichen et al., 1996).

How could the sorting phenotype found in FRT cells be explained? Sorting of the GPI-anchored proteins seems to follow the transport of NBD-GlcCer, but is diverged from the apical pathway which is assumed to use raft association and carbohydrates for sorting. Raft lipids in detergent-insoluble assemblies exist in FRT cells, but obviously the analysed GPI-anchored proteins cannot interact with rafts (Zurzolo et al., 1994). One explanation for this would be that the GPI-anchor added has a different structure; for example it could contain unsaturated fatty acids and therefore not partition into rafts. Indeed the GPI structure can differ in different cell lines (McConville and Ferguson, 1993) but has not yet been analysed in FRT cells. Another explanation would be that a protein factor is required to insert GPI-anchored proteins into rafts during biosynthetic traffic. Transfer of GPI-anchored
PLAP in raft lipid containing liposomes results in detergent insolubility of the molecule (Schroeder et al., 1994). However, when isolated GPI-anchored proteins are transplanted into living cells incubation for prolonged times is required before they acquire detergent insolubility (van den Berg et al., 1995). This demonstrates that accommodating a GPI-anchored protein in the raft lipid environment is not a trivial process but might indeed need chaperone like cellular protein factors. One exciting observation was that FRT cells lack caveolin-1 (Zurzolo et al., 1994), a cholesterol binding protein implicated in vesicular transport and structural component of cell surface caveolae (Murata et al., 1995; Fra et al., 1995; Scheiffele et al., 1998). However, expression of caveolin-1 in FRT cells did not restore apical sorting or raft association of GPI-anchored proteins (Lipardi et al., 1998). Therefore, other or additional components could be lacking. While this problem still remains to be resolved it emphasizes again that apical components can be diverged into different routes.

Caco-2 Cells

One system where apical signals seem to be more stringently decoded than in MDCK cells is the human intestinal cell line Caco-2. Secretory proteins without signals are almost exclusively released in the basolateral medium which demonstrates that the capacity of the apical pathway is small (Rindler and Traber, 1988). In MDCK cells the same proteins are secreted in a nonpolarized fashion (Gottlieb et al., 1986). However, a direct apical pathway for secretory proteins exists as gp80, the apical marker protein in MDCK cells, is secreted from the apical surface of Caco-2 cells (Appel and Koch-Brandr, 1994). The distribution of most membrane proteins at steady state in Caco-2 cells is the same as in MDCK cells. However, not all apical membrane proteins are transported via a direct route from the TGN, but they are first delivered to the basolateral membrane and then subsequently transcytosed (Matter et al., 1990; Le Bivic et al., 1990). GPI-anchored proteins which have an optimal membrane anchor for raft association reach the apical membrane by the direct pathway and they have been shown to enter DIDs during transport (Garcia et al., 1993). Also sucrose-isomaltase which is partially insoluble can be transported by the direct route (Garcia et al., 1993). We speculate that proteins which can associate avidly with rafts will be transported via the direct route, whereas proteins which only loosely associate with rafts travel via the indirect route as they do not have sufficient affinity to the apical sorting machinery in the TGN. These proteins might be included without signals and tolerated as "stow-aways" in the basolateral vesicles (see also below). As the capacity of the basolateral route is much higher than that of the direct apical route this passive forward transport could be sufficient for effective surface delivery. An other explanation would be that apical and basolateral proteins are efficiently sorted in the TGN but that some apical vesicles dock and fuse with the basolateral membrane. However, as some proteins mainly travel along the direct route whereas others only use the indirect route this is not very likely.
Hepatocytes

Hepatocytes appear not to use a direct apical targeting route. Instead all proteins are first delivered to the basolateral membrane and apical components then transcytosed (Hubbard et al., 1989). So far no apical secretion from hepatocytes has been detected and also raft associated proteins like the GPI-anchored 5'-nucleotidase are delivered to the apical membrane via an indirect route (Schell et al., 1992). As endocytosis of raft proteins from the basolateral membrane occurs only slowly, this creates considerable delay in apical surface delivery. Two scenarios can be imagined for this sorting phenotype: either all proteins are transported in the same carriers from the TGN to the basolateral plasma membrane or apical and basolateral proteins are packaged into different vesicles as in MDCK cells, but they are all targeted to the basolateral plasma membrane. This could be achieved by placing the docking and fusion machinery for both routes to the basolateral membrane. The involvement of different vesicle populations in basolateral transport has been suggested recently (Nickel et al., 1994), however, it is not yet understood if they separate apically or basolaterally directed cargo.

