Polarized biosynthetic traffic in renal epithelial cells: sorting, sorting, everywhere

Mark A. Ellis, Beth A. Potter, Kerry O. Cresawn and Ora A. Weisz

Am J Physiol Renal Physiol 291:707-713, 2006. First published Jun 20, 2006;
doi:10.1152/ajprenal.00161.2006

Am J Physiol Renal Physiol 291: F707–F713, 2006.
First published June 20, 2006; doi:10.1152/ajprenal.00161.2006.

Invited Review

Polarized biosynthetic traffic in renal epithelial cells: sorting, sorting, everywhere

Mark A. Ellis, Beth A. Potter, Kerry O. Cresawn, and Ora A. Weisz

Laboratory of Epithelial Cell Biology, Renal-Electrolyte Division,
University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Ellis, Mark A., Beth A. Potter, Kerry O. Cresawn, and Ora A. Weisz. Polarized biosynthetic traffic in renal epithelial cells: sorting, sorting, everywhere. *Am J Physiol Renal Physiol* 291: F707–F713, 2006. First published June 20, 2006; doi:10.1152/ajprenal.00161.2006.—The maintenance of apical and basolateral membrane domains with distinct protein and lipid compositions is necessary for the proper function of polarized epithelial cells. Delivery of cargo to the basolateral surface is thought to be mediated by the interaction of cytoplasmically disposed sorting signals with sorting receptors, whereas apically destined cargoes are sorted via mechanisms dependent on cytoplasmic, glycan-mediated, or lipid-interacting sorting signals. Apical and basolateral cargo are delivered to the surface in discrete tubular and vesicular carriers that bud from the *trans*-Golgi network (TGN). While it has long been thought that the TGN is the primary compartment in which apical and basolateral cargoes are segregated, recent studies suggest that sorting may begin earlier along the biosynthetic pathway. Moreover, rather than being delivered directly from the TGN to the cell surface, at least a subset of biosynthetic cargo appears to transit recycling endosomes en route to the plasma membrane. The implications and limitations of these challenges to the conventional model for how proteins are sorted and trafficked along the biosynthetic pathway are discussed.

trans-Golgi network; Madin-Darby canine kidney; membrane traffic; apical; basolateral

THE IDENTITY OF CELLULAR ORGANELLES and compartments is determined by their unique steady-state protein and lipid composition, which is maintained despite continuous addition and removal of membrane. In the case of the plasma membrane, new membrane is added on the exocytic fusion of intracellular vesicles, whereas membranes are retrieved via several internalization pathways, collectively referred to as endocytosis. In polarized epithelial cells such as renal, intestinal, or hepatic cells, the subdivision of proteins and lipids is more pronounced in that the cell surface itself is divided into distinct domains. In these cells, the apical surface faces tubular lumens that are in continuity with the external environment of the organism, whereas the basolateral surface maintains contact with adjacent cells and the substratum. The protein and lipid compositions of these surfaces are tailored to the particular functions of each domain, which include protecting against invasion by toxins and pathogens, absorption or secretion of nutrients, signaling, and ion transport. The generation and maintenance of distinct apical and basolateral domain identities are accomplished largely via the preferential targeting of newly synthesized and recycling proteins to one or the other domain (reviewed in Refs. 6 and 43). The observation that nonpolarized cells such as fibroblasts are also capable of sorting newly synthesized "apical" and "basolateral" cargo (22, 31, 57) has been taken to suggest that the biosynthetic sorting machinery is universally expressed in all cell types. The identity of targeting signals and how they are decoded by the cell to effect selective apical or basolateral delivery of a given protein have been the subjects of intensive study, but a comprehensive model for polarized protein sorting remains elusive.

Address for reprint requests and other correspondence: O. A. Weisz, 978 Scaife Hall, 3550 Terrace St., Pittsburgh, PA 15261 (e-mail: weisz@pitt.edu).

Madin-Darby canine kidney (MDCK)-derived cell lines have provided an outstanding model system in which to dissect the trafficking signals and mechanisms that direct the polarized delivery of apical and basolateral proteins (28). These cells form polarized monolayers in culture and are easily grown on permeable filter supports that allow selective access to the apical or basolateral membrane domains. Morphological and biochemical studies based on the ability to stage proteins in the *trans*-Golgi network (TGN) with a low-temperature block and subsequently image vesicular transport to the plasma membrane or biochemically resolve distinct vesicles containing apical and basolateral cargo by immunoisolation have provided us with a basic model for protein sorting along the biosynthetic pathway. In this model, newly synthesized apical and basolateral proteins traverse the Golgi complex together until they are segregated in the TGN into post-Golgi vesicles that are directly delivered to the plasma membrane.

In the past several years, this relatively simple model has been challenged by evidence of sorting in pre-TGN compartments and by the observation that biosynthetic cargo may traverse intermediate compartments en route from the TGN to the plasma membrane (Fig. 1). Moreover, recent studies implicating a role for epithelial-specific adaptor protein complexes and for endocytic compartments in biosynthetic membrane traffic suggest that key differences exist in post-Golgi sorting mechanisms between polarized and nonpolarized cells. These findings have led to the speculation that sorting of some proteins is not confined to the Golgi complex but instead may occur at multiple locations along the biosynthetic pathway. These observations and their impact on our current appreciation of biosynthetic sorting mechanisms are discussed in more detail below.

