An Anchor-minus Form of the Polymeric Immunoglobulin Receptor is Secreted Predominantly Apically in Madin–Darby Canine Kidney Cells

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Abstract. The polymeric immunoglobulin receptor is expressed in a variety of polarized epithelial cells. Newly made receptor travels first to the basolateral surface. The receptor is then endocytosed, transported across the cell in vesicles, and exocytosed at the apical surface. We have now deleted the membrane spanning and cytoplasmic portions of the receptor by site-directed mutagenesis, thus converting the receptor to a secretory protein. When expressed in polarized Madin–Darby canine kidney (MDCK) cells the truncated protein is secreted at both surfaces, with a ratio of apical-to-basal secretion of 3.4. In contrast, when the exogenous secretory protein chicken lysozyme is expressed in these cells, it is released at both sides with a ratio of apical-to-basal secretion of 0.43. (Koder-Koch, C., R. Bravo, S. Fuller, D. Cutler, and H. Garoff, 1985, *J. Cell Biol.*, 43:297–306). Lysozyme is thought to lack a signal that targets it to one surface or the other, and so its secretion may represent a default, bulk flow pathway to both surfaces. When compared with lysozyme, the truncated polymeric immunoglobulin receptor is preferentially secreted apically by a factor of 3.4:0.43 or 7.8. We suggest that the lumenal portion of the polymeric immunoglobulin receptor contains a signal that targets it to the apical surface.

In polarized epithelial cells, the plasma membrane is divided into distinct apical and basolateral domains (reviewed in references 13 and 24). Many plasma membrane proteins are located exclusively at one surface or the other. It has been shown that at least in some cases newly synthesized plasma membrane proteins are sent directly from the Golgi apparatus to the appropriate surface (3, 14, 15, 21). Proteins destined for the two surfaces traverse the Golgi stacks together and are segregated only at the level of the *trans*-Golgi network (7, 10, 22). Moreover, many secretory proteins produced by polarized cells are released exclusively at one surface or the other (9, 12), and these are presumably also sorted in the *trans*-Golgi network.

How are these proteins targeted to the correct surface? A plausible model is that receptors in the *trans*-Golgi network recognize structural features or signals of the protein and direct it to the proper location (2, 13). Certain proteins may not contain any such signals. For instance, when a variety of secretory proteins (such as lysozyme, growth hormone, prochymosin, $\alpha 2\mu$ globulin, or immunoglobulin κ chain) are expressed in polarized Madin–Darby canine kidney (MDCK) cells which normally do not produce these proteins, the proteins are released in roughly equal amounts at both surfaces (9, 12). A likely interpretation is that these proteins are not recognized by receptors that direct them to a particular surface. Instead, they are randomly packaged by default into vesicles leaving the *trans*-Golgi network for both cell surfaces (13). The relative secretion of these proteins at the two surfaces would then reflect the relative total volume of the vesicles traveling to the two surfaces. The approximately equal secretion apically and basolaterally suggests that the volumes of vesicles traveling to the two surfaces are roughly equal. This is true even though the basolateral surface of MDCK cells has four times the area of the apical surface (26). In contrast to these exogenous secretory proteins, certain endogenous secretory proteins in MDCK cells are released exclusively apically or basolaterally (9, 12, 13).

As a model system for studying sorting, we have used the polymeric immunoglobulin receptor (poly-Ig-R) (18). Normally this receptor travels first to the basolateral surface where it can bind its ligand (17). The receptor (with or without ligand) is then endocytosed, transported across the cell, and is exocytosed at the apical surface. During transcytosis or after reaching the cell surface, the receptor is proteolytically cleaved and a large fragment (known as secretory component [SC][1]) is released into the apical medium. We expressed the poly-Ig-R cDNA in MDCK cells, which normally do not produce this molecule (17). The receptor functioned

1. *Abbreviation used in this paper*: SC, secretory component.

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as in in vivo, traveling first to the basolateral surface and then to the apical, and was cleaved to SC, which was released into the apical medium.

