An Endogenous MDCK Lysosomal Membrane Glycoprotein Is Targeted Basolaterally before Delivery to Lysosomes

Ivan R. Nabi, André Le Bivic,* Douglas Fambrough,‡ and Enrique Rodriguez-Boulan

Department of Cell Biology and Anatomy, Cornell University Medical College, New York 10021; *Laboratoire de Differenciation Cellulaire, Faculté des Sciences, Luminy, Marseilles Cedex 9, France; and ‡Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Abstract. Using surface immunoprecipitation at 37°C to "catch" the transient apical or basolateral appearance of an endogenous MDCK lysosomal membrane glycoprotein, the AC17 antigen, we demonstrate that the bulk of newly synthesized AC17 antigen is polarly targeted from the Golgi apparatus to the basolateral plasma membrane or early endosomes and is then transported to lysosomes via the endocytic pathway. The AC17 antigen exhibits very similar properties to members of the family of lysosomal-associated membrane glycoproteins (LAMPs). Parallel studies of an avian LAMP, LEP100, transfected into MDCK cells revealed colocalization of the two proteins to lysosomes, identical biosynthetic and degradation rates, and similar low levels of steady-state expression on both the apical (0.8%) and basolateral (2.1%) membranes. After treatment of the cells with chloroquine, newly synthesized AC17 antigen, while still initially targeted basolaterally, appears stably in both the apical and basolateral domains, consistent with the depletion of the AC17 antigen from lysosomes and its recycling in a nonpolar fashion to the cell surface.

POLARIZED epithelial cells form a cohesive monolayer in which the apical membrane, facing the lumen, has a protein and lipid composition distinct from the basolateral membrane which faces the internal medium. These differences are crucial to the role of epithelia in regulating the flow of water, ions, and other solutes between biological compartments of different composition. The two distinct surface domains are separated by the zonula occludens, or tight junction, which constitutes the main permeability barrier of the epithelial monolayer (53). Recent work has identified two sites where apical and basolateral proteins are sorted during biogenesis: the *trans*-Golgi network (TGN)[1] and the basolateral surface. Viral, endogenous, and transfected proteins are delivered directly from the TGN, via carrier vesicles, to the apical and basolateral surfaces of MDCK cells (33, 42, 45, 49, 51, 54, 64). In the hepatocyte, the only route demonstrated for apical protein delivery is indirect, via the basolateral surface (1, 25). An intermediate phenotype with both direct and indirect delivery of apical proteins was found in intestinal cells (34, 43). An indirect pathway can also exist in MDCK cells, since the polymeric Ig receptor, which transports IgA or IgM produced in the lamina propria, follows a transcytotic (indirect) pathway in all epithelial cells studied to date (46). No indirect pathway has yet been described for basolateral proteins.

The signals that direct proteins along these pathways are currently the object of intense study (53, 58). Apical targeting information may be present in the ectodomain of viral glycoproteins (11, 44, 55) and in the glycolipid anchor of proteins attached to the membrane via a glycosyl phosphatidyl inositol linkage (6, 38, 40, 66). Many rapidly endocytosed proteins are basolaterally distributed (16–18, 50), and recent work has suggested a correlation between the presence of signals for coated pit internalization in the cytoplasmic domain and basolateral targeting in MDCK cells. An F_c receptor isoform containing an internalization signal is targeted basolaterally, while another isoform differing solely in the cytoplasmic domain and lacking an internalization signal is targeted apically in MDCK cells (26). A cytoplasmic deletion that confers endocytic properties to the nerve growth factor receptor reverses its polarity from apical to basolateral in transfected MDCK cells (35). Insertion of a tyrosine in the cytoplasmic domain of apical influenza hemagglutinin (HA), normally excluded from coated pits and poorly endocytosed, results in both coated pit internalization and basolateral targeting (5, 30). However, the basolateral targeting signal appears to be distinct from the endocytic signal for the polymeric Ig receptor (7).

In addition to sorting apical and basolateral proteins in epithelia, the TGN also segregates soluble lysosomal enzymes from the secretory pathway. Mannose-6-phosphate (M6P) containing lysosomal enzymes interacts with M6P receptors in the TGN and are then transported within clathrin-coated vesicles to an acidic prelysosomal compartment; here the enzyme dissociates from the receptor and is

1. *Abbreviations used in this paper*: endo F and H, endoglycosidase F and H, respectively; LAMP, lysosomal associated membrane glycoproteins; LAP, lysosomal acid phosphatase; M6P, mannose-6-phosphate; TGN, *trans*-Golgi network.

