Mini-Review

Plasma Membrane Protein Sorting in Polarized Epithelial Cells

Keith Mostov, Gerard Apodaca, Benjamin Aroeti, and Curtis Okamoto

Departments of Anatomy, and Biochemistry and Biophysics, and Cardiovascular Research Institute, Box 0452, University of California, San Francisco, California 94143-0452

SPATIAL asymmetry is fundamental to the structure and function of most eukaryotic cells. A basic aspect of this polarity is that the cell's plasma membrane is divided into discrete domains. Examples include the membranes of axons and dendrites in neurons, the growing bud and mating projection in the yeast *Saccharomyces cerevisiae*, and lamellipodia in fibroblasts. However, the best-studied type of polarity is found in epithelia, which is the most common type of tissue in animals (52, 56). In simple epithelia the cells form a monolayer where one surface of the cell—the apical—faces the outside world or a topologically equivalent space, such as the lumen of the intestines. This is separated by tight junctions from the basolateral surface, which contacts adjacent cells and the underlying connective tissue.

Apical and basolateral surfaces have very different protein and lipid compositions (4, 10, 57). For example, the basolateral surface contains receptors for adhering to other cells and the basement membrane, and for uptake of nutrients from the circulation. The apical surface contains transporters for exchanging small molecules with the outside world. As epithelial cells divide and differentiate they must send proteins to the correct surface to establish their polarity (25). Moreover, in a differentiated epithelial cell, polarity is maintained despite an enormous flux of membrane components traveling to and from each surface.

Pathways to the Cell Surface

As shown in Fig. 1, epithelial cells use two pathways to send proteins to the correct surface. Newly made proteins can travel directly from the trans-Golgi network (TGN)[1] to either the apical or basolateral surface. Alternatively, proteins can use an indirect pathway traveling first from the TGN to the basolateral surface, followed by endocytosis and transcytosis to the apical surface (46, 55). The former pathway was discovered in MDCK cells which form a well-polarized monolayer when cultured on a permeable support. In pioneering work, Rodriguez-Boulan and Sabatini (53) showed that influenza virus buds solely from the apical surface, whereas vesicular stomatitis virus (VSV) buds from the basolateral surface (53). Viral envelope proteins, hemagglutinin (HA) of influenza and G protein of VSV (VSVG), travel through the secretory pathway together to the trans-Golgi network (TGN). Here they are packaged into separate vesicles that carry these proteins to the apical and basolateral surfaces, respectively (24, 51). Newly made endogenous MDCK proteins are also delivered directly from the TGN to their final destination (33). Hepatocytes, in contrast, lack a pathway from the TGN to the apical (canalicular) surface and rely entirely on the indirect, transcytotic route (3). The enterocyte-like CaCo-2 cell line uses a mixture of the direct and transcytotic strategies (32, 42). For several apical proteins a fraction is delivered directly from the TGN to the apical surface, while the balance follows the transcytotic route. MDCK cells can also use the transcytotic route, as was shown for the polymeric immunoglobulin receptor (pIgR) transfected into these cells (45), as well as for endogenous proteins (5).

Both the cell type and the protein influence the route taken to the apical surface. For example, dipeptidyl peptidase IV (DPP-IV) and aminopeptidase N (APN) use the transcytotic route in liver and both routes in CaCo2 cells. When transfected into MDCK cells, they mainly use the direct route from the TGN to the apical surface (39, 66). However, about 20% of both travel to the MDCK basolateral surface. In the case of APN, this was judged to be a non-transcytosing pool, although transcytosis was evaluated indirectly (66). If true, this would imply that in MDCK cells, basolateral APN is unable to be transcytosed and that transcytosis depends on the cell type. In contrast, we found that the 20% of DPP-IV reaching the basolateral surface is then transcytosed (11). The fraction of DPP-IV going to the basolateral surface varies with the cell type, but it is transcytosed efficiently in all cells (40). In contrast the pIgR exclusively uses the transcytotic route regardless of the cell type.