The cytoplasmic domain of the poly-Ig receptor consists of 103 amino acids located at the carboxy terminus (16, 20). Using oligonucleotide-directed mutagenesis, we previously deleted 101 of these amino acids (18). This tail-minus mutant did not travel to the basolateral surface, but rather was sent directly from the Golgi network to the apical surface. This result suggested, but did not prove, that the cytoplasmic domain contains a basolateral signal and that the membrane-spanning or lumenal domain contains an apical signal. However, one could imagine, for instance, that all membrane proteins that lack a signal for basolateral targeting are sent by "default" to the apical surface.

We now further truncated the poly-Ig-R to remove the membrane-spanning domain, converting the receptor to a secretory protein. We observed that it is still predominantly secreted at the apical surface. This provides direct evidence that the lumenal domain of the poly-Ig-R contains an apical targeting signal. Furthermore, it suggests that the same signal can direct both a membrane-anchored and a secreted protein to the apical surface, and that the receptor involved may be the same.

Materials and Methods

Materials

DNA restriction enzymes, polymerase, ligase, and kinase were from Pharmacia Fine Chemicals (Piscataway, NJ) or New England Biolabs (Beverly, MA) and used according to the manufacturer's directions. All radioactive compounds were the highest specific activity available from New England Nuclear (Boston, MA). FBS and G418 were from Gibco (Grand Island, NY); Millicells and HATF nitrocellulose filters were from Millipore/Continental Water Systems (Bedford, MA). Tetramethylammonium chloride was from Aldrich Chemical Co. (Milwaukee, WI). Other materials were from previously described sources (4, 18, 19).

In Vitro Mutagenesis

We followed the same protocol as was used in our earlier work (18). We began with the full-length poly-Ig-R cDNA cloned into M13 Mp8 phage. An oligonucleotide, 5′ ATCAGTACTTAGGCACT 3′ was synthesized on an Applied Biosystems (Foster City, CA) synthesizer and purified according to the manufacturer's directions. This is complementary to nucleotides 2056–2072, except for a single change that converts the lys at 629 to a stop codon (20). The standard oligonucleotide mutagenesis procedure was followed (28). This involved, in brief, hybridization of the mutagenic oligonucleotide to a single-stranded template, synthesis of a second strand with the Klenow fragment of DNA polymerase, ligation, and transformation into the *Escherichia coli* strain TG1. Plaques containing the mutant phage were identified by hybridization with the ^{32}P-labeled mutagenic oligonucleotide. Hybridization and washing were carried out exactly as described (27). This procedure uses washes at 50°C in 3 M tetramethylammonium chloride and allows unambiguous discrimination between mutant and nonmutant clones. As previously described (18), we resequenced the entire insert to insure that no extraneous mutations were introduced.

Expression in MDCK Cells

We followed our previously described procedure to express the anchor-minus poly-Ig receptor in MDCK cells (16). In brief, we removed the poly-Ig receptor coding region from the M13 replication form by Bgl II digestion and inserted the fragment into the Bam HI site of the retroviral vector DO-L (4). The resulting plasmid DNA (10 μg) was transfected by the calcium phosphate procedure into ψAM cells. After 18 h, the medium, which contained virus, was removed and added to a 60-mm dish of MDCK cells at 25% of confluence. As previously described (16) cells were grown in MEM with 10% FBS for 3 d. The now confluent dish was trypsinized and cells distributed into six 10-cm-diam tissue culture dishes (Corning Glass Works, Corning Science Products, Corning, NY). Cells were grown in media containing 0.25 mg/ml G418 (16) for 3 wk. Colonies were picked using cloning rings and expanded.

