Polarized epithelial cells separate an organism’s internal milieu from its external environment. To perform this barrier function, these cells possess an asymmetric design, with an apical membrane facing an “outside” lumen and a basolateral membrane facing neighboring cells and the basal lamina. These two distinct membrane domains are separated by intercellular junctional complexes, called tight junctions, which render the epithelial cell monolayer selectively permeable to solutes and fluid. A transporting epithelial tissue’s functional properties are determined in large measure by the inventories of ion channels, transporters, and pumps that are differentially localized to their apical and basolateral membranes and that account for the tissue’s capacity to mediate the net secretion or absorption of fluid and solutes. Differential sorting and directed targeting of membrane proteins to specific membrane domains in epithelial cells are thus necessary for the generation and maintenance of the biochemical polarity that is a prerequisite for their physiologic function.

ABSTRACT
Renal epithelial cells must maintain distinct protein compositions in their apical and basolateral membranes in order to perform their transport functions. The creation of these polarized protein distributions depends on sorting signals that designate the trafficking route and site of ultimate functional residence for each protein. Segregation of newly synthesized apical and basolateral proteins into distinct carrier vesicles can occur at the trans-Golgi network, recycling endosomes, or a growing assortment of stations along the cellular trafficking pathway. The nature of the specific sorting signal and the mechanism through which it is interpreted can influence the route a protein takes through the cell. Cell type–specific variations in the targeting motifs of a protein, as are evident for Na,K-ATPase, demonstrate a remarkable capacity to adapt sorting pathways to different developmental states or physiologic requirements. This review summarizes our current understanding of apical and basolateral trafficking routes in polarized epithelial cells.

Polarized epithelial cells are thus selectively permeable to solutes and render the epithelial cell monolayer selectively permeable to solutes and render the epithelial cell monolayer selective.

Polarized Epithelial Cells

ABSTRACT
Renal epithelial cells must maintain distinct protein compositions in their apical and basolateral membranes in order to perform their transport functions. The creation of these polarized protein distributions depends on sorting signals that designate the trafficking route and site of ultimate functional residence for each protein. Segregation of newly synthesized apical and basolateral proteins into distinct carrier vesicles can occur at the trans-Golgi network, recycling endosomes, or a growing assortment of stations along the cellular trafficking pathway. The nature of the specific sorting signal and the mechanism through which it is interpreted can influence the route a protein takes through the cell. Cell type–specific variations in the targeting motifs of a protein, as are evident for Na,K-ATPase, demonstrate a remarkable capacity to adapt sorting pathways to different developmental states or physiologic requirements. This review summarizes our current understanding of apical and basolateral trafficking routes in polarized epithelial cells.


Polarized epithelial cells separate an organism’s internal milieu from its external environment. To perform this barrier function, these cells possess an asymmetric design, with an apical membrane facing an “outside” lumen and a basolateral membrane facing neighboring cells and the basal lamina. These two distinct membrane domains are separated by intercellular junctional complexes, called tight junctions, which render the epithelial cell monolayer selectively permeable to solutes and fluid. A transporting epithelial tissue’s functional properties are determined in large measure by the inventories of ion channels, transporters, and pumps that are differentially localized to their apical and basolateral membranes and that account for the tissue’s capacity to mediate the net secretion or absorption of fluid and solutes. Differential sorting and directed targeting of membrane proteins to specific membrane domains in epithelial cells are thus necessary for the generation and maintenance of the biochemical polarity that is a prerequisite for their physiologic function.

ABSTRACT
Renal epithelial cells must maintain distinct protein compositions in their apical and basolateral membranes in order to perform their transport functions. The creation of these polarized protein distributions depends on sorting signals that designate the trafficking route and site of ultimate functional residence for each protein. Segregation of newly synthesized apical and basolateral proteins into distinct carrier vesicles can occur at the trans-Golgi network, recycling endosomes, or a growing assortment of stations along the cellular trafficking pathway. The nature of the specific sorting signal and the mechanism through which it is interpreted can influence the route a protein takes through the cell. Cell type–specific variations in the targeting motifs of a protein, as are evident for Na,K-ATPase, demonstrate a remarkable capacity to adapt sorting pathways to different developmental states or physiologic requirements. This review summarizes our current understanding of apical and basolateral trafficking routes in polarized epithelial cells.

Polarized epithelial cells separate an organism’s internal milieu from its external environment. To perform this barrier function, these cells possess an asymmetric design, with an apical membrane facing an “outside” lumen and a basolateral membrane facing neighboring cells and the basal lamina. These two distinct membrane domains are separated by intercellular junctional complexes, called tight junctions, which render the epithelial cell monolayer selectively permeable to solutes and fluid. A transporting epithelial tissue’s functional properties are determined in large measure by the inventories of ion channels, transporters, and pumps that are differentially localized to their apical and basolateral membranes and that account for the tissue’s capacity to mediate the net secretion or absorption of fluid and solutes. Differential sorting and directed targeting of membrane proteins to specific membrane domains in epithelial cells are thus necessary for the generation and maintenance of the biochemical polarity that is a prerequisite for their physiologic function.

