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Invited Review

Regulation of protein traffic in polarized epithelial cells

K.E. Mostov
Department of Anatomy, Biochemistry and Biophysics, and Cardiovascular Research Institute, School of Medicine, University of California, San Francisco, California, USA

Summary. The plasma membrane of polarized epithelial cells is divided into apical and basolateral surfaces with different compositions. Proteins can be sent directly from the trans Golgi network (TGN) to either surface, or can be sent first to one surface and then transcytosed to the other. The glycosyl phosphatidylinositol anchor is a signal for apical targeting. Signals in the cytoplasmic domain containing a ß-turn determine basolateral targeting and retrieval, and are related to other shorting signals. Transcytosed proteins, such as the polymeric immunoglobulin receptor (pIgR) are endocytosed from the basolateral surface and delivered to the apical recycling compartment underneath the apical surface. This compartment is a central sorting station, as it receives material from both surfaces and sorts them to the correct surface. Delivery to the apical surface from both the TGN and the apical recycling compartment is regulated by protein kinase A and protein kinase C, and endocytosis from the apical surface is also regulated by kinases. Transcytosis of the pIgR is additionally regulated by phosphorylation of the pIgR and by ligand binding to the pIgR. Regulation of traffic in polarized epithelial cells plays a central role in cellular homeostasis, response to external signals, and differentiation.

Key words: Golgi, Endosome, Transcytosis, Sorting signal, Recycling

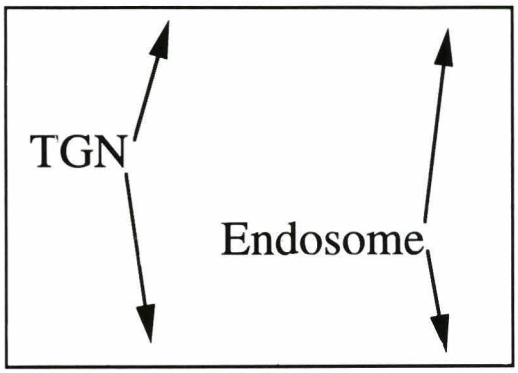
Introduction

Most eukaryotic cells are spatially asymmetric or polar. Understanding how the complex three-dimensional organization of eukaryotic cells is established and maintained is a central question in cell biology. One of the simplest types of polarized cells is the epithelial cell. Epithelial cells form a layer that lines many cavities of the body, such as the lining of the digestive, respiratory and urinary systems. The plasma membrane of these cells can be generally divided into two domains, an apical or free surface that faces adjacent cells and the underlying basement membrane (reviewed in Mostov et al., 1992; Rodriguez-Boulan and Powell, 1992). The two domains are separated by tight junctions, which help to prevent mixing of the surface and provide a tight seal between cells, thereby preventing leakage across the monolayer. The two plasma membrane domains serve very different functions, and therefore have quite disparate protein and lipid compositions. For instance, in intestinal absorptive epithelia the apical surface contains enzymes involved in breaking down the food contents of the intestine, while the basolateral surface contains molecules involved in cell-cell and cell substrate adhesion. The differences between apical and basolateral surface are only one aspect to underlying polarity of the cell; however, they provide a powerful experimental window into understanding the fundamental question of cell polarity. The principles that underly polarity of epithelial cells also seem to apply to other, more difficult to study cells. For instance, neurons are perhaps the most dramatically polarized of all cells, as they have axons that can extend meters from the cell body. If a protein that is normally found in the apical cell surface of an epithelial cell is exogenously expressed in neurons, the protein is usually transported to the axonal plasma membrane. Conversely, a protein ordinarily found in the basolateral surface of epithelial cells will usually be transported to the plasma membrane of the neuronal cell body and dendrites when expressed exogeneously in neurons (reviewed in Rodriguez-Boulan and Powell, 1992).