RPE

The retinal pigment epithelium (RPE) is derived from the neuroectoderm. The particularity of this cell type is that its apical surface is not free: microvilli and lamellipodia interact with photoreceptor outer segments directly and through the interphotoreceptor matrix (Zinn and Marmor, 1979). A stable cell line, termed RPE-J, has been created which forms polarized monolayers and resembles primary cultures in vitro (Nabi et al., 1993). Budding of influenza and vesicular stomatitis virus occurs with the same polarity as in MDCK cells, suggesting that the envelope proteins are sorted with the same polarity (Nabi et al., 1993). The absence of the interphotoreceptor matrix in cultured cells accounts for several differences in the steady state localization of membrane proteins in vitro vs. in vivo (Gundersen et al., 1993) (see below).

DEVELOPMENT OF POLARITY

During the establishment of cell surface polarity epithelial cells undergo dramatic changes. At areas of cell-cell contacts adherens junctions, tight junctions, and desmosomes are formed which are crucial to build up a tight monolayer. Two factors appear to determine initial polarity: interactions with the substratum and cell-cell contacts (Eaton and Simons, 1995). Single cells attached to a substratum exhibit polarity as they promote polarized apical and basolateral budding of influenza or vesicular stomatitis virus, respectively (Rodriguez-Boulan et al., 1983). Such polarized release of viral particles is also observed in absence of a substratum when cells are grown as small aggregates in suspension. Influenza viruses bud from the free surface whereas VSV preferentially buds from cell-cell contact sites (Rodriguez-Boulan et al., 1983). This demonstrates that cells are able to distinguish apical and basolateral components already in the initial stages of polarization. MDCK cells
secrete extracellular matrix components from their basolateral side where also integrins for substratum adhesion are expressed (Caplan and Jamieson, 1987; Ojakian and Schwimmer, 1994). This allows cells in suspension culture to form cysts with the apical membrane outside and the basolateral inside, binding to the selfproduced matrix (Wang et al., 1990).

Cell-cell contacts are mediated by cadherins (Kemler, 1992). In MDCK cells the basolateral E-cadherin forms Ca\(^{2+}\)-dependent contacts with the neighbouring cells and thereby fixes the cytoskeleton (Gumbiner et al., 1988). After adsorption to the substratum MDCK cells in culture form E-cadherin mediated contacts. Already two hours later the apical protein gp135 is transported polarized to the apical pole (Wollner et al., 1992). Instead exclusive basolateral delivery of proteins occurs first 48 hours after the initial cell-cell contacts (Wollner et al., 1992). However, a polarized distribution of the basolateral proteins is achieved earlier by selective stabilization in the basolateral plasma membrane. It appears that interactions with the cytoskeleton facilitate early establishment of basolateral polarity, whereas first in fully polarized cells exocytosis occurs with high fidelity to the right target domain. When cells during polarization are cultured in medium with low Ca\(^{2+}\) concentrations (1–5 μM) apical polarization is maintained whereas no polarity of basolateral proteins can be achieved (Vega-Salas et al., 1987). This highlights the importance of the assembly of the cytoskeleton for the basolateral pathway.

The organization of the cytoskeleton changes during polarization. In unpolarized MDCK cells most microtubules are emerging from the centrioles in the perinuclear region and extend their plus-ends to the cell periphery. After cell-cell contacts are established the two centrioles separate and move to the apical pole of the cells (Buendia et al., 1989). This process depends on the cell-cell contacts as disruption of the intercellular junctions with low Ca\(^{2+}\) medium causes the centrioles to move back to the center of the cell (Buendia et al., 1989). In fully polarized cells microtubules span the apical-basal axis with their minus ends underneath the apical membranes. These polar microtubular tracks are used for exocytosis and different motor proteins are involved in apical and basolateral transport (Lafont et al., 1994). The microtubules have an increased stability in the fully polarized cells and do not only emerge from centrioles. Consistently, two pools of γ-tubulin have been detected, one non-centriolar and another associated to centrioles (Meads and Schroer, 1995).

