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Sorting of a secretory protein (gp80) to the apical surface of Caco-2 cells

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SUMMARY

We have investigated the synthesis and polarized secretion of the exogenous gp80 glycoprotein complex in the human epithelial adenocarcinoma cell line, Caco-2. gp80 is secreted at the apical surface of Madin-Darby canine kidney (MDCK) cells and should, therefore, display the signal(s) required for sorting into the apical exocytic pathway. In Caco-2 cells, no bona fide secretory protein released preferentially at the apical surface has been described so far. To address the question of whether Caco-2 cells possess a machinery capable of delivery of secretory proteins at the apical surface, we stably transfected the cells with a recombinant gene coding for the gp80 glycoprotein complex. Pulse-chase analysis showed that stably transfected Caco-2 cells secrete gp80 quantitatively into the medium. In polarized layers of filter-grown Caco-2 cells, the protein was secreted predominantly at the apical surface, demonstrating the ability of the cells to efficiently sort secretory proteins directly into the apical exocytic pathway. Our results further demonstrate that the apical targeting information of gp80 recognized by MDCK cells is also recognized by Caco-2 cells.

Key words: caco-2 cells, polarized secretion, protein sorting, epithelial cells

INTRODUCTION

Epithelial cells generate and maintain boundaries between the inside and outside of the organism and perform a variety of vectorial functions such as the transport of nutrients, ions and antibodies. The structural basis for these functions is the differentiation of the plasma membrane into two distinct domains that are separated by tight junctions (Rodriguez-Boulan and Nelson, 1989). The basolateral membrane contacts the underlying connective tissue and neighboring cells; the apical membrane faces the exterior of the organism or the epithelial lumen. The two plasma membrane domains differ in their protein and lipid composition, reflecting the ability of the cells to vectorially transport new plasma membrane constituents (Simons and Fuller, 1985; Simons and van Meer, 1988). Depending on the cell type, the characteristic protein composition of the two membranes is generated by a direct exocytic or an indirect endocytic pathway (Simons and Wandinger-Ness, 1990). In Madin-Darby canine kidney (MDCK) cells, newly synthesized plasma membrane proteins are sorted efficiently at the exit from the *trans*-Golgi network (TGN) and are delivered directly to either the apical or the basolateral surface (Le Bivic et al., 1990b; Mostov et al., 1992). In contrast, hepatocytes appear to lack a direct apical exocytic pathway. In these cells, all apical plasma membrane proteins are sorted exclusively by the endocytic pathway. The proteins are initially transported to the basolateral membrane followed by transcytosis to the apical surface (Bartles and Hubbard, 1988). The enterocyte-like Caco-2 cell line uses both pathways. Some endogenous apical membrane proteins, such as sucrase-isomaltase, are sorted efficiently in the TGN and are routed directly to the apical surface analogous to the route found in MDCK cells. Other endogenous apical membrane proteins, such as aminopeptidase N and dipeptidylpeptidase IV, are in part delivered by the direct route to the apical membrane and in part by the transcytotic pathway via the basolateral membrane (Le Bivic et al., 1990a; Matter et al., 1990).

Secreted proteins may also be targeted to either the apical or the basolateral surface domain in polarized epithelial cells (Simons and Fuller, 1985). In addition, these proteins may be released continuously (constitutive secretion) or discontinuously (regulated secretion) from the cells (Kelly, 1985). While it is clear that the sorting of secretory proteins has to occur intracellularly, the molecular mechanisms underlying the sorting process are poorly defined. For MDCK cells, it is well established that newly synthesized secretory proteins are constitutively released in a polarized manner at both surfaces. Laminin and heparan sulfate proteoglycan are secreted at the basolateral side (Caplan et al., 1987), while the gp80 glycoprotein complex and a 20 kDa osteopontin-related polypeptide are predominantly secreted at the apical cell surface (Ullrich et al., 1991; Urban et al., 1987). In contrast to MDCK cells, hepatocytes only have a basolaterally directed secretory pathway (Bartles and Hubbard, 1988). Caco-2 cells, which use exocytic and endocytic sorting for plasma membrane proteins, release all known endogenous secretory proteins predominantly into the basolateral medium (Traber et al., 1987). In addition, a number of exogenous secretory proteins that should be devoid of sorting signals for polarized secretion in epithelial cells are also released predominantly at the basolateral cell surface (Hughson et al., 1989; Rindler and Traber, 1988; Soole et al., 1992). So far, no bona fide secretory protein released prefer-

entially from the apical surface has been described in these cells. These data led to the proposal that, in Caco-2 cells, the basolateral route is signal-independent representing the default pathway in these cells. These results also raised the question of whether this cell line is at all capable of protein secretion at the apical cell surface. Apical secretion would depend entirely on the efficiency of one of the two sorting mechanisms present in these cells: namely, on the efficiency of intracellular sorting in the TGN.