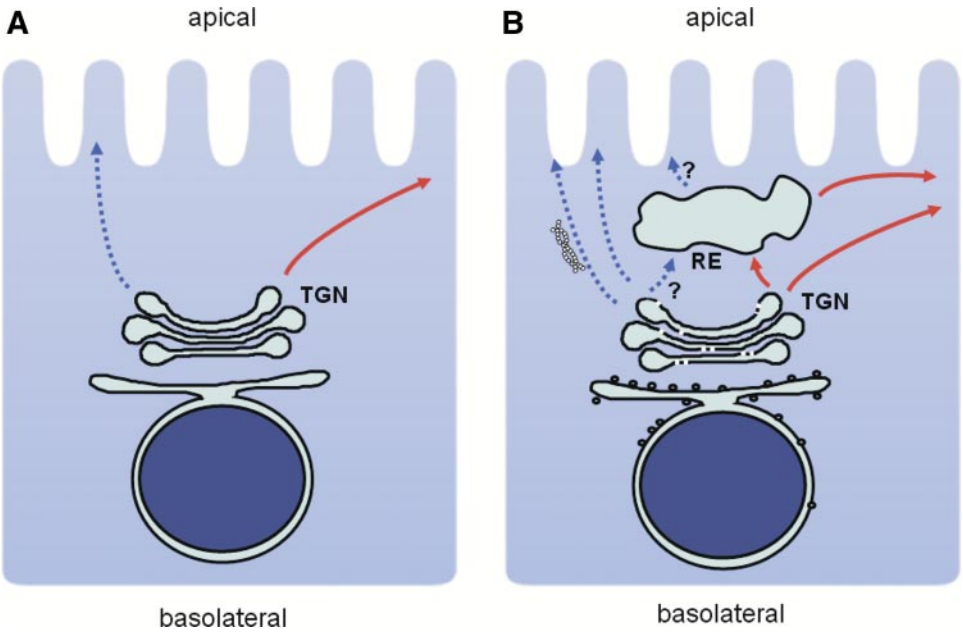

Fig. 1. Conventional and updated model for polarized biosynthetic sorting in renal epithelial cells. *A*: standard model for biosynthetic delivery in which apical and basolateral proteins are segregated into distinct vesicles in the *trans*-Golgi network (TGN) and delivered vectorially to their respective cell surface domains (dashed blue and solid red arrows, respectively). *B*: updated version of this model. In this scenario, segregation into distinct microdomains that may allow differential processing of apical and basolateral proteins may begin far earlier than the TGN. On reaching the TGN, a significant fraction of basolaterally destined proteins are directed to the common recycling endosome (RE) before surface delivery. Apical proteins are sorted into distinct types of transport carriers that use actin-dependent or -independent mechanisms to reach the plasma membrane. Whether some apical proteins traverse the RE or a different endocytic intermediate (not depicted here for simplicity; see text for details) before cell surface delivery remains unresolved.

Biosynthetic Sorting Signals

Basolateral sorting motifs, with some exceptions (41), are found within cytoplasmically disposed portions of proteins and are composed of amino acid primary sequences, many of which fit the consensus sequence for binding to adaptor protein (AP) complexes. The AP complex family consists of four heterotetrameric complexes that mediate cargo sorting at the cell surface and at various intracellular sites (52). AP-2 is localized exclusively to the plasma membrane, whereas the remaining complexes are distributed on both TGN and endosomal compartments. Indeed, each of the TGN-localized AP complexes (AP-1, AP-3, and AP-4) has been implicated in basolateral delivery (2, 10, 32, 35, 48). However, a longstanding paradox is that all of these AP complexes are known to mediate transport between the TGN and endosomal compartments rather than with the plasma membrane. This quandary may have been resolved by recent observations which suggest that at least some newly synthesized basolateral proteins indeed traverse recycling endosomes before reaching the cell surface. Thus it is possible that some polarized trafficking signals are interpreted at this site in addition to or in lieu of functioning at the TGN.

In contrast to basolateral sorting signals, apical targeting motifs have been localized to domains that are exposed to the lumen, membrane, or cytosol. Furthermore, apical sorting signals are often not discrete amino acid sequences, but rather posttranslation modifications [such as glycans or glycosylphosphatidylinositol (GPI) linkages] or transmembrane domain properties that enable preferential association with glycolipid-enriched microdomains (a.k.a. lipid rafts) (44). The role of lipid rafts in apical sorting remains unclear, as lipid raft association can be uncoupled from apical delivery (50) and the presence of a GPI anchor does not necessarily impart apical targeting of a given protein (38). Both N- and O-linked glycans have also been described as sorting motifs for a number of proteins (reviewed in Refs. 40 and 42). Glycan-dependent sorting could be mediated by association of glycosylated proteins with a sorting receptor, similar to lectins (glycan-binding proteins) that are thought to mediate export of some proteins from the endoplasmic reticulum (46). Alternatively, glycans may cause proteins to aggregate into preexport complexes. Finally, cytoplasmic tail sequences that target newly synthesized proteins to the apical surface have also been reported for megalin and for an increasing array of polytopic proteins (7, 18, 49). However, no consensus amino acid sequence or motif that directs apical targeting has yet been described.

Role of Pre-TGN Sorting in Polarized Delivery

Several recent reports using very different approaches have suggested the intriguing possibility that biosynthetic sorting of apical and basolateral cargo may actually occur earlier in the secretory pathway than previously thought. These studies are consistent with the possibility that newly synthesized proteins may access different sorting platforms or microdomains shortly after biosynthesis. Indeed, there is some precedence for this idea, as prior evidence in yeast has suggested that GPI-anchored proteins are sorted into a distinct class of vesicles leaving the endoplasmic reticulum (30).