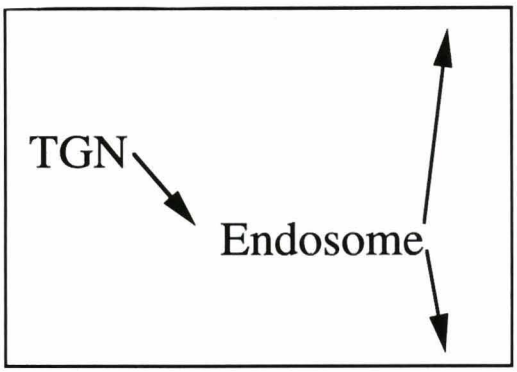
Offprint requests to: Keith Mostov, Department of Anatomy, Biochemistry and Biophysics, and Cardiovascular Research Institute, University of California, San Francisco, California 94143-0452, USA

Sorting pathways

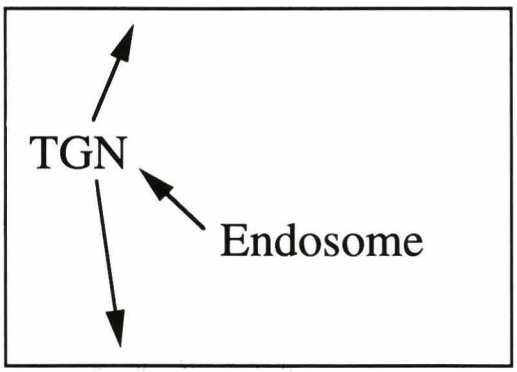
Epithelial cells can use a variety of mechanisms to localize proteins to the correct surface. Newly made plasma membrane proteins are made on the rough endoplasmic reticulum and the transverse the Golgi apparatus to the trans-Golgi network (TGN). There they can be packaged into vesicles that deliver them to the appropriate surface, either apical or basolateral (Fig. 1,



A. Similar machinery operates in TGN and endosomes.



B. Polarized sorting is in endosomes.



C. Polarized sorting is in TGN.

panel A). This pathway was originally characterized in Madin-Darby canine kidney (MDCK) cells. These cells form a well-polarized, tight monolayer when grown on permeable filters and have been a widely used cell culture model for studying polarity (reviewed in Mostov et al., 1992; Rodriguez-Boulan and Powell, 1992). However, it was subsequently discovered that some cells, such as hepatocytes do not have a pathway for direct delivery of plasma membrane proteins from the TGN to the apical surface. Other cells, such as the Caco2 human colon carcinoma cell line (another popular cultured cell model) use direct TGN to apical delivery to only a very limited extent. Instead, hepatocytes and Caco2 cells rely on an indirect pathway, in which proteins are first delivered from the TGN to the basolateral surface (Fig. 1, panel A). Subsequently, the proteins are endocytosed and delivered to endosomes. Most endocytosed proteins recycle back to the basolateral surface. However, selected proteins are transcytosed to the apical surface. Transcytosis is now recognized to be the only pathway for apical delivery of membrane proteins found in all epithelial cells examined so far and in some cells, such as hepatocytes, it is the only pathway for apical delivery.

A further mechanism for achieving the polarized distribution of proteins in epithelial cells is differential retention at one particular surface. For example, in some strains of MDCK cells the Na^+, K^+-ATPase is randomly targeted from the TGN to both the apical and basolateral surfaces (Hammertom et al., 1991). Those molecules reaching the apical surface have a short residence time there and are probably degraded, whereas molecules delivered to basolateral surface remain there for long periods.

Sorting signals and sorting machinery

Proteins are generally believed to contain shorting signals that specify their intracellular trafficking. The first signal to be identified for polarized targeting was a post-translational modification, rather than part of the peptide backbone of the protein (reviewed in Lisanti and Rodriguez-Boulan, 1990). Some integral plasma membrane proteins do not span the membrane but instead are anchored to the extracellular leaflet of the membrane by a glycosylphosphatidylinositol (GPI) anchor. In most epithelial cells these proteins are found exclusively at the apical surface. Moreover, if a GPI anchor is attached to an otherwise basolateral protein, the protein can be redirected to the apical surface,

Fig. 1. Possible locations for polarized sorting. In each panel a highly simplified epithelial cells is shown, with the apical surface at the top, and the basolateral surface at the bottom. Panel A is the classical view, in which proteins are sorted either in the TGN or the endosomes for delivery to the correct surface. Panels B and C present alternative models. In panel B proteins move from the TGN to some portion of the endosomal system, where they are sorted to the correct surface. In Panel C, endocytosed proteins move from the endosomes to the TGN, and are then sorted to the correct surface.

