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Functional Expression of the Polymeric Immunoglobulin Receptor from Cloned cDNA in Fibroblasts

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Abstract. The polymeric immunoglobulin receptor, a transmembrane protein, is made by a variety of polarized epithelial cells. After synthesis, the receptor is sent to the basolateral surface where it binds polymeric IgA and IgM. The receptor–ligand complex is endocytosed, transported across the cell in vesicles, and re-exocytosed at the apical surface. At some point the receptor is proteolytically cleaved so that its extracellular ligand binding portion (known as secretory component) is severed from the membrane and released together with the polymeric immunoglobulin at the apical surface. We have used a cDNA clone coding for the rabbit receptor and a retroviral expression system to express the receptor in a nonpolarized mouse fibroblast cell line, $\psi 2$, that normally does not synthesize the receptor. The receptor is glycosylated and sent to the cell surface. The cell cleaves the receptor to a group of polypeptides that are released into the medium and co-migrate with authentic rabbit secretory component. Cleavage and release of secretory component do not depend on the presence of ligand. The cells express on their surface 9,600 binding sites for the ligand, dimeric IgA. The ligand can be rapidly endocytosed and then re-exocytosed, all within ~10 min. Very little ligand is degraded. At least some of the ligand that is released from the cells is bound to secretory component. The results presented indicate that we have established a powerful new system for analyzing the complex steps in the transport of poly-Ig and the general problem of membrane protein sorting.

Of all the pathways of intracellular protein traffic, one of the most complex is that of the receptor for transcellular transport of the polymeric immunoglobulins, IgA and IgM (poly-Ig)[1] (reviewed in reference 19). The poly-Ig receptor, a transmembrane protein, is synthesized by various epithelial cells and sent first to the basolateral surface. Here it binds poly-Ig, and the receptor–ligand complex is endocytosed via coated pits. The complex is transported in smooth vesicles across the cell and exocytosed at the apical surface. During transcytosis or shortly after exocytosis at the apical surface, the receptor is proteolytically cleaved so that the extracellular ligand-binding domain is freed from the membrane and released together with the poly-Ig into the external secretion (18, 21, 26, 27). This cleaved portion is known as secretory component (SC). The protease responsible for cleavage has not been identified.

Three aspects of the poly-Ig receptor pathway make it a unique system for studying membrane traffic. First, unlike many epithelial cell plasma membrane proteins that are found either on the basolateral or apical surface of a polarized cell (24), the poly-Ig receptor goes first to the basolateral and then to the apical surface. Second, although the poly-Ig receptor is endocytosed together with several other receptors at the basolateral surface, it is then sorted away from these receptors (which go either to the lysosomes or recycle to the original cell surface) and is sent to the surface of the cell opposite to that at which it entered (9). Third, the receptor is used for only one round of transport and then cleaved. Little is known, however, about the "signals" on the poly-Ig receptor or other membrane proteins that direct them to the appropriate destinations, or about the cellular machinery for recognizing these signals (1). The degree to which these signals and recognition systems are specific to particular membrane proteins and cell types (or are universal) has not been explored.

One recent approach to analyzing membrane protein traffic has been to introduce and express cloned genes that code for foreign membrane proteins into various cell types (see for example reference 8). We have used the same strategy with the poly-Ig receptor by using a cDNA clone that contains the entire coding region of the receptor (20) and have expressed the poly-Ig receptor in mouse fibroblasts. This allows us to examine how a cell that is not polarized and normally never makes the poly-Ig receptor handles a receptor that was designed to function in polarized cells. Will the receptor reach the cell surface? If so, will the receptor be endocytosed and where will it be sent? Finally, will the receptor be proteolytically cleaved by the fibroblast? Since the fibroblast lacks epithelial-type polarity, one might predict that it would simply return the poly-Ig receptor to any cell surface. On the other hand, if fibroblasts also lack the machinery that recognizes

[1] *Abbreviations used in this paper:* dIgA, dimeric IgA; DME, Dulbecco's modified Eagle's medium; poly-Ig, polymeric immunoglobulin; sc, secretory component.

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receptors specifically coded for transcytosis, the poly-Ig receptor may not be endocytosed, or may be endocytosed and directed to some other compartment, such as the lysosome, or accumulate in endosomes. The experiments reported here were designed to test the universality of the signals for transport present in the poly-Ig receptor and the cellular machinery for recognizing them.

Materials and Methods

Construction of DO-L-poly-Ig-R

All DNA manipulations involved standard techniques (15). The plasmid p21.53 contains the entire reading frame of the poly-Ig-receptor cDNA (20). The initiator AUG begins at nucleotide 124, and the stop codon is at nucleotide 2,442. There are sites for the restriction enzyme Stu I at positions 96, 1,059, 2,438, 2,638, and 2,967, and a site for the enzyme Nae I at position 2,451. Using partial digestion with Stu I and complete digestion with Nae I (both from New England Biolabs, Beverly, MA), we isolated the fragment from 96 to 2,451, which contains the entire coding region. The fragment was blunt-ended with T4 polymerase I (New England Biolabs), and prephosphorylated Bgl II linkers (Pharmacia Inc., Piscataway, NJ) were added. The vector DO-L was kindly provided by Drs. A. Korman, C. Cepko, and R. C. Mulligan of the Whitehead Institute, and will be described in detail in a later publication. It is similar to the previously described vector SV(X) (4). However, the neomycin resistance gene is driven by its own promoter, the SV40 early promoter. Furthermore, the vector contains the early region of the polyoma virus to enhance transient replication. There is a unique Bam HI site, located downstream from the 5′ retroviral long terminal repeat. The Bgl II linkered poly-Ig-receptor insert was ligated into this site, in the proper orientation. The construction was verified by restriction mapping and DNA sequencing.

Introduction of the Poly-Ig-R into Cells

10 μg of DO-L-poly-Ig-R or DO-L without insert DNA in 10 μl H_2O was added to 0.5 ml of solution containing 8 g NaCl, 0.37 g KCl, 0.1 g Na_2HPO_4, 1 g dextrose, and 5 g Hepes per liter (pH adjusted to 7.05 at 20°C with NaOH). 32 μl of 2 M $CaCl_2$ was added. The tube was gently mixed for 20 s and then incubated at 22°C for 45 min. The solution was then added to a 10-cm dish of ψam cells (gift of R. Mulligan) (6), which had been split 1:5 from a confluent culture 24 h earlier. After 20 min, 10 ml of Dulbecco's modified Eagle's medium (DME) containing 10% calf serum was added. The dish was incubated at 37°C for 4 h. The medium was removed, and the cells were shocked with 15% glycerol in DME for 3.5 min. This was removed and 5 ml of fresh medium was then added. This medium (designated transient viral supernatant) was removed after 18 h, filtered through an 0.45 μm filter (Millipore Corp., Bedford, MA), supplemented with polybrene (Sigma Chemical Co., St. Louis, MO) to 8 μg/ml, and frozen at −70°C.