In this study, we introduced a recombinant gene coding for the gp80 glycoprotein complex (Hartmann et al., 1991) into Caco-2 cells. The protein is a species homolog of the rat testes protein, SGP-2 (clusterin, TRPM-2 product), of the human plasma protein, apolipoprotein J (SP40,40, complement lysis inhibitor (CLI)), and the bovine chromaffin granule protein, glycoprotein III (GPIII) (De Silva et al., 1990; Griswold et al., 1986; Leger et al., 1987; Murphy et al., 1988; Palmer and Christie, 1990). gp80 is synthesized as a single-chain precursor protein. After intracellular proteolytic maturation, it is secreted as a disulfide-linked, glycosylated and sulfated, heterodimeric complex. In MDCK cells, the glycoprotein complex is sorted into the apical exocytic pathway, a process that has been shown to be dependent on the attachment of N-linked carbohydrate chains (Urban et al., 1987). The analysis of the transport of the protein in Caco-2 cells should yield information on the presence of a machinery responsible for efficient polarized delivery of secretory proteins at the apical surface and on the cell-type specificity of the signal and the decoding apparatus. Our results demonstrate that secretion of gp80 in Caco-2 cells is polar with 80% of the protein released at the apical surface.

MATERIALS AND METHODS

Caco-2 cells were obtained from Dr A. Zweibaum (INSERM U 178, Villejuif, France). The generation and characterization of polyclonal anti-gp80 antibodies have been described (Urban et al., 1987). The anti-basolateral membrane glycoprotein (blMg)-monoclonal antibody G1/136 (Eilers et al., 1989) was a gift from Dr Hans-Peter Hauri, Basel, Switzerland. The monoclonal antibody against the human gp80 variant complement lysis inhibitor (CLI) was kindly provided by Dr J. Tschopp, Lausanne, Switzerland. Media and reagents for cell culture were obtained from Seromed, Berlin, Germany, and Gibco, Eggenstein, Germany. The cells were cultured on polycarbonate filters (Transwell ™ 3412, Costar, Cambridge, MA). Reagents for transfection, protease inhibitors, alkaline phosphatase-conjugated anti-rabbit IgG-antibody and peroxidase were obtained from Sigma Chemical, Deisenhofen, Germany. Protein A-Sepharose was obtained from Pharmacia Systems, Freiburg, Germany. [^{35}S]methionine (>800 Ci/mmol) was purchased from Amersham Buchler, Braunschweig, Germany. The vector pRSV, carrying the RSV-LTR promotor and SV40 splicing and poly(A)$^+$ signals, was obtained from I. Carras, Genentech, San Francisco, CA. A full-length gp80 cDNA was inserted into the *Bam*HI restriction site of pRSV to yield pRSV/gp80.

Cell culture

The human colon carcinoma-derived cell line, Caco-2 (Pinto et al., 1983), was grown in Dulbecco's modified Eagle's medium (DMEM, 4.5 g glucose/l) with 1% non-essential amino acids, 2 mM glutamine and 20% foetal calf serum (FCS) in an atmosphere of 95% air and 5% CO_2. Cells between passages 80 and 110 were used for experiments. For the study of polarized secretion, cells were seeded on polycarbonate filters (1×10^6 to 1.5×10^6 cells/filter), 24.5 mm in diameter, with 2 ml medium in the upper and 3 ml in the lower chamber. The medium was changed the next day and then every 2-3 days. Under these conditions, the cells were cultured for 7-14 days after confluency to permit formation of differentiated monolayers. Transepithelial resistance (TER) was measured using a Millicell^R-ERS voltohmmeter (Millipore, Eschborn, Germany). Filters having an electrical resistance across the monolayer of at least 450 ohms·cm^2 were chosen for experiments. Strain II MDCK cells were used for control experiments and cultured as described before (Urban et al., 1987).

Transfection and selection

To generate stable transformants of Caco-2 cells, the plasmids pRSV/gp80 and pSV-2 neo (confering resistance against the selecting drug G418) were cotransfected by calcium phosphate-mediated transfection of adherent cells in suspension (Sambrook et al., 1989). Briefly, exponentially growing Caco-2 cells were harvested by trypsinization and resuspended in culture medium containing serum at a concentration of 2×10^6 cells/ml. One milliliter of cell suspension and 1 ml of the calcium phosphate-DNA coprecipitate, containing 20 μg of DNA with a 1:7 ratio of selectable to nonselectabe gene, were mixed and added to a 94 mm Petri dish. After 20 minutes at room temperature, 8 ml of medium were added, and the cells were incubated for a total of 8 hours at 37°C and in 5% CO_2. Cells were then glycerol-shocked with 15% glycerol for 2 minutes at 37°C and returned to 37°C for 48 hours. Trypsinized cells were split 1:2 into new dishes containing 500 μg/ml G418 sulphate (Geneticin, actual drug concentration). After 4 weeks, individual colonies of stable transfectants were picked and expanded in culture medium containing 250 μg/ml G418 sulphate.