E-cadherin expressing cells localize the ankyrin-spectrin membrane cytoskel-
ton to the basolateral membrane. Anchoring of Na\(^{+}\),K\(^{+}\)-ATPase to spectrin is used to stabilize the protein selectively in the basolateral plasma membrane (Hammerton et al., 1991), and ectopic expression of E-cadherin in nonpolarized fibroblasts is sufficient to localize Na\(^{+}\),K\(^{+}\)-ATPase to cell-cell contact sites (McNeill et al., 1990) (see also below). Similarly, the apical cytoskeleton can be used to localize an epithelial Na\(^{+}\)-channel via a SH3-domain interaction to the apical membrane (Rotin et al., 1994).

PLASTICITY OF EPITHELIAL POLARITY

The polarity of proteins in vivo can change during differentiation or depending on growth conditions. A reversal of polarity can be induced by external cues like the
electrical potential across the membrane and extracellular matrix components. For example the polarity of band 3, a Cl/HCO₃ exchanger in intercalated epithelial cells of the renal collecting tubule, reverses when animals are fed an acid diet (Schwarz et al., 1985). This change in polarity can be mimicked in cultured cells depending on the density they are plated. It turned out that a 230 kDa extracellular matrix protein was capable of inducing the change of polarity (van Adelsberg et al., 1994). If this is achieved by domain specific stabilization or by including band 3 into different transport vesicles is not known. Also targeting of the LDL-receptor varies in different tissues. Using transgenic mice it has been demonstrated that basolateral delivery in hepatocytes depends on the same cytoplasmic signal as identified in cultured cells (Yokode et al., 1992). However, in kidney the protein was apically expressed (Pathak et al., 1990). The presence of the described hierarchically acting signals for polarized sorting might allow to modulate polarity. Basolateral signals could be inactivated by cell-specific post-translational modifications, thereby activating the recessive apical signal (Casanova et al., 1990; Simons, 1995). Such post-translational modifications could be induced by cell-cell signalling or extracellular cues as mentioned above.

Cell-cell contacts and adhesion can give crucial cues for epithelial polarization. The cell adhesion molecule N-CAM is apically polarized in the RPE but found on the basolateral membrane in cultured cells. The contact with the neural retina which is lacking in vitro was suggested to be required for the apical localisation (Gundersen et al., 1993). Also Na⁺,K⁺-ATPase is restricted to the apical membrane of the RPE but is distributed in a nonpolarized fashion in RPE-J cells in vitro. Expression of E-cadherin results in basolateral accumulation of the ankyrin-spectrin cytoskeleton and thereby the Na⁺,K⁺-ATPase is also moved basolaterally. Concomitantly, expression of an ankyrin isoform is induced (Marrs et al., 1995). Therefore, cell adhesion molecules can be employed to determine epithelial polarity.

As described before apical or basolateral pathways can be selectively influenced by regulatory components as trimeric G proteins and protein kinases (Mostov and Cardone, 1995) and therefore developmental regulation of polarity could also be achieved by stimulation of one pathway increasing its capacity.

SORTING IN ENDOSONMES

Sorting signals are not only decoded in the TGN. As obvious in the case of Caco-2 cells, the basolateral endosomes are capable of distinguishing apical and basolateral proteins because apical proteins on the indirect route are efficiently transcytosed (Matter et al., 1990). Dissection of the signals present in the LDL-receptor demonstrated that the same signals mediating sorting in the TGN of MDCK cells also function in endosomes (Matter et al., 1993). When the distal signal in the cytoplasmic tail of the receptor was inactivated the weaker proximal signal mediated basolateral sorting and subsequently endocytosis (see Figure 3). From the basolateral endosomes the receptor was then efficiently transcytosed to the apical recycling endosome and plasma membrane. The endocytic pathways originating from the basolateral and apical surface meet in the apical recycling compartment (Oдоризzi et al., 1996). From here transferrin receptors can be sorted back to the basolateral surface and apically recycling proteins are redirected to the apical plasma membrane.
Also morphological studies by electron microscopy support the idea that endosomes are capable of sorting apical and basolateral components because morphologically different types of vesicles can bud from the compartment (Odorizzi et al., 1996). However, the main sorting event in the exocytic pathway of MDCK cells occurs in the TGN from where two distinct apical and basolateral vesicle populations are released (Wandinger-Ness et al., 1990). Those vesicles have been postulated to be directly delivered to the respective plasma membrane domain, but recently evidence was provided that the basolateral transport occurs via endosomes. The transferrin receptor could be recovered before surface arrival from an endosomal fraction labeled with the fluid-phase marker horseradish peroxidase, whereas the (apical) GPI-anchored protein PLAP was excluded from this fraction (Futter et al., 1995). Secondly, after release from the TGN the basolateral asialoglycoprotein receptor H1 was transiently detected in an immunoisolated endosomal fraction using the mannose 6-phosphate receptor as a marker (Leitinger et al., 1995). The passage of the basolateral route through an acidic compartment has also been suggested when it was observed that weak bases like NH₄Cl block basolateral secretion (Caplan and Jamieson, 1987). Under these conditions also nonpolarized secreted proteins like rat growth hormone and lysozyme are directed to the apical membrane (Scheiffele and Simons, unpublished), possibly because of a general block of vesicle budding from the basolateral endosomes (Aniento et al., 1996). The similarity of basolateral sorting signals and endocytosis signals further suggests that the clathrin-mediated endocytic pathway and the basolateral route could converge in endosomes.