ABSTRACT
Renal epithelial cells must maintain distinct protein compositions in their apical and basolateral membranes in order to perform their transport functions. The creation of these polarized protein distributions depends on sorting signals that designate the trafficking route and site of ultimate functional residence for each protein. Segregation of newly synthesized apical and basolateral proteins into distinct carrier vesicles can occur at the trans-Golgi network, recycling endosomes, or a growing assortment of stations along the cellular trafficking pathway. The nature of the specific sorting signal and the mechanism through which it is interpreted can influence the route a protein takes through the cell. Cell type–specific variations in the targeting motifs of a protein, as are evident for Na,K-ATPase, demonstrate a remarkable capacity to adapt sorting pathways to different developmental states or physiologic requirements. This review summarizes our current understanding of apical and basolateral trafficking routes in polarized epithelial cells.

Polarized epithelial cells separate an organism’s internal milieu from its external environment. To perform this barrier function, these cells possess an asymmetric design, with an apical membrane facing an “outside” lumen and a basolateral membrane facing neighboring cells and the basal lamina. These two distinct membrane domains are separated by intercellular junctional complexes, called tight junctions, which render the epithelial cell monolayer selectively permeable to solutes and fluid. A transporting epithelial tissue’s functional properties are determined in large measure by the inventories of ion channels, transporters, and pumps that are differentially localized to their apical and basolateral membranes and that account for the tissue’s capacity to mediate the net secretion or absorption of fluid and solutes. Differential sorting and directed targeting of membrane proteins to specific membrane domains in epithelial cells are thus necessary for the generation and maintenance of the biochemical polarity that is a prerequisite for their physiologic function.

ABSTRACT
Renal epithelial cells must maintain distinct protein compositions in their apical and basolateral membranes in order to perform their transport functions. The creation of these polarized protein distributions depends on sorting signals that designate the trafficking route and site of ultimate functional residence for each protein. Segregation of newly synthesized apical and basolateral proteins into distinct carrier vesicles can occur at the trans-Golgi network, recycling endosomes, or a growing assortment of stations along the cellular trafficking pathway. The nature of the specific sorting signal and the mechanism through which it is interpreted can influence the route a protein takes through the cell. Cell type–specific variations in the targeting motifs of a protein, as are evident for Na,K-ATPase, demonstrate a remarkable capacity to adapt sorting pathways to different developmental states or physiologic requirements. This review summarizes our current understanding of apical and basolateral trafficking routes in polarized epithelial cells.
proteins is directed primarily by signals embedded within the sorted protein’s primary structure (Table 1). The most common types of signals involved in the sorting of basolateral membrane proteins are tyrosine-based (NPxY or YxxØ) or dileucine (D/ExxxLL) motifs.8–12 These sequences are embedded within these proteins’ cytosol-facing domains and are similar in composition and geometry to the signals that drive endocytosis. In fact, basolateral signals can overlap with or serve as endocytosis signals, although this is not always the case.15,17,18 Additionally, basolateral sorting motifs involving a single leucine residue have been identified in several proteins, including stem cell factor,19 CD147,20 and amphiregulin (EExxxL).21 Protein components of the cellular trafficking machinery, including subunits of the clathrin adaptor complexes, mediate the interpretation of both tyrosine-based and dileucine targeting sequences.22–24 The adaptor protein (AP) complexes function as cargo receptors in the formation of clathrin-coated pits and vesicles.25–31 Tyrosine-based basolateral signals interact with μ1B, an epithelial-specific isoform of the μ1 subunit of the heterotetrameric AP complex.32–34 Less is known about the nature of signals that drive the targeting of proteins with multiple transmembrane domains, although some of these proteins are known to dimerize with single-pass membrane proteins that convey sorting information.35,36