© The Rockefeller University Press, 0021-9525/91/12/1573/12 $2.00

then transported to lysosomes, whereas the free M6P receptors are recycled back to the TGN (13, 62). The M6P receptor-rich compartment is referred to as both the prelysosome and the late endosome and the two terms will be used interchangeably throughout the text. At steady state, M6P receptors are predominantly localized to prelysosomes but are also found in the TGN, peripheral endosomes, and the plasma membrane (22).

Integral membrane lysosomal glycoproteins lack the M6P signal and are still targeted to lysosomes (28). Lysosomal acid phosphatase (LAP), a transmembrane glycoprotein lacking an M6P signal, is transported via the cell surface to the lysosome, where it is proteolytically cleaved to release soluble enzyme (4, 48, 63). A family of lysosomal associated membrane glycoproteins (LAMPs) including avian LEP100 (14), human lamp-1 and lamp-2 (15, 61), mouse LAMP-1 and LAMP-2 (8, 10), and rat lgp110 and lgp120 (20, 24) has been described. The surface expression of LAMPs is low (36, 37) or undetectable (28). However, deletion of the cytoplasmic tyrosine from the short (11 amino acids) cytoplasmic tail of human lamp-1 inhibits its transport to lysosomes such that it accumulates at the cell surface (65). It has been proposed that the route of LAMPs to lysosomes is via the same M6P receptor-rich prelysosomal compartment described for proteins containing the M6P lysosomal targeting signal (21). We show here that an endogenous MDCK lysosomal membrane glycoprotein, recognized by the AC17 monoclonal antibody, exhibits identical properties to transfected LEP100 and transits the basolateral surface and/or early endosomes before delivery to lysosomes.

Materials and Methods

Cells and Materials

MDCK II cells were grown in Dulbecco's minimum essential medium (DMEM) (Gibco Laboratories, Grand Island, NY) supplemented with 5% fetal bovine serum (Hyclone Laboratories Inc., Logan, UT), glutamine, and nonessential amino acids (Gibco Laboratories) in an air–5% CO_2 atmosphere at constant humidity. When grown on filters, 2×10^6 cells were seeded on 24.5-mm Transwell polycarbonate filters (Costar Corp., Cambridge, MA) and cultured for 5–7 d with changes of medium every other day. Transepithelial resistance was measured using a Millipore device (Millipore Corp., Bedford, MA) and was typically 200–300 $\Omega \cdot cm^2$. Primary cultures of quail fibroblasts were the kind gift of Dr. Takashi Mikawa (Department of Cell Biology, Cornell University Medical College, New York) and were cultured in DMEM supplemented with 10% fetal bovine serum, glutamine, and nonessential amino acids. All chemicals were from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. The Stella 2.1 modeling program was purchased from High Performance Systems (Hanover, NH).

Transfection of LEP100 cDNA into MDCK Cells

The major EcoRI fragment encompassing the LEP100 cDNA (14) was inserted into the EcoRI cloning site of the pVcos expression vector (41). Transfection of MDCK cells was performed by a modification of the calcium-phosphate precipitation procedure as described previously (18, 19). LEP100-expressing clones were selected by immunofluorescence with the CV24 anti-LEP100 mAb (36). Over 90% of the cells from the LEP-3F6 clone expressed LEP100 and this clone was used for all subsequent studies.

Antibodies

The mouse AC17 mAb (IgG_1) was produced by immunization of BALB/c mice with MDCK cells detached with PBS/EDTA and injected intraperitoneally (10^6 cells/mouse). Boosts with the same material were done at days 20, 40, 51, 52, and 53 and the mouse was killed at day 54. Hybridomas between spleen cells and NS1 myeloma cells were produced as previously described (31). Hybridoma supernatants were screened by immunofluorescence of wounded MDCK monolayers and positive clones were subcloned twice by limiting dilution. Ascites fluid was produced from the intraperitoneal injection of 3×10^6 AC17 hybridoma cells into pristane treated BALB/c mice. Scatchard analysis of binding to AC17 antigen in fixed permeabilized cells shows a K_d of ~5 nM for the AC17 mAb. For some experiments monoclonal IgGs were purified from ascites fluids and ^{125}I-iodinated by the iodogen method (56). A polyclonal rabbit antiserum was generated by immunizing rabbits with the peptide KSHAGYQTI, corresponding to the COOH terminus of the cytosolic tail of avian LEP100 and mammalian LAMP1, covalently linked to keyhole limpet hemocyanin. Anti-peptide antibody was isolated by affinity chromatography on a peptide column.