Metabolic labelling of cells and immunoprecipitation

To screen for gp80 expression, transformed Caco-2 cells were metabolically labelled as subconfluent monolayers in 35 mm plastic dishes. Labelling was performed with 300 μCi of [^{35}S]methionine in 1.5 ml labelling medium (MEM, lacking methionine, supplemented with 2.2 g/l $NaHCO_3$, 10 mM Hepes (pH 7.4), 0.2% BSA and protease inhibitors antipain 1 μg/ml, trypsin inhibitor 10 μg/ml and benzamidine 1.75 μg/ml) overnight at 37°C. Media were collected and immunoprecipitations were performed as described (Kondor-Koch et al., 1985) using our polyclonal anti-gp80 antibody or a monoclonal antibody (CLI-9) directed against the human variant. For the study of polarized secretion, cells were seeded on polycarbonate filters and grown for 7-14 days after confluency to develop a high transepithelial resistance.

For continuous labelling, Caco-2 and Caco-2/gp80 cells were washed twice with PBS containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBS$^+$). A 1 ml sample of labelling medium was added to the apical and 2 ml of labelling medium containing 0.5 mCi [^{35}S]methionine was added to the basolateral chamber. Labelling continued for 6 hours or overnight. Pulse labelling of filter-grown Caco-2/gp80 high resistance monolayers and 3-day-old filter cultures of MDCK cells was performed as described (Urban et al., 1987). Briefly, cells were starved for 30 minutes in methionine-free medium, and then 200 μCi [^{35}S]methionine in 150 μl labelling medium was applied to the basolateral side of the inverted filter for 25 minutes at 37°C. Filters were chased with 1 ml labelling medium containing methionine (15 mg/l) in the apical and 2 ml in the basolateral chamber for various periods of time. The entire cell lysate and the separately collected apical and basolateral media of one filter-grown monolayer were used and immunoprecipitated with the gp80 antibody. Immunoprecipitated proteins were reduced with 10 μM DTT when indicated and analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Piccioni et al., 1982). Fluorography was performed as described by Bonner and Laskey (1974), and bands were visualized using Kodak X-Omat films. TCA-precipitations were done by adding TCA to a concentration of 10% to the samples. After incubation overnight at 4°C, the precipitate was collected by centrifugation, washed with ice-cold 10% TCA

and resuspended in sample buffer. Specific bands were quantitated on appropriate exposures by densitometry scanning with a DESAGEA CD 50 (DESAGA, Heidelberg, Germany).

Immunofluorescence

Immunofluorescence analysis of high-resistance monolayers of Caco-2 and Caco-2/gp80 cells grown on filters was performed by an indirect technique (Fuller et al., 1984). Briefly, cells were fixed in 3% paraformaldehyde for 30 minutes at RT, quenched with 50 mM NH_4Cl in PBS^+ and permeabilized with 0.2% Triton X-100 in PBS for 5 minutes if indicated. The cell-layers were incubated with a mouse monoclonal antibody (diluted 1:300) against the 120 kDa basolateral membrane glycoprotein (blMg) followed by a Texas Red-conjugated anti-mouse Ig antibody (diluted 1:50) for 30 minutes; 300 µl of antibody solution were applied to the apical side of each filter. The filters were cut out of the holders and mounted in PBS/glycerol. Immunofluorescence analysis of subconfluent Caco-2 cells grown on coverslips was performed with our anti-gp 80 antibody (diluted 1:500) or a monoclonal antibody (CLI-9) against the human variant (diluted 1:500). The second antibody was a Texas Red-conjugated anti-rabbit or anti-mouse Ig antibody, respectively.