Analysis of Protein Secretion

We followed our earlier procedures (16, 18). Initially, six clones were assayed by continuous labeling for 90 min with [^{35}S]cysteine of cells grown on 35-mm plastic dishes. These cells were then lysed with SDS and immunoprecipitated with goat antiserum to rabbit SC. Immunoprecipitates were analyzed on 20 × 20 × 0.15-cm gels of 7% acrylamide. After electrophoresis (generally for 5 h at 7 W) gels were soaked for 15 min in glacial acetic acid, 30 min in 20% diphenol oxidase/80% glacial acetic acid (wt/wt) and 5 min in 3% glycerol/97% H_2O (vol/vol), dried, and exposed to Kodak XAR-5 film. We found this fluorography procedure to be more convenient and of higher resolution than the diphenol oxidase/dimethyl sulfoxide method, and more sensitive than using various commercial reagents. Of the six clones screened, three produced very low levels of poly-Ig receptor and were not analyzed further. Most work was performed with a clone, designated 18I, that produced the greatest level of poly-Ig receptor. Some experiments were also carried out with the two other clones, designated 18H and 18J, which produced ~30 and 50% as much poly-Ig receptor as the 18I clone, respectively.

After the initial screening, subsequent experiments used cells grown on 1-cm diameter Millicells. Cells were starved for 15 min with MEM lacking cysteine and containing 10% dialyzed FBS. Cells were then labeled by placing the Millicell on top of a 75-μl drop of this medium containing 30 μCi of [^{35}S]cysteine. The drop was on a sheet of parafilm. Labeling was for 10–20 min. The Millicell was then placed in a 24-well plate and washed twice with MEM. In some cases, cells were chased for variable times using 0.4 ml of MEM/10% FBS both inside and outside of the Millicell. Cells and media were analyzed as previously described (16). The filters containing the cells were boiled in an SDS-containing buffer, while the media were adjusted to 0.8% SDS and boiled. A fivefold excess of Triton X-100 was then added and the samples immunoprecipitated (16).

Endo H Digestion

In some cases after immunoprecipitation, the immunoprecipitates were eluted from the protein A–Sepharose beads by boiling with 50 μl of 1% SDS in 0.3 M Na citrate, pH 5.5. The sample was divided in half, and one portion received 5 mU of cloned Endo H, obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN). Both samples were incubated overnight at 37° and then analyzed by SDS-PAGE and fluorography.

Quantitation of [^{35}S]Methionine Uptake

This was assayed by a slight modification of a published procedure (1). Millicells were incubated for 10 min with 200 μl of MEM containing one-tenth the normal amount of methionine and 10% dialyzed serum on both the inside and outside of the Millicell. The medium on either the inside or outside of the Millicell cell was then replaced with similar medium containing 25 μC/ml [^{35}S]methionine. After 5 min at 37°C, the Millicell was cut out with a scalpel and the cells solubilized by boiling in 0.5 ml 0.5% SDS. 100 μl of solubilized material was then precipitated with 10% TCA at 4°C for 30 min, collected on a filter, dried, and counted in Liquifluor (Dupont Co. Diagnostic & BioResearch Systems, Wilmington, DE). Five Millicells were used for each cell type assayed.

Immunofluorescence

Immunofluorescence on Millicells was performed as previously described (16). The primary antibodies were a goat anti-rabbit SC from J.-P. Kraehenbuhl (Universite de Epalinges) (whole serum, diluted 1:250) and a monoclonal against an endogenous 60-kD basolateral MDCK antigen (reference 11, culture supernatant diluted 1:5). Secondary antibodies were FITC-conjugated Fab fragments of rabbit anti-goat IgG or goat anti-mouse IgG, from Cooper Biomedical Inc. (Malvern, PA) (used at 10 μg/ml).

We also obtained an MDCK cell line expressing chicken lysozyme (12). This line was constructed by Dr. C. Kondor Koch and colleagues and was provided by Dr. L. Roman (University of Texas at Dallas). The lysozyme was immunoprecipitated by a rabbit anti-lysozyme antisera provided by Drs. D. Sabatini, M. Rindler, and T. Gottlieb (New York University Medi-

cal School, New York, NY) using our standard protocol. The immunoprecipitations with either the anti-SC or anti-lysozyme were quantitative, as shown by the failure of a second round of immunoprecipitation to recover further material. We quantitated the bands on fluorographs with a laser densitometer (LKB Instruments, Inc., Gaithersburg, MD) and integrator. Multiple exposures of each gel were used to check that the results were linear.

