# Regulation of Protein Traffic in Polarized Epithelial Cells: The Polymeric Immunoglobulin Receptor Model

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Most eukaryotic cells are spatially asymmetric or polarized. One of the most basic types of polarized cell organization found in multicellular animals is the epithelial cell (for review, see Mostov et al. 1992; Rodriguez-Boulan and Powell 1992; Mostov and Cardone 1995). Epithelial cells form sheets that line cavities, or they externally cover an organism. In this manner, these cells constitute the fundamental interface between the organism and the outside world. A simple epithelium is formed by a monolayer of cells. A circumferential tight junction divides the plasma membrane of each cell into two domains: The apical plasma membrane faces the lumen of the cavity, and the basolateral plasma membrane faces adjoining cells and the underlying basement membrane and connective tissue. These two domains of the plasma membrane differ in their protein and lipid compositions, reflecting the different functions served by the two surfaces in a polarized cell.

Newly synthesized plasma membrane proteins can be targeted to the correct surface by a direct or indirect route. In the direct route, the proteins pass through the Golgi apparatus and the *trans*-Golgi network (TGN) and are then targeted directly to either the apical or basolateral surface. Direct TGN-to-apical targeting does not occur in certain types of cells, such as hepatocytes. In the indirect route, proteins are first targeted to one surface, which is usually the basolateral surface. Subsequently, they are endocytosed and then either recycled back to the basolateral surface or transcytosed to the apical surface. Unlike direct TGN-to-apical targeting, indirect targeting to the apical surface via transcytosis occurs in every type of epithelial cell examined to date. In some types of cells, such as hepatocytes, indirect targeting via transcytosis is the only pathway for plasma membrane proteins to reach the apical surface. The steady-state concentration of a protein at a given surface depends not only on its rate of delivery, but also on its rate of removal from that surface (Hammerton et al. 1991).

As a model system for studying these trafficking events, we use the polymeric immunoglobulin receptor (pIgR) (for review, see Mostov 1994). This receptor is expressed in a wide variety of epithelial cells, such as those lining the gastrointestinal, respiratory, and genitourinary tracts. Newly synthesized pIgR is first sent from the TGN to the basolateral surface (Fig. 1). At the basolateral surface, pIgR can bind its ligand, dimeric IgA (dIgA), and in some species, pentameric IgM. Whether or not pIgR binds its ligand, this receptor is then endocytosed and transcytosed to the apical surface. At the apical surface, the extracellular, ligand-binding domain of the pIgR is cleaved and released either alone or together with the dIgA. This released fragment of the pIgR is called secretory component (SC). SC remains bound to the polymeric immunoglobulin and helps to protect it against degradation.

We have expressed the cloned cDNA for pIgR in Madin-Darby canine kidney (MDCK) cells, which form a well-polarized monolayer in culture. The transfected pIgR behaves in the MDCK cells as in vivo and serves as a useful model to study protein trafficking.

## RESULTS AND DISCUSSION

### Basolateral Sorting Signal of the pIgR

In a systematic effort to understand the signals that direct the pIgR during its complex pathway through the cell, we have made several mutations in the cytoplasmic domain of the pIgR (Fig. 2). Until recently, it was hypothesized that sorting of membrane proteins to the apical surface required a sorting signal, whereas sorting to the basolateral surface did not (for review, see Simons and Wandinger-Ness 1990). Studies on the pIgR have shown that basolateral sorting requires a sorting signal in the cytoplasmic domain of the membrane protein (Casanova et al. 1991a,b; Mostov et al. 1992). The pIgR spans the membrane once and contains a carboxy-terminal, cytoplasmic domain of 103 amino acids. The 17 amino acids of this cytoplasmic domain that lie closest to the membrane comprise a signal that is necessary and sufficient for targeting of the pIgR from the TGN to the basolateral surface. This 17-residue segment can be transplanted to a heterologous reporter molecule and direct the delivery of this molecule to the basolateral surface. Studies on other basolaterally targeted proteins have demonstrated that mutations in their cytoplasmic domain can