Whereas the TGN to apical pathway is present only in certain cells, transcytosis is found in all epithelial cells examined. The importance of transcytotic sorting is emphasized by the fact that while roughly 5% of the cell surface is newly made per hour, the equivalent of about 50% of the cell surface is endocytosed per hour (63). Most endocytosed proteins simply recycle, but without exquisite selectivity for entry into the transcytotic pathway, the two surfaces of the cell would be rapidly scrambled.

The amount of a protein at a surface depends on the rates of delivery and removal due to endocytosis and/or degrada-

1. *Abbreviations used in this paper*: APN, aminopeptidase N; ASGPR, asialoglycoprotein receptor; DPP-IV, dipeptidyl peptidase IV; FCR II, immunoglobulin Fc receptor II; GPI, glycosyl-phosphatidylinositol; HA, influenza virus hemagglutinin; LDLR, low density lipoprotein receptor; LGP 120, lysosomal membrane glycoprotein 120; M6PR, mannose-6-phosphate receptor; NGFR, nerve growth factor receptor; pIgR, polymeric immunoglobulin receptor; TfR, transferrin receptor; TGN, *trans*-Golgi network; VSVG, vesicular stomatitis virus G protein.

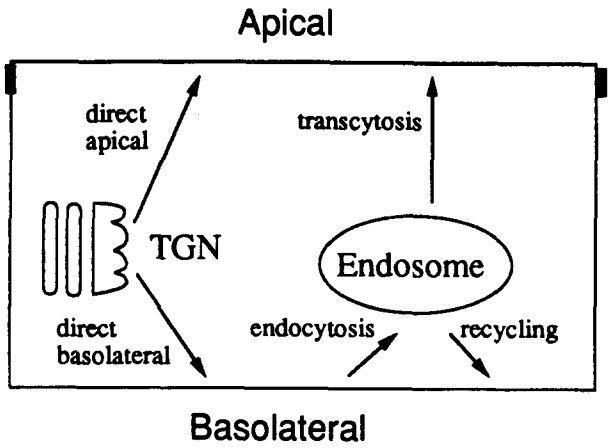

Figure 1. Pathways of protein traffic in polarized epithelial cells. For details see text. Only selected pathways are shown.

tion. Proteins can be selectively retained at one surface by interaction with other proteins, such as the cytoskeleton, extracellular matrix, or adhesion proteins on adjacent cells. For example, in MDCK cells the Na-K ATPase is delivered to both surfaces. The basolateral molecules form a long-lived complex with the submembranous cytoskeleton, whereas the apical ones are rapidly removed (26). Selective retention adds another level of regulation of the polarized distribution of membrane components.

Sorting Information: The Default Pathway

What information is needed to direct molecules to the proper location? Some molecules may not have any sorting information, but travel instead by a default pathway. Soluble secretory proteins that lack any sorting information are believed to simply follow the movement of fluid through the entire secretory pathway to the surface of non-polarized cells (67). To be a marker for the default pathway, a molecule must have no interaction with the sorting machinery or other molecule that is being sorted. Even so, such a marker can be excluded from vesicles by a tight packing of other proteins, such as apparently occurs in dense core secretory vesicles (9).

Finding a default pathway marker for membrane components is difficult. Molecules in a bilayer are effectively at a much higher concentration than those in solution, so the effects of weak interactions and excluded volume can be much greater. For example, in MDCK cells the outer leaflet of the apical surface is comprised mainly of glycosphingolipids, which are hydrogen bonded to each other and apparently exclude glycerol-based phospholipids (63). While water may be the ideal marker for the fluid-phase default pathway, it is unclear what the marker for the membrane default pathway should be. In the absence of such a marker, less-direct arguments have been made as to whether traffic of proteins to either epithelial surface occurs by default. Until recently the most widely accepted model was that basolateral sorting is by default (57). The principal reasons advanced were: (*a*) Hepatocytes lack a direct apical pathway; (*b*) "housekeeping" proteins found on the surface of non-epithelial cells (e.g., transferrin receptor in fibroblasts) are on the basolateral surface of epithelial cells; and (*c*) the apical surface is a differentiated domain or organelle specialized for interacting with the outside world.