Results

The intact rabbit poly-Ig-R consists of 755 amino acids. Starting at the NH_2 terminus, residues 1–629 are extracellular, residues 630–652 span the membrane, and residues 653–755 are cytoplasmic (20). We used an oligonucleotide to convert the Lys at position 629 to a stop codon, thus truncating the poly-Ig-R immediately before the membrane spanning segment. This anchor-minus poly-Ig-R was then expressed in MDCK cells using a retroviral expression system. Cells were grown on Millipore filters (Millicells) to yield confluent monolayers.

Most of our studies were conducted with one clone, 18I, which produced the largest amount of the anchor-minus receptor. Quantitatively similar results were obtained with two other clones (see below), while three clones produced unusably small amounts of the receptor.

When 18I cells are metabolically pulse-labeled with [^{35}S]Cys for 20 min and then solubilized, immunoprecipitated with antiserum against the receptor, and analyzed by SDS-PAGE and fluorography, a poorly resolved group of species of 65–70 kD is observed. (Fig. 1). Within a few hours this material is converted to two species of slightly different molecular masses and almost entirely secreted into the medium (Fig. 1). This is analogous to the secretion of anchor-minus forms of several viral membrane proteins (6, 8).

We were puzzled by the heterogeneity of the receptor immunoprecipitated at various timepoints. One possibility is that because a 20-min pulse labeling was used to label sufficient material to permit very accurate quantitation, some of the receptor may have already had its oligosaccharides modified by the Golgi apparatus. Another possible explanation is that there could be variable numbers of oligosaccharides added, and/or heterogenous processing of the oligosaccharides. To clarify this, we pulse labeled for only 5 min (Fig. 2, lane *1*) to minimize oligosaccharide processing. Two closely spaced species were observed. (This gel was subjected to electrophoresis for 50% longer than usual, to increase the resolution of bands). However, when digested with Endo H, both species are converted to a single species (Fig. 2, lane 2), indicating that there is only one type of polypeptide chain. The differences observed were therefore due only to variations in glycosylation. When the cells were chased for 1 h, two major species are observed (Fig. 2, lane *3*). These are largely resistant to Endo H, although a small amount of Endo H–sensitive material is still present (Fig. 2, lane *4*, *arrowhead*). The two species seen at 1 h have therefore largely acquired complex carbohydrates which are resistant to Endo H. These species comigrate with the material released into the medium (Fig. 1), which is also Endo H resistant (data not shown). The heterogeneity thus appears to be due to variations in the number of oligosaccharides added, possibly to heterogenous processing of the sugars. We cannot strictly rule out other possible causes, such as a variable proteolytic cleavage of the receptor (5). Attempts to remove the complex carbohydrates with Endo F or *N*-glyconase have not been successful, so we cannot prove that the two bands seen in the chase samples represent only one type of polypeptide chain. In any case, the two species that are released into the medium behave identically with regard to polarity of release, which is the focus of this study. The observed heterogeneity does not detract from the analysis found below.

It should also be mentioned that a similar heterogeneity was previously observed when we expressed both the wild-