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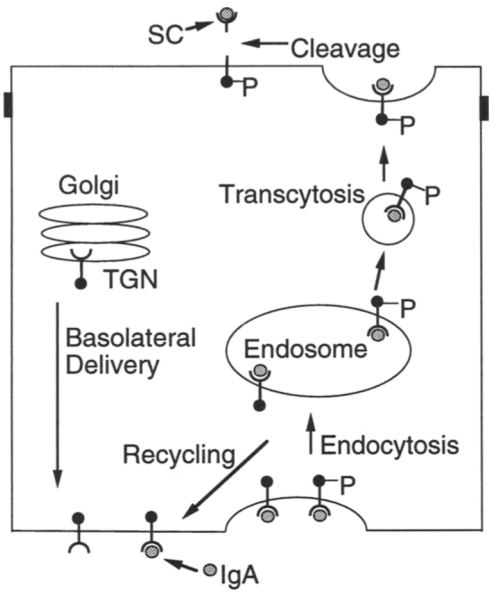


**Figure 1.** Pathway of pIgR through an epithelial cell. A simplified epithelial cell is illustrated, with the apical surface at the top and basolateral surface at the bottom.

cause mistargeting to the apical surface, presumably by inactivation of a basolateral signal (for review, see Mostov et al. 1992). One particularly interesting example is the LDL receptor, whose cytoplasmic domain contains two basolateral targeting signals. It has recently been shown that these signals can be transplanted to a heterologous reporter molecule, causing re-targeting of the chimera to the basolateral surface (Matter et al. 1993, 1994). This result constitutes very strong evidence that, like the pIgR, the cytoplasmic domain of the LDL receptor also contains basolateral sorting signals.

Analysis of the pIgR's 17-residue basolateral sorting signal by alanine scanning mutagenesis indicates that three residues appear to be crucial for basolateral sorting (Fig. 3) (Aroeti et al. 1993). In addition, the three-dimensional solution structure of a synthetic peptide corresponding to this 17-residue signal has been determined by nuclear magnetic resonance spectroscopy. One of the three crucial residues (Val-660) is in position 3 of a type 1 β turn, whereas the other two crucial residues (His-656 and Arg-657) precede the turn. This β turn is particularly interesting because signals for clathrin-mediated endocytosis (and probably for several other sorting events) generally contain a type 1 β turn (Mostov et al. 1992). Usually a tyrosine residue occupies position 1 or 4 of the turn. We suggest that a type 1 β turn is a conserved feature of a large family of sorting signals. Depending on the particular sequence, the signal might serve one or more functions. For example, in several proteins (other than the pIgR) the same short segment of amino acids can function as both a basolateral sorting signal and a signal for clathrin-mediated endocytosis. However, it should be noted that basolateral signals in different proteins do not fit into an obvious consensus motif, so the rules that determine if a given type 1 β turn can function as a basolateral sorting signal are not yet clear.

Polarized sorting occurs in both the biosynthetic (TGN) pathway and in the endocytotic pathway. We found that the 17-residue basolateral sorting signal controls basolateral sorting in both pathways (Aroeti and Mostov 1994). Alanine point mutations that decrease TGN-to-basolateral sorting and correspondingly

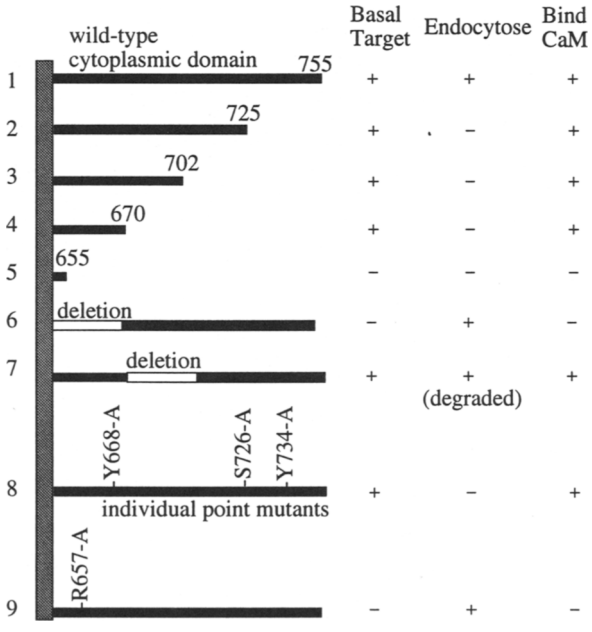


**Figure 2.** Summary of mutations in the cytoplasmic domain of the pIgR. The 103-amino-acid cytoplasmic domain is depicted as projecting from the membrane at the left. The ability of each mutant to be targeted from the TGN to the basolateral surface, to be rapidly endocytosed, or to bind CaM is indicated.