### Table 1. Signals and mechanisms for sorting to the apical and basolateral membranes

<table>
<thead>
<tr>
<th>Sorting Signal</th>
<th>Protein</th>
<th>Presumed Sorting Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basolateral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine-based</td>
<td>Low-density lipoprotein receptor</td>
<td>Recycling, but not delivery, μ1b-dependent</td>
<td>10, 152</td>
</tr>
<tr>
<td></td>
<td>Vesicular stomatitis virus G protein</td>
<td>μ1b-dependent</td>
<td>153, 154</td>
</tr>
<tr>
<td></td>
<td>Igp120</td>
<td>μ3a-dependent</td>
<td>9, 155</td>
</tr>
<tr>
<td>Dileucine</td>
<td>Fc receptor FcRII-B2</td>
<td>μ1b-independent</td>
<td>11, 15, 156</td>
</tr>
<tr>
<td></td>
<td>Mannose 6-phosphate receptor</td>
<td>μ1b-independent</td>
<td>157, 158</td>
</tr>
<tr>
<td></td>
<td>E-cadherin</td>
<td>Rab11-mediated</td>
<td>3, 12</td>
</tr>
<tr>
<td>Monoleucine</td>
<td>Stem cell factor</td>
<td>Clathrin-mediated; μ1b-dependent</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>CD147</td>
<td>Recycling, but not delivery, μ1b-dependent</td>
<td>21</td>
</tr>
<tr>
<td><strong>Apical</strong></td>
<td>GPI-anchor</td>
<td>Lipid raft-associated</td>
<td>40, 41</td>
</tr>
<tr>
<td></td>
<td>Folate receptor</td>
<td>Lipid raft-associated</td>
<td>43</td>
</tr>
<tr>
<td>N-Glycans</td>
<td>Clusterin (gp80)</td>
<td>Raft-independent</td>
<td>46, 159</td>
</tr>
<tr>
<td></td>
<td>gp114</td>
<td>Galectin-3-mediated, raft-independent</td>
<td>47, 61</td>
</tr>
<tr>
<td></td>
<td>Growth hormone</td>
<td>Independent of galectins 3 and 4</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Erythropoietin</td>
<td>Cholesterol-dependent</td>
<td>49, 160</td>
</tr>
<tr>
<td></td>
<td>Endolyn</td>
<td>Raft-independent</td>
<td>50</td>
</tr>
<tr>
<td>O-Glycans</td>
<td>p75 neurotrophin receptor</td>
<td>Galectin-3-mediated, raft-independent</td>
<td>53, 61</td>
</tr>
<tr>
<td></td>
<td>Lactase phlorizin hydrolase</td>
<td>Galectin-3-mediated, raft-independent</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>MUC1</td>
<td>Raft-independent</td>
<td>99, 161, 162</td>
</tr>
<tr>
<td></td>
<td>Podocalyxin</td>
<td>Transient lipid raft association</td>
<td>52</td>
</tr>
<tr>
<td>Transmembrane domain</td>
<td>Dipeptidyl peptidase IV</td>
<td>Lipid raft-associated</td>
<td>55, 58</td>
</tr>
<tr>
<td></td>
<td>Sucrase isomaltase</td>
<td>Lipid raft-associated</td>
<td>55, 56</td>
</tr>
<tr>
<td></td>
<td>Neuraminidase</td>
<td>Lipid raft-associated</td>
<td>67, 70</td>
</tr>
<tr>
<td></td>
<td>Influenza hemagglutinin virus F protein</td>
<td>Lipid raft-associated</td>
<td>69, 163</td>
</tr>
<tr>
<td></td>
<td>Respiratory syncytial virus</td>
<td>Lipid raft-associated</td>
<td>71, 164</td>
</tr>
<tr>
<td></td>
<td>Sucrase isomaltase</td>
<td>Lipid raft-associated</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>H,K-ATPase</td>
<td>Raft-independent</td>
<td>72</td>
</tr>
</tbody>
</table>

**APICAL SORTING SIGNALS**

Apical signals are more diverse than their basolateral counterparts. Topologically, apical signals can reside in any domain of an apically sorted protein’s structure—extracellular, transmembrane, or cytosolic. Compositionally, apical signals can be based on amino acids, lipids, or carbohydrates. The apical membrane is divided into the ciliary region (which includes the periciliary base and the primary cilium itself) and the noncilary membrane, and these domains are compositionally distinct from one another. This division adds further complexity to the process of apical sorting.37

The recognition that multiple glycosylphosphatidylinositol-anchored proteins (GPI-AP) are localized to the apical membranes of MDCK cells provided a first clue to the existence and nature of apical sorting signals.38 The sorting behaviors of chimeric proteins suggest that the GPI anchor is sufficient to specify sorting to the apical membrane.39,40 However, other reports indicate that the anchor is necessary to ensure apical sorting of GPI-APs, but is not in itself sufficient.41 The finding that GPI-APs oligomerize and partition into detergent-resistant membranes while trafficking to the apical membrane is consistent with the hypothesis that lipid rafts play critical roles in apical protein sorting.42,43 Affinity of GPI-APs for glycosphingolipid-enriched rafts is thought to cause their preferential
incorporation into vesicles bound for the apical membrane.