One line of evidence in support of pre-TGN sorting comes from analysis of the glycan structures on apical vs. basolateral glycoconjugates. Prydz et al. (54) reported differential processing of apical and basolateral populations of the proteoglycan serglycin, including some modifications that occur early in the Golgi complex. Proteoglycans are modified by the addition of glycosaminoglycan chains (GAGs). GAGs are composed of a core tetrasaccharide linker followed by repeating dissacharide units of glucuronic acid and either *N*-acetyl-glucosamine (heparin sulfate chains) or *N*-acetyl-galactosamine (chondroitan sulfate chains). Further modification of GAGs by sulfation of the core linker region occurs in the early Golgi, whereas sulfation of the polysaccharide chains occurs in the *trans*-Golgi. Interestingly, Prydz et al. (54, 55) found that sulfation of both the core linker region and the polysaccharide chains of serglycin was different between basolateral and apical pools of

this proteoglycan, implicating differential modification of this pool early in the secretory pathway. These results suggest the possibility that apically and basolaterally destined populations of serglycin have differential access to GAG-processing machinery before reaching the TGN.

A second line of evidence supporting the sequestration of apical from basolateral cargo in pre-TGN compartments comes from a recent study by Alfalah et al. (1) demonstrating the ability to segregate both fully and incompletely processed forms of apical cargo from basolateral cargo based on their solubility in the nonionic detergent Tween 20. In these experiments, several apically destined proteins were found to be insoluble in Tween 20, whereas basolateral proteins remained soluble (1). The incorporation of newly synthesized apical proteins into Tween 20-insoluble complexes was observed even when cells were incubated at 15°C, which prevents newly synthesized proteins from leaving the endoplasmic reticulum/intermediate compartment. Together with the studies by Prydz et al. (54, 55) described above, these results suggest that apical and basolateral cargoes are sorted into distinct microdomains early in the biosynthetic pathway.

A third line of evidence for pre-TGN sorting comes from studies by the Zurzolo laboratory (37) on the mechanism that drives sorting of apically vs. basolaterally delivered GPI-anchored proteins. Comparison of the behavior of these proteins on velocity and flotation gradients revealed that all GPI-anchored proteins associated with Triton X-100-insoluble microdomains, but only apically destined proteins formed higher-order oligomers. Oligomerization occurred concomitantly with incorporation into Triton X-100-insoluble oligomers, with the acquisition of resistance to endoglycosidase H, and with terminal sialylation, consistent with a time frame between passage through the medial-Golgi and arrival at the TGN. Moreover, when oligomerization of the apically sorted marker GPI-GFP (green fluorescent protein linked to GPI) was disrupted by site-directed mutagenesis of cysteine residues required for GFP oligomerization, the resulting monomers retained their Triton X-100 insolubility but were now delivered basolaterally (37).

Polarized Protein Sorting in the TGN

During export from the TGN, proteins are packaged into vesicular and tubular cargo carriers. A number of studies have focused on identifying key factors involved in polarized protein exit from the TGN. In general, these studies have taken advantage of the ability to accumulate mature, newly synthesized proteins in the TGN by a 20°C temperature block (29). On warming the cells to 37°C, the cargo proteins are rapidly exported from the TGN in transport carriers that can be visualized by live cell imaging or isolated biochemically. This section will highlight a few of the recent findings that have advanced our understanding of the regulatory mechanisms that mediate the export of apical and basolateral cargo from this compartment.

The temperature-sensitive variant tsO45 of the vesicular stomatitis virus glycoprotein (VSV-G) has been commonly used as a basolateral marker due to the ease of accumulating and staging large amounts of this protein in the endoplasmic reticulum or TGN by varying incubation temperature. In nonpolarized MDCK and COS-7 cells, VSV-G fused to GFP was found to exit the TGN in highly dynamic tubules and vesicles that fused with each other and fragmented as they traveled along microtubules (17, 51). Keller et al. (22) advanced these studies by examining TGN export of coexpressed basolateral VSV-G-YFP and apical (CFP linked to a GPI anchor) markers in nonpolarized MDCK cells. In these studies, VSV-G-YFP and GPI-CFP were observed to be sorted into distinct tubular and vesicular carriers that emanated from the TGN and were vectorially delivered to the plasma membrane without apparently detouring through endosomes. Interestingly, fission of these carriers from the TGN may be differentially regulated by distinct machineries. Dynamin, a GTPase initially identified as a component of the endocytic machinery, has also been implicated in membrane fission steps along the biosynthetic pathway. Two groups have shown that dynamin-2, a ubiquitously expressed isoform that mediates the fission of clathrin-coated vesicles and caveolae from the plasma membrane, also functions in TGN release of vesicles containing the apical cargo p75-neurotrophin receptor (4, 23). In contrast, CtBP3/BARS, a protein that contains a curvature-inducing membrane association BAR domain, has been suggested to be responsible for the fission of basolateral transport intermediates (4). Fission of basolaterally destined transport carriers has also been shown to require protein kinase D (56).

A relatively recent complication of the model for apical and basolateral protein segregation in the TGN comes from growing evidence suggesting the existence of different pathways for the TGN export of apical proteins with distinct sorting signals. Jacob and Naim (21) followed the TGN export of two fluorescently tagged apical proteins that utilize distinct targeting mechanisms, the lipid raft-associated protein sucrase-isomaltase and the non-raft-associated protein lactase-phlorizin hydrolase, in Cos-1 and nonpolarized MDCK cells. These two apical proteins initially exited the TGN together in large vesicular compartments that subsequently gave rise to smaller vesicles that preferentially contained either of the two cargoes (21). Subsequent studies revealed that post-TGN trafficking of sucrase-isomaltase but not lactase-phlorizin hydrolase was found to be actin dependent, although both required microtubules for efficient surface delivery in polarized MDCK cells (20). Vesicles containing sucrase-isomaltase were subsequently immunoisolated and, consistent with a role for actin in transport of raft-associated cargo, proteomic analysis identified the motor protein myosin I and its regulator, α-kinase 1, on these vesicles (16). Another mechanism for actin involvement in biosynthetic trafficking may be via the generation of actin comets that function to propel transport carriers through the cytoplasm (9). Indeed, vesicles containing raft-associated but not raft-independent apical markers have been observed in association with actin comets in MDCK cells (15, 45).