Other methods

For immunoblot analyis, confluent cells in 35 mm dishes were incubated overnight in 1 ml FCS-free, normal growth medium supplemented with protease inhibitors. Media were collected and immunoblots were done as described (Burnette, 1981) using our polyclonal anti-gp80 antiserum from rabbit as first antibody and an antibody against rabbit IgG conjugated to alkaline phosphatase as second antibody. Northern blot analysis was performed according to described methods (Sambrook et al., 1989). For testing the tightness of filter-grown, high-resistance monolayers, horseradish peroxidase (HRP) type II was diluted at a concentration of 100 µg/ml in normal growth medium and applied to the apical side of the filter. After 6 hours of incubation, apical and basolateral media were collected separately. Enzyme concentration was determined by adding 100 µl of samples to 900 µl of substrate solution in a microcuvette. The sample was mixed carefully and measured after 1 minute in a HITACHI/U-1100 Spectrophotometer at 495 nm.

RESULTS

Caco-2 cells do not express the endogenous gp80 gene

Before transfection, Caco-2 cells were tested for endogenous gp80 expression. We were unable to detect the protein in Caco-2 cells either by immunofluorescence staining or by immunoprecipitation of metabolically labelled cells using a polyclonal anti-canine gp80 antiserum or a monoclonal anti-human CLI antibody (data not shown). The latter antibody was used to exclude ambiguities originating from the use of a canine antiserum for the analysis of human cells. Finally, the gp80 cDNA did not hybridize with poly(A)$^+$ RNA extracted from Caco-2 cells (Fig. 1) despite an identity of 82 % in the coding region of the cDNAs of the human and dog homologs. We conclude, therefore, that Caco-2 cells do not express the endogenous gp80 gene.

Transfected Caco-2 cells synthesize and secrete the gp80 glycoprotein complex

To be able to analyse the intracellular transport of the gp80 glycoprotein complex, Caco-2 cells were cotransfected with pRSV/gp80 and pSV2-neo to yield stable transformants. Transfection was performed by the calcium phosphate/DNA coprecipitation method as described in Materials and Methods. After 4 weeks, neomycin-resistant colonies were picked and expanded. Individual clones were screened for gp80 expression by immunoblot-analysis.

Four clones (Caco-2/gp80 2.4, 2.5, 4.11 and 6.4) out of 25 displayed immunoreactive proteins in the medium and were selected for further analysis. Nearly confluent monolayers of the clones, untransformed Caco-2 cells and MDCK cells were labelled overnight with [^{35}S]methionine (Fig. 2). MDCK cells secrete gp80 as a disulfide-linked heterodimeric complex of 80 kDa into the medium. When subjected to SDS-PAGE under reducing conditions, fluorographies show a protein triplet made up of the 35 kDa and the 45 kDa subunits and a band at 40 kDa that is composed of both subunits exhibiting varying degrees of *N*-glycosylation (Urban et al., 1987).

As shown in Fig. 2, all clones analysed secrete the characteristic 35-45 kDa protein triplet of the dissociated gp80 glycoprotein complex into the medium, though in different amounts. A small portion of the uncleaved 80 kDa precursor is also visible. No proteins could be detected in the medium of untransformed Caco-2 cells. The analysis demonstrates that the four Caco-2/gp80 clones secrete correctly synthesized and processed gp80 into the medium, though with a somewhat different ratio of the 35 kDa form to the 40 kDa form, suggesting that the smaller subunit in Caco-2 cells occurs predominantely as the 40 kDa species rather than the 35 kDa species.

Clone Caco-2/gp80 2.4 yielded the highest amount of immunoprecipitable protein in the medium. This clone was named Caco-2/gp80 and chosen for further analyses.

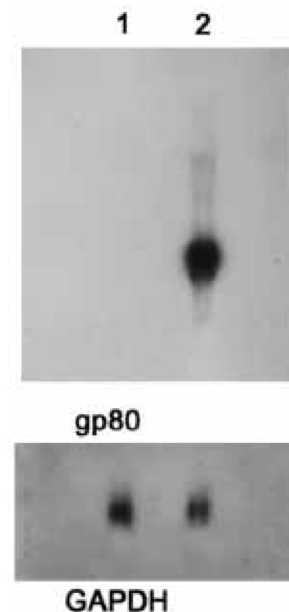

Fig. 1. Northern blot analysis of mRNA from Caco-2 cells. A 5 µg sample of poly(A)$^+$ RNA isolated from Caco-2 cells (lane 1) and MDCK cells (lane 2) was loaded on each lane. The blots were hybridized with the 1327 bp gp80/*Sac*I fragment (upper panel). Hybridization to a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (Fort et al., 1985) was used as control for the amount of mRNA loaded (lower panel).