MACHINERY FOR DOCKING AND FUSION

Not only the nature of the sorting signals differs in the two TGN-surface routes of MDCK cells. Dissection of the transport machinery has revealed further dramatic differences. Basolateral transport depends on the same machinery for docking and fusion as so far characterised for synaptic vesicles and ER-Golgi transport (Ilonen et al., 1995; Rothman, 1994). The involvement of NEM-sensitive factor (NSF), the soluble NSF attachment proteins (SNAPs) and the SNAP receptors (SNAREs) was demonstrated by sensitivity of basolateral delivery to NEM, to NSF antibodies and to neurotoxins which cleave and thereby inactivate SNAREs. Removal of rab proteins from cells, which might regulate the assembly of the SNARE complexes, also caused an inhibition of basolateral transport. On the other hand apical transport was insensitive to these treatments revealing different requirements for docking and fusion (Ilonen et al., 1995). The following considerations might explain the need for a different machinery in apical transport: The lipid composition assumed to reside in the apical vesicles is very different than that found in COP coated vesicles which use the NSF/SNAP/SNARE system (Brügger et al., 1997). Also apical proteins do not use cytoplasmic signals which in the NSF/SNAP/SNARE system are required to recruit coat proteins (Fiedler et al., 1996). Finally, the existence of two different principles for docking and fusion would strictly prevent delivery of the content of mistargeted vesicles with the wrong plasma membrane.

But how is specific docking and fusion of apical vesicles achieved? This process is so far not understood. However, a member of the annexin protein family, annexin
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13b, has been isolated from MDCK cells which localizes to apical vesicles and the apical plasma membrane (Lafont et al., 1998). Annexins have been postulated to perform multiple functions, one of them being membrane fusion (Moss, 1992). As annexins are calcium-dependent phospholipid binding proteins and able to form oligomers (Luecke et al., 1995), one working model is that an annexin complex on the transport vesicle would bind a second complex attached to the plasma membrane. After this docking step fusion could occur, also mediated by the coupled annexins. While this model is still speculative it has been demonstrated that annexin 13b can associate to sphingolipid-cholesterol rafts independently of calcium (Lafont et al., 1998). Antibodies to annexin 13b and recombinant annexin 13b specifically block apical transport of HA whereas basolateral transport of VSV G is not affected (Lafont et al., 1998). Therefore, annexin 13b could indeed function in the apical pathway.

ANALOGY OF NEURONAL AND EPITHELIAL POLARITY

Neurons are a striking example of polarized cells. Comparison of neuronal and epithelial polarity suggested that the same sorting signals and mechanism for transport could be used, with the axonal pathway representing an apical route and the dendritical a basolateral one (Rodríguez-Boulan and Powell, 1992; De Hoop and Dotti, 1993). In agreement with this hypothesis influenza and Semliki Forest viruses bud from the axons or the dendrites, respectively (Dotti and Simons, 1990), and GPI-anchored proteins are targeted preferentially to the axons (Dotti et al., 1991). Rab8, the GTPase regulating basolateral transport in MDCK cells, is involved in traffic to the dendrites (Huber et al., 1993) suggesting that also the machinery for transport is similar. Like the apical pathway, also axonal transport employs sphingolipid-cholesterol rafts. Thy-1, a GPI-anchored protein, and influenza virus hemagglutinin are found in DIGs during axonal transport (Ledesma et al., 1998). As hippocampal neurons in culture pass through different stages of polarity, sorting can be analysed and compared between premature and mature cells. After establishment of an axon stage three neurons sort dendritical proteins efficiently but deliver HA and Thy-1 unpolarized and fail to associate them with DIGs (Dotti and Simons, 1990; Ledesma et al., 1998). In fully mature stage 5 neurons HA and Thy-1 are sorted and found in DIGs. It is possible that the raft pathway is not or only in a premature form present in stage 3 neurons and therefore Thy-1 and HA are delivered unsorted. Interestingly, the lack of DIG association in the stage 3 cells correlates with the presence of only low amounts of the raft lipid sphingomyelin which seems to be much more abundant in stage 5 cells (Ledesma et al., 1998).