ψ2 cells (16) were split 1:10 24 h before use. The media was removed, and 2.5 ml of the transient viral supernatant was added. After 3 h at 37°C, 10 ml of fresh medium was then added without first removing the viral supernatant. After a 3-d incubation the cells were split 1:50 into medium containing 0.31 mg/ml (active drug concentration) of G418 from Gibco (Grand Island, NY). Cells were grown in this for 12 d, at which time colonies were visible. Well separated colonies were picked using cloning cylinders that were cut from 200-μl yellow pipet tips (Rainin Instrument Co. Inc., Woburn, MA), and clones were expanded.

Cell Culture

All cells were initially grown in DME containing 10% calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO_2, 95% air. However, since calf serum contains significant levels of IgA, clone 1 and clone 1C cells were adapted to media containing 10% fetal calf serum (which contains only trace levels of IgA) instead of calf serum over a period of 4 wk. The experiments reported here were all performed with these adapted cells.

Clone 1C cells were observed to detach easily from plastic tissue culture vessels. To minimize this, the surfaces were precoated for 1 min with a solution of fibronectin (20 μg/ml in phosphate-buffered saline [PBS], from New York Blood Center; we thank R. Hynes for this suggestion).

Metabolic Labeling and Analysis

Labeling of cells with [^{35}S]cysteine, lysis, immunoprecipitation, SDS PAGE, and fluorography were done as previously described (18). The antiserum used was goat antiserum to rabbit secretory IgA (which contains antibodies to SC) from Cooper Biomedical, Inc., Malvern, PA. When indicated, cells were rinsed well with PBS and grown overnight in serum-free medium containing DME, 0.2% ovalbumin, 40 μg/ml insulin, 5 μg/ml human transferrin, 25 ng/ml prostaglandin E1, 1 nM 3,3,5-triiodo-L-thyronine, 50 nM Na_2SeO_3, 50 nM hydrocortisone, and 10 mM Hepes (pH 7.3). All supplements were from Sigma Chemical Co.

Cell-Surface Iodination

35-mm dishes of confluent monolayers were placed on ice and rinsed three times with cold PBS. 0.25 ml of PBS containing 20 mM glucose, 25 μl Enzymobeads (Bio-Rad Laboratories, Richmond, CA), and 1 mCi/ml Na ^{125}I (Amersham Corp., Arlington Heights, IL; highest specific activity available) was added. The dishes were gently agitated every 5 min for a 30-min period. Cells were then washed four times with cold PBS containing 20 mM KI, and then twice with cold DME containing 10% fetal calf serum and 20 mM Hepes, pH 7.3. 1 ml of this medium (prewarmed to 37°C) was added, and the dishes were floated in a 37°C water bath for various times. Cells and media were then processed for immunoprecipitation in the same manner as metabolically labeled cells.

Immunofluorescence

Cells were grown on fibronectin-coated coverslips. They were fixed in 3% paraformaldehyde (freshly made up in PBS) for 30 min at room temperature. After washing three times with PBS, cells were quenched with PBS containing 50 mM NH_4Cl for 30 min (all washes were for at least 5 min at room temperature). Cells were then washed with PBS containing 0.2% gelatin. In certain cases, cells were then permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature. Cells were incubated with antiserum to SC (1:250 dilution in PBS) for 30 min at room temperature and washed four times. Cells were then incubated with fluorescein isothiocyanate-conjugated, affinity purified Fab fragments of rabbit anti-goat IgG (Cooper Biomedical, Inc.; diluted to 10 μg/ml in PBS containing 0.2% gelatin) for 30 min. Cells were again washed four times and mounted in 90% glycerol. Cells were examined in a Zeiss PM3 microscope (Carl Zeiss, Inc., Thornwood, NY) using a 63× oil immersion lens, epifluorescence, and fluorescein isothiocyanate filters. Photographs were taken using Kodak Tri-X film, exposed, and developed at 400 ASA.

For live cell experiments, cells were incubated at 4°C with antibodies to SC for 1 h. Cells were washed at 4°C three times with PBS containing gelatin followed by PBS alone and fixed in 3% paraformaldehyde for 1 h at 4°C. Quenching, washing, and second antibody incubation were as above.

dIgA Uptake Studies

Purified human dIgA was the kind gift of Drs. J. M. Schiff and B. Underdown, University of Toronto. The dIgA was iodinated using the iodine monochloride method (10), to a specific activity of 4×10^6 cpm/μg. Unincorporated ^{125}I was removed by dialysis. Unless otherwise indicated, cell binding studies used cells grown on 35-mm plastic dishes (Corning Glass Works, Corning, NY) and were performed in 0.5 ml of medium. The media used for binding and washing was generally DME containing 1% fetal bovine serum, and buffered to pH 7.3 with 20 mM Hepes. Unbound material was removed by washing with six 1-ml portions of media over a 20-min period at 4°C. Cells were then solubilized with two 0.9-ml portions of 0.1 N NaOH. A 0.1-ml aliquot of this was used for protein determination, using the BCA kit from Pierce Chemical Co. (Rockford, IL), with bovine serum albumin (BSA) as a standard. A standard curve of cell number versus protein was constructed using a Coulter counter. The remainder of the solubilized material was counted in a Packard gamma counter (Packard Instrument Co., Downers Grove, IL).

In the experiment described in Fig. 8, after cells were warmed up for brief intervals, they were re-cooled and incubated with 0.5 ml of PBS containing 0.25% pronase (Boehringer Mannheim Diagnostics, Inc.) at 4°C for 1 h (11). 1 ml of media containing 10% fetal calf serum was added, and the cells (which had detached from the plate) were transferred to a 1.5-ml centrifuge tube. The tubes were subjected to centrifugation for 2 min at 4°C in an Eppendorf microfuge. The cell pellet and supernatant were counted separately.