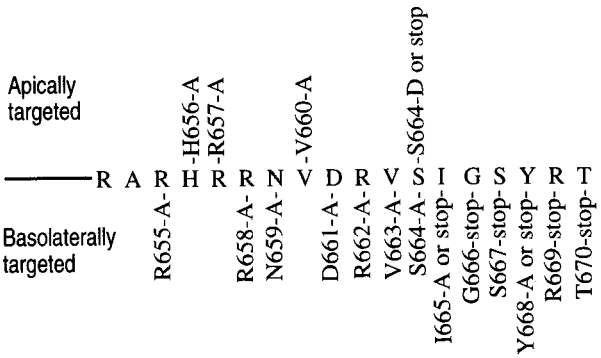


**Figure 3.** Summary of point mutations in the 17-residue basolateral targeting signal. The amino acid sequence of this signal is given in the single letter code. Point mutations or truncations that result in apical targeting are illustrated above, and mutations that result in basolateral targeting are indicated below.

increase TGN-to-apical sorting have the same effect on sorting that occurs after endocytosis; i.e., these point mutations decrease recycling in the endocytotic pathway back to the basolateral surface, and correspondingly increase transcytosis to the apical surface. Similar results have been obtained from studies with the LDL receptor system (Matter et al. 1993). These data suggest that polarized sorting in both the biosynthetic and endocytotic pathways might occur in the same location (Fig. 4). However, we hypothesize that molecules are first delivered from the TGN to some compartment in the endosomal system and are then sorted to the basolateral surface. This is consistent with previous observations that several other classes of molecules, including lysosomal enzymes and MHC class II proteins, are delivered from the TGN to endosomes.

### The Pathway of Transcytosis

Until recently, polarized epithelial cells were thought to contain separate populations of basolateral and apical early endosomes (Bomsel et al. 1989). Fluid-phase markers endocytosed from the apical or basolateral surface entered their respective early endosomes and then recycled to the original surface. A fraction of each fluid-phase marker was transferred to a common pre-lysosomal compartment. However, no direct connection was believed to exist between apical and basolateral early endosomes. The pIgR that is undergoing transcytosis was thought to be sorted in basolateral early endosomes into vesicles that directly delivered the pIgR to the apical surface, bypassing the apical early endosome.

We have carefully analyzed the transcytosis of pIgR and found that this model is not accurate (Apodaca et al. 1994a,b). Our results from both biochemical and morphological methods indicate that pIgR transcytosis can be divided into at least three steps (Fig. 5). In step 1, pIgR undergoes clathrin-mediated endocytosis from the basolateral plasma membrane to basolateral early endosomes. In step 2, a microtubule-dependent event transports pIgR to a tubular compartment located immediately beneath the center of the apical plasma membrane. We call this tubular compartment the apical recycling compartment. In step 3, pIgR is delivered from the apical recycling compartment to the apical plasma membrane. The apical recycling compartment appears to play a critical role in polarized sorting. This compartment receives very little fluid-phase material from either surface of the cell, which may be the reason it was not detected in earlier studies on the