While it is not clear whether multiple sorting pathways may exist for basolateral proteins as appears to be the case for apical cargo, the number and diversity of cytoplasmic proteins implicated in basolateral sorting have increased substantially over the past several years. In particular, the identification of several AP complexes as key participants in basolateral sorting has generated some controversy regarding the itinerary and sorting sites for newly synthesized basolaterally destined proteins. Individual studies have recently implicated both AP-3 and AP-4 in surface delivery of basolateral cargo proteins (32, 48). An added wrinkle that has received more attention is the

identification of an epithelial-specific adaptor protein complex (AP1B) that differs from the ubiquitously expressed AP-1 by the incorporation of a distinct medium subunit (μ1b) and which is expressed in a subset of polarized cell lines, including MDCK cells (10, 33). Heterologous expression of μ1b in LLC-PK_1 cells resulted in basolateral delivery of cargo proteins that are usually missorted apically in this cell line (10). However, subsequent studies revealed that a significant fraction of AP1B complexes localize to recycling endosomes rather than the TGN (11, 12) and mediate the basolateral redistribution of proteins only after they have initially been delivered (in a mispolarized manner) to the plasma membrane (14). A "hybrid" model suggesting that different cargo proteins differentially utilize the direct vs. indirect pathways has been proposed (53); however, in the absence of direct evidence to reconcile the published data, the role of AP1B in delivery of newly synthesized as opposed to recycling proteins remains controversial.

Post-TGN Sorting Along the Biosynthetic Pathway

As described above, the conventional model for biosynthetic trafficking of membrane proteins in polarized epithelial cells has been that apical and basolateral proteins are sorted in the TGN into post-Golgi vesicles that fuse directly with the plasma membrane. However, growing evidence supporting an involvement of endosomal compartments in biosynthetic traffic has forced us to rethink and refine the basic principles of this model.

Early studies demonstrated that newly synthesized transferrin receptor (TfR) and asialoglycoprotein receptor H1 pass through endosomal compartments en route from the TGN to the plasma membrane in nonpolarized cells (13, 24, 25); however, delivery of GPI-anchored proteins appears to proceed via an alternate pathway (13). Similarly, Orzech et al. (34) observed that polymeric immunoglobulin receptors traversed endocytic compartments before basolateral delivery in polarized MDCK cells. More recently, Ang et al. (3) extended these studies using a combination of biochemical and live-cell techniques to investigate the significance and extent of endosomal transit of the basolateral marker, VSV-G. VSV-G-YFP was staged in the TGN of nonpolarized MDCK cells stably expressing the human TfR, and the cells were imaged after being warmed in the presence of fluorescently labeled human transferrin. Although initially segregated, within 5–10 min of warm-up a small fraction of VSV-G-YFP released from the TGN appeared in transferrin-positive structures, presumably recycling endosomes. These findings were supported biochemically by immunoisolation experiments demonstrating the recovery of labeled transferrin in VSV-G-YFP-containing compartments (3). To determine whether trafficking through recycling endosomes was required for basolateral delivery of VSV-G, Ang et al. internalized transferrin conjugated to horseradish peroxidase and then treated cells with diaminobenzidine and hydrogen peroxide to form an insoluble precipitate in peroxidase-containing compartments, thus effectively inactivating transferrin-positive endosomes. Delivery of VSV-G to the cell surface was inhibited by roughly 85% after inactivation of recycling endosomes, suggesting that traffic through this compartment is an obligate step in surface delivery of VSV-G (3).

Similar findings were reported by Lock and Stow (27), who examined the biosynthetic pathway of basolaterally expressed E-cadherin in nonpolarized HeLa cells using live-cell imaging. Wild-type E-cadherin was found to exit the TGN in post-Golgi carriers that fused with rab11-positive recycling endosomes in these cells. Interestingly, in polarized MDCK cells, the authors found that basolateral delivery of wild-type E-cadherin was dependent on rab11 function, whereas apical delivery of a cadherin mutant lacking its dileucine-based sorting motif was not (27).

Several studies, including many of those described above, have also investigated the biosynthetic trafficking route of "apically destined" proteins with mixed conclusions. A caveat in all of these cases is that the apical proteins examined represent mutants of the original basolateral markers used. For example, Orzech et al. (34, 35) concluded that an apically directed mutant of pIgR that lacks a casein kinase phosphorylation site traffics indirectly to the cell surface; however, this mutant contains an intact AP-1 interaction motif that could account for the post-TGN itinerary of the protein. Similarly, Ang et al. (3) coisolated labeled transferrin in compartments that were positive for an apically delivered mutant of VSV-G; however, the AP-interacting sequence in this poorly-defined VSV-G variant also remains intact. Other studies have recently challenged the intracellular route taken by more conventional apical markers, namely, GPI-anchored proteins. As noted above, GPI-anchored proteins were found not to enter transferrin-positive compartments en route to the cell surface (13). This observation is consistent with findings by other groups that GPI-anchored proteins are delivered directly (i.e., not via an endosomal intermediate) and vectorially (i.e., not via transcytosis) from the TGN to the apical domain (22, 26, 37). This conclusion has recently been challenged by Polishchuk et al. (39), who demonstrated using live-cell imaging that GPI-anchored YFP exits the TGN in the same population of post-Golgi tubules that contain newly synthesized VSV-G-CFP. Moreover, both cargos then appeared at the basolateral surface, and GPI-YFP was subsequently internalized and delivered to the apical domain by transcytosis via an endosomal intermediate. In contrast, another apical marker, p75, was delivered directly to the apical membrane. The conclusions of these experiments were largely based on the innovative use of the membrane-impermeant fixative tannic acid to inhibit exocytic traffic to a given plasma membrane domain. In these studies, treatment with basolaterally applied tannic acid disrupted surface delivery of GPI-YFP and VSV-G-CFP but did not affect apical delivery of tagged p75 (39). In contrast, apically applied tannic acid impeded p75 delivery and GPI-YFP delivery but did not affect VSV-G transport (39). These results not only challenged the conventional model for direct TGN-to-plasma membrane delivery of GPI-anchored proteins but also suggested that the primary sorting site of these proteins may be at the basolateral membrane or in endosomal compartments rather than the TGN.