However, evolutionary and developmental considerations suggest that the basolateral surface is in some ways the more specialized domain and it is plausible that apical sorting is by default. In a single-cell organism the entire plasma membrane interacts with the outside world and is in that sense apical. Multicellular animals develop from a single-cell "organism", the fertilized egg. When a fertilized Xenopus egg divides into two cells it forms de novo a basolateral surface, sealed by tight junctions (21, 50). (In mammalian embryos, which develop in a protected environment, epithelialization takes place later at the compaction stage.) An essential feature of the evolution of multicellular animals is not an external surface, but rather an internal, i.e., basolateral surface that interacts with the underlying connective tissue. From this perspective, the apical surface would seem to be the more primitive domain, rather than a specialization. The notion that epithelia develop from primitive cells which lack the apparently specialized apical surface has resulted from studies of events such as induction of kidney tubules from loose mesenchyme (52). In fact, the more common developmental process is the conversion of epithelia into other tissue types, such as the induction of mesoderm and of neural tissue (1). This may involve the loss or modification of the apical surface, e.g., into axons in neurons.

One experimental model for epithelial development is to culture MDCK cells in low calcium medium where the cells lack intracellular contact and are poorly polarized. When normal levels of calcium are added one can monitor the development of polarity (52). Apical proteins are correctly delivered to the apical surface very early after adding calcium. In contrast, the basolateral markers are delivered to both surfaces, although the apically missorted molecules are short-lived (67a). One interpretation of these results is that the machinery for TGN to apical sorting is present even in non-polarized cells, whereas the machinery for basolateral sorting only develops when polarity is induced. Although these arguments support the hypothesis that apical sorting might be by default, the issue is clearly not settled.

Sorting Signals

Traffic to one or possibly both surfaces of the epithelial cell requires information. In several other sorting processes this information resides in a sorting signal, i.e., a structural feature of the protein undergoing sorting (6). Sorting signals can act positively to direct a protein to a pathway, or negatively by retaining a protein in a compartment or preventing entry into a vesicle. Direct demonstration of a sorting sequence requires both inactivation by mutation, as well as transferring the signal to a heterologous protein and showing that the construct is predictably sorted to a new location.

The first signal for polarized sorting to be rigorously established was the glycosyl-phosphatidylinositol (GPI) anchor which anchors the carboxy terminus of certain proteins to the outer leaflet of the lipid bilayer. GPI-anchored proteins are found exclusively on the apical surface of a variety of epithelial cells (37). Replacing the transmembrane peptide anchor of a normally basolateral protein with a GPI anchor will redirect the protein to the apical surface (8, 38). This clearly shows that the GPI anchor is an apical sorting signal, although it does not rule out the possibility that apical targeting of proteins with transmembrane peptide anchors is by default.

The observations that both GPI-anchored proteins and glycosphingolipids are apically disposed has suggested a mechanism for apical sorting (38, 57). Hydrogen bonding may cause these molecules to cluster together in microdomains in the TGN, which resemble the apical surface. Some apical proteins (e.g., HA) become resistant to solubilization by non-ionic detergents shortly before reaching the apical surface, perhaps due to interaction of their membrane-spanning segments with clustered glycosphingolipids (58). This model postulates that unknown proteins interact with these clusters and sort them into vesicles leading to the apical surface.