indicating that the signal is sufficient for apical sorting. Glycosphingolipids are enriched at the apical surface and it appears that GPI anchored proteins, glycosphingolipids, and a few transmembrane proteins (e.g. the hemagglutinin or HA protein of influenza virus) form hydrogen-bonded «rafts» or microdomains in the TGN. These rafts are then shorted to the apical membrane (Simons and van Meer, 1988). These rafts are resistants to solubilization by non-ionic detergents, so treatment of cells with such detergents, followed by flotation of the extract on sucrose density gradients permits the isolation of a low density membranous fraction enriched in these molecules. These rafts appear to be related to caveolae, which are specialized, often invaginated, regions of the plasma membrane that contain clusters of GPI-anchored proteins (reviewed in Lisanti et al., 1994). A biochemical marker of caveolae is caveolin, an integral membrane protein which may form the morphologically distinguishable coat on the cytoplasmic surface of caveolae. Interestingly, vesicles that have budded off from the TGN and carry HA bound for the apical surface also contain caveolin, suggesting that caveolin might be involved in formation and/or targeting of rafts to the apical plasma membrane and in organization of caveolae. Furthermore, the thyroid FRT cell line lacks caveolin and mis-sorts some GPI-anchored proteins to the basolateral surface (Lisanti et al., 1994; Zurzolo et al., 1994). However, Caco2 cells lack caveolin, but correctly sort GPI-anchored proteins (Le Bivic, personal communication). VIP36 is another component of glycosphingolipid rafts and exocytotic carrier vesicles and may be involved in sorting (Fiedler et al., 1994).

Signals have been identified in the cytoplasmic domains of proteins that are targeted to the basolateral surface (reviewed in Mostov et al., 1992). The first such signal was found in the cytoplasmic domain of the polymeric immunoglobulin receptor (pIgR) (Casanova et al., 1991). The protein is normally transcytosed to the basolateral surface, where it can bind its ligand, dimeric IgA (dIgA) (reviewed in Mostov, 1994). With or without bound ligand, the pIgR is then endocytosed and then transcytosed to the apical surface. There the extracellular ligand binding portion of the receptor is cleaved off and released into secretions with the IgA. This cleaved fragment is termed secretory component (SC) and also serves to protect the transcytosed dIgA from degradation in the often hostile envrionment outside the epithelial cell, e.g. the lumen of the intestine. The pIgR has been exogenously expressed in MDCK cells, where it appears to be trafficked as in vivo (Mostov et al., 1986; Mostov and Deitcher, 1986). This has given a powerful model system to study potential sorting signals in the pIgR. The pIgR has a C-terminal cytoplasmic domain of 103 amino acids. We have systematically mutated this region and examined the mutant pIgRs for altered trafficking (Mostov, 1994). One of our principal results is that the 17 residues of the cytoplasmic domain that are closest to the membrane are a signal for targeting from the TGN to the basolateral surface (Casanova et al., 1991). Removal of this segment by truncation or in frame deletion produces a receptor that is mistargeted from the TGN to the apical surface. More importantly, these 17 residues can be transplanted to a heterologous reported molecule and redirect the reporter to the basolateral surface.

A wide variety of other proteins are now thought to contain basolateral signals, i.e. mutations in their cytoplasmic domain cause the protein to mistargeted to the apical surface (reviewed in Mostov et al., 1992). One of the best studied examples is the low density lipoprotein receptor (LDLR), whose 50 amino acid cytoplasmic domain contains two independent basolateral targeting signals (Matter et al., 1992).

We have performed a detailed mutational and structural analysis of the basolateral targeting signal of the pIgR (Aroeti et al., 1993). Alanine scanning mutagenesis reveals that three residues (His656, Arg657, and Val660) are particularly important for the basolateral targeting signal. We have also synthesized a 17 amino acid peptide corresponding to the signal and analyzed its conformation in solution by two dimensional NMR spectroscopy. The peptide contains a type I ß-turn, encompassing residues 658-661, followed by a nascent helical structure.