Metabolic Labeling

Confluent monolayers of LEP-3F6 MDCK cells grown on filters for 5–7 d were incubated in methionine/cysteine-free DMEM containing 0.2% BSA for 30 min and then pulse-labeled with ^{35}S-Express (160 μCi/filter; New England Nuclear, Boston, MA) for 20 min on the basolateral surface. Steady-state metabolic labeling was performed by the incubation of filters with ^{35}S-Express (80 μCi/filter) in methionine/cysteine-free DMEM supplemented with 0.1 vol complete medium overnight. Cells were chased in complete medium supplemented with cold methionine and cysteine as described for individual experiments before lysis of the cells.

Immunoprecipitation and Immunoblots

The AC17 antigen and LEP100 were immunoprecipitated by incubation of cell lysates with protein A–Sepharose beads (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) coated sequentially with rabbit anti-mouse antibodies (Cappel Laboratories, Malvern, PA) and the AC17 or CV24 mAbs, respectively, as previously described (32). For endoglycosidase F (endo F) and endoglycosidase H (endo H) (Boehringer Mannheim Corp., Indianapolis, IN) treatments, the immunoprecipitates were eluted by boiling for 5 min in 0.1% SDS and 0.1 M sodium citrate containing a protease inhibitor mix and digested with 0.1 U endo F for 6 h or 1 mU of endo H for 16 h at 37°C. The immunoprecipitated samples were analyzed by SDS-PAGE 8% (29) and the dried gels were processed for fluorography (9). Where indicated, MDCK cells were extracted with 1% Triton X-114 (3) and the detergent and aqueous phases formed at 37°C were analyzed by SDS-PAGE and protein blotting onto nitrocellulose as previously described (60) and AC17 mAb binding revealed with ^{125}I-protein A (New England Nuclear). Autoradiographs were obtained from radiolabeled gels and blots using preflashed film (Eastman Kodak Co., Rochester, NY). Quantitation of the intensity of labeled bands was taken from the average of three passes through the left, middle, and right areas of an individual band using a scanning densitometer (model GS300; Hoefer Scientific Instruments, San Francisco, CA). Comparisons were made between bands of intensities falling within the linear response range of the densitometer.

Surface Immunoprecipitation

Cells on filters pulse-labeled with ^{35}S-Express for 20 min in methionine/cysteine-free medium were chased at 37°C in the presence of normal medium containing AC17 mAb (50× diluted ascites fluid, final mAb concentration ~100 μg/ml) in the apical (0.5 ml) or basolateral (1 ml) medium for the indicated period of time. The filters were washed five times for 15 min with PBS/CM containing 0.5% BSA at 4°C. The cells were lysed at 4°C in lysis buffer (1% Triton X-100, 20 mM Tris, pH 8, 150 mM NaCl, 5 mM EDTA, 0.2% BSA, and a mix of protease inhibitors) and precleared for 1 h with BSA conjugated to CNBr-activated Sepharose 4B (Pharmacia LKB Biotechnology Inc.). Antigen–antibody complexes were precipitated at 4°C for 2 h with rabbit anti-mouse protein A–Sepharose beads and analyzed by SDS-PAGE and fluorography as described above.

Biotin Hydrazide Labeling of Surface Glycoproteins

Exposed glycoproteins on the surface of MDCK cells were labeled as previously described with minor modifications (39). To enhance detection, confluent monolayers of LEP-3F6 MDCK cells were incubated twice for 30 min with 10 mM periodate and then twice for 30 min with 2 mM biotin-X-hydrazide (Calbiochem Corp., La Jolla, CA) added at 4°C to either the apical or basolateral surfaces before immunoprecipitation, blotting, and ^{125}I-streptavidin labeling.