The search for peptide-sorting signals and for basolateral-sorting signals has until recently been frustrating. The basic strategies have been to mutate or delete various portions of molecules, especially cytoplasmic domains, and to construct chimeric molecules. Much of the earlier work gave confusing and even directly contradictory results (10). There are several likely explanations for these problems. Almost all earlier studies used viral spike glycoproteins. Their oligomeric structure is necessary for proper transport and easily disrupted by mutations. Many studies did not directly examine the polarity of delivery from the TGN to the surface but only steady state distribution, which is complicated by instability, endocytosis, and transcytosis. Distribution was often assessed by fluorescence microscopy and seldom quantitated. Finally, the cells expressing the construct may not have been fully polarized. This may have especially been true in transient expression systems where either only a small fraction of cells express a transfected gene or cells lose polarity due to a viral expression vector. We have also frequently found stable, clonal MDCK cell lines that are poorly polarized. In our hands, polarized apical delivery of the endogenous gp80 is the most reliable indicator that the cells are well polarized (62).

The first studies to use a cellular, rather than viral, protein and to overcome these problems used the pIgR. Normally this protein travels first to the basolateral surface and then is transcytosed to the apical surface (45). Deletion of either the cytoplasmic domain or this domain and the membrane anchor produces a molecule that travels directly from the TGN to the apical surface (43, 44). This suggests that the cytoplasmic domain is needed for basolateral sorting. If just the membrane-proximal 17 residues of the 103 amino acid carboxy-terminal cytoplasmic domain are present, the pIgR is sorted to the basolateral surface (12). In contrast, a deletion of this 17-mer that leaves the remainder of the cytoplasmic domain fused in frame is sorted apically. Most importantly, this 17-mer can be transplanted to a normally apical protein and redirect it to the basolateral surface.

In recent months quite a few examples of cellular proteins have been found that are normally basolateral, but can be converted to apical proteins by mutating or deleting their cytoplasmic domain (Table I) (27). The simplest interpretation is that these domains contain basolateral sorting signals. However, only in the case of the pIgR has it been demonstrated that the signal can function when transferred to a heterologous protein. The cytoplasmic domains of these proteins have no obvious homology or structural feature in common, so it is difficult to discern what the basolateral signals are. A tantalizing hint is that in several cases there is a correlation (first noted for HA; 7) between rapid endocytosis via clathrin-coated pits and basolateral targeting. In most endocytosed receptors, the signal for clustering in coated pits involves a Tyr residue, although in some cases (but not all) an aromatic Phe or Trp can substitute for the Tyr (48). Mutagenesis studies have shown that residues near the Tyr are important and have led to the proposal of several different, but related consensus sequences of four to six residues including the Tyr (61). The internalization signals from the LDLR, M6PR, and pIgR can substitute for the signal in the TfR (16). Computer modeling and biophysical studies have indicated that the signal consists of a particular type of reverse β turn and that several side chains, including the Tyr, are exposed on one side of the turn and presumably interact with some other molecule (17) (C. Peters and K. von Figura, personal communication).

Table I. Cellular Plasma Membrane Proteins in Which Mutation of Their Cytoplasmic Domain Affects Polarity

Protein	Reference
pIgR	44, 12
HA*	7
LDLR	28
TfR	28
LGP 120	28
FcR II	28
NGFR	34
lysosomal acid phosphatase	C. Peters and K. von Figura (personal communication)
ASGPR	I. Geffen and M. Spiess (personal communication)

* The influenza virus coat protein, HA, is not a cellular protein, but is included here as it serves to illustrate principles discussed in the text.

In at least five cases there is a correlation between endocytosis and basolateral sorting. Particularly revealing is the HA, which is normally apical and not endocytosed. Replacing a particular residue in its 10 amino acid cytoplasmic domain with a Tyr yields a molecule that is oligomerized, rapidly endocytosed and basolaterally sorted (7). In LGP120 (28) and ASGPR (I. Geffen and M. Spiess, personal communication) mutation of a cytoplasmic Tyr reduces endocytosis and produces an apical, rather than basolateral molecule. The IgG FcRII lacks a Tyr internalization signal. Nevertheless, a deletion in its cytoplasmic domain blocks endocytosis and redirects the molecule from the basolateral to the apical surface (28). The NGFR is normally apical and not endocytosed. An in-frame deletion of part of the cytoplasmic domain creates a basolateral, rapidly endocytosed molecule (34).