The relationship of basolateral targeting signals to other types of sorting signals is intriguing (reviewed in Mostov et al., 1992). The best studied sorting signal is that for rapid internalization by clathrin-coated pits. These signals are generally a stretch of 4-6 amino acids and usually contain a tyrosine (or sometimes other aromatic amino acid) (Thomas and Roth, 1994). In at least three cases the structure of the endocytosis signal has been shown to be a type I-ß turn, with the tyrosine at the first or last (i.e. fourth) position of the turn (Trowbridge et al., 1993). For a number of proteins (although not the pIgR) the same short peptide segment seems to function as both a basolateral targeting signal and an internalization signal. Several of these signals have been analyzed by detailed mutagenesis and in almost every case it has been possible to find mutations that diminish one function (e.g. basolateral targeting) without affecting the other function (Matter et al., 1993; Prill et al., 1993; Thomas and Roth, 1994). Sorting signals that contain tyrosine and that are similar (or identical) to internalization signals have also been found to mediate other sorting events, such as delivery of membrane proteins to lysosomes and recycling of proteins to the TGN (for review see Thomas and Roth, 1994). We propose that the fundamental feature of all of these sorting signals is a type I ß-turn. Depending on the exact amino acid sequence the signal can serve as a basolateral targeting signal, internalization signal, TGN retrieval signal, lysosomal targeting signal, or some combination of these (and possibly other) functions. This hypothesis implies that these various ß-turns signals are recognized by similar proteins, which comprise part of the sorting machinery in the cell. So far there is good evidence that internalization signals are recognized by

the AP2 adapter proteins that are components of plasma membrane clathrin coated pits (Pearse and Robinson, 1990; Chang et al., 1993). Clathrin adapter proteins are structurally related to the «coatomer» protein complex, which forms a coat on vesicles traveling in the secretory pathway. It is likely that there is a family of such proteins, which recognize different subsets of these sorting signals. A candidate for a protein that recognizes the basolateral targeting signal of the vesicular stomatitis virus G protein has recently been identified (Pimplikar et al., 1994).

Recently, a second type of sorting signal has been discovered, which consists of the sequence LeuLeu. Occasionally other hydrophobic aliphatic residues can substitute for one of the leucines. Originally, this signal was implicated in targeting of proteins from the TGN to endosomes and lysosomes (Johnson and Kornfeld, 1992; Letourneur and Klausner, 1992). Recently, however, LeuLeu has also been shown to function in internalization and in basolateral targeting, so this signal can apparently serve all of the functions ascribed to tyrosine based signals (Hunziker and Fumey, 1994). The LeuLeu motif also serves to direct the MCH class II invariant chain to lysosomes (Odorizzi et al., 1994). So far there is no structural data on the conformation of LeuLeu. However, it has been suggested that LeuLeu may be contained in a ß-turn, and that it may resemble the crucial valine in the third position of the ß-turn in the basolateral signal of the pIgR (Hunzinker and Fumey, 1994).

Polarized sorting in endosomes

In most epithelial cells polarized sorting can potentially occur both in the TGN and in endosomes. Recent evidence suggests that sorting in both pathways is controlled by the same signals. For instance, the mutations in the pIgR basolateral signal described above decreased TGN to basolateral sorting and increase TGN to apical sorting. However, some molecules do reach the basolateral surface and are internalized. The mutations have the same effect on sorting in the endosomes as they do in the TGN, i.e. endosome to basolateral sorting (otherwise known as recycling) is decreased, while endosome to apical sorting (otherwise known as transcytosis) is increased (Aroeti and Mostov, 1994). Similar results have been observed in the LDLR, where mutations that weaken one of the basolateral targeting signals causes transcytosis of the mutant receptor (Matter et al., 1993). The basolateral targeting signals therefore appear to act as basolateral retrieval signals, ie. when a protein is internalized the signal directs it back to the basolateral surface. This would be analogous to retrieval signals found in various parts of the secretory pathway. In the case of the pIgR this signal can be inactivated by phosphorylation of a serine near the signal, which causes the molecule to be transcytosed to the apical surface (see below).

The conventional interpretation of these data is that the same basolateral signal acts in both the TGN and in endosomes, and implies that the machinery found in these two locations that recognizes the signal is similar or identical (Fig. 1, panel A). However, we cannot rule out a different (but not mutally exclusive) hypothesis that most or all the sorting takes place in one location. One possibility is that some proteins travel first from the TGN to the endosome before reaching the cell surface (Fig. 1, panel B). There is ample precedent for this, in that certain proteins, such as MHC class II molecules are delivered from the TGN to endosomes before reaching the surface (Odorizzi et al., 1994). An alternative possibility is that proteins are delivered from the endosome to the TGN, where they are sorted to the correct surface (Fig. 1, panel C). This seems less likely, as endocytosed receptors that have been desialylated at the cell surface are not detectably resialylated during trancytosis, whereas if these proteins had returned to the TGN, one would expect resialylation to occur (Matter et al., 1993; Aroeti and Mostov, 1994).