More recent work from two groups has questioned the validity of these conclusions with respect to the itinerary of GPI-anchored proteins in polarized cells. Using a combination of live-cell imaging and biochemical approaches, Paladino et al. (36) and Hua et al. (19) found that GPI-anchored proteins are directly delivered from the TGN to the apical membrane in fully polarized MDCK cells. In contrast, GPI-anchored proteins used the transcytotic pathway to reach the apical surface in nonpolarized MDCK cells, and the fraction of protein that

was transcytosed decreased when MDCK cells were grown on permeable supports for 4 days instead of 2 days, the condition used by Polishchuk et al. (39). Moreover, treatment with tannic acid did not disrupt direct apical delivery of GPI-anchored proteins but led to rapid redistribution of the surface pool, apparently as a result of a breach in the integrity of tight junctions (36). These results are consistent with the original model for vectorial delivery of GPI-anchored proteins from the TGN to the apical surface; however, they do not rule out the possibility that transport occurs via an endosomal intermediate rather than directly.

Summary and Future Directions

Clearly, even basic issues regarding the route and mechanisms used by newly synthesized proteins to reach their ultimate destination remain controversial or unknown. The studies described above have forced us to rethink many of the basic principles of the conventional model for biosynthetic protein sorting in polarized epithelial cells, but they continue to pose many unresolved questions.

With respect to the growing evidence for pre-TGN sorting, how does this reconcile with the growing evidence for post-Golgi segregation of apical proteins into distinct classes of vesicles (15, 20, 21)? What would be the purpose or function of segregating all apical proteins from basolateral cargo at an early stage in transport if apical proteins are subsequently reassorted into distinct carriers? One possibility is that incorporation into Tween 20-insoluble complexes provides selective access or protection from particular modifications that are important for subsequent polarized sorting. Some obvious questions are whether the differential sulfation of apically destined serglycin reflects association with Tween 20-insoluble complexes in the early Golgi and whether association of Triton X-100-soluble proteins with Tween 20-insoluble microdomains is required for the eventual apical delivery of these cargoes. In this regard, it should be noted that inhibition of sulfation did not appear to affect the polarity of serglycin secretion, suggesting that this modification is not a critical sorting determinant (55).

Another obvious question concerns the relationship between Tween 20- and Triton X-100-insoluble microdomains. Shuck et al. (47) have reported that Tween 20-insoluble complexes isolated from MDCK cells contain 10-fold more proteins than Triton X-100-insoluble complexes, raising some concern about the selectivity of this detergent. Does lipid processing in the Golgi complex result in conversion of some Tween 20-insoluble complexes into Triton X-100-resistant forms? What regulates the selective association of only a subset of apically destined proteins with these domains? Additionally, what mechanisms might mediate the apparent post-Golgi segregation of Triton X-100-soluble and -insoluble proteins? As observed for GPI-anchored proteins, perhaps oligomerization plays a role in this process for other types of proteins as well. Indeed, the concept that "clustering" is key for efficient apical sorting of non-raft-associated proteins as well as GPI-anchored proteins in the TGN or post-TGN compartments has recently gained momentum with the publication of a provocative study suggesting a role for galectin-3-mediated clustering in post-Golgi apical targeting of non-raft-associated cargo in MDCK cells (8).

Similarly, a number of issues remain to be resolved with respect to the role of endosomes in TGN to surface delivery. While the studies described above provide convincing evidence that endosomes play a more important role in biosynthetic traffic than previously thought, several uncertainties have yet to be resolved. For one, the role of AP1B complexes in basolateral sorting of biosynthetic vs. postendocytic traffic remains controversial. In addition, because many of the conclusions of the studies described above are based on observations in nonpolarized or semipolarized cells, a primary issue to be considered is the relationship between these studies and protein traffic in fully polarized cells. While TGN sorting may be regulated in a similar manner in undifferentiated and differentiated cells, the post-Golgi itinerary of biosynthetic proteins can change during cell polarization, as the studies of Paladino et al. (36) clearly demonstrate. Moreover, as the endocytic pathway is organized differently in plastic-grown vs. fully polarized cells (Fig. 2), the identity of the endosomes that receive biosynthetic cargo is not yet clear. In nonpolarized