Autoradiography

Cells were incubated with 1 μg/ml ^{125}I-labeled dIgA for 4 h at 4°C or 37°C, washed extensively with DME containing 1% fetal calf serum and then with PBS, and fixed at 4°C for 2 h in a solution containing 1% freshly depolymerized paraformaldehyde, 2.5% glutaraldehyde, and 2 mM $CaCl_2$ in 0.1 M sodium

cacodylate buffer, pH 7.4. Cells were rinsed with 0.1 M cacodylate buffer, postfixed for 1 h in 1% OsO_4, and dehydrated in graded ethanols. They were then scraped from the dishes, collected in 1.5-ml centrifuge tubes, and embedded in EPON. 0.5-μm sections were stained and coated by dipping in Ilford L4 emulsion, diluted 1:1, exposed for 3 wk at 4°C, and developed for 4 min in Kodak D19 at 18°C (23).

Immunoprecipitation of dIgA-SC Complexes

Cells were surface iodinated as described above. After thorough washing, they were incubated at 4°C in Hepes-buffered DME without serum, containing 50 μg/ml dIgA, for 2 h. Cells were then washed six times at 4°C in Hepes-buffered DME lacking dIgA. Cells were incubated at 37°C in this media for 20 min. 2 μg of unlabeled rabbit SC and an equal volume of 2.5% Triton X-100, 150 mM NaCl, 50 mM triethanolamine-HCl (pH 8.6), 5 mM EDTA was added to the media, followed by 1 μl of either goat antiserum to human serum IgA (Cooper Biomedical, Inc.) or non-immune goat serum. The samples were incubated at 4°C for 4 h. Immunoprecipitates were then collected, washed, and analyzed as described above.

Results

Introduction of Poly-Ig-Receptor cDNA

We began with our previously described cDNA clone coding for the poly-Ig-receptor and by use of the restriction enzymes Stu I and Nae I removed most of the 5′ and 3′ noncoding regions. This was inserted into the retroviral expression vector, DO-L, as described in Materials and Methods. This vector provides the signals necessary for transcription and processing of mRNA, integration of a DNA copy into the genome, and carries as a selectable marker the neomycin resistance gene. We transfected this construct (designated DO-L–poly-Ig-R) into ψam cells by the calcium phosphate co-precipitation method. The ψam cells provide the helper functions necessary to replicate and package the DO-L–poly-Ig-R into infectious particles (6). The virus transiently produced by the ψam cells was used to infect ψ2 cells (16), which, like ψam cells, were derived from NIH 3T3 cells.

Expression of the Poly-Ig-Receptor

Clones of stably infected ψ2 cells were selected by their resistance to the neomycin analogue, G418. We examined 12 clones for production of the poly-Ig-receptor by metabolic labeling with [^{35}S]cysteine, followed by immunoprecipitation with antiserum to rabbit SC, SDS PAGE, and fluorography. All 12 clones produced the receptor, although they varied in the extent to which the receptor's oligosaccharides were processed to the mature, complex type (data not shown) (18, 21, 26). Four of the clones gave apparently normal processing of the oligosaccharides of the poly-Ig-receptor. One such clone was selected for further study and designated clone 1C. For control purposes, a clone of cells infected with virus derived from the DO-L vector alone, without a cDNA insert, was selected and designated clone 1.

When 1C cells were pulse-labeled for 15 min with [^{35}S]-cysteine, a single polypeptide with an apparent M_r of 90 kD was seen (Fig. 1, lane *1*). In a pulse-chase experiment, this was converted to a broad band of ~110 kD (Fig. 1, lanes *2–6*). Digestion with endoglycosidase H showed that the 90-kD band contained endoglycosidase H–sensitive, high mannose type oligosaccharides, whereas the 110-kD band contained the endoglycosidase H–resistant, complex type oligosaccharides (data not shown).

With increasing chase time, the 110-kD band diminished in amount (Fig. 1, lanes *2–6*). A new group of bands of ~70 kD appeared (Fig. 1, lanes *7–11*), which roughly co-migrated with authentic rabbit SC (Fig. 1, lane *12*). The 70-kD bands were predominantly found in the medium, although traces were cell-associated. Although the diffuseness of the bands prevented precise quantitation, the disappearance of the 110-kD band roughly correlated with the appearance of the 70-kD bands. This suggests a precursor–product relationship between the 110-kD band and the 70-kD bands.

It is useful here to compare the forms of the poly-Ig-receptor seen here with those observed in vivo and in cell-free translations. The considerable heterogeneity of the rabbit poly-Ig-receptor is the result of several processes. First, three alleles of the receptor have been defined serologically (12), and most of the earlier work was apparently done with heterozygous rabbits. In cell-free translations of mRNA from heterozygous rabbits, four primary translation products are immunoprecipitated with antiserum to rabbit SC: a doublet of 90–95 kD and a doublet of 70–75 kD (21). The 70–75-kD doublet arises by alternative splicing of mRNAs coding for the 90–95-kD doublet (Deitcher, D., and K. Mostov, manuscript in preparation). The two polypeptides comprising each doublet are apparently encoded for by the two alleles of the poly-Ig-receptor in heterozygous rabbits (Deitcher, D., and K. Mostov, unpublished observations). In the experiments reported here we used a cDNA derived from an mRNA coding for one of the 90–95-kD primary translation products. Further heterogeneity may also be generated by variability in oligosaccharide processing and by the cleavage to SC. It has recently been shown that the carboxy terminus of human SC is heterogeneous (7). Cleavage to SC may not occur at a unique amino acid sequence, but instead at one of several sites in a particularly accessible part of the molecule. Alternatively, cleavage may take place at one site, followed by a variable amount of digestion by an exopeptidase. In any case, the heterogeneous group of SC immunoreactive molecules released into the medium by the clone 1C cells approximately co-migrates with authentic rabbit milk SC, suggesting that the cleavage is at least roughly correct (13). Furthermore, we have found that the material secreted by the 1C cells can specifically bind to human polymeric IgA (see below).

We also labeled the poly-Ig-receptor on the cell surface by lactoperoxidase iodination at 4°C, followed by immunoprecipitation, SDS PAGE, and autoradiography. The major species labeled is the intact poly-Ig receptor (Fig. 2, lane *1*). However, if after labeling, the cells are briefly warmed to 37°C and then both cells and media are immunoprecipitated, much of the receptor is rapidly converted to SC and released into the media (Fig. 2, lanes *2–10*) in as little as 1 min. The diffuseness of the bands made quantitation difficult. Taken together with the data of Fig. 1, it appears that newly synthesized poly-Ig-receptor reaches the cell surface slowly, but once there much of it is rapidly cleaved to SC and released into the medium. We cannot rule out that cleavage is accelerated by damage due to iodination.