To add to the complexity, it now appears that soluble, secretory proteins use a different route from membrane proteins to travel from the TGN to the cell surface (Boll et al., 1991). In rat liver, two populations of TGN to basolateral surface vesicles have been isolated; one population is enriched in soluble, secretory proteins, while the other is enriched in newly made membrane proteins (Saucan and Palade, 1994).

So far, we have little direct data on other components of the machinery responsible for polarized traffic. Small GTPases of the rab family are crucial in almost all membrane traffic steps. Rab 8 is involved in TGN to basolateral delivery, and in the analogous TGN to cell body and dendritic delivery in neurons (Huber et al., 1993). An unidentified rab(s) appears to be involved in apical delivery. Loss of cell polarity by transformation down regulates rab 8 and several other rabs, and upregulates one unidentified rab proteins (Huber et al., 1994). Rab17 is an epithelial-specific rab and may be involved in trancytosis (Lütcke et al., 1993).

The mechanisms underlying polarity have been analyzed genetically in other systems, such as the budding yeast, *S. cerevisiae*. A recent insight may tie together polarity in epithelial cells and yeast. Transformation of MDCK cells by the ras oncogene causes a relatively selective loss of correct apical sorting of plasma membrane proteins, whereas basolateral sorting remains largely intact (Schoenenberger et al., 1991). Transformation by ras can usually be antagonized by rap1, another small GTPase. Mammalian rap1 is homologous to RSR1, a gene product in yeast that is involved in bud formation (Ruggieri, 1992). Taken together, these data raise the possibility that rap1/RSR1 are involved in apical polarity in mammalian cells, and in the plausibly homologous polarity of the bud in yeast.

The apical recycling compartment - A new location for polarized sorting

Studies using fluid phase markers have shown that such markers endocytosed from either the apical or basolateral surface enter separate apical or basolateral early endosomes, located underneath their respective surfaces (Bomsel et al., 1989). Fluid phase markers endocytosed at one surface do not enter the early endosomes underlying the opposite surface. A portion of the fluid phase markers endocytosed from both surfaces enters a common late endosome or prelysosome located deeper in the cell. Recent work has shown that the route taken by membrane bound markers is quite different and has given new insight into the pathway taken by transcytosing molecules, in particular the pIgR (Quintart et al., 1989; Apodaca et al., 1994a; Barroso and Sztul, 1994). Transcytosis of the pIgR can be divided into three experimentally distinguishable steps: 1) Internalization from the basolateral surface and delivery to basolateral early endosomes; 2) Transport to a tubulo-vesicular compartment, which is located immediately beneath the apical plasma membrane and is termed to the apical recycling compartment; 3) Transport from the apical recycling compartment to the apical plasma membrane.

The apical recycling compartment appears to be a central location for polarized sorting (Fig. 2). In addition to being a way-station for transcytosing pIgR, it also receives membrane-bound (but not fluid-phase) markers that have been endocytosed from the apical surface and that will recycle to that surface. Even more importantly, a significant portion of the transferrin (Tf) that has been endocytosed from the basolateral surface and that will recycle back to the basolateral surface reaches the apical recycling compartment (Hughson and Hopkins, 1990; Apodaca et al., 1994a). Therefore, this compartment receives membrane-bound material from both surfaces of the cell and correctly sorts these materials back to the correct surfaces. Delivery from basolateral early endosomes to the apical recycling compartment is greatly slowed by depolymerizing microtubules with nocodazole, indicating that this process is largely microtubule-dependent (Breitfeld et al., 1990; Hunziker et al., 1990). Moreover, the apical recycling compartment appears to be clustered around the microtubule organizing center, which in MDCK cells is immediately underneath the center of the apical plasma membrane and forms the basal body of a cilium. Depolymerization of microtubules with nocodazole also causes the apical recycling compartment to disperse throughout the apical region of the cytoplasm, indicating that microtubules are important in the organization of this compartment. The apical recycling compartment may contain rabs 18 and 20 (Zerial, personal communication), which may be essential to its function.