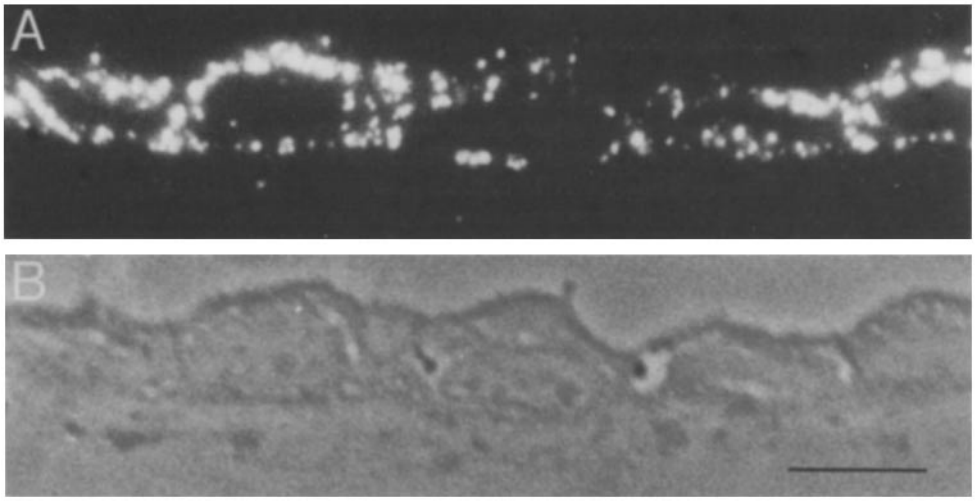

Figure 1. Localization of the AC17 antigen in MDCK cells. Semi-thin frozen sections of MDCK cells were immunofluorescently labeled with the AC17 mAb (*A*). The corresponding phase image is shown in *B*. Bar = 5 μm.

Immunofluorescence

Procedures for indirect immunofluorescence of MDCK cells were as described (52). For intracellular staining, cells fixed with 3% paraformaldehyde were permeabilized with 0.075% (wt/vol) saponin. 0.5-μm frozen sections of MDCK cells on collagen (32) were processed for immunofluorescence as described (59). Fluorescent double labeling of cells was performed with the AC17 and CV24 mAbs using FITC-conjugated CV24 mAb or with the AC17 mouse mAb and polyclonal rabbit anti-tail peptide antibody. Endocytosis of the CV24 and AC17 mAbs was assayed as previously described (65). All fluorescent secondary antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Fluorescently labeled cells on cover slides were mounted in gelvatol (Monsanto Chemical Co., St. Louis, MO) and photographed with a Leitz Ortholux epifluorescence microscope using 400 ASA TMAX film (Kodak).

Immunoperoxidase Labeling and Electron Microscopy

Immunoperoxidase localization of the AC17 antigen and LEP100 to lysosomes was performed as previously described (36). Immunolabeled samples were postfixed in 1% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in Epon. Thin sections were observed in an electron microscope (JEOL U.S.A. Inc., Peabody, MA).

Results

Expression of LAMPs in MDCK Cells

Monoclonal antibodies generated against MDCK proteins were screened by immunofluorescent labeling of wounded MDCK monolayers. One monoclonal antibody, designated AC17 (IgG_1), recognized distinct vesicular structures characteristic of lysosomes (Fig. 1). To characterize the expression of this protein, an exogenous lysosomal glycoprotein, avian LEP100, was transfected into MDCK cells (14, 36, 37). Gentomycin-resistant clones were selected for LEP100 expression by immunofluorescent labeling with the CV24 anti-LEP100 mAb. All expressing clones exhibited a typical lysosomal fluorescent staining pattern and one such clone, labeled LEP-3F6, was used for all subsequent studies. LEP100 expressed in MDCK cells has a molecular mass of 110 kD compared with the 100 kD of the endogenous protein expressed in quail fibroblasts (Fig. 2). Endo F digestion to remove N-linked oligosaccharides revealed that the polypeptide core of both exogenously expressed LEP100 in MDCK cells and endogenous LEP100 in quail fibroblasts had an identical size of 46 kD, as previously reported (36). The increased apparent molecular weight of LEP100 in MDCK cells is probably the result of differences in the structure of the complex N-linked oligosaccharide chains. The AC17 mAb recognizes a glycoprotein of 95 kD in MDCK cells, which when digested by endo F yields a product of 40 kD (Fig. 2), different from LEP100 but characteristic of lysosomal membrane glycoproteins (28). There is no crossreactivity between the two antibodies: the AC17 antibody does not react with chicken fibroblasts (data not shown) and CV24 does not crossreact with MDCK cells (Fig. 3). After extraction with Triton X-114 the AC17 antigen partitions predominantly in the detergent phase, indicating that it is an integral membrane glycoprotein (Fig. 2 *C*).