We next examined whether the cleavage and release of SC requires the presence of dIgA. It has previously been reported that if isolated rat liver is perfused with medium lacking IgA, SC continues to be released into the bile (22). However, IgA is produced locally by plasma cells in the liver, which could account for at least some SC release. To eliminate all IgA and IgM in our system, we grew cells in serum-free medium. As

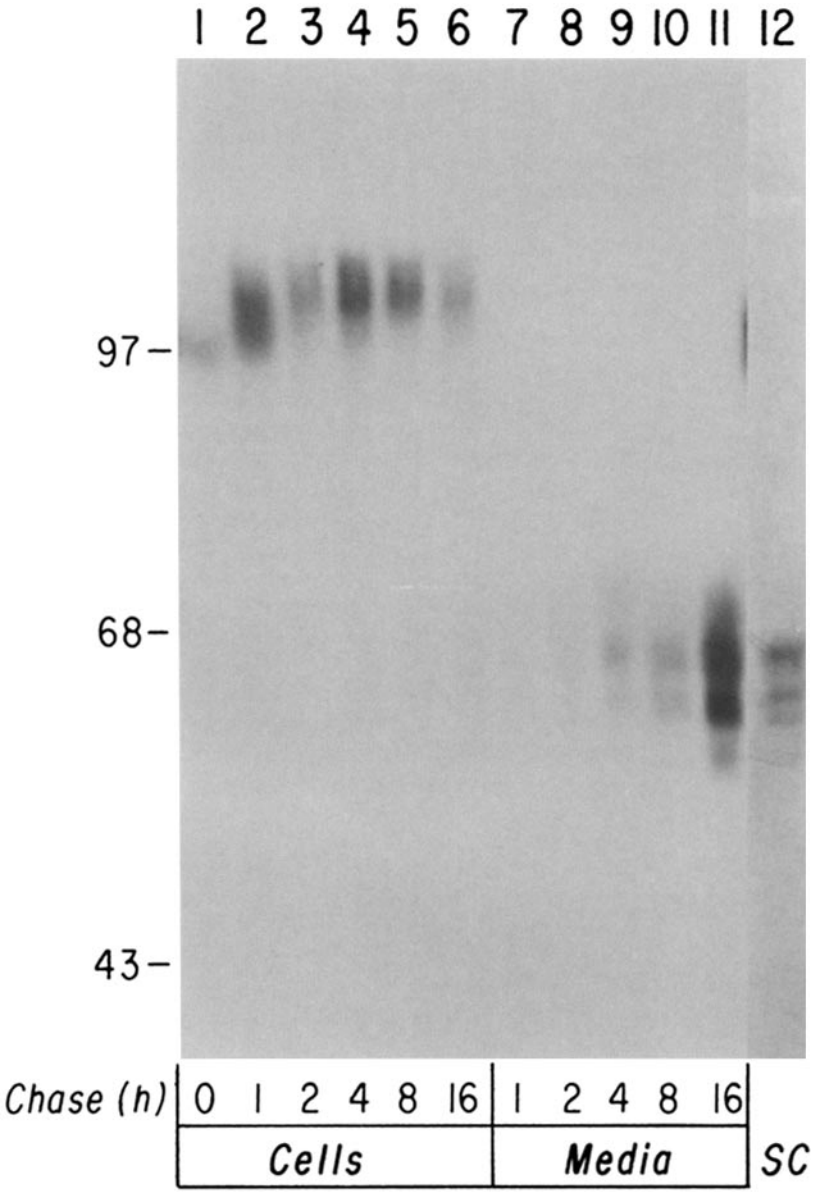


Figure 1. Pulse-chase analysis of poly-Ig receptor synthesis and processing. Cells were labeled with [^{35}S]cysteine for 15 min, and then chased for indicated times with medium containing excess unlabeled cysteine. Cells and medium were then immunoprecipitated and analyzed as described in Materials and Methods. Lanes *1–11* are a fluorographed gel. Lane *12* is a Coomassie Blue–stained gel of SC purified from rabbit milk. Positions of molecular weight markers are indicated at left (phosphorylase A, 97,000; BSA, 68,000; ovalbumin, 43,000).

seen in Fig. 3, the kinetics of cleavage and release of SC are virtually identical in the complete absence of poly-Ig (lanes *1–9*), or when dIgA is added in saturating amounts (lanes *10–18*).

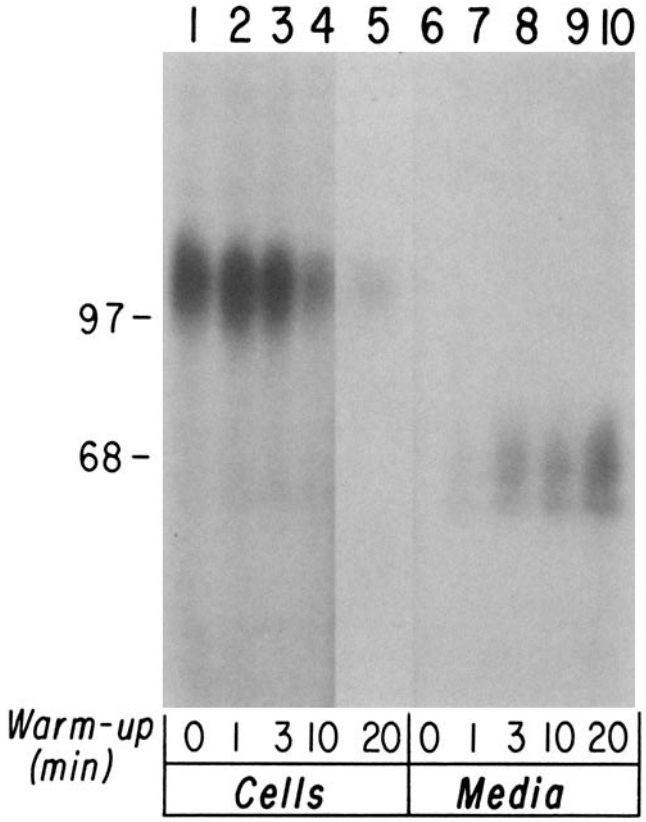
Localization of the Poly-Ig-Receptor

We next studied the location of the poly-Ig-receptor in clone 1C cells by immunofluorescence microscopy. Cells were gently fixed in paraformaldehyde, in an effort to avoid permeabilizing the cell membrane. Cells were stained with goat antiserum to rabbit SC, followed by fluorescein isothiocyanate–conjugated rabbit antibody to goat IgG. This showed an immunofluorescence pattern strongly suggestive of surface labeling (Fig. 4*A*), with diffuse labeling over the entire cell and stronger labeling at the edges. To ensure that surface labeling occurred exclusively, we also used live cells. Live cells incubated with the antibodies at 4°C gave a punctate pattern of fluorescence on the cell surface, suggesting clustering of receptors, perhaps in coated pits (Fig. 4*C*). Finally, if fixed cells were permeabilized with Triton X-100, numerous bright spots of various sizes, presumably vesicles, were seen throughout the cell (Fig. 4*B*). We could not identify these as any particular subcellular organelle. In all of these experiments, use of control cells (clone 1) resulted in almost no fluorescent signal (data not shown).