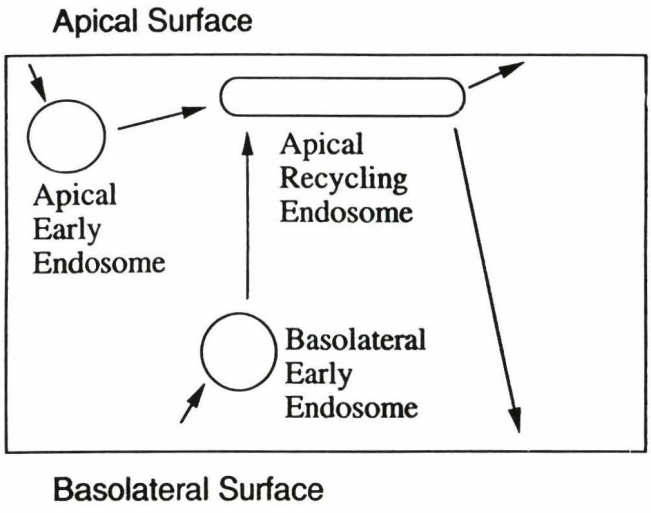


Fig. 2. The apical recycling compartment. The apical recycling compartment receives endocytosed proteins coming from both apical early endosomes and basolateral early endosomes. The apical recycling compartment then sorts the proteins to the correct destination.

In some less well polarized cells, such as CHO cells, recycling transferrin is found in a pericentriolar, perinuclear compartment, termed the recycling compartment (Mayor et al., 1993). We suggest that the apical recycling compartment is the polarized cell homologue of this recycling compartment. An obvious differences is that in polarized epithelial cells the microtubule organizing center moves away from the nucleus to a position underneath the apical plasma membrane. This is consistent with the notion that many of the pathways involved in cell polarization pre-exist in non-polarized cells and are modified as cell polarity develops (see below).

Regulation of polarized membrane traffic

Classically, most secretory and endocytotic pathways were considered to be constitutive, that is they had a constant rate, not particularly affected by outside influences. The major exceptions were dense core regulated secretory granules and synaptic vesicles, whose rapid release is triggered by secretagogues or neurotransmitters. Secretoy granules are often found in polarized epithelial cells, such as the exocrine pancreas or goblet cells. Synaptic vesicles are generally found in neurons, although similar vesicles are found in some endocrine cells.

Recent evidence indicates that «constitutive» traffic is actually subject to regulation at multiple levels (Bomsel and Mostov, 1992). One of the earliest indications was the finding that basolateral secretion of soluble proteins by cultured kidney cells was stimulated by inhibition of a pertussis toxin-sensitive heterotrimeric G protein, probably Gi (Stow et al., 1991). Subsequently, it was observed that activation of the heterotrimeric Gs protein stimulated TGN to apical delivery in MDCK cells (Pimplikar and Simons, 1993). Similarly, activation of Gs stimulated basolateral to apical transcytosis of the pIgR, as well as bidirectional transcytosis of non-specific markers, such as ricin (Bomsel and Mostov,

1993; Hansen and Casanova, 1994). However, transcytosis of $(Fab')_2$ fragments of polyclonal antibodies directed against the SC portion of the pIgR are inhibited by activation of Gs (Barroso and Sztul, 1994). Classically, activation of Gs causes activation of adenylate cyclase, production of cAMP, and activation of the cAMP dependent kinase, protein kinase A (PKA), although Gs can also directly act on other signaling pathways. Very recently it was shown that directly increasing the intracellular concentration of cAMP stimulates TGN to apical delivery as well as transcytosis of pIgR and ricin (Eker et al., 1994; Hansen and Casanova, 1994; Pimplikar and Simons, 1994). These effects are inhibited by H89, which is thought to be a relatively specific inhibitor of PKA, implying that PKA is involved. Although it is quite likely that the effects of Gs activation are at least in part due to production of cAMP, it is not yet clear if Gs may have some cAMP-independent effects on traffic.