Both N- and O-linked glycosylation have also been shown to serve as sorting signals for many apical proteins. The potential for N-glycans to act as apical sorting signals was first recognized for the glycoprotein gp80, which is missorted after treatment with N-glycosylation inhibitors. N-Glycans have since been shown to fulfill this function for many proteins in studies using glycosylation-deficient cell lines or mutagenesis. Insertion of a site for addition of N-linked glycans to the sequence of nonglycosylated rat growth hormone resulted in this glycans to the sequence of nonglycosylated rat growth hormone. The potential for O-linked glycans to act as structural supports that enhance protein folding or inhibit aggregation, thus allowing protein sequence-based signals to interact with protein receptors or lipid domains.

Transmembrane domain sequences are responsible for the apical sorting of multiple viral single-pass membrane proteins, including hemagglutinin, neuraminidase, and the respiratory syncytial virus F protein. A transmembrane domain also appears to play a key role in apical sorting of the polytopic gastric H,K-ATPase protein. The α-subunits of the H,K-ATPase and Na,K-ATPase pumps share approximately 65% sequence identity, permitting the construction of structurally intact and functionally active chimeric pump proteins. A chimera in which the fourth transmembrane domain of the H,K-ATPase α-subunit was inserted into the complementary position of the basolateral Na,K-ATPase α-subunit was targeted to the apical membrane in transfected epithelial cells.

As is the case for GPI-APs, all major classes of apical sorting signals have been implicated in inducing clustering of their associated proteins into detergent-resistant membrane domains. The role of clustering in driving biosynthetic apical sorting, however, is murky at best. Mutational analysis of the influenza virus hemagglutinin (HA) protein transmembrane domain showed that this sequence is required for the HA protein’s raft association, but not for its apical sorting. Furthermore, GPI-APs lose raft association when cells are subjected to mild levels of cholesterol depletion, but are still sorted to the apical membrane. Secreted forms of GPI-APs lacking the GPI linkage are targeted apically despite having lost both their raft association and the apical influence previously attributed to GPI. A 2005 review of epithelial trafficking suggested the novel unifying hypothesis that apical signals function as oligomerization-promoting factors. Support for this conjecture is supplied by data showing that a GPI-linked green fluorescent protein (GFP) construct was sorted to the apical membrane unless the GFP contained mutations known to abolish its tendency to oligomerize.

The variable outcomes of seemingly straightforward experiments designed to test the activities of specific types of apical signals complicate the establishment of the role of any particular signal or class of signals in apical sorting. Some of this complexity may be attributable to the existence of redundant or multipart signals. One example of such a bipartite apical signal is found in podocalyxin. Individual removal of a heavily glycosylated region in the ectodomain or of a cytosolic region containing a C-terminal postepithelial density 95/disc-large/zona occludens domain interaction motif produced only minimal effects on the protein's steady state localization. In contrast, removing both of these apical signal domains resulted in podocalyxin protein that exhibits an entirely nonpolarized localization. The multiplicity of studies exploring the various classes of apical signals and the roles of lipid rafts clearly demonstrate that apical targeting is not the product of a simple binary “on-or-off switch” process. Further studies will be required to explore the individual functions of specific signal types, as well as the roles for oligomerization and for hierarchical and cooperative interplay in determining the mechanisms through which complex signals are interpreted.

**SORTING SITE FOR BIOSYNTHETIC CARGO**

To understand the mechanisms mediating trafficking in polarized epithelial cells, it is necessary to elucidate the site at which basolateral proteins and apical proteins are separated from one another as they pursue their postsynthetic trafficking itineraries. Several studies address this issue using biochemical techniques or microscopy-based assays. By assessing the sialic acid content of the sugars attached to the basolaterally directed vesicular stomatitis virus glycoprotein (VSV-G), Fuller et al. demonstrated that this protein remains in physical contact with the apically directed influenza neuraminidase (whose enzymatic activity is capable of desialating VSV-G) through the late Golgi.
Similarly, following infection of MDCK cells with two viruses that bud from opposite membranes—VSV-G from the basolateral membrane and HA from the apical membrane—Rindler and colleagues observed colocalization of VSV-G and HA within the same Golgi cisternae by colloidal gold immuno-electron microscopy. In contrast, work with GFP-tagged serglynin protein has suggested apical/basolateral separation as early as the cis Golgi or ER. Furthermore, studies suggest the existence of a novel class of detergent-resistant membrane domains that act as aggregators of apical proteins in the ER. Further research will be necessary to determine whether this type of early separation plays an obligate functional role in epithelial protein sorting.

One case of well established early segregation involves a specific class of apical proteins. GPI-APs have been shown to exit the ER in different vesicular carriers than other apical proteins. Most recently, the p24 family of transmembrane proteins was identified in yeast as the receptor adaptor for GPI-APs, facilitating their incorporation into coat protein complex II-coated vesicles in the ER and regulating coat protein complex I-mediated retrograde transport of escaped, unremodeled GPI-APs. Because GPI-AP signals are chemically distinct from other sorting signals, which tend to be short amino acid sequences or post-translational modifications, it is perhaps not surprising that their sorting would be mechanistically and temporally distinct.