The stably transfected Caco-2 cells have maintained their epithelial phenotype

Several assays were performed to compare Caco-2/gp80 cells with wild-type Caco-2 cells with regard to the maintainance of the epithelial character. When grown to confluency on impermeable plastic supports, Caco-2/gp80 cells showed the characteristic polygonal shape and the typical dome formation of the parent cell line. When grown on porous polycarbonate filters for 7-14 days after confluency, the transfected cell line formed tight monolayers that displayed transepithelial resistances higher than 450 ohms·cm^2. The transepithelial resistance developed was similar to that of the parent cell line. The plasma membrane polarity of filter-grown Caco-2/gp80 cells was tested by indirect immunofluorescence using an antibody against the endogenous 120 kDa basolateral membrane glycoprotein (blMg). The expression of this protein has been described as being restricted to the basolateral membrane in Caco-2 cells (Eilers et al., 1989). Anti-blMg antibodies were applied to the apical surface of either unpermeabilized or Triton X-100-permeabilized monolayers followed by a secondary Texas Red-conjugated anti-mouse Ig antibody. In unpermeabilized cells, the antibodies had access only to apical antigens, whereas, in permeabilized cells, apical, basolateral and internal antigens could be reached. As shown in Fig. 3B, the staining of the unpermeabilized cells resulted in only background fluorescence, whereas in Fig. 3A the permeabilized cells show the typical polygonal staining pattern that is characteristic of a basolateral antigen.

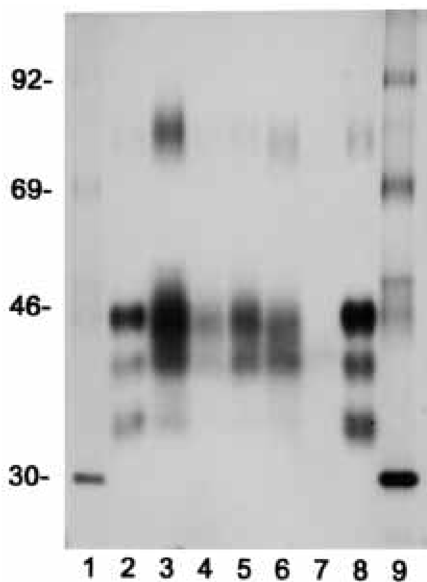

Fig. 2. Analysis of gp80 secreted by transformed Caco-2 clones, untransformed Caco-2 cells and MDCK cells. The cells were grown to near confluency in 35 mm plastic dishes and labelled overnight with 300 μCi [^{35}S]methionine in 1.5 ml labelling medium. Radiolabelled proteins of media samples were immunoprecipitated with anti-gp80 antibodies, reduced and analysed by SDS/10%PAGE. Lanes 2 and 8, MDCK cells; lanes 3-6, Caco-2/gp80 clones 2.4, 2.5, 4.11 and 6.4, respectively; lane 7, untransformed Caco-2 cells; lanes 1 and 9, molecular mass standards (indicated in kDa).

Caco-2 cells are known to secrete their endogenous secretory products predominantly at the basolateral surface (Hughson et al., 1989; Rindler and Traber, 1988). To confirm that Caco-2/gp80 cells retained the described polarity of secretion of endogenous proteins, filter-grown monolayers were continously labelled with [^{35}S]methionine. As shown in Fig. 4, TCA-precipitable proteins from separately collected apical and basolateral media demonstrate the described polarity of secretion. Quantitation of the individual lanes by densitometry proved that 85% of endogenous secretory proteins are released into the basolateral medium of Caco-2/gp 80 cells.

The gp80 glycoprotein-complex expressed in the transfected cells is released predominantly at the apical cell surface

For a more detailed characterization of the biogenesis of the glycoprotein complex in Caco-2/gp80 cells, and to make sure that the majority of the protein is secreted into the medium, pulse-chase experiments were performed. Caco-2/gp80 cells were grown 7-14 days post-confluency on polycarbonate filters to allow separate analysis of the apical and basolateral media. Binding of gp80 to the Transwell filters has been described to be minimal (Urban et al., 1987). Because errors due to paracellular leaks are often a problem in studies of this type, the tightness of the monolayer was evaluated before each experi-