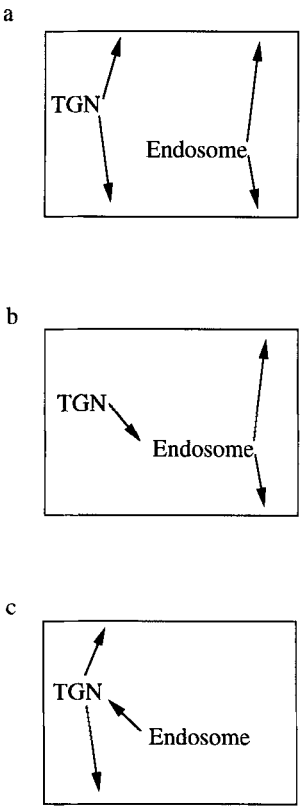


**Figure 4.** Possible locations for polarized sorting. Panel *a* is the conventional view that sorting in the exocytotic pathway occurs in the TGN, and sorting in the endocytotic pathway occurs in endosomes. Panel *b* illustrates an alternative view that appears likely in light of very recent data. In this model, proteins travel from the TGN to the endosomal pathway, where they are sorted to the correct surface. Panel *c* presents a related possibility that endocytosed proteins are delivered from the endosomal pathway to the TGN for polarized sorting. There is little evidence to support this last possibility.

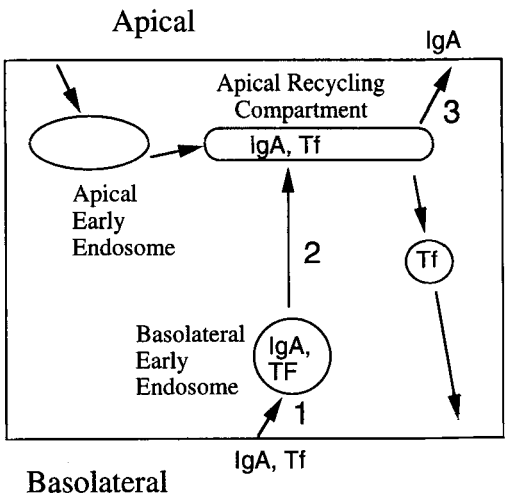


**Figure 5.** Pathway of transcytosis of pIgR through the apical recycling compartment. In step 1, IgA bound to the pIgR and transferrin (Tf) bound to its receptor are endocytosed from the basolateral surface and delivered to basolateral early endosomes. In step 2, both ligands and their receptors are delivered to the apical recycling compartment. Apically endocytosed membrane proteins are delivered to apical early endosomes and then to the apical recycling compartment. Receptors and ligands are sorted in the apical recycling compartment, so that in step 3, IgA and pIgR are delivered to the apical surface.

endocytosis of fluid-phase markers. In contrast to fluid-phase markers, membrane-bound proteins endocytosed from either surface of the cell are delivered to this apical compartment, where they can be sorted for transport to the apical or basolateral surface. For instance, transferrin is endocytosed by its receptor from the basolateral surface and very efficiently recycles to that surface. It was previously assumed that recycling occurred entirely from the basolateral early endosomes. Although some transferrin may recycle from the basolateral early endosomes, we find that a significant fraction reaches the apical recycling compartment before recycling to the basolateral surface. This result shows that the apical recycling compartment is able to perform polarized sorting to either surface, directing transferrin to the basolateral surface and pIgR to the apical surface.

In nonpolarized cells, such as Chinese hamster ovary (CHO) cells, transferrin is sent from peripheral early endosomes to a pericentriolar recycling compartment before returning to the plasma membrane (Mayor et al. 1993). We suggest that the apical recycling compartment in the polarized cell is the equivalent of this pericentriolar recycling compartment seen in nonpolarized CHO cells. In CHO cells and many other nonpolarized cells, the centriole and the pericentriolar recycling compartment are located very close to the nucleus. This can make it difficult to distinguish the pericentriolar recycling compartment from other organelles that are often near the nucleus, such as the TGN and late endosomes. In contrast, in MDCK cells and many other polarized epithelial cells, the centriole is located immediately underneath the center of the apical plasma membrane, where the centriole can form the basal body of a cilium. The apical recycling compartment tends to cluster around the centriole and is therefore clearly distant from the basally located nucleus and other organelles that cluster near the nucleus, such as the TGN.

The organization of the apical recycling compartment around the centriole is dependent on microtubules (Apodaca et al. 1994b). Depolymerization of the microtubules with nocodazole causes this compartment to disperse throughout the apical portion of the cytoplasm. Protein traffic to both the apical and basolateral surfaces is slowed to varying degrees by nocodazole, suggesting that microtubules play a role in delivery to both surfaces (Breitfeld et al. 1990).