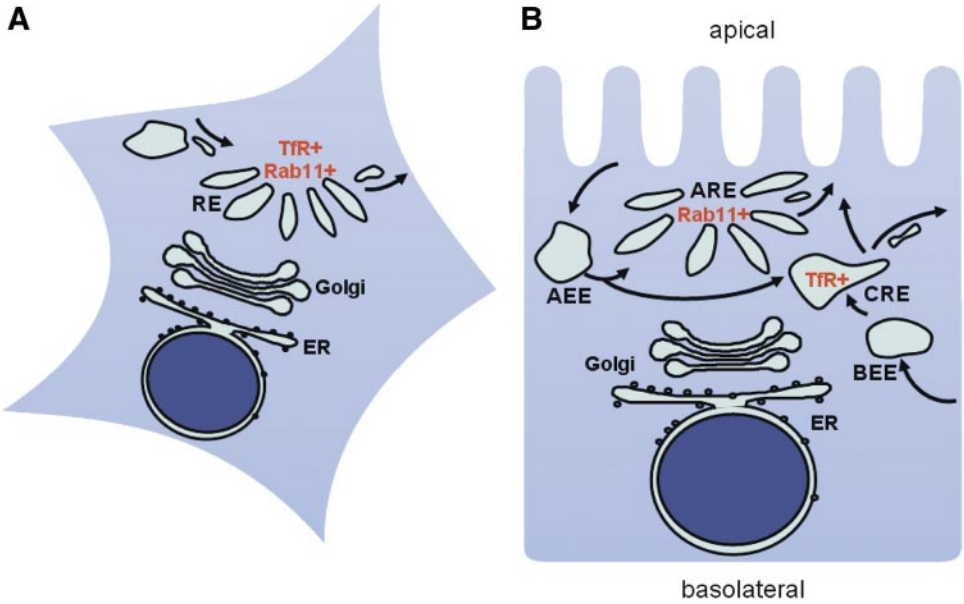

Fig. 2. Organization of the endocytic pathway in nonpolarized vs. polarized cells. In nonpolarized cells (*A*), recycling proteins such as the transferrin receptor (TfR) traffic through recycling endosomes that are positive for rab11. In contrast, in polarized cells (*B*), TfR internalized through basolateral early endosomes (BEE) traffics via the rab11-negative common recycling endosome (CRE) but does not access rab11-positive apical recycling endosomes (ARE). The ARE receive transcytosing cargo as well as proteins internalized though apical early endosomes (AEE). The implications of this differential organization of the endocytic pathway with respect to the identification of polarized biosynthetic trafficking routes are discussed in the text. ER, endoplasmic reticulum.

cells, TfR passes through juxtanuclear recycling endosomes that are positive for rab11 staining. In contrast, TfR in polarized cells passes through the common recycling endosome, a compartment that receives internalized apical and basolateral cargo but is rab11 negative (5) Rab11 in polarized cells is localized instead to the apical recycling endosome, a subapical tubular cluster dedicated to apical sorting along the endocytic and transcytotic pathways, and is not present on common recycling endosomes. (5). Thus, whereas the recycling endosome identified in nonpolarized cell studies as TfR positive by Ang et al. (3) and as rab11 positive by Lock and Stow (27) represents the same compartment, these two markers identify separate compartments in polarized MDCK cells. Future studies will be required to dissect whether biosynthetic cargoes traverse the common recycling endosome or the apical recycling endosome en route to the plasma membrane.

A related issue is whether newly synthesized apical proteins also detour through endosomes. As described above, results from the studies to date are contradictory. This confusion likely reflects differences in cell type and polarity and the use of apically destined proteins that are derived from basolateral markers in some studies. An intriguing possibility that could potentially reconcile these results is that apically destined cargo may pass through Rab11-positive apical recycling endosomes en route to the cell surface in polarized cells, whereas basolaterally destined cargo might traverse TfR-positive common recycling endosomes. Indeed, a recent report examining biosynthetic delivery in polarized MDCK cells suggests that apically destined GPI-YFP may be waylaid in a subapical compartment before reaching the cell surface, although this step was not a rate-limiting step for delivery (19). In contrast, VSV-G-YFP did not appear in this compartment (19).

A corollary to consider is whether a direct pathway from the TGN to the surface exists at all. Futter et al. (13) have demonstrated that like GPI-anchored proteins, newly synthesized secretory proteins do not traverse TfR-positive compartments before reaching the cell surface. Whether polarized cells also maintain a direct pathway to either plasma membrane domain remains unknown.

In summary, the conventional model for biosynthetic sorting clearly needs to be updated. Although segregation of apical and basolateral proteins from one another can clearly occur in the TGN, there is growing evidence that sorting also occurs at additional intracellular sites. Given the diversity of proteins that must be expressed at either cell surface domain of differentiated cells to maintain organ function, it is perhaps not surprising that multiple intracellular sites participate in interpreting the array of sorting signals that determine protein localization. The growing intricacy of the mechanisms and pathways utilized by the cell to accomplish this speaks to the complexity of the task at hand.

ACKNOWLEDGMENTS

We thank Rebecca Hughey and the members of the Weisz laboratory for helpful comments on the manuscript. B. A. Potter is supported by a predoctoral fellowship from the Pennsylvania-Delaware Affiliate of the American Heart Association.

We acknowledge those whose work could not be cited or discussed directly due to space limitations.

GRANTS

Studies on polarized membrane traffic sorting are supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-54407 and DK-064613 (to O. A.Weisz).