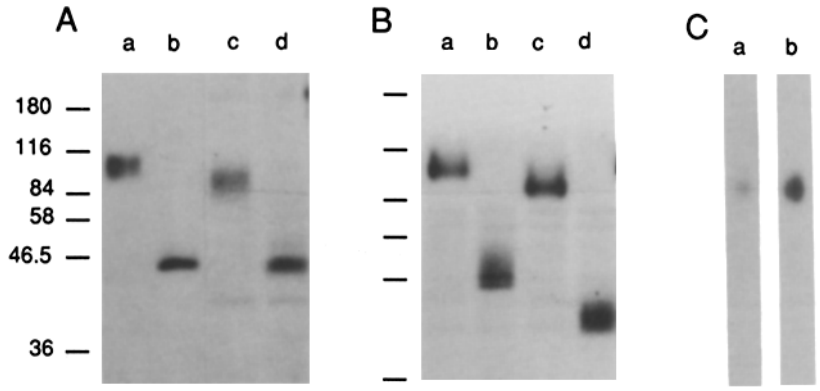

Figure 2. Expression of LEP100 and the AC17 antigen in MDCK cells. (*A*) LEP100 was immunoprecipitated from [^{35}S]methionine/cysteine pulse-labeled MDCK LEP-3F6 cells (lanes *a* and *b*) or quail fibroblasts (lanes *c* and *d*) and the samples in lanes *b* and *d* were treated with endo F. (*B*) LEP100 (lanes *a* and *b*) and the AC17 antigen (lanes *c* and *d*) were immunoprecipitated from pulse-labeled LEP100 transfected MDCK cells and the samples in lanes *b* and *d* were treated with endo F. (*C*) MDCK cells were extracted with Triton X-114 and the aqueous (*a*) and detergent (*b*) phases were blotted with the AC17 mAb.