On the other hand, there are clear examples where endocytosis and basolateral sorting are not correlated. In both the LDLR and TfR mutation of the cytoplasmic Tyr blocks endocytosis but not basolateral sorting (28). Other mutations in their cytoplasmic domains redirect the proteins from the basolateral to the apical surface. In the pIgR, the mutant with only the 17-mer basolateral sorting sequence is basolateral and not detectably endocytosed, while the mutant with an in-frame deletion of this signal is apical and endocytosed at a near wild-type rate (12). Moreover, mutation of both Tyr residues virtually eliminates endocytosis, but does not alter basolateral sorting (C. Okamoto and K. Mostov, unpublished data).

How can we reconcile these observations? One possibility is that the Tyr internalization signal is closely related structurally to one type of basolateral sorting signal. These might both involve β turns and some β turns might be able to serve both functions. An exposed turn is a suitable structure for recognition by other molecules. However, not all Tyr internalization signals function as basolateral sorting signals, because the mutant pIgR with a deletion of its 17-mer residue basolateral targeting signal is apically targeted and has a functional Tyr internalization signal (12). Conversely, some basolateral targeting signals (TfR, LDLR, pIgR) are not endocytotic signals. Given the wide range of sequences that work as internalization sequences, it is not surprising that some sequences form turns that serve only as one signal or the other or both. TfR and LDLR might have redundant basolateral sorting signals, one identical to the endocytotic signal and the other acting as a backup. Finally, in cases where the two types of signals appear to be identical (LGP120, NGFR, HA), we may have simply not yet found mutations that separate the two functions. For example, several mutations (including Tyr to Ala) in lysosomal acid phosphatase eliminated both endocytosis and basolateral targeting. However, a recently made Tyr to Phe mutation blocked endocytosis but not basolateral sorting (C. Peters and K. von Figura, personal communication).

The numerous examples of the involvement of the cytoplasmic domain in basolateral sorting are most simply explained by a model in which this step is signal mediated. However, one could imagine that all these proteins have cryptic apical signals and that the cytoplasmic domain (or a protein that binds to it) acts by preventing entry into apical vesicles. Proteins would then enter the supposed default pathway to the basolateral surface. It has been suggested (28) that this mechanism might particularly occur in the pIgR, which is specialized for transcytosis and is targeted first to the basolateral and then to the apical surface. However, the ability of the pIgR to appear sequentially on both surfaces of the cell is not unusual. In fact, many of the proteins considered above as basolateral are in some circumstances found at the apical surface or are transcytosed. The FcR II transcytoses IgG from apical to basolateral in MDCK cells and possibly in placenta (29, 59). The LGP120 can undergo basolateral to apical transcytosis in MDCK cells (28). The LDLR is found on the apical surface of kidney tubules in transgenic mice (47) and may be on the apical surface of MDCK cells (35). The TfR is on both surfaces of the placental-derived BeWo cell line (14). Both the M6PR (a rapidly endocytosed basolateral protein in MDCK cells (49)) as well as a 100-kD lysosomal membrane protein are on the apical surface of osteoclasts (2). Whatever mechanism is responsible for basolateral sorting must be flexible enough to direct these proteins to the apical surface when appropriate.