Despite these examples of early segregation, most sorting appears to occur at some point after proteins have passed through the late Golgi and reached the TGN. Evidence in favor of this argument comes from live imaging studies, which have revealed the separation of proteins into different vesicles upon or directly after their export from the TGN. In these experiments, progressive lateral separation of representative apical and basolateral proteins, concurrent with the exclusion of resident Golgi proteins from these segregated domains, culminated in the proteins exiting the Golgi in distinct carriers.

In addition to separation of apical and basolateral cargo, distinct sorting of some proteins bound for the same destination has also been observed within the TGN. Basolateral proteins are often classified according to the dependence of their sorting on the α1B clathrin adaptor subunit. Two basolateral proteins—the α1B-dependent VSV-G protein and α1B-independent Na,K-ATPase—exit the TGN in separate carriers, demonstrating that the role for the TGN in epithelial sorting extends beyond the strict apical/basolateral dichotomy. μ1B is expressed in a subset of epithelial tissues and, as part of the AP1 clathrin coat adaptor complex, facilitates sorting of proteins that use tyrosine-based sorting signals (such as VSV-G) through the Rab8-positive common recycling endosome (CRE). As a complement to this pathway, the TGN-localized μ1A isoform was recently shown to facilitate TGN export of a GFP-tagged LDL-R construct, which contains a nonconventional tyrosine-based basolateral signal and does not typically traverse the endosomal system.

A nonconventional route for apical transit that bypasses the Golgi apparatus has also been proposed for a subset of apical proteins. A GFP-tagged version of the serglycin protein was shown to traffic to the apical membrane in the presence of the Golgi transport-blocking drug brefeldin. Apical delivery was also observed when a KDEL ER retrieval sequence was appended to serglycin to prevent the protein’s progress along the secretory pathway from the ER to the Golgi. Recently, analysis of the glycosylation patterns of newly synthesized polycystin-2 suggested that the cilary pool of this protein exits the Golgi at the cis compartment, rather than from the TGN. Additional studies focusing on newly synthesized cohorts of native proteins expressed at wild-type levels in untreated cells will be necessary to establish whether there is indeed a Golgi bypass route that functions as a bona fide apical transport pathway.

Sorting can also occur after proteins exit the TGN. Lipid raft–dependent and –independent classes of apical cargo segregate from one another by vesicle fusion following TGN exit. Furthermore, as discussed below, a substantial number of post-Golgi protein sorting processes occur in the endosomal system. Finally, some proteins are sorted via a transcellular route. Although few proteins appear to pursue such pathways in renal epithelial cells, many apical proteins in hepatic cells are sent first to the basolateral membrane, followed by endocytosis and transport to the apical/canalicular membrane.

**SORTING THROUGH ENDOSONES**

The endosomal system is an essential component of a cell’s trafficking network—both endocytic and exocytic—for membrane proteins, lipids, receptor-bound ligands, and various solutes (Figure 1). In polarized cells, proteins and lipids endocytosed from the apical and basolateral surface enter the apical (AEE) and basolateral early endosomes, respectively. From there, proteins can be sorted to the surface, targeted to the lysosomes for ultimate degradation, or transported to the CRE or apical recycling endosome (ARE). The ARE is a cup-shaped compartment in the subapical region of epithelial cells characterized by the presence of the small GTPase Rab11a and the motor protein Myosin Vb (MyoVb), and the absence of rapidly recycling transferrin receptor.

Multiple biosynthetic pathways passing through the endosomal system en route to the apical membrane have been observed. An elegant demonstration of trafficking of newly synthesized raft-dependent and raft-independent proteins through separate endosomal compartments was obtained by functionally inactivating the apical early and apical recycling endosomes. Overexpression of the dominant-negative acting MyoVb-tail domain selectively inhibited apical trafficking of the raft-independent apical protein endolyn, while horseradish peroxidase–mediated ablation of the AEE selectively inhibited apical trafficking of the raft-dependent influenza HA
Proteins with glycan-dependent signals are thought to traverse the ARE. Glycosylated growth hormone, which relies on N-glycans for its sorting, colocalizes with ARE markers en route to the apical surface, and its apical expression is decreased by overexpression of MyoVb-tail. In contrast, nonglycosylated GH did not localize to the ARE. In the case of the disaccharidases lactase phlorizin hydrolase and sucrase isomaltase—a raft-dependent protein and raft-independent protein, respectively—separation does not occur immediately after release from the TGN, but rather after sequential movement through Rab4-, Rab8-, and Rab11-positive endosomal compartments (or perhaps subcompartments).