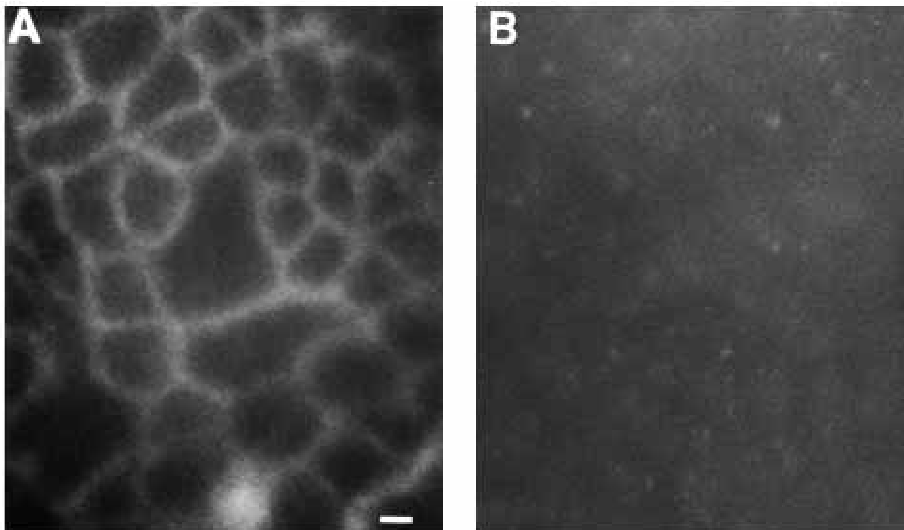

Fig. 3. Analysis of cell surface distribution of the endogenous basolateral membrane glycoprotein (blMg) by indirect immunofluorecence. Cells of clone Caco-2/gp80 were grown to high resistance monolayers on polycarbonate filters. After fixation in 3% paraformaldehyde, one of the two filters was permeabilized with Triton X-100. Specific antibodies against the blMg of 120 kDa were applied apically to both filter cultures followed by a secondary Texas Red-conjugated antibody. (A) Staining of the blMg in cells treated with Triton X-100 to allow the antibodies access to basolateral and internal antigens. (B) Staining of the blMg in unpermeabilized cells. Bar, 12 μm.

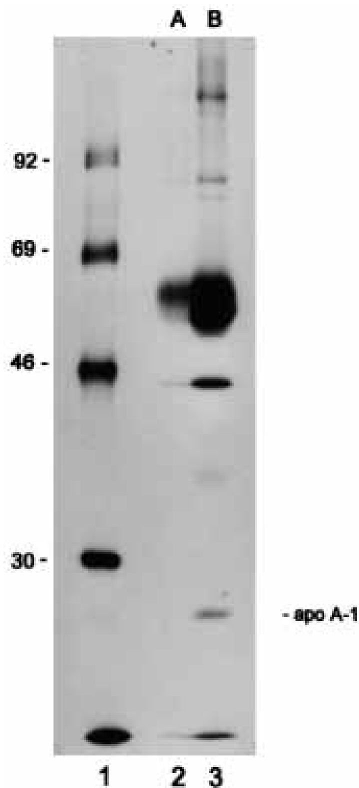

Fig. 4. Fluorography showing the polarized secretion of endogenous proteins by Caco-2/gp80 cells. Filter-grown high resistance monolayers of Caco-2/gp80 cells were continously labelled for 6 hours with 500 μCi [^{35}S]methionine. Apical (lane 2) and basolateral (lane 3) media were collected separately and precipitated in 10% TCA. Aliquots of the samples were analysed by SDS-10%PAGE and visualized by fluography. Molecular mass standards (in kDa) were run in lane 1. The position of apolipoprotein A-1 is indicated on the right.

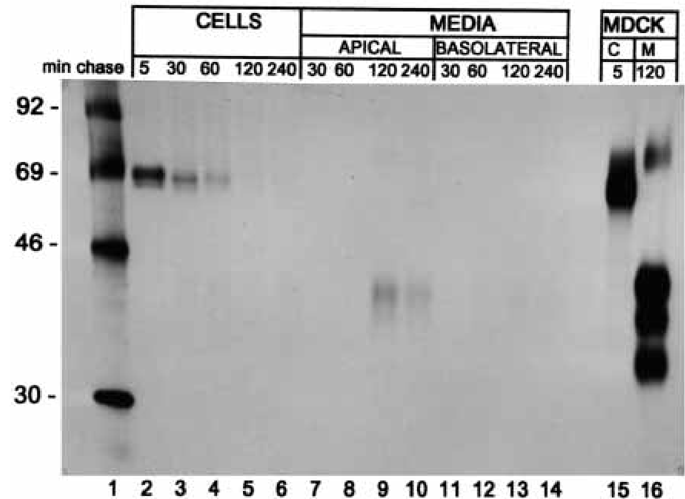

Fig. 5. Pulse-chase analysis of processing and secretion of the gp80 glycoprotein complex in Caco-2/gp80 cells. High resistance cell monolayers grown on polycarbonat filters were pulse labelled for 25 minutes with 200 μCi [^{35}S]methionine and chased for the indicated periods of time. Proteins in the cell lysates and separately collected apical and basolateral media were immunoprecipitated and run under reducing conditions on SDS-10%PAGE. Labelling of MDCK cells was included in the experiment for comparison of endogenously secreted gp80 and gp80 secreted from cDNA. Lanes 2-6 show the immunoprecipitated protein in the cell lysates; lanes 7-10 in the apical and lanes 11-14 in the basolateral media of Caco-2/gp80 cells; lanes 15 and 16 show gp80 immunoprecipitated from cell lysate and medium of MDCK cells. gp80 is depicted either as the 65 kDa precursor protein or as the subunits of 35-45 kDa. Molecular mass standards (in kDa) were run in lane 1.