Sorting in neurons will not be identical to sorting in MDCK cells. As already indicated by the heterogeneity among different epithelial cell types, variations will occur. However, we expect that neurons, like epithelial cells, make use of two post-Golgi routes which are functionally similar to the apical and basolateral pathways (Simons et al., 1993).
APICAL AND BASOLATERAL COGNATE ROUTES

The two targeting pathways of polarized cells could be generated in two different ways. A new epithelial route could be induced upon differentiation to the polar phenotype or two previously existing routes could be diverged and focused to different domains by reorganization of the cytoskeleton and establishment of the tight junctions (Rodriguez-Boulan and Powell, 1992). When signals for transport and the mechanisms for docking and fusion had been identified in the polarized cell system this provided the tools to distinguish the two pathways experimentally in nonpolarized cells. It turned out that surface transport of a basolateral protein was also signal mediated in nonpolarized fibroblasts as peptides encoding the basolateral signal of VSV G could specifically block its delivery (Miisch et al., 1996). Furthermore, docking and fusion of the basolateral marker in nonpolarized cells required the NSF/SNAP/SNARE machinery as in MDCK cells. Instead surface transport of the apical marker HA in nonpolarized cells was independent of these factors, but the protein was found associated with sphingolipid-cholesterol rafts (Yoshimori et al., 1996). Therefore, apical and basolateral cognate routes do exist in nonpolarized cells and function similarly as in epithelial cells.

Why should cells with a continuous plasma membrane have two different routes to the surface? The signals identified in polarized cells will allow directed surface delivery. It has been observed that some proteins are preferentially targeted to the leading edge of fibroblasts where they might be used for locomotion (Singer and Kupfer, 1986; Bretscher, 1996; Bretscher, 1992a). This polarized exocytosis on the biosynthetic pathway and from endosomes could provide components required for extension of the cell: new membrane and receptors for attachment to the extracellular matrix. It appears that the basolateral cognate route is used for this targeting step (Figure 5A,B) as so far all molecules identified are basolateral proteins: the transferrin receptor (Hopkins et al., 1994), VSV G (Bergmann et al., 1983) and some integrins (Bretscher, 1992b). Interestingly, not all integrins are recycling efficiently. While α5β1 and α6β4 are circulating, α5β1 and α4β1 do not follow the same route (Bretscher, 1992b). This could be explained by the fact that α5β1 and α4β1 are found in big complexes with the detergent insoluble proteins CD9 and CD81 which should allow segregation from the basolateral cognate endocytic route (Berdichevski et al., 1996; Mannion et al., 1996; Shevchenko et al., 1997).

When rab8, the GTPase regulating basolateral transport in epithelial cells, is expressed as constitutively active mutant in fibroblasts, the cells rearrange their cytoskeleton and form long extensions (Peränen et al., 1996). The basolateral cognate vesicles are targeted into these processes and accumulate their cargo in the tip. Like in the leading edge new membrane and the basolateral integrins are required for outgrowth and will now be directed to the place of their function. Also molecules on the biosynthetic route are transported in a polarized fashion. While VSV G is concentrated in the extensions, influenza virus HA is transported without preference (Peränen et al., 1996).