Mounting evidence demonstrates that, in addition to being separated into different endosomal compartments, cargoes are segregated into different subdomains within endosomes and that this lateral segregation facilitates cargo sorting (reviewed by Miczynska and Zerial). Using immuno-electron microscopy, Sonnichsen et al. demonstrated separation of resident Rab GTPases into distinct endosomal subdomains. Recycling endosomes exhibited Rab4- and Rab11-positive subdomains, and early endosomes possessed distinct Rab4 and Rab5 subdomains. Within early endosomes, recycling cargo segregates to tubular domains, while cargo headed for degradation remains in the spherical portion of the endosome. More recently, enrichment of recycling β2-adrenergic receptor was observed in tubular microdomains of endosomes that are stabilized by a local actin network. This localization depended on sorting information embedded within the β2-adrenergic receptor sequence, and it took place in different tubules and with different kinetics than did the bulk sorting of the rapidly recycling transferrin receptor. In polarized cells, apical and basolateral cargo segregated laterally in recycling endosomes in a phosphoinositide 3-kinase-dependent manner. This segregation was not observed when the same proteins were studied in nonpolarized cells. To fully establish the extent to which lateral segregation is operative.

**Figure 1.** Apical and basolateral proteins pursue multiple biosynthetic routes to the plasma membrane following their exit from the TGN. This model depicts these routes as well as some endocytic pathways. Proteins targeted to the basolateral membrane can traffic directly from the TGN (1), or traffic through endosomes (2) before surface delivery (3). A similar direct route for apical proteins has not been observed. Endocytosed basolateral proteins traffic through the basolateral early endosome (BEE, 3) and CRE before recycling or degradation. Apically directed proteins traverse the apical early endosome (AEE, 4a and b) or the apical recycling endosome (ARE, 5a and b) before apical delivery. GPI-linked proteins, influenza HA protein, and other raft-dependent proteins traverse the AEE, while proteins such as endolyn and MUC1, whose trafficking depends on glycan residues, traffic through the ARE. Conversely, lactase phlorizin hydrolase and sucrase isomaltase traffic between the apical early and apical recycling endosomes (6). Proteins internalized from the apical membrane to the AEE can traffic to the ARE for recycling back to the surface, or to the CRE and late endosomes if they are targeted for degradation (unlabeled arrows). LE, late endosome; lys, lysosome; MVB, multivesicular body.
and functionally important in sorting, it will be necessary to couple higher-resolution imaging of newly synthesized proteins with innovative assays capable of revealing and perturbing domain separations within endosomes, the Golgi network, and earlier trafficking compartments.

CELL-SPECIFIC VARIATIONS IN TRAFFICKING

Even when expressed in nonpolarized cells, apical and basolateral proteins can be separated into different cargo vesicles.86,106,107 This suggests that the machinery required to accomplish polarized sorting is expressed ubiquitously. However, polarized sorting can manifest highly cell type–specific patterns. The multiplicity of signal classes that are involved in apical and basolateral sorting, as well as the complex array of vesicular compartments that participate in the segregation of apical and basolateral proteins bearing these signals, allows for a plastic system in which distinct subsets of proteins can be redirected in response to environmental cues. Several proteins show cell-specific variations in trafficking.108–112 To demonstrate the remarkable capacity of the protein trafficking system to adapt to the physiologic requirements of particular cell states and tissues, we will focus on cell types in which the Na,K-ATPase complex exhibits an apical localization. As previously mentioned, the Na,K-ATPase complex localizes to the basolateral membrane of most polarized cells, where it plays an important role in the regulation of ion transport.

Cell Type–Specific Variations in Sorting

The Na,K-ATPase localizes to the apical membranes of cells of the retinal pigment epithelium (RPE)113–116 and the choroid plexus.117–120 Although these cells exhibit an apical distribution of this canonical basolateral protein, other proteins that serve as standard apical and basolateral markers retain their characteristic distributions.

Consistent with the vital role of the sodium pump and other transport proteins in determining the direction of solute flow, these distinct distributions of the Na,K-ATPase both reflect and determine the physiologic properties of their respective cell types. In renal tubular epithelial cells, basolateral sodium pump generates the transepithelial sodium gradients that drive most of the kidney’s absorptive fluxes of solutes and water.121 In the choroid plexus, the apical pool of Na,K-ATPase produces the secretory flux that is necessary for the generation of cerebral spinal fluid.122