Function of the Poly-Ig-Receptor

We next examined whether the poly-Ig-receptor could bind and internalize its ligand, human dimeric IgA (dIgA) (25). In these experiments we used clone 1 cells as a control for nonspecific binding. Similar control data were obtained by including a large excess of unlabeled dIgA. Incubation of cells with 0.2 μg/ml ^{125}I-dIgA at 4°C resulted in specific binding that plateaued by ~2 h at a level of ~200 pg per 10^6 cells and did not change substantially over the next 6 h (data not shown).

We carried out saturation binding experiments and Scatchard plot analysis, which showed that there was one class of binding sites with an apparent K_d of 8×10^{-9} M and 9.6×10^3 sites per cell (Fig. 5, *A* and *B*).



We also studied degradation of ^{125}I-dIgA by the cells. Conversion of ^{125}I-dIgA to TCA-soluble material by the clone 1 control cells was almost as great as by the clone 1C cells (Fig. 6). Specific degradation attributed to the poly-Ig-receptor was very small, amounting to ~4% of the material that became bound to the cells in 8 h at 37°C (data not shown).

Internalization of d-IgA

We next used light microscopic autoradiography to determine whether the bound dIgA was internalized by the cells (29). Semithin (0.5 μm) sections of cells incubated with ^{125}I-dIgA at 4°C for 4 h and then processed for light microscopic autoradiography showed predominantly surface labeling (Fig. 7*A*). Cells incubated at 37°C, in contrast, showed labeling within the cytoplasm, which suggests that internalization had occurred (Fig. 7*B*). Light microscopic ^{125}I autoradiography does not have the resolution to indicate where in the cell the internalized ligand is located. Autoradiography at the electron microscope level was not feasible because of insufficient radioactive signal.

We also examined the kinetics of internalization biochemically. Cells were incubated with ^{125}I-dIgA for 2 h at 4°C, and

Figure 2. Processing of surface-iodinated poly-Ig receptor. Cells were iodinated at 4°C. Warm (37°) medium was added for indicated periods. Cold media was used in lanes *2* and *6*. Cells and media were immunoprecipitated.

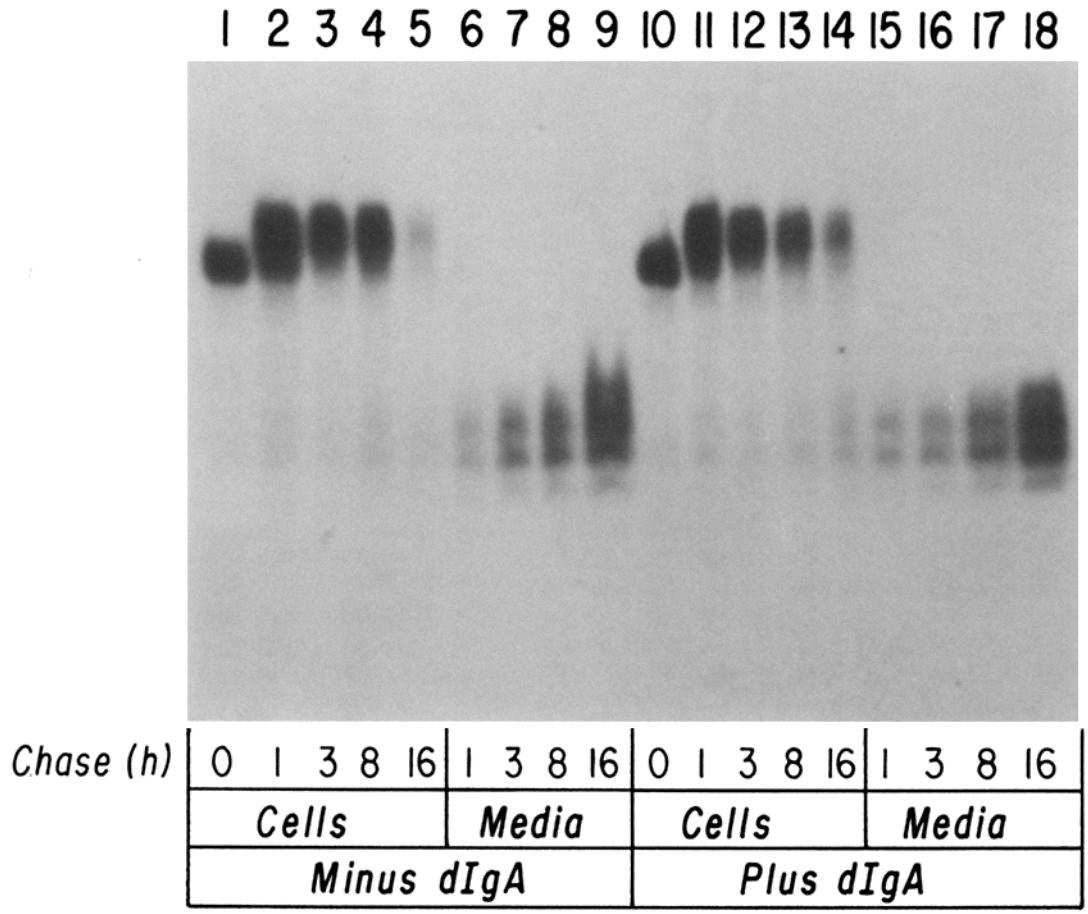


Figure 3. Processing of poly-Ig receptor in the absence or presence of dIgA. Cells were grown in serum free medium as described in Materials and Methods. Pulse chase experiments similar to that of Fig. 1 were performed. Lanes *10–18* were performed with 50 μg/ml dIgA present during the experiment.