Activation of protein kinase C (PKC) by phorbol esters also stimulates TGN to apical delivery, as well as basolateral to apical transcytosis of pIgR and transferrin (Cardone et al., 1994; Pimplikar and Simons, 1994). PKC is a family of kinases, and in MDCK cells it appears that the α and/or ε forms are responsible, at least for pIgR transcytosis. The target of PKC is not known. However, it has recently been reported that PKC activation enhances binding of coat proteins to the Golgi and trafficking through the Golgi (Luini and De Matteis, 1993).

For both PKA and PKC it appears that a principal site of stimulation of transcytosis is delivery from the apical recycling compartment to the apical plasma membrane (Cardone et al., 1994; Hansen and Casanova, 1994). The apical recycling compartment may therefore be a principal site where membrane traffic, especially to the apical surface, is regulated. We suggest that this may be generally true for many cell types. For example cells in the collecting duct of rat kidney and the toad urinary bladder respond to antidiuretic hormone by transporting water channels from subapical vesicles to the apical plasma membrane, and then retrieving the channels when the hormone is withdrawn (Zeidel et al., 1992). Adipocytes (not obviously epithelial-like) respond to insulin by increasing plasma membrane delivery of glucose transporters from a compartment that may be homologous to the apical recycling compartment (James et al., 1994). Although glucose transporter movement is specific for insulin-responsive tissues, many cells increase recycling of other molecules (e.g. transferrin receptors) to the cell surface in response to hormones.

Activation of Gs or a direct increase in cAMP also leads to an increase in endocytosis specifically from the apical surface, whereas basolateral endocytosis is largely unaffected (Eker et al., 1994). This suggests that all of the steps in membrane traffic between the apical surface and the apical recycling compartment are coordinately regulated by PKA (and perhaps by PKC?). Apical endocytosis, but not basolateral endocytosis, is specifically inhibited by cytochalasin (Gottlieb et al., 1993), which is consistent with apical endocytosis having unusual properties. Clathrin coated pits at the apical side seem to be very slow to bud off (Roth, personal communication); this could be a point of regulation. It is not known what the relevant target(s) of the PKA is, but one possibility is the cystic fibrosis transmembrane conductance regulator, which is a PKA-regulated chloride channel (Bradbury et al., 1992). Increased activity of this channel increases apical endocytosis and recycling. Mastoporan, a peptide toxin that can stimulate some heterotrimeric G proteins as well as many other targets, stimulates apical endocytosis without raising cAMP, implying that some other regulatory mechanism also exists (Eker et al., 1994).

Regulation of transcytosis of the pIgR

In addition to the general regulation of apical traffic by PKA and PKC, transcytosis of pIgR is regulated by several additional mechanisms that are more specific for this molecule. First, phosphorylation of Ser664 in the basolateral targeting signal stimulates transcytosis, apparently by inactivating the signal (Casanova et al., 1990). Mutation of this residue to an alanine (pIgR-A664) decreases transcytosis and increases recycling. Conversely, mutation to an aspartic acid, whose negative charge might mimic the phosphoserine, increases trancytosis, decreases recycling, and also increases TGN to apical delivery. The pIgR-A664 is slowed both in movement from the basolateral early endosome to the apical recycling compartment, and from the apical recycling compartment ot the apical surface (Song and Mostov, unpublished). This is consistent with the idea that basolateral retrieval of the pIgR and other proteins (e.g. transferrin) operates at both of these stages. Second, binding of the ligand, dIgA, to the pIgR stimulates transcytosis. It was first reported that dIgA binding to the pIgR-A664 mutant stimulated trancytosis (Hirt et al., 1993), and it was subsequently found that transcytosis of the wild-type pIgR is also stimulated (Song et al., 1994). Although either phosphorylation or ligand binding provides some stimulation, maximal transcytosis is obtained only when both are present. Both signals are probably physiologically relevant. Regulation by dIgA binding insures that if the amount of dIgA presented to a cell is increased (e.g. in response to infection) all of the dIgA will be quickly transported across the cell to the site where it is needed. Phosphorylation of Ser664 is the only mechanism whereby the pIgR can be transported without dIgA bound (Mostov, 1994). Many mammalian secretions contain a considerable excess of «free» SC, i.e. SC that is not bound to dIgA. This free SC must have resulted from trancytosis of pIgR without dIgA bound. SC binding to dIgA protects the dIgA against proteolysis in secretions. Presumably, a larger excess of free SC drives its binding to dIgA, affording a greater degree of protection. We do not know the kinase involved or how it is regulated, but phosphorylation provides the

organism with a way to regulate the amount of free SC and thus the stability of the dIgA.