ment by determining the transepithelial resistance. In addition, leakage across the monolayer was tested by adding horseradish peroxidase into the apical medium of filter-grown cells. After incubation for 6 hours at 37°C, apical and basolateral media were separately collected and peroxidase concentration was quantitated in the individual samples as described in Material and Methods. Filter-grown Caco-2/gp80 cells with a resistance across the monolayer higher than 450 ohms·cm^2 showed no leakage of HRP into the basolateral medium and were used for experiments.

Five filter-grown monolayers were labelled with [^{35}S]methionine from the basolateral side and then chased for various periods of time at 37°C. gp80 present in the cell lysates and the apical and basolateral media were immunoprecipitated, reduced with 10 μM DTT and analysed by SDS-PAGE. A similar experiment was performed with filter-grown monolayers of MDCK cells. In MDCK cells, gp80 is first detectable as a protein of approximately 65 kDa, representing the high-mannose form present in the endoplasmic reticulum. This precursor is converted into a protein of 80 kDa, which is only present in minor amounts in the cells, since it is rapidly cleaved into the 35-45 kDa polypeptides that are linked together by disulfide bonds (Urban et al., 1987). As shown in Fig. 5, after 5 minutes of chase, the 65 kDa high-mannose form of gp80 was detected in the lysate of Caco-2/gp80 cells. After 120 minutes of chase, only minor amounts of the protein were still present in the cell lysate, and the 35-45 kDa subunits appear in the media. gp80 secretion in Caco-2/gp80 cells was polar, with most of the protein being secreted into the apical medium. The results show that Caco-2/gp80 cells secrete gp80 quantitatively into the medium, and that this secretion occurs predominantly at the apical cell surface.

To be able to compare the ratio of gp80 secreted into apical and basolateral media more precisely, filter-grown Caco-2/gp80 monolayers, continuoulsy labelled for 6 hours or overnight, were analysed. Proteins from cell lysates and separately collected apical and basolateral media were immunoprecipitated with anti-gp80 antibodies and separated by SDS-PAGE. Bands were visualized by fluorography and quantitated by densitometry. As shown in Fig. 6A,B for the 6-hour labelling period, Caco-2/gp80 cells secrete gp80 in a polarized manner with 80% of the protein appearing in the apical and 20% in the basolateral medium. The same result was obtained when the cells were labelled overnight. The standard deviation was 5% as determined from eight independent experiments. Because the cells were not chased with cold methionine, a small amount of the 65 kDa precursor is visible in the cell lysate. Similar experiments performed with clone Caco-2/gp80 4.11 confirmed that also in this clone the secretion of the gp80 glycoprotein complex occurred predominantly at the apical cell surface (data not shown). Therefore the distribution of gp80 in

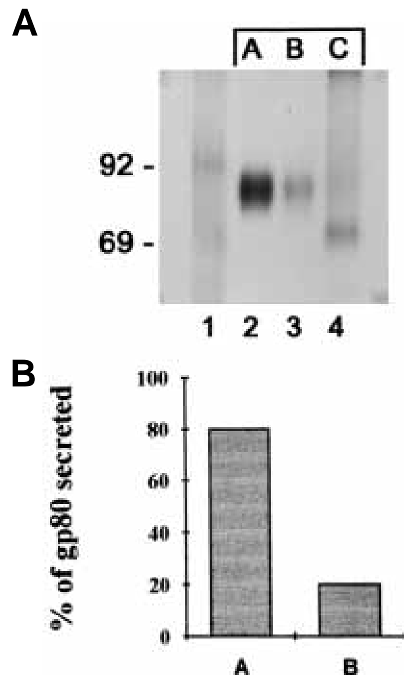

Fig. 6. Polarized secretion of gp80 by Caco-2/gp80 cells. (A) Filter-grown high resistance monolayers were labelled for 6 hours with 500 μCi [^{35}S]methionine. Immunoprecipitated proteins from cell lysate and separately collected apical and basolateral media were run under nonreducing conditions on SDS-12.5%PAGE followed by fluorography. Lanes 2 and 3, apical (A) and basolateral (B) media, respectively; lane 4, cells (C). Molecular mass standards were run in lane 1 and indicated (in kDa). (B) The graph shows the distribution of gp80 over the apical (A) and basolateral (B) media as quantitated by densitometry.

the apical and basolateral media of the transfected cells is unlikely to be due to clonal variability.