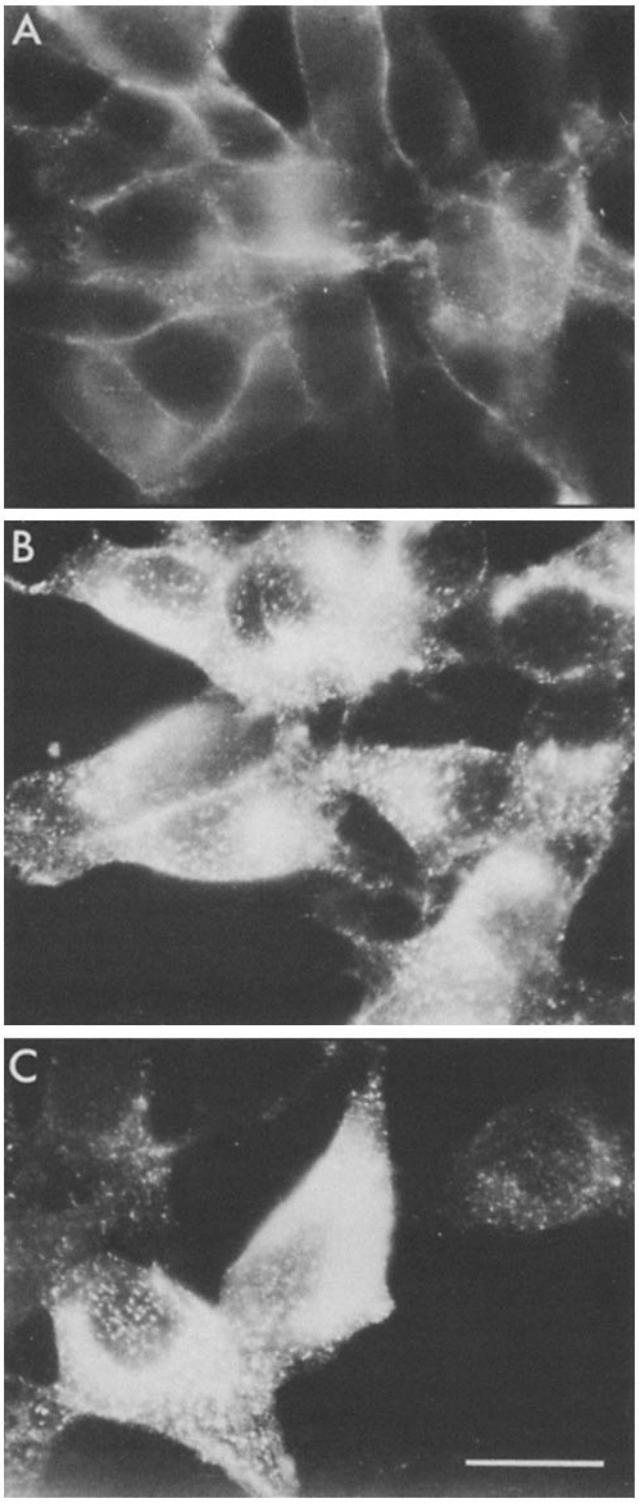


Figure 4. Immunofluorescent microscopy of the poly-Ig receptor. Cells were treated as described in Materials and Methods. (*A*) Paraformaldehyde-fixed cells; (*B*) paraformaldehyde-fixed, Triton X-100–permeabilized cells; (*C*) live cells stained at 4°C. Bar, 10 μm.

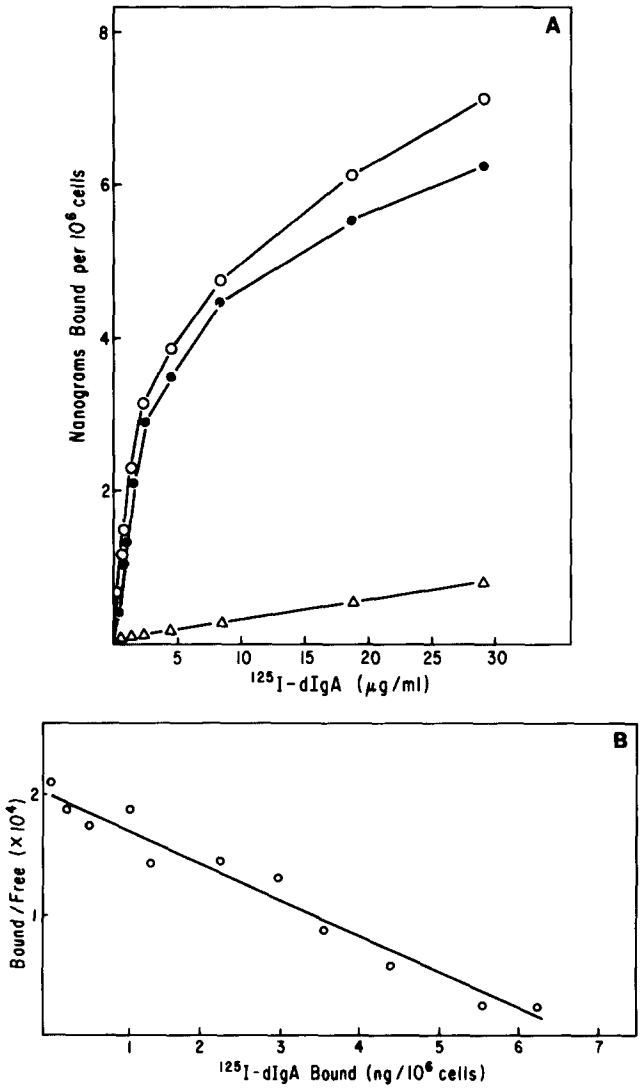


Figure 5. Saturation binding analysis. (*A*) Cells were incubated with varying concentrations of ^{125}I-dIgA at 4°C for 2 h and cell-associated radioactivity determined. ○, binding to clone 1C cells; △, binding to clone 1 cells; ●, net binding to poly-Ig receptor (i.e., clone 1C cells minus clone 1 cells). (*B*) Data are replotted in a Scatchard plot. Assuming a molecular weight of 400,000 for dIgA, the data suggests a single class of 9,600 binding sites per cell with a K_d of 8×10^{-9} M. All points are means of triplicates, and standard errors of the means were generally <10%.

unbound dIgA was washed away. Cells were then warmed for brief intervals and recooled. After recooling, the cells were digested with pronase at 4°C and separated into pellet and supernatant by centrifugation. Radioactivity remaining in the pellet was defined as internalized dIgA (5, 11). Points were corrected for the approximately 6% of the bound ^{125}I-dIgA that could not be released by pronase at time = 0. A similar phenomenon was seen with the transferrin receptor. Counts released into the supernatant by pronase were taken to indicate dIgA on the cell surface. When warming began, most of the material was rapidly released into the medium (Fig. 8). We do not know if this is due to dissociation from the receptor or receptor cleavage to SC. However, a significant fraction of the dIgA is internalized, reaching a peak at 3 min of ~30% of the total bound dIgA. This is only transient, as the material is rapidly re-externalized and released into the medium (5).