The glycolipid clustering mechanism mentioned above may also play a role in sorting lipids, GPI-anchored proteins, and perhaps other proteins (such as the detergent-insoluble HA) to the apical surface (57). Perhaps all of the apical forms of the proteins listed in Table I cluster with glycolipids and the normal function of their cytoplasmic domains is to prevent this. We have so far not observed any detergent insolubility of the apically targeted mutant pIgR's. It would be worthwhile to check this for other apical mutants. The glycolipid clustering model probably cannot account for all apical sorting, as in several cell lines some GPI-anchored proteins are basolateral (37) (C. Zurzolo, L. Nitsch, E. Rodriguez-Boulan, and M. Lisanti. 1990. *J. Cell Biol.* 111: 327). We think it more likely that as a minimum, cells have both a basolateral sorting mechanism and a glycolipid mechanism for apical sorting.

Only one signal has been identified for transcytosis. Phosphorylation of a particular Ser in the cytoplasmic domain of the pIgR signals transcytosis (13). Replacement of the Ser with a non-phosphorylatable Ala prevents transcytosis, while mutation to an Asp (whose negative charge mimics phosphate) stimulates transcytosis. The negative charge does not simply inactivate the basolateral sorting signal, as the Asp mutant is basolaterally sorted before transcytosis (12). The effects of the charge on transcytosis are also seen if the Ala and Asp-containing mutant cytoplasmic domains are fused with a heterologous protein. This suggests that this signal for transcytosis acts in the cytoplasmic domain, and not via an effect on the extracellular domain (G. Apodaca and K. Mostov, unpublished data).

The connection between endocytotic and basolateral sorting signals has led to the suggestion that proteins travel from the TGN first to endosomes and from there to the basolateral surface (27, 28). Brefeldin A causes the TGN and early endosomes to fuse into a tubular network in several cell types (36, 68), although this has not been shown in MDCK cells (30). This result suggests that normally there is a significant connection between the TGN and endosomes. One indirect argument against this model is that the Asp-mutant pIgR (and other efficiently transcytosed proteins) would traverse the same endosomal compartment first on the way to the basolateral surface, and again on the way to the apical surface. As this mutant is not phosphorylated, the endosome would not have an obvious way to efficiently sort the apparently identical molecules to two different destinations.

Sorting Machinery

There is little data on the cellular proteins that recognize sorting signals and direct proteins to the correct surface, although understanding this machinery is probably the best way to settle the argument about default pathways. The possible relationship of endocytotic and basolateral sorting signals suggests a possible involvement of clathrin and the adaptor family of proteins (28). In plasma membrane coated pits the Tyr internalization signals in receptors are believed to interact with the HA-2/AP-2 adaptor complex (48). Clathrin-coated regions of the TGN contain only the HA-1/AP-1 adaptor, which does not bind to the Tyr internalization signal on endocytosed receptors. TGN clathrin-coated vesicles function in delivery of proteins to lysosomes and formation of regulated secretory granules (31, 48). The basolaterally sorted VSVG is not seen in clathrin coated regions of the TGN (24). Also the ASGPR (a basolateral protein in MDCK cells) (65) is excluded from HA1/AP-1-coated regions of the TGN in HepG2 cells (H. Geuze, personal communication). These observations make it unlikely that clathrin in the TGN is involved in basolateral sorting.

Many transport steps use non-clathrin-coated vesicles.

The coat proteins of intra-Golgi cisternae vesicles have been isolated and one component, β-COP, is homologous to the 100-116 kD family of proteins that are the major components of the HA1 and HA2 adaptors (20). There is thus a family of adaptor-like molecules used in both clathrin- and non-clathrin–coated vesicles. It is not known if the β-COP protein recognizes sorting signals or is just involved in forming vesicles. A 108 kD protein has been found in transcytotic vesicles and may also be a member of this family (30, 60). Perhaps TGN-derived vesicles going to the basolateral and/or apical surfaces have their own adaptor-like molecule(s). These adaptors may recognize the signals for basolateral (and apical?) sorting. The known Golgi-associated adaptors (HA-1/AP-1 and β-COP) are rapidly dissociated from the membrane by brefeldin A (20) (F. Brodsky, personal communication). In MDCK cells brefeldin A blocks transport to the apical surface from both the TGN and the basolateral surface, suggesting that adaptors are involved in these processes (30, 41).