Similar polarized exocytosis is observed in the budding yeast *Saccharomyces cerevisiae*. Polarity is developed by assembly of an asymmetric cytoskeleton which directs the secretory pathway to the site of bud formation (Drubin and Nelson, 1996). Secretion and addition of new membrane occurs preferentially at the bud.
This is probably achieved by transporting the vesicles along the cytoskeleton (Drubin et al., 1993) and by placing the docking machinery to the growth site (TerBush and Novick, 1995). In analogy to the apical and basolateral cognate routes in fibroblasts this route would represent a basolateral cognate pathway (Figure 5D). However, it is still very speculative if in yeast a raft pathway with similar principles as in mammalian cells exists. Parallel biosynthetic pathways to the plasma membrane have been described. From the Golgi complex two populations of exocytic vesicles are produced, one carrying cell-surface components and another transporting the periplasmic enzymes inverterase and acid phosphatase (Harsay and Bretscher, 1995). However, in late secretary mutants (sec1, sec4 and sec6) delivery of both vesicle populations is blocked, suggesting that they are transported by functionally similar pathways and not apical and basolateral cognate routes (Harsay and Bretscher, 1995). However, rafts might also exist in yeast. Digs can be isolated as in mamm-
malian cells (Kübler et al., 1996), but their composition has not yet been carefully analysed. Interestingly, the rate of ER to Golgi transport of a GPI-anchored protein was shown to be enhanced by ceramide synthesis whereas transport of soluble and transmembrane proteins was not changed (Horvath et al., 1994). Therefore, GPI-anchored proteins in yeast might be transported in raft-like lipid microdomains.

Another example of polarization of nonepithelial cells is observed for osteoclasts (Salo et al., 1996). Resorbing osteoclasts secrete protons and enzymes from their ruffled membrane to degrade bone matrix components. Analysis of the targeting of the viral marker proteins VSV G and influenza virus HA demonstrated that the continuous basal membrane facing the bone marrow and extracellular fluid is divided into two different subdomains, receiving apical and basolateral cognate traffic (Figure 3C) (Salo et al., 1996). Bone degradation products are specifically transcytosed from the ruffled membrane to the apical cognate domain (Salo et al., 1997; Nesbitt and Horron, 1997). Whether sphingolipid-cholesterol rafts operate in the biosynthetic or transcytotic routes to this domain is not known.

TWO CIRCUITS?

Analysis of the post-Golgi membrane traffic in polarized and nonpolarized cells revealed two functionally distinct routes. It has been proposed that these two exocytic routes represent parts of two general circuits which operate in intracellular transport (Simons and Ikonen, 1997). One would be the basolateral circuit employing cytoplasmic signals and the other the apical circuit using raft association and carbohydrates. The exocytic routes are balanced by apical and basolateral endocytosis processes. The clathrin-pathway, like the basolateral endocytosis, would make use of cytoplasmic signals and adaptors whereas the apical route would be raft-mediated, depending on clustering of the internalized proteins, cholesterol and actin (Simons and Ikonen, 1997; Rothberg et al., 1990; Deckert et al., 1996). Although named "apical" and "basolateral" the circuits are not anymore restricted to apical and basolateral surfaces in epithelial cells, where they were defined as the preferred mechanism used for the respective exocytosis route. Instead the circuits meet in most compartments of the cell as clathrin-coated pits and clathrin-mediated endocytosis also occur on the apical plasma membrane and raft associated proteins can be found on the basolateral surface (Melkonian et al., 1995; Simons and Ikonen, 1997).

Proteins transported on one route would be excluded from the other, as raft markers like GPI-anchored proteins or influenza virus HA do not interact with clathrin-coated pits (Lazarovits and Roth, 1988; Bretscher et al., 1980). When raft association is weakened by mutation of the HA TMD determinant the mutant protein can be endocytosed through clathrin-coated pits, although it does not contain a cytoplasmic endocytosis signal (Lazarovits et al., 1996). Probably, the mutant HA can be tolerated rather than being actively included. Also the GPI-anchored prion protein can switch to the basolateral circuit. It contains a protease-resistant signal which is required for its clathrin-mediated endocytosis (Shyng et al., 1995), probably by attaching it to a receptor with a cytoplasmic internalization signal. The pIgAR changes for transcytosis from the basolateral to the apical route, controlled by inactivation of its cytoplasmic signal (Aroeti and Mostov, 1994).
SUMMARY

Identification of signals and transport mechanisms in epithelial cells demonstrated that the apical and basolateral routes use different principles. While basolateral sorting depends on cytoplasmic proteinaceous signals, apical targeting is based on carbohydrate-protein and lipid-protein interactions. The observations made in non-epithelial cells demonstrate that none of the pathways is a unique and specialized mechanism of epithelial cells. It rather appears that the principles of apical and basolateral transport are used to build up two independent circuits in the cell which are used to create surface polarity. As the signals directing proteins into the different circuits function hierarchically, proteins can be switched from one to the other pathway. This allows plasticity at the level of intracellular sorting which can be employed to serve the needs of different cell types.

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