The finding that dIgA binding to the pIgR stimulates transcytosis alters the classic view that the pIgR is constitutively transcytosing, and leads us to consider that the pIgR is a signaling receptor. In fact recent data from our laboratory suggests that the pIgR uses a conventional transmembrane signaling pathway (Cardone and Mostov, unpublished). Binding of dIgA to pIgR causes a rapid activation of PKC, and the production of inositol 1,4,5-triphosphate (IP3). This implies that the pIgR activates a phosphatidylinositol phosphate specific phospholipase C. The PKC stimulates transcytosis, as described above. The IP3 is predicted to lead to an increase in intracellular free Ca^{2+}. Ca^{2+} probably has many effects on membrane traffic, but one effect may be specific for the pIgR: we have found that in the presence of Ca^{2+}, calmodulin binds to the basolateral targeting signal of the pIgR (Enrich and Chapin, unpublished). We suggest that when dIgA binds, the resultant Ca^{2+} signal causes calmodulin to bind to and sequester the basolateral signal, thereby allowing trancytosis to occur. We hypothesize that these signaling pathways occur in the apical recycling compartment, which is consistent with the observation that IP3 dependent calcium release starts in the apical region of polarized epithelial cells (Berridge and Dupont, 1994). Calmodulin is probably also involved in other roles in polarized membrane traffic. We recently found that inhibition of calmodulin by specific drugs blocks transcytosis of pIgR, recycling of transferrin, and causes all material endocytosed from both surfaces to be delivered to exceptionally large, novel endosomal structures (Apodaca et al., 1994b).

Finally, it should be noted that internalization of the pIgR is determined by two tyrosine-based internalization motifs that are similar to such signals found in other receptors. Surprisingly, internalization also is stimulated more than three-fold by phosphorylation of Ser726, the second major site of phosphorylation of the pIgR (Okamoto et al., 1994). This may be a novel mechanism to regulate internalization. Contrary to a previous report (Hirt et al., 1993), phosphorylation of Ser726 does not appear to be involved in polarized sorting of the pIgR.

Why regulate traffic?

Taken together, all of these recent results indicate that «constitutive» membrane traffic is actually subject to many levels of regulation (Bomsel and Mostov, 1992). We can speculate that there are at least three broad classes of underlying reasons for this regulation.

First, the cell must maintain the steady-state size and composition of each of its membranous compartments, including the apical and basolateral surfaces. This requires continual regulation of all traffic to and from each compartment. One general way to do this is to monitor specific «cargo» molecules traveling through each pathway. If the amount of cargo builds up in a particular location, traffic out of a compartment is stimulated. A model of this process might be the stimulation of transcytosis of pIgR by binding of the cargo dIgA.

Second, the cell must respond to outside signals, such as hormones, neurotransmitters, and growth factors. Both "constitutive" and classically regulated secretory pathways must be regulated. For instance, as a cell grows the size and/or number of each membranous compartment (e.g. the plasma membrane) must increase.

Third, differentiated phenotypes may require active regulation of specialized membrane traffic pathways. As mentioned above, it is possible that many of the pathways that seem specific for differentiated, polarized cells may preexist in less-well differentiated cells. As the cells differentiates these pathways may be modified, in part by using intracellular signaling systems. This also provides the cell with a way to flexibly tailor its polarity, especially as it participates in complex morphological process. For example, when MDCK cells are exposed to hepatocyte growth factor (also known as scatter factor) the cells transiently become partially unpolarized, and then reorganize themselves into tubular structures. This transient loss of polarity could be regulated by intracellular signaling systems, such as those described in this review.

Finally, we suggest that the process of membrane traffic and signaling are much more tightly interconnected that has been traditionally appreciated. One aspect that is rapidly becoming apparent is that the specific intracellular localization of many signaling molecules is crucial to their correct function. For instance, many signaling molecules, including several heterotrimeric G proteins and cytoplasmic tyrosine kinases, can be localized to caveolae (Lisanti et al., 1994). The localization of some of these molecules is determined by post-translational modification with fatty acids.

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