The polarized organization of the endocytotic pathway also depends on calmodulin (CaM). Treatment of MDCK cells with CaM antagonists, such as W13, causes fluid-phase and membrane-bound material endocytosed from both surfaces of the cell to be delivered to a common compartment, consisting of abnormally large vesicles (Apodaca et al. 1994a). This phenomenon appears to be the result of fusion of apical and basolateral early endosomes, a process that is normally prevented by a CaM-mediated process.

### Regulation of Transcytosis

One can imagine several reasons that virtually all membrane traffic events appear to be tightly regulated (Bomsel and Mostov 1992; Mostov and Cardone 1995). First, homeostatic maintenance of the size and composition of each compartment requires that traffic into and out of the compartment be balanced. Second, membrane traffic must be altered as the cell grows, divides, and responds to changing extracellular conditions and signals. Third, differentiation of the cell requires alterations in membrane traffic.

Transcytosis of the pIgR is regulated at multiple levels and provides an excellent model to study the regulation of membrane traffic (Fig. 6). The internalization of pIgR from the basolateral plasma membrane (step 1 of transcytosis) is regulated by phos-

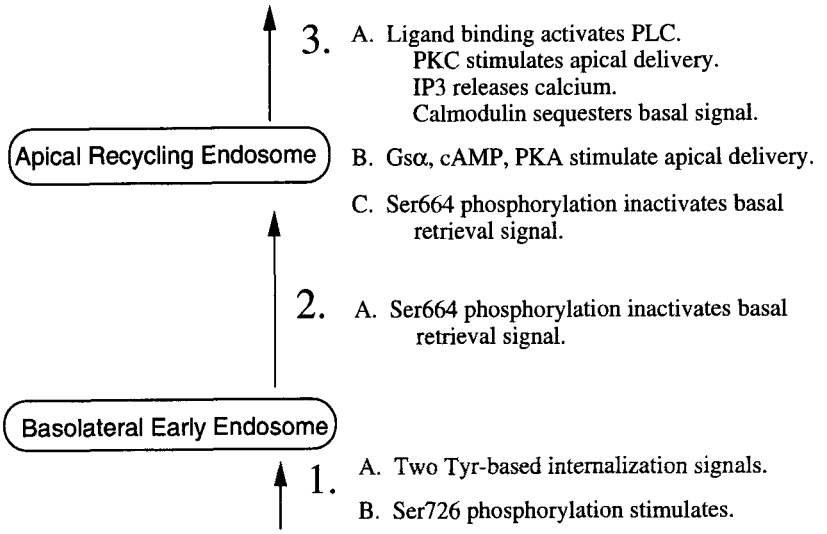


**Figure 6.** Regulation of pIgR transcytosis at each of the three steps of transcytosis.

phorylation (Okamoto et al. 1994). The pIgR contains two tyrosine-based internalization signals, which resemble internalization signals found in other rapidly endocytosed receptors (Okamoto et al. 1992). However, phosphorylation of Ser-726, which is distantly located from both of these tyrosine-based signals, is required for rapid endocytosis of the pIgR. It is possible that phosphorylation of Ser-726 alters the folding of the cytoplasmic domain of the pIgR, thereby exposing one of the tyrosine-based internalization signals to the endocytotic machinery.

From both the biosynthetic and endocytotic pathways, the pIgR basolateral sorting signal helps direct the pIgR to the basolateral surface. This sorting signal can be thought of as a "basolateral retrieval signal," analogous to the KDEL/HDEL signal for retrieval of proteins to the endoplasmic reticulum (ER). If this basolateral retrieval signal were permanently active, the pIgR might continuously recycle to the basolateral surface and never be transcytosed to the apical surface. However, we have found at least three mechanisms that promote transcytosis. First, Ser-664, which is located in the 17-residue basolateral sorting signal, is phosphorylated (Casanova et al. 1990). Mutation of Ser-664 to a nonphosphorylatable alanine decreases transcytosis and increases recycling. Mutation to an aspartic acid, whose negative charge may mimic a phosphate, increases transcytosis and also increases TGN-to-apical sorting. (The effect of this aspartic acid mutation is very similar to the alanine scanning mutations described above that weaken the basolateral targeting signal.) We suggest that the nonphosphorylated pIgR is initially targeted to the basolateral surface. Once reaching that surface (or perhaps after endocytosis), the pIgR is phosphorylated on Ser-664, thereby weakening the basolateral sorting signal and allowing the pIgR to be transcytosed. Phosphorylation of the pIgR seems to stimulate both steps 2 and 3 of transcytosis, which suggests that the basolateral sorting signal may work to basolaterally recycle pIgR from both the basolateral early endosome and the apical recycling compartment.