One possible mechanism for differential localization may relate to ankyrin-fodrin complexes, which have been demonstrated to help link the Na,K-ATPase to the cytoskeleton and thus influence its localization. A role for basolateral ankyrin-fodrin in facilitating Na,K-ATPase basolateral localization has been suggested in MDCK and renal tubule cells.123,124 Conversely, ankyrin and fodrin were both found at the apical surface of choroid plexus and RPE cells, where ankyrin directly interacts with Na,K-ATPase.125,126 Fodrin and ankyrin also localize to the lateral membrane of choroid plexus cells, however,125 suggesting that the presence of these cytoskeletal linkers is not sufficient to specify Na,K-ATPase targeting. It is more likely that this cytoskeletal attachment plays a role in retention at the membrane, rather than directly determining the pump’s initial trafficking or ultimate distribution. It will be informative to more closely follow the biosynthetic route of native Na,K-ATPase protein in both renal and choroid plexus epithelial cells to parse out the alternate roles of selective targeting and selective retention in creating the distinct localizations of Na,K-ATPase these cell types exhibit. Recent advances in the generation of an immortalized choroid plexus cell line for use in in vitro experiments may prove extremely valuable in efforts to understand Na,K-ATPase trafficking in this tissue.127

In the case of RPE cells, the absence of epithelial-specific μ1B AP expression is associated with the aberrant apical localization of the coxsackie and adenovirus receptor protein.128 Interestingly, knockdown experiments in MDCK cells demonstrated that μ1B is required for correct recycling of coxsackie and adenovirus receptor to the basolateral membrane, but not for its initial biosynthetic delivery.128,129 Absence of μ1B cannot explain the apical localization of the Na,K-ATPase or the similarly “mis-sorted” CD147 in RPE cells, as the trafficking of these proteins is μ1B independent. While the underlying mechanism responsible for the apical accumulation of CD147 in RPE remains a mystery, apical CD147 appears to facilitate the apical accumulation of another protein, monocarboxylate transporter-1.130 In the case of the Na,K-ATPase, varying levels of the adherens junction protein E-cadherin were shown to affect the sodium pump’s apical versus basolateral localization in RPE cells. RPE cells expressing lower levels of E-cadherin exhibit more apical Na,K-ATPase than do cells possessing higher E-cadherin levels.131 The level of ankyrin and fodrin expression did not correlate with Na,K-ATPase localization in these cells.

Developmental Changes in Sorting

Studies performed on fetal kidneys revealed the presence of the Na,K-ATPase α1-subunit at the apical and lateral membranes (or distributed in a nonpolarized manner) of renal vesicles and newly formed collecting ducts in early stages of kidney organogenesis (Figure 2).132 This change in localization of active sodium pump is thought to play an important role in the formation of the lumen during tubulogenesis.133 Burrow and colleagues further showed that the timing of the postnatal switch to basolateral α1 distribution closely mirrored a switch in the expression of β-subunit isoforms. The sodium pump consists of two obligate subunits (α and β), as well as a regulatory component (γ). The catalytic α-subunit must assemble with the heavily glycosylated β-subunit in the ER in order to become catalytically active and to reach the cell surface.36,134–136 There are three α-subunit and two β-subunit isoforms; in mature tubules, the predominant heterodimer is α1β1. While β2 was
expressed in the developing kidney, \( \beta_1 \) protein was not observed, despite the presence of equivalent levels of \( \beta_1 \) and \( \beta_2 \) mRNA. \(^{133} \) \( \beta_2 \) is also expressed in RPE, \(^{137} \) in the choroid plexus, \(^{138} \) and in tissue from patients with autosomal dominant polycystic kidney disease, \(^{139} \) where apical localization of Na,K-ATPase has been observed. These data, combined with the virtual lack of endogenous \( \beta_2 \) expression in the adult kidney, suggest a role for \( \beta_2 \) in guiding the apical accumulation of the Na,K-ATPase. The mechanism through which the \( \beta_2 \)-subunit might exert these effects remains to be determined.

Epithelial cells can manifest tissue type–specific or developmental stage–dependent idiosyncrasies in their sorting behaviors. The mechanisms responsible for these behaviors remain largely uncharacterized. Clearly, however, while much of the polarized sorting machinery is present in all cell types, the regulated expression of distinct effector proteins can play a role in fine-tuning protein trafficking to a cell’s specific physiology and developmental state.

**IMPAIRED PROTEIN TRAFFICKING AND DISEASE**

Perturbations in the complicated network of subcellular trafficking pathways are implicated in many diseases. \(^{140} \) Pathogenic defects in trafficking include inverted polarity, disrupted recycling and intracellular retention, as highlighted in the following examples of renal pathology.