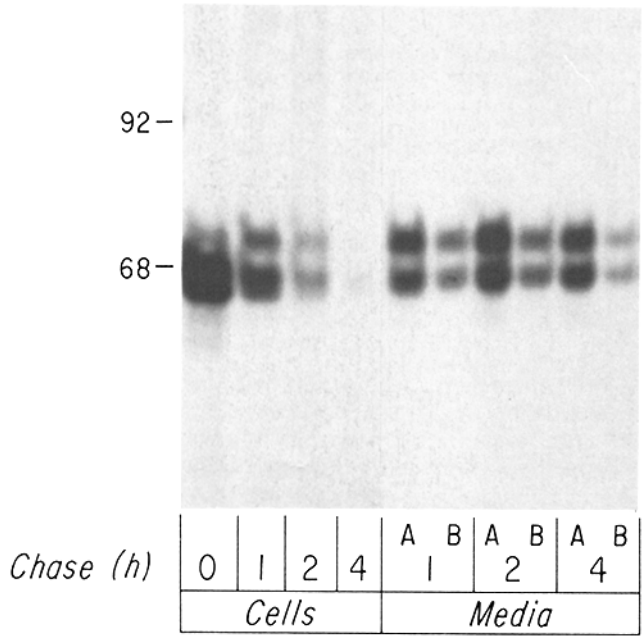

Figure 1. Pulse-chase analysis of release of anchor-minus poly-Ig-R by MDCK cells. MDCK cells were grown on Millipore filters, pulse-labeled for 20 min with [^{35}S]Cys, and chased for the indicated times. Cells and apical and basal media were harvested, immunoprecipitated with goat antiserum to SC, and analyzed by SDS-PAGE and fluorography. The positions of molecular weight markers (β-galactosidase, BSA) are indicated at left. (*A*) apical medium; (*B*) basal medium.

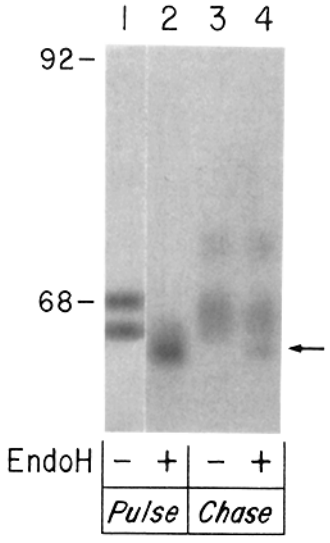

Figure 2. Endo H digestion of anchor-minus poly-Ig-R. MDCK cells were pulse-labeled for 5 min and then chased for the indicated time. Immunoprecipitates were then digested with Endo H and analyzed by SDS-PAGE and fluorography.

Table I. Comparison of Polarity of Release of Anchor-minus Poly-Ig-R and Lysozyme

	Fraction of material (± SEM)			
	Anchor-minus Poly-Ig-R			Lysozyme
Cell line	*18I*	*18H*	*18J*	
Cells	0.04 (0.02)	0.06 (0.04)	0.05 (0.03)	0.10 (0.04)
Apical medium	0.74 (0.08)	0.67 (0.16)	0.77 (0.23)	0.27 (0.06)
Basal medium	0.22 (0.05)	0.27 (0.09)	0.18 (0.10)	0.63 (0.10)
Ratio apical/ basal	3.36	2.48	4.27	0.43

Quantitation of polarity of release of anchor-minus poly-Ig-R and chicken lysozyme. Cells were pulse labeled for 20 min and then chased for 4 h. The data for clone 18I is the mean of six experiments, while the data for the other cell lines are the means of three experiments.

type (16) and tail-minus (18) forms of the poly-Ig-R in MDCK cells. Much like the present work, the various species also behaved identically with regard to polarity, and did not interfere with making useful conclusions.

We carefully quantitated the amount of anchor-minus poly-Ig-R released into the apical and basolateral media (Table I). By 4 h of chase, the bulk of the receptor was released apically.

The material in the cells is harvested by boiling the filter with attached cells in SDS, and then immunoprecipitating. Hence, any anchor-minus receptor that is released at the basolateral surface and trapped by the filter would be included in this fraction. At 4 h, only 4% of the receptor is found in this fraction. Assuming that all of this material is in the filter, rather than cell associated, then at most 4% of the material is trapped by the filter.