The second major control mechanism for transcytosis is binding of the ligand, dIgA (Song et al. 1994b). Although a significant rate of transcytosis occurs when the pIgR is not bound to its ligand, binding of dIgA augments this rate of transcytosis. This stimulation does not depend on phosphorylation of Ser-664. In fact, because of the low baseline rate of transcytosis of the Ala-664 mutant without dIgA bound, binding of dIgA to this mutant gives a proportionately greater stimulation of transcytosis. However, maximal transcytosis of pIgR is achieved only when dIgA is bound and phosphorylation of Ser-664 occurs.

The finding that dIgA binding to the pIgR stimulates transcytosis suggests that the pIgR is capable of transducing a signal across the plasma membrane to the cytoplasmic sorting machinery (Fig. 7). We have recently found that binding of dIgA to the pIgR very rapidly causes the tyrosine-phosphorylation of several proteins, including a phosphatidylinositol-specific phospholipase Cγ1 (PLCγ1). This enzyme causes hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) to diacylglyceride (DAG) and inositol 1,4,5-trisphosphate (IP3). The DAG in turn leads to activation of protein kinase C (PKC). We have previously shown that activation of PKC by phorbol esters stimulates transcytosis, so it is likely that activation of PKC by dIgA binding to pIgR also stimulates transcytosis (Cardone et al. 1994).

The production of IP3 probably causes the release of $Ca^{++}$ from intracellular stores and an increase in in-

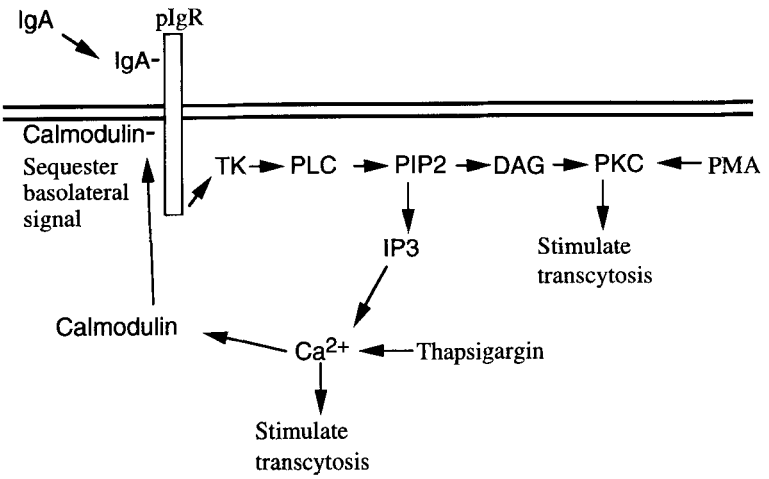


**Figure 7.** A model for how IgA binding to the pIgR activates intracellular signaling pathways and stimulates transcytosis. When IgA binds to the pIgR, the pIgR interacts with a tyrosine kinase (TK). The tyrosine kinase phosphorylates a phosphatidylinositol-specific phospholipase Cγ1 (PLC), which then hydrolyzes PIP2, yielding DAG and IP3. The DAG activates PKC, which stimulates transcytosis. The IP3 causes an increase in $[Ca^{++}]_i$, which activates CaM. The CaM binds to and sequesters the basolateral targeting signal of the pIgR. This may promote transcytosis and/or act as a negative feedback inhibition of signaling.