DISCUSSION

This report provides the first description of a bona fide secretory protein released predominantely at the apical surface of Caco-2 cells, and thus demonstrates that these cells possess the required machinery for polarized apical secretion.

Caco-2 cells secrete endogenous secretory proteins predominantly into the basolateral medium. Exogenous secretory proteins that should lack sorting signals are also predominantly released at the basolateral surface (Rindler and Traber, 1988; Hughson et al., 1989; Soole et al., 1992). Therefore, proteins secreted preferentially at the apical plasma membrane domain should carry specific sorting information. The gp80 glycoprotein complex is a well-characterized marker for polarized apical secretion in MDCK cells (Urban et al., 1987). By analysing the biogenesis of the protein in transfected Caco-2 cells, we could demonstrate that the efficiency of sorting is identical in these cells and MDCK cells, with 80% and 79% of gp80 found in the apical medium, respectively. Thus, the apical targeting information present in the protein is recognized with comparable efficiency in both cell lines.

Only two other examples of a preferential apical release in enterocytes or enterocyte-like cells have been described. A 110 kDa form of the lysosomal enzyme, acid α-glucosidase, is released predominantly at the apical plasma membrane in Caco-2 cells (Eilers et al., 1989; Klumperman et al., 1991). Since the enzyme is synthesized as a membrane-bound precursor protein, it is unclear if sorting into the apical exocytic pathway occurs on the membrane-bound or the soluble form of the enzyme that is detected in the medium (Wisselaar et al., 1993). In contrast to α-glucosidase, another lysosomal enzyme, β-hexosaminidase, which is synthesized as a soluble precursor protein, is released predominantly at the basolateral cell surface (Rindler and Traber, 1988).

The second example is the recent demonstration that apolipoprotein A-1 (apo A-1) is released predominantly (>85%) at the apical surface in the epithelial cells of jejunal segments (Danielsen et al., 1993), while in Caco-2 cells, apo A-1 is secreted preferentially at the basolateral cell surface (Traber et al., 1987; this paper, Fig. 4). Because Caco-2 cells synthesize α-fetoprotein and express more apo B-100 than apo B-48 (Rindler and Traber, 1988; Traber et al., 1987), it has been argued that these cells do not resemble mature enterocytes, but rather represent intestinal cells at an early fetal stage of development in which a direct apical secretory pathway has not yet been established. Our data reveal the presence of a direct apical secretory route in Caco-2 cells and demonstrate the capability of these cells to sort secretory proteins into this pathway. Nevertheless, the ability to sort apo A-1 into the apical exoytic pathway may well be a property that either has not yet been established in Caco-2 cells or has been lost during adaption to cell culture.

Evidence has been presented from the study of human growth hormone transport after ectopic expression in transgenic mice that a regulated route of exocytosis may be present in the enterocytes of the small intestine (Trahair et al., 1989). Rindler and Traber (1988) examined the transport of rat growth hormone in Caco-2 cells and found it to be secreted predominantly from the basolateral side. They assumed that the secretion was constitutive; however, they did not determine what fraction of the metabolically labelled hormone resided inside the cells after the chase period. The bovine homolog of gp80, named GPIII, has been isolated from secretory granules of adrenal medulla chromaffin cells, suggesting that, in cells featuring a regulated pathway of secretion, the protein is sorted into that pathway (Palmer and Christie, 1990). Using the phaeochromocytoma cell line PC12 as a model system, we have shown that the rat homologue of gp80 is indeed sorted into the regulated pathway (C. Pilarsky et al., unpublished data). If a regulated pathway is present in Caco-2 cells, we should have been able to detect it with the gp80 probe. However, we obtained no evidence for a regulated route of exocytosis. The mature gp80 glycoprotein complex was quantitatively secreted from Caco-2 cells, and only trace amounts of the 65 kDa high mannose precursor could be detected inside the cells. We conclude that either no regulated pathway is present in Caco-2 cells, or gp80 is not the appropriate probe to detect it in these cells.

Previous to this report there was no evidence for direct, intracellular apical sorting of soluble proteins in Caco-2 cells, although many membrane-bound proteins have been shown to be directly targeted to the apical membrane. Thus, one might have assumed that different pathways were needed to sort soluble and membrane-bound proteins. However, our data are

consistent with a model in which soluble and membrane-bound proteins are sorted together. The gp80-expressing Caco-2 cells can in future be used as a model system to characterize the molecular mechanisms underlying intracellular sorting in intestinal cells.

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