Moreover, we previously observed that mature SC could freely diffuse through the Millipore filter (16). The anchor-minus form of the poly-Ig receptor produced here is somewhat larger than mature SC and therefore might not pass as easily through the filter. To test this, we studied secretion from nonpolarized MDCK cells. We plated cells on Millicells, and after only 5 h, performed a pulse-chase experiment. It has been shown that although the cells can attach during the 5 h, a much longer time is required for them to become polarized (1). When we previously carried out this protocol with cells expressing the wild-type receptor, the mature SC was released with a slightly basolateral preponderance (16). We now carried out this experiment on the cells producing the anchor-minus receptor. We again observed that a slight preponderance of the receptor was secreted into the basal medium (Table II). This indicates that the anchor-minus receptor is intrinsically capable of passing through the filter. When the cells have had the opportunity to become fully polarized, most of the receptor is released apically.

Another possible artifact is that some of the anchor-minus receptor that is targeted to the basolateral surface is selectively degraded. We therefore compared the amount of receptor present after 0 h chase with that recovered in all fractions (cells and media) at 4 h of chase. We found that 94 ± 16% was recovered, suggesting that degradation was not a significant factor.

Most of our studies used the clone 18I which produced the greatest amount of the receptor. We carried some quantitative studies with two other clones, 18H and 18J, which produced less receptor. Although the lower levels made quantitation less reliable, the results on polarized secretion were very similar (Table I), indicating that the polarized secretion is not restricted to one unusual clone.

Table II. Comparison of Polarity of Anchor-minus Poly-Ig-R Release from Confluent and Subconfluent Cultures of Clone 18I Cells

	Fraction of material (± SEM)	
	Confluent 4-d cultures	Subconfluent 5-h cultures
Cells	0.04 (0.02)	0.07 (0.04)
Apical medium	0.74 (0.08)	0.39 (0.11)
Basal medium	0.22 (0.05)	0.54 (0.10)

We previously observed that an advantage of the retroviral expression system is that all cells in any one clone express uniform levels of the receptor (16). We examined clone 18I by immunofluorescence, using antiserum to SC. After fixation, cells were permeabilized with 0.1% Triton X-100 for 5 min. Much as we observed previously (16), we found that all the cells were producing uniform amounts of the receptor (data not shown). This indicates that the polarized secretion we observe is not due to a mixture of polarized cells that produce the receptor and nonpolarized cells that do not.

In contrast to the anchor-minus poly-Ig-R, when several exogenous secretory proteins such as chicken lysozyme are expressed in MDCK cells, they are released in approximately equal amounts at both surfaces (9, 12). We obtained the MDCK cell line producing lysozyme (12) and repeated the pulse-chase experiment using an anti-lysozyme antiserum to verify our methodology. We actually found moderately more lysozyme secreted basolaterally, rather than apically (Table I). We do not understand the small discrepancy between our data and the previous reports, but it strengthens our conclusion that the soluble poly-Ig-R is preferentially secreted apically.

The ratio of apical-to-basal secretion is 3.37 (mean of three clones) for the anchor-minus poly-Ig-R and 0.43 for lysozyme. This indicates that the anchor-minus poly-Ig-R is preferentially targeted to the apical surface by a factor of 3.37:0.43 = 7.8. This calculation ignores the material associated with the cells. If we assume that all of this material is secreted at the basolateral surface and trapped by the filter, the preferential targeting factor is 8.8.

The comparison of the MDCK clones expressing the anchor-minus poly-Ig receptor and lysozyme depends on both clones having the same degree of overall polarity. The parent MDCK cell line that we used was obtained (via Karl Matlin) from Kai Simons at the European Molecular Biology Laboratory (Heidelberg, FRG) and is the same cloned MDCK line used to construct the lysozyme clone. We compared the degree of polarity of the parent MDCK line with the anchor-minus poly-Ig receptor and lysozyme clones, using polarity of [^{35}S]Met uptake as a quantitative assay. This has previously been shown to be a reliable marker of the polarity of MDCK cells (1). As shown in Table III the polarity of Met uptake was similar in all cases. The degree of polarity was less than that reported for type I MDCK cells (1). This appears to be a result of differences between type I and type II MDCK cells (Mostov, K., unpublished data).