tracellular free $Ca^{++}$ ($[Ca^{++}]_i$). Artificially increasing $[Ca^{++}]_i$ with the drug thapsigargin rapidly stimulates transcytosis, so it is likely that the increase in $[Ca^{++}]_i$ caused by dIgA binding to pIgR also stimulates transcytosis. The increased $[Ca^{++}]_i$ has many possible targets, including CaM. Binding of $Ca^{++}$ to CaM could stimulate transcytosis in several possible ways. Recently, we found that CaM binds directly to the 17-residue basolateral sorting signal of the pIgR (Apodaca et al. 1994a). Binding is highly specific and completely dependent on $Ca^{++}$. However, a detailed analysis of CaM binding to various point mutations in the basolateral signal indicates that the requirements for basolateral sorting are not precisely the same as the requirements for CaM binding. We suggest, instead, that CaM binding may regulate transcytosis. One possibility is that CaM binding masks the basolateral signal, preventing it from interacting with the basolateral sorting machinery and thereby allowing the pIgR to be transcytosed. A non-mutually exclusive possibility is that CaM binding prevents the pIgR from causing the tyrosine-phosphorylation of PLCγ1 and thus provides a negative feedback control on signaling.

The stimulation of transcytosis by dIgA binding, activation of PKC, or increase in $[Ca^{++}]_i$ all seem to act on step 3 of transcytosis, i.e., the delivery from the apical recycling compartment to the apical plasma membrane (Song et al. 1994a). One partial explanation for this is that the IP3-sensitive pool of $Ca^{++}$ may reside primarily in organelles in the apical region of the cytoplasm.

A third mechanism for regulating transcytosis comprises extracellular signals other than dIgA. For instance, binding of bradykinin or cholinergic drugs to their receptors stimulates transcytosis, possibly by activation of PKC and increasing $[Ca^{++}]_i$. Activation of the heterotrimeric $G_s$ protein also stimulates transcytosis, as well as delivery from the TGN to the apical surface (Bomsel and Mostov 1993). Part of this stimulation is apparently due to activation of adenylate cyclase by $G_s$. However, in a perforated cell system that reconstitutes a portion of the transcytotic pathway, $G_s$ appears to control transcytosis by a mechanism that is independent of adenylate cyclase and cAMP. For instance, the Gβγ subunit acts synergistically with the $G\alpha_s$ subunit to promote transcytosis. Depletion of $G\alpha_s$ from the cytosol inhibits transport. Most surprisingly, replacement of the depleted $G\alpha_s$ with recombinant $G\alpha_s$ is effective only when the recombinant $G\alpha_s$ is in the GDP-bound form. These results suggest that the Gαβγ heterotrimer plays an essential role in transcytosis. The heterotrimer may promote binding of a coat to vesicles, perhaps via an interaction with ARF.

## CONCLUSIONS

The pIgR provides an excellent model for analyzing the regulation of membrane traffic in polarized epithelial cells. In particular, our scheme for signaling by the pIgR provides an example of how traffic can be regulated by using well-known signaling pathways. Transport of the pIgR may be further regulated by other signaling pathways that have not yet been investigated. Many other receptors (e.g., for EGF, PDGF, and IgG) transduce ligand-dependent signals across the membrane. Although these receptors are generally regarded as having primarily a signaling function, the trafficking of these receptors is also influenced by ligand binding. In contrast, the pIgR is classically considered as a trafficking receptor, designed for ferrying its cargo, dIgA, across the cell. However, it is clear that the pIgR also functions as a signaling receptor, so the distinction between receptors specialized for signaling and those specialized for trafficking becomes somewhat artificial.

Virtually every membrane-trafficking step in the cell is highly regulated, yet several ligands, receptors, and signaling pathways remain largely unknown. We suggest that trafficking of many of these other molecules may be regulated by binding of ligand to a receptor, in a fashion similar to that of the pIgR. In some cases, the ligand may be cargo moving through a particular trafficking pathway, e.g., the secretory or endocytotic pathway, analogous to dIgA moving through the transcytotic pathway (Bomsel and Mostov 1992). Regulation by cargo binding to a receptor offers an attractive mechanism to maintain the homeostasis of each compartment. The high degree of regulated movement of the pIgR illustrates how membrane traffic processes are closely intertwined with the regulatory and signaling mechanisms of the cell.

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