Taken together with the data presented in Figs. 2 and 7, the most likely conclusion is that when a newly synthesized poly-Ig-receptor molecule reaches the cell surface, it can follow one of two competing pathways. About 70% is cleaved and released directly. However, ~30% is first internalized, and then rapidly re-externalized and released. We do not

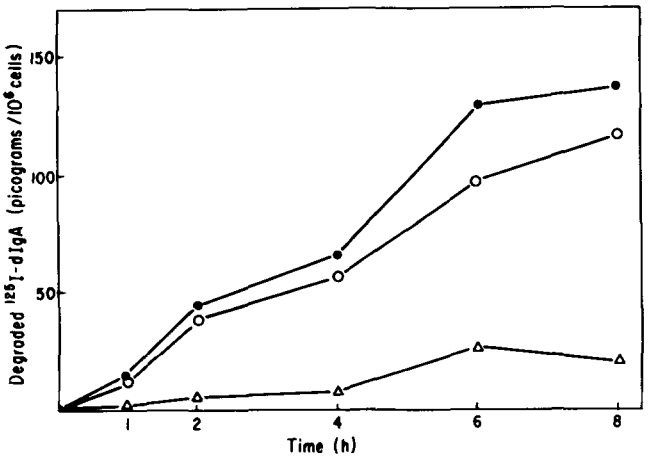


Figure 6. Degradation of ^{125}I-dIgA. Cells were incubated with 0.2 μg/ml ^{125}I-dIgA for the indicated times. TCA soluble radioactivity was determined as described (10). ●, clone 1C cells; ○, clone 1 cells; △, degradation specifically attributable to the poly-Ig receptor (i.e., clone 1C cells minus clone 1 cells). Points are means of triplicates, and standard errors of means varied by <10%.

know whether cleavage occurs only at the cell surface or also inside endocytotic vesicles. A less likely explanation is that upon warming, the dIgA-poly-Ig-receptor complex is not internalized, but becomes pronase resistant because of a conformation or other change in binding induced by warming. This has not been seen in other systems and would also require that the change be reversed within ~10 min at 37°C, but remain stable during the 1 h digestion with pronase at 4°C.

We next considered whether SC released from the cell is complexed with dIgA. We iodinated the cells and allowed unlabeled dIgA to bind at 4°C. Cells were then warmed to 37°C to allow cleavage of the poly-Ig-receptor to SC to occur. The media was then immunoprecipitated with antiserum to IgA which does not react with SC. Although the immunoprecipitation contains several nonspecific bands that are also precipitated by non-immune serum, material co-migrating with SC is specifically precipitated by the anti-IgA antibody (Fig. 9, compare lanes *1* and *2*). We conclude that at least some of the released d-IgA is complexed with SC. We could not quantitate this because we observed fairly rapid dissociation of the SC-dIgA complex (data not shown).

Discussion

Using a cDNA clone containing the coding region of the rabbit poly-Ig-receptor and a retroviral expression system, we have introduced and expressed a functional poly-Ig-receptor in fibroblasts. The receptor is transported to the cell surface and binds dIgA. At least 30% of the bound dIgA is endocytosed and then rapidly released into the medium together with the cleaved fragment of the receptor, SC.

We do not know whether cleavage takes place at the cell surface or in endosomes. A significant fraction of the poly-Ig receptor that has been surface iodinated at 4°C is cleaved and released after warming to 37°C for just 1 min. dIgA that has been bound at 4°C is released with similar kinetics upon warming. This rapid time course suggests that at least some cleavage occurs at the cell surface without prior endocytosis,

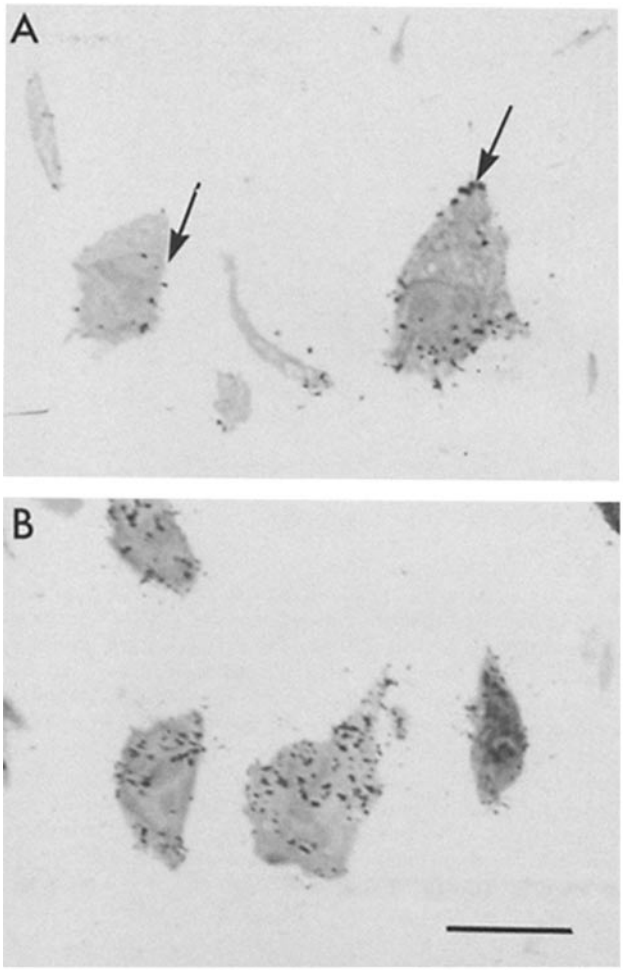


Figure 7. Light microscopic autoradiography of bound ^{125}I-dIgA. Clone 1C cells were incubated with 2.0 μg/ml ^{125}I-dIgA for 4 h at either 4°C (*A*) or 37°C (*B*). 0.5-μm sections were processed for autoradiography. Exposure was for 3 wk. (*A*) Within the resolution of ^{125}I light microscopic autoradiography, labeled IgA appears to be present predominantly on the surfaces of cells incubated at 4°C. (*B*) At 37°C, labeled IgA has accumulated inside the cells. Bar, 10 μm.

since endocytosis and re-exocytosis usually do not occur this quickly (3). However, at least 30% of the bound dIgA is internalized. Presumably it is bound to uncleaved receptor, as otherwise the dIgA-SC complex would probably dissociate from the cell surface. Whether the internalized receptor was cleaved while inside the fibroblast or after re-exocytosis is unknown. There is evidence from normal cells to support either alternative: in liver cleavage is believed to occur after exocytosis at the bile canaliculus (27) which is the equivalent of the apical domain, whereas in intestines cleavage appears to occur in transcytotic vesicles (18).