Table III. Comparison of Polarity of [^{35}S]Methionine Uptake in Various MDCK Clones

Cell line	Parent MDCK	181	Lysozyme
Ratio apical/basal Uptake (± SEM)	7.7 (2.3)	9.2 (3.6)	8.3 (3.5)

We used type II cells in this study precisely so that we could compare our clone with the lysozyme clone. Despite the lower degree of polarity of [^{35}S]Met uptake in type II cells as opposed to type I, both of the clones studied here are similarly polarized and so the comparisons made in this report are valid. As a further measure of polarity, we examined the distribution of an endogenous 60-kD basolateral surface antigen, which is defined by a monoclonal antibody (11). By immunofluorescence of filter-grown cells, we found similar, moderately bright fluorescence at the basolateral surface of the parent MDCK cells and both clones. Apical staining in contrast was very faint and not convincingly above background (data not shown). We regard this qualitative, subjective assay as a less satisfactory but useful confirmation of polarity.

Discussion

We expressed an anchor-minus form of the poly-Ig receptor in polarized MDCK monolayers grown on Millipore filters. This secretory protein was secreted predominantly (74%) at the apical surface, although a substantial minority (22%) was released basolaterally. In contrast, lysozyme was secreted with a slight basolateral preponderance (63%). Lysozyme is representative of a group of exogenous secretory proteins that is not thought to interact with a receptor(s) in MDCK cells that targets them to a particular surface (9, 12). Lysozyme secretion may therefore represent the default, bulk-flow pathway (13). Compared with lysozyme, the anchor-minus poly-Ig-R is preferentially targeted apically by a factor of 7.8.

We hypothesize that the anchor-minus poly-Ig-R contains a signal that interacts with a receptor in the lumen of the secretory pathway that directs the anchor-minus poly-Ig-R to the apical surface. It is quite likely that the same signal directs the tail-minus and wild-type poly-Ig-R to the apical surface. This result suggests that the same basic mechanism may direct both membrane and secretory proteins to the apical surface. The generality of this conclusion remains to be tested. Of course the signals could work negatively, by preventing delivery to the basolateral surface.

We wish to emphasize that this interpretation rests crucially on the hypothesis that lysozyme (and the several other exogenous secretory proteins that have been tested) are true bulk flow markers that are not preferentially targeted to one surface or the other (9, 12). It would be desirable to further substantiate this hypothesis using completely synthetic bulk flow markers.

Another possibility is that there is a default pathway for membrane proteins that in the absence of a signal for basolateral localization sends them to the apical surface. The cytoplasmic tail-deleted poly-Ig-R could travel by this pathway. However, this would require that the soluble poly-Ig-R fortuitously expresses a cryptic signal that directs it to the apical surface, rather than to the default pathway for soluble proteins that leads equally to both surfaces. This seems much less likely.

Delivery of the full-length poly-Ig-R to the apical surface (17) is somewhat more precise than for the anchor-minus poly-Ig-R reported here (92% apical vs. 74%). The targeting receptor is most likely an integral membrane protein. When the poly-Ig-R is anchored in the membrane, it is therefore probably at a high local concentration relative to the targeting receptor. When the poly-Ig-R is no longer anchored, it may be a a lower local concentration. More molecules may fail to bind to the targeting receptor and thus could be missorted to the basolateral surface.

Results similar to those reported here have been obtained with the influenza virus hemagglutinin which is normally found on the apical surface. When truncated to produce a secreted protein, it is secreted into the apical medium (23).

In contrast to these results, when a retroviral glycoprotein, which is normally located basolaterally, is truncated and converted to a secretory protein, it is secreted roughly equally from both surfaces of MDCK cells (25). This might imply that basolateral transport of this protein uses a signal that either requires membrane anchorage or is located in the membrane-spanning or cytoplasmic portion of the molecule. However, the nonpolarized secretion may be secondary to another effect, such as an alteration of oligomeric structure in the truncated vs. a full-length molecule.

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