In Dent disease, mutations in the chloride channel CIC-5 result in redistribution of H\(^+\)-ATPase to the basolateral membrane rather than the apical membrane of proximal tubule cells. \(^{141,142} \) The CIC-5 mutation also impairs the function of the endocytic system, contributing to proteinuria. Bartter syndrome can be caused by mutations that perturb the trafficking or the distribution of the CIC-Kb channel or the renal outer medullary K\(^+\) channel. \(^{143} \) Mutations resulting in decreased internalization and downregulation of the epithelial sodium channel cause Liddle syndrome. \(^{144} \) The resulting constitutive activity of the epithelial sodium channel at the apical membrane leads to excessive sodium retention and pseudohyperaldosteronism. \(^{145} \) In the case of nephrogenic diabetes insipidus, mutations in the

---

**Figure 2.** Na,K-ATPase displays distinct polarized localization in different tissues and developmental stages. (A–C) Immunolocalization of sodium pump \( \alpha \)-subunit in murine choroid plexus (A), developing kidney (B), and adult kidney (C). Arrowheads indicate apical localization of \( \alpha \)-subunit. Arrows indicate basolateral localization of \( \alpha \)-subunit. (A) Sodium pump \( \alpha \)-subunit localizes to the apical membrane of choroid plexus cells. (B) In developing kidneys harvested from embryonic day 17 mice, sodium pump \( \alpha \)-subunit localizes to the apical membrane of the epithelial cells of the renal vesicles, which are the earliest structures in nephrogenesis. In newly developing collecting ducts, sodium pump \( \alpha \)-subunit localizes to both apical and basolateral membranes or—in more mature collecting ducts—to basolateral membranes only. (C) Sodium pump \( \alpha \)-subunit localizes to the basolateral infoldings of adult kidney tubules. (D–F) Schematic views of sodium pump localization in choroid plexus (D), the developing kidney (E), and the adult kidney (F). Sodium pump \( \alpha \)-subunit is depicted in red. Bar=10 \( \mu \)m.
gene encoding the collecting duct water channel aquaporin-2 can produce a functional but misrouted channel that fails to reach the plasma membrane.\textsuperscript{146,147} Without aquaporin-2 at the apical membrane, the water permeability of collecting tubule epithelial cells is decreased and excess water is lost in urine. Disruption of apico-basal polarity has been associated with polycystic kidney disease.\textsuperscript{148} Mislocalization of Na, K-ATPase, the epidermal growth factor receptor, polycystin-1 and other proteins has been observed, although these trafficking defects may be consequences rather than causes of polycystic kidney disease pathogenesis.\textsuperscript{149–151}

CONCLUSION

Polarized sorting of membrane proteins in epithelial cells is vital for the generation and maintenance of the cell polarity, which is in turn necessary for numerous aspects of epithelial cell function. Recent data contribute to our understanding of the vast complexity of sorting signals, regulators, and compartments that together control sorting to the apical and basolateral membranes. The ER, Golgi, and endosomal structures have all been observed to function as sorting sites along the biosynthetic pathway, with increasing evidence for cargo being separated into distinct subdomains within these compartments. Future studies will continue to uncover and further define apical and basolateral trafficking pathways and will reveal how they are altered to fit the physiology of different tissues. Given the importance of apico-basal polarity for normal kidney function, this research holds important implications for our understanding of the pathogenesis and therapy of renal disease.

ACKNOWLEDGMENTS

The authors would like to thank all of the members of the Caplan Laboratory for helpful discussions. This work is supported by National Science Foundation Graduate Research Fellowship Grant No. DGE-1122492 (E.H.S.) and National Institutes of Health grants DK17433 and DK072612 (M.J.C.).

REFERENCES

18. Thomas DC, Roth MG: The basolateral targeting signal in the cytoplasmic domain of glycoprotein G from vesicular stomatitis virus resembles a variety of intracellular targeting motifs related by primary sequence but having diverse targeting activities. J Biol Chem 269: 15732–15739, 1994
60. Mattila PE, Youker RT, Mo D, Burns JR, Cresawn KO, Hughey RP, Ihle G, Weisz OA: Multiple biosynthetic trafficking routes


74. Tall RD, Alonso MA, Roth MG: Features of influenza HA required for apical sorting differ from those required for association with DRMs or MAL. Traffic 4: 838–849, 2003

75. Lipardi C, Nitsch L, Zurzolo C: Detergent-insoluble GPI-anchored proteins are apically sorted in fischer rat thyroid cells, but interference with cholesterol or sphingolipids differentially affects detergent insolubility and apical sorting. Mol Biol Cell 11: 531–542, 2000


78. Fuller SD, Bravo R, Simons K: An enzymatic assay reveals that proteins destined for the apical or basolateral domains of an epithelial cell line share the same late Golgi compartments. EMBO J 4: 297–307, 1985


81. Vuong TT, Prydz K, Tveit H: Differences in the apical and basolateral pathways for glycosaminoglycan biosynthesis in Madin-Darby canine kidney cells. Glycobiology 16: 326–332, 2004


proteins by actin-stabilized endosomal microdomains. Cell 143: 761–773, 2010