Permeabilized cells and cell-free systems have been used to reconstitute budding of vesicles from the TGN (54, 64), and budding of transcytotic vesicle from early endosomes (M. Bomsel and K. Mostov, unpublished results). Delivery to the apical surface has been reconstituted in cells permeabilized with streptolysin-O (23). This approach should allow biochemical dissection of the steps involved in sorting and transport. A complementary approach is to isolate transport vesicles, either by fractionation of whole cells (60) or after production in a reconstituted system, and analyze their composition. HA-containing apical vesicles and VSVG containing basolateral vesicles produced from permeabilized MDCK cells have distinct but overlapping compositions (64).

Several studies have shown that microtubules play a role in transporting both TGN-derived and transcytotic vesicles to the apical, but not the basolateral surface (4). Disruption of microtubules by nocodazole or colchicine reduces the rate of apical delivery, but has less effect on the ultimate extent of apical delivery. In epithelial cells microtubules are largely arrayed with their plus ends near the basolateral surface and their minus ends near the apical surface. A minus end–directed motor is probably involved although this has not been demonstrated.

As MDCK cells acquire polarity after switching from low Ca to normal Ca media, basolateral markers are sent from the TGN to both surfaces (D. Wollner and J. Nelson, personal communication). One interpretation is that the machinery to form basolateral vesicles in the TGN develops slowly after polarity is induced. Another possibility is instead that the correctly polarized acceptor or docking sites develop only slowly on the basolateral plasma membrane (52). As they become polarized, MDCK cells gradually acquire a submembranous cytoskeleton containing fodrin linked to the basolateral membrane. Perhaps this must be fully assembled in order for TGN-derived vesicles meant for the basolateral surface to be correctly targeted. Indeed, one can further speculate that in a non-polarized cell (e.g., fibroblast) the TGN segregates potentially apical and basolateral proteins into separate vesicles, but because the plasma membrane is not divided into appropriate domains both classes of vesicles simply fuse with the undifferentiated surface. In such cells early endosomes might also segregate potentially transcytosed proteins (e.g., pIgR) from recycling ones (e.g., TfR) but then both vesicle populations fuse with the surface.

Small GTP-binding proteins of the ras superfamily control a wide variety of membrane transport steps. In mammalian cells there are a large number of ras related proteins known as rab proteins that are each localized to a specific membrane compartment (22). This has led to the suggestion that rab proteins identify individual compartments and insure that vesicular traffic is properly directed from one compartment to the next. It is therefore surprising that no epithelial-specific rab proteins have so far been reported (15) despite an extensive search. The absence of such proteins would be consistent with the notion that the TGN in non-polarized cells segregates apical and basolateral proteins. Furthermore, we have recently found the segregation of the pIgR into transcytotic vesicles is apparently mediated by the heterotrimeric G_s protein (M. Bomsel and K. Mostov, unpublished). Although we originally expected that this process would use an epithelial-specific GTP–binding protein, this may not be the case.

Other Cell Types

In neurons the axon may correspond to the apical surface, while the cell body and dendrites correspond to the basolateral surface (18). The apical proteins, HA, and GPI-anchored Thy-1, are found only in axons, while the basolateral VSVG is found only in the cell body and dendrites. This raises the exciting possibility that the principles involved in epithelial polarity may be used in a wide variety of other cell types.

The highly polarized budding yeast *S. cerevisiae* offers the best opportunity to analyze polarity genetically (19). During budding all traffic to the surface is directed to the bud. In response to mating pheromones they produce an elongate projection. Choosing a bud site and directing membrane traffic into the bud requires a number of genes, including GTPases and a GDP–GTP exchange protein. Combining results and insights from yeast and mammalian systems has been an extremely powerful approach in analyzing membrane traffic and other aspects of cell biology. We hope to use this strategy to achieve a deeper understanding of cell polarity.

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