REFERENCES

1. **Alfalah M, Wetzel G, Fischer I, Busche R, Sterchi EE, Zimmer KP, Sallmann HP, and Naim HY.** A novel type of detergent-resistant membranes may contribute to an early protein sorting event in epithelial cells. *J Biol Chem* 280: 42636–42643, 2005.
2. **Ang AL, Folsch H, Koivisto UM, Pypaert M, and Mellman I.** The Rab8 GTPase selectively regulates AP-1B-dependent basolateral transport in polarized Madin-Darby canine kidney cells. *J Cell Biol* 163: 339–350, 2003.
3. **Ang AL, Taguchi T, Francis S, Folsch H, Murrells LJ, Pypaert M, Warren G, and Mellman I.** Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. *J Cell Biol* 167: 531–543, 2004.
4. **Bonazzi M, Spano S, Turacchio G, Cericola C, Valente C, Colanzi A, Kweon HS, Hsu VW, Polishchuck EV, Polishchuck RS, Sallese M, Pulvirenti T, Corda D, and Luini A.** CtBP3/BARS drives membrane fission in dynamin-independent transport pathways. *Nat Cell Biol* 7: 570–580, 2005.
5. **Brown PS, Wang E, Aroeti B, Chapin SJ, Mostov KE, and Dunn KW.** Definition of distinct compartments in polarized Madin-Darby canine kidney (MDCK) cells for membrane-volume sorting, polarized sorting and apical recycling. *Traffic* 1: 124–140, 2000.
6. **Campo C, Mason A, Maouyo D, Olsen O, Yoo D, and Welling PA.** Molecular mechanisms of membrane polarity in renal epithelial cells. *Rev Physiol Biochem Pharmacol* 153: 47–99, 2005.
7. **Chuang JZ and Sung CH.** The cytoplasmic tail of rhodopsin acts as a novel apical sorting signal in polarized MDCK cells. *J Cell Biol* 142: 1245–1256, 1998.
8. **Delacour D, Cramm-Behrens CI, Drobecq H, Le Bivic A, Naim HY, and Jacob R.** Requirement for galectin-3 in apical protein sorting. *Curr Biol* 16: 408–414, 2006.
9. **Fehrenbacher KL, Boldogh IR, and Pon LA.** Taking the A-train: actin-based force generators and organelle targeting. *Trends Cell Biol* 13: 472–477, 2003.
10. **Folsch H, Ohno H, Bonifacino JS, and Mellman I.** A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. *Cell* 99: 189–198, 1999.
11. **Folsch H, Pypaert M, Maday S, Pelletier L, and Mellman I.** The AP-1A and AP-1B clathrin adaptor complexes define biochemically and functionally distinct membrane domains. *J Cell Biol* 163: 351–362, 2003.
12. **Folsch H, Pypaert M, Schu P, and Mellman I.** Distribution and function of AP-1 clathrin adaptor complexes in polarized epithelial cells. *J Cell Biol* 152: 595–606, 2001.
13. **Futter CE, Connolly CN, Cutler DF, and Hopkins CR.** Newly synthesized transferrin receptors can be detected in the endosome before they appear on the cell surface. *J Biol Chem* 270: 10999–11003, 1995.
14. **Gan Y, McGraw TE, and Rodriguez-Boulan E.** The epithelial-specific adaptor AP1B mediates post-endocytic recycling to the basolateral membrane. *Nat Cell Biol* 4: 605–609, 2002.
15. **Guerriero CJ, Weixel KM, Bruns JR, and Weisz OA.** Phosphatidylinositol 5-kinase stimulates apical biosynthetic delivery via an Arp2/3-dependent mechanism. *J Biol Chem* 281: 15376–15384, 2006.
16. **Heine M, Cramm-Behrens CI, Ansari A, Chu HP, Ryazanov AG, Naim HY, and Jacob R.** α-Kinase 1, a new component in apical protein transport. *J Biol Chem* 280: 25637–25643, 2005.
17. **Hirschberg K, Miller CM, Ellenberg J, Presley JF, Siggia ED, Phair RD, and Lippincott-Schwartz J.** Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. *J Cell Biol* 143: 1485–1503, 1998.
18. **Hodson CA, Ambrogi IG, Scott RO, Mohler PJ, and Milgram SL.** Polarized apical sorting of guanylyl cyclase C is specified by a cytosolic signal. *Traffic* 7: 456–464, 2006.
19. **Hua W, Sheff D, Toomre D, and Mellman I.** Vectorial insertion of apical and basolateral membrane proteins in polarized epithelial cells revealed by quantitative 3D live cell imaging. *J Cell Biol* 172: 1035–1044, 2006.