Except for polarized transcytosis, the fibroblasts correctly carry out all of the transport and processing steps of the poly-Ig receptor. This indicates that fibroblasts possess the machinery to recognize the signals on the receptor for transport and cleavage. This is surprising since the very nature of the receptor's pathway is intimately involved with epithelial cell polarity and since fibroblasts lack apical and basolateral surfaces and the transcytotic pathway connecting them. Fibroblasts transport many molecules to the plasma membrane and

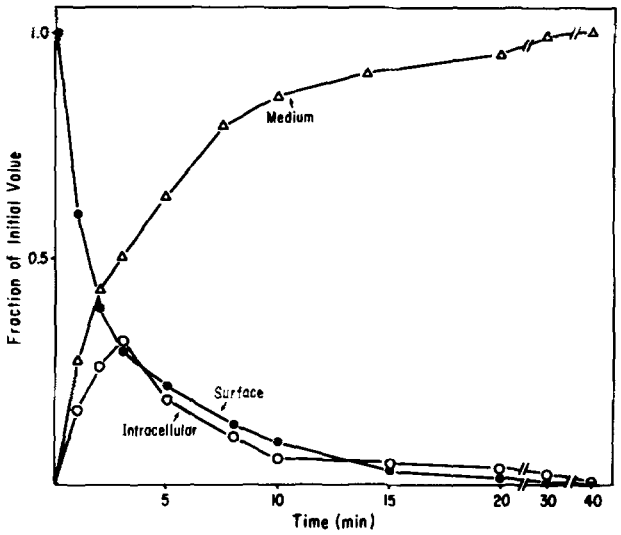


Figure 8. Internalization and re-externalization of ^{125}I-dIgA. Cells were incubated with 0.4 μg/ml ^{125}I-dIgA for 2 h at 4°C, and then washed at 4°C. For warmup, 1 ml of media (DME, 10% fetal calf serum, 20 mM Hepes, pH 7.3) at 37°C was added and the dishes floated in a 37°C water bath. After the indicated time, media was removed and the cells rinsed quickly with 1 ml cold PBS. The warm-up media and PBS rinse were pooled and counted and taken to indicate dIgA released into the media (△). Cells were digested with pronase as described in Materials and Methods. ●, pronase accessible cell surface material; ○, pronase resistant intracellular material. Points are corrected for ~6% of the bound dIgA that could not be digested at time 0. Values are expressed as the percentage of total dIgA initially bound, which averaged 1,172 pg per 35-mm dish. Points are means of triplicates. The standard error of the means was <15%.

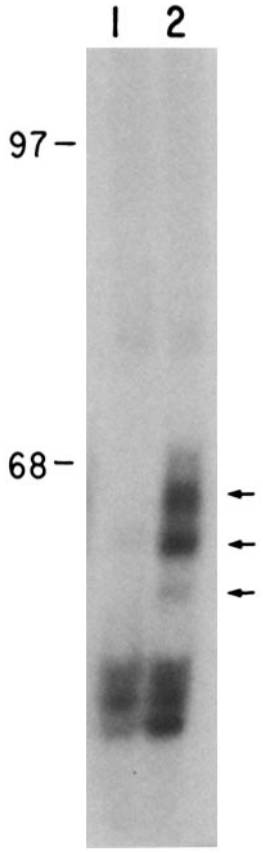


Figure 9. Binding of released SC to dIgA. Cells were iodinated and then incubated with 50 μg/ml unlabeled dIgA for 2 h at 4°C. After washing, cells were warmed to 37°C for 20 min. Media was immunoprecipitated as described in Materials and Methods. Lane *1*, immunoprecipitation with normal goat serum; lane *2*, immunoprecipitation with antiserum to human IgA.

endocytose many receptors. One might therefore reasonably expect that the poly-Ig receptor would be similarly transported to the cell surface and be endocytosed. Our results suggest that since fibroblasts cannot sort the poly-Ig receptor into a non-existent transcytotic pathway, by default they recycle it back to the cell surface, presumably in the same vesicles carrying other recycling receptors.

Of course, fibroblasts do have some degree of polarity. In culture, one surface is exposed to the medium, while the other attaches to the plastic substrate. Furthermore, various processes extend from the cell body and it has been proposed that at least under conditions of cell locomotion, endocytosis and exocytosis are concentrated at different regions of the fibroblast cell surface (2). It is possible that polarized insertion or endocytosis of the poly-Ig receptor occurs in our fibroblast line. However, neither of the types of polarity observed in fibroblasts is related in any obvious way to the division of epithelial cell surfaces into apical and basolateral domains.

The fibroblasts can also cleave the poly-Ig receptor into SC, although we do not know if cleavage is at exactly the correct position(s). Little is known about the protease involved, but the data presented here indicate either that the requisite protease is found in fibroblasts and is thus probably fairly universal, or that cleavage is autocatalytic. By way of comparison, when proinsulin is expressed in mouse fibroblasts, uncleaved proinsulin is released into the medium. If proinsulin is expressed instead in a mouse pituitary cell line which makes and correctly processes ACTH, the proinsulin is sorted into a regulated secretion pathway and processed to insulin (17). On the other hand, when the somatostatin precursor was expressed in monkey fibroblasts (COS cells), it was apparently processed to somatostatin (28). More recently, the somatostatin precursor was expressed in many tissues in transgenic mice and processed in a variety of ways, some of which were aberrant (14). These data indicate that correct processing of these hormone precursors is often tissue specific, whereas our data suggest that processing of the poly-Ig-receptor apparently is not.

The results presented here suggest that the signals and cellular machinery needed for transporting the poly-Ig-receptor to the cell surface, endocytosis, and re-exocytosis are common to many cell types (1). The signals that send this receptor first to the basolateral surface, and then after endocytosis sort it into the transcytotic pathway leading to exocytosis at the apical surface, can presumably only be decoded by polarized cells in which this pathway exists. The approach described here allows us to separate the transport steps common to most cells from those limited to polarized epithelial cells. We are currently working to express the poly-Ig-receptor in polarized cell lines. Our goal is to study the transport of normal and mutant receptors in both polarized and nonpolarized cells to determine the nature of the signals involved in various transport processes. The results of this investigation indicate that we have a powerful new approach to studying the transport of poly-Ig and the general problem of membrane protein sorting.

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