20. **Jacob R, Heine M, Alfalah M, and Naim HY.** Distinct cytoskeletal tracks direct individual vesicle populations to the apical membrane of epithelial cells. *Curr Biol* 13: 607–612, 2003.
21. **Jacob R and Naim H.** Apical membrane proteins are transported in distinct vesicular carriers. *Curr Biol* 11: 1444–1450, 2001.
22. **Keller P, Toomre D, Diaz E, White J, and Simons K.** Multicolour imaging of post-Golgi sorting and trafficking in live cells. *Nature Cell Biol* 3: 140–149, 2001.
23. **Kreitzer G, Marmorstein A, Okamoto P, Vallee R, and Rodriguez-Boulan E.** Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. *Nature Cell Biol* 2: 125–127, 2000.
24. **Laird V and Spiess M.** A novel assay to demonstrate an intersection of the exocytic and endocytic pathways at early endosomes. *Exp Cell Res* 260: 340–345, 2000.
25. **Leitinger B, Hille-Rehfeld A, and Spiess M.** Biosynthetic transport of the asialoglycoprotein receptor H1 to the cell surface occurs via endosomes. *Proc Natl Acad Sci USA* 92: 10109–10113, 1995.
26. **Lisanti MP, Caras IW, Gilbert T, Hanzel D, and Rodriguez-Boulan E.** Vectorial apical delivery and slow endocytosis of a glycolipid-anchored fusion protein in transfected MDCK cells. *Proc Natl Acad Sci USA* 87: 7419–7423, 1990.
27. **Lock JG and Stow JL.** Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. *Mol Biol Cell* 16: 1744–1755, 2005.
28. **Madin SH, Andriese PC, and Darby NB.** The in vitro cultivation of tissues of domestic and laboratory animals. *Am J Vet Res* 18: 932–941, 1957.
29. **Matlin KS and Simons K.** Reduced temperature prevents transfer of a membrane glycoprotein to the cell surface but does not prevent terminal glycosylation. *Cell* 34: 233–243, 1983.
30. **Muniz M, Morsomme P, and Riezman H.** Protein sorting upon exit from the endoplasmic reticulum. *Cell* 104: 313–320, 2001.
31. **Müsch A, Xu H, Shields D, and Rodriguez-Boulan E.** Transport of vesicular stomatitis virus G protein to the cell surface is signal mediated in polarized and nonpolarized cells. *J Cell Biol* 133: 543–558, 1996.
32. **Nishimura N, Plutner H, Hahn K, and Balch WE.** The δ subunit of AP-3 is required for efficient transport of VSV-G from the *trans*-Golgi network to the cell surface. *Proc Natl Acad Sci USA* 99: 6755–6760, 2002.
33. **Ohno H, Tomemori T, Nakatsu F, Okazaki Y, Aguilar RC, Foelsch H, Mellman I, Saito T, Shirasawa T, and Bonifacino JS.** μ1B, a novel adaptor medium chain expressed in polarized epithelial cells. *FEBS Lett* 449: 215–220, 1999.
34. **Orzech E, Cohen S, Weiss A, and Aroeti B.** Interactions between the exocytic and endocytic pathways in polarized Madin-Darby canine kidney cells. *J Biol Chem* 275: 15207–15219, 2000.
35. **Orzech E, Schlessinger K, Weiss A, Okamoto CT, and Aroeti B.** Interactions of the AP-1 Golgi adaptor with the polymeric immunoglobulin receptor and their possible role in mediating brefeldin A-sensitive basolateral targeting from the *trans*-Golgi network. *J Biol Chem* 274: 2201–2215, 1999.
36. **Paladino S, Pocard T, Catino MA, and Zurzolo C.** GPI-anchored proteins are directly targeted to the apical surface in fully polarized MDCK cells. *J Cell Biol* 172: 1023–1034, 2006.
37. **Paladino S, Sarnataro D, Pillich R, Tivodar S, Nitsch L, and Zurzolo C.** Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins. *J Cell Biol* 167: 699–709, 2004.
38. **Paladino S, Sarnataro D, and Zurzolo C.** Detergent-resistant membrane microdomains and apical sorting of GPI-anchored proteins in polarized epithelial cells. *Int J Med Microbiol* 291: 439–445, 2002.
39. **Polishchuk R, Di Pentima A, and Lippincott-Schwartz J.** Delivery of raft-associated, GPI-anchored proteins to the apical surface of polarized MDCK cells by a transcytotic pathway. *Nat Cell Biol* 6: 297–307, 2004.
40. **Potter BA, Hughey RP, and Weisz OA.** Role of N- and O-glycans in polarized biosynthetic sorting. *Am J Physiol Cell Physiol* 290: C1–C10, 2006.
41. **Puthenveedu MA, Bruns JR, Weisz OA, and Linstedt AD.** Basolateral cycling mediated by a lumenal domain targeting determinant. *Mol Biol Cell* 14: 1801–1807, 2003.
42. **Rodriguez-Boulan E and Gonzalez A.** Glycans in post-Golgi apical targeting: sorting signals or structural props? *Trends Cell Biol* 9: 291–294, 1999.
43. **Rodriguez-Boulan E, Kreitzer G, and Müsch A.** Organization of vesicular trafficking in epithelia. *Nat Rev Mol Cell Biol* 6: 233–247, 2005.
44. **Rodriguez-Boulan E and Musch A.** Protein sorting in the Golgi complex: shifting paradigms. *Biochim Biophys Acta* 1744: 455–464, 2005.
45. **Rozelle AL, Machesky LM, Yamamoto M, Driessens MHE, Insall RH, Roth MG, Luby-Phelps K, Marriott G, Hall A, and Yin HL.** Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP-Arp2/3. *Curr Biol* 10: 311–320, 2000.
46. **Schrag JD, Procopio DO, Cygler M, Thomas DY, and Bergeron JJ.** Lectin control of protein folding and sorting in the secretory pathway. *Trends Biochem Sci* 28: 49–57, 2003.
47. **Schuck S, Honsho M, Ekroos K, Shevchenko A, and Simons K.** Resistance of cell membranes to different detergents. *Proc Natl Acad Sci USA* 100: 5795–5800, 2003.
48. **Simmen T, Honing S, Icking A, Tikkanen R, and Hunziker W.** AP-4 binds basolateral signals and participates in basolateral sorting in epithelial MDCK cells. *Nat Cell Biol* 4: 154–159, 2002.
49. **Takeda T, Yamazaki H, and Farquhar MG.** Identification of an apical sorting determinant in the cytoplasmic tail of megalin. *Am J Physiol Cell Physiol* 284: C1105–C1113, 2003.
50. **Tall RD, Alonso MA, and 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.
51. **Toomre D, Keller P, White J, Olivo JC, and Simons K.** Dual-color visualization of *trans*-Golgi network to plasma membrane traffic along microtubules in living cells. *J Cell Sci* 112: 21–33, 1999.
52. **Traub LM.** Common principles in clathrin-mediated sorting at the Golgi and the plasma membrane. *Biochim Biophys Acta* 1744: 415–437, 2005.
53. **Traub LM and Apodaca G.** AP-1B: polarized sorting at the endosome. *Nat Cell Biol* 5: 1045–1047, 2003.
54. **Tveit H, Dick G, Skibeli V, and Prydz K.** A proteoglycan undergoes different modifications en route to the apical and basolateral surfaces of Madin-Darby canine kidney cells. *J Biol Chem* 280: 29596–29603, 2005.
55. **Vuong TT, Prydz K, and Tveit H.** Differences in the apical and basolateral pathways for glycosaminoglycan biosynthesis in Madin-Darby canine kidney cells. *Glycobiology* 16: 326–332, 2006.
56. **Yeaman C, Ayala MI, Wright JR, Bard F, Bossard C, Ang A, Maeda Y, Seufferlein T, Mellman I, Nelson WJ, and Malhotra V.** Protein kinase D regulates basolateral membrane protein exit from *trans*-Golgi network. *Nat Cell Biol* 6: 106–112, 2004.
57. **Yoshimori T, Keller P, Roth MG, and Simons K.** Different biosynthetic transport routes to the plasma membrane in BHK and CHO cells. *J Cell Biol* 133: 247–256, 1996.