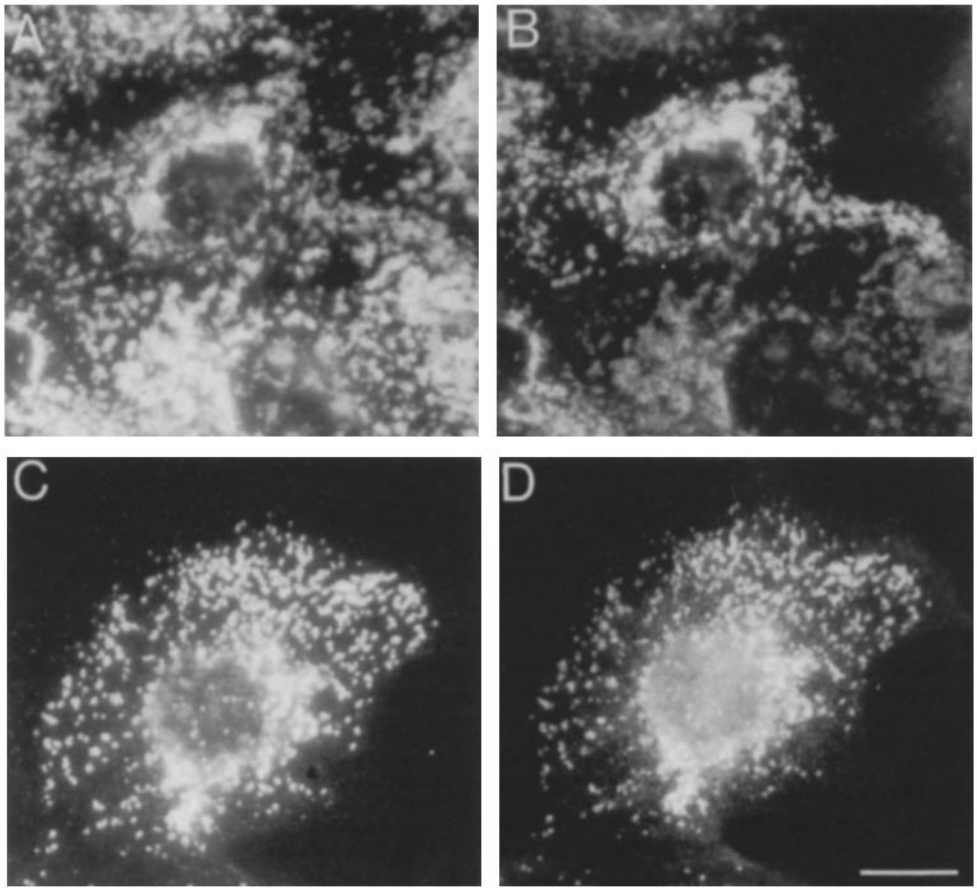

Figure 3. Colocalization of the AC17 antigen with lysosomal membrane glycoproteins. LEP-100 transfected MDCK cells were fixed, permeabilized, and immunofluorescently labeled with the AC17 mAb followed by Texas Red–conjugated anti-mouse secondary antibody (*A*) and then with FITC-conjugated CV24 anti-LEP100 mAb (*B*). Alternatively, untransfected MDCK cells were labeled with the AC17 mAb followed by Texas Red anti-mouse secondary antibody (*C*) and then with a rabbit polyclonal anti-peptide antibody against the cytoplasmic tail of LEP100 followed by FITC-conjugated anti-rabbit secondary antibody (*D*). Identical fields were photographed in *A* and *B* and in *C* and *D* using filters for rhodamine (*A*, *C*) or fluorescein (*B*, *D*) fluorescence. No crossover of fluorescence was detected between the rhodamine and fluorescein channels. The lack of fluorescein fluorescence in the non-expressing cell in the upper right-hand corners of *A* and *B* demonstrates the specificity of the double labeling with the two mouse monoclonal antibodies. Bar = 5 μm.

The vesicular structures immunofluorescently labeled by the AC17 mAb were determined to be lysosomes by the complete colocalization of the AC17 antigen with transfected LEP100 as well as with the endogenous antigen recognized by a rabbit polyclonal antibody raised against a synthetic peptide corresponding to the conserved carboxy-terminal amino sequence of the cytoplasmic tail of mammalian LAMP-1 (Fig. 3). Immunoperoxidase electron microscopy with the CV24 and AC17 mAbs revealed characteristic lysosomal structures (Fig. 4).

Processing and Lifetime of Lysosomal Glycoproteins in MDCK Cells

To determine the rate of passage of newly synthesized AC17 antigen and LEP100 through the Golgi, pulse–chase experiments were carried out on LEP-3F6 cells (Fig. 5 *A*). The immunoprecipitated proteins were treated overnight with endo H to remove unprocessed high-mannose oligosaccharide chains. As previously described, both proteins acquire endo H resistance with a $t_{1/2}$ of 25 min, reflecting the kinetics of their transport to the Golgi apparatus (36).

The extensive glycosylation of lysosomal glycoproteins endows them with resistance to lysosomal degradation and an extremely long lifetime within the cell (28). Pulse–chase labeling of LEP-3F6 MDCK cells with [^{35}S]methionine and cysteine followed by chases of varying lengths of time in isotope-free medium revealed that in MDCK cells both transfected LEP100 and endogenous AC17 antigen are extremely stable proteins. Both proteins are synthesized as precursors of lower molecular weight and acquire their mature size within 1 h of chase (Fig. 5 *B*). Approximately 70% of the pulse-labeled LEP100 and AC17 antigen are still present after 23 h of chase, which, when extrapolated, indicates a $t_{1/2}$ of 120–150 h for both proteins.

Surface Expression of the AC17 Antigen and LEP100 in MDCK Cells

The extensive glycosylation of lysosomal glycoproteins allowed us to detect both LEP100 and AC17 antigen by periodate oxidation of cell surface carbohydrates and reaction with biotin-X-hydrazide followed by immunoprecipitation and ^{125}I-streptavidin blotting (Fig. 6). Equivalent amounts of the two proteins were detected on the apical and basolateral surfaces. In chick embryo fibroblasts 2–3% of total cellular LEP100 is found on the cell surface by radioimmunoassay (37). Using a similar approach with iodinated AC17 and CV24 mAbs we determined the apical, basolateral, and intracellular distribution of AC17 antigen and LEP100 in LEP-3F6 MDCK cells (Table I). Both proteins were localized predominantly intracellularly (97%) with 0.8% of the total cellular protein found on the apical surface and 2.1% on the basolateral surface. Pretreatment of the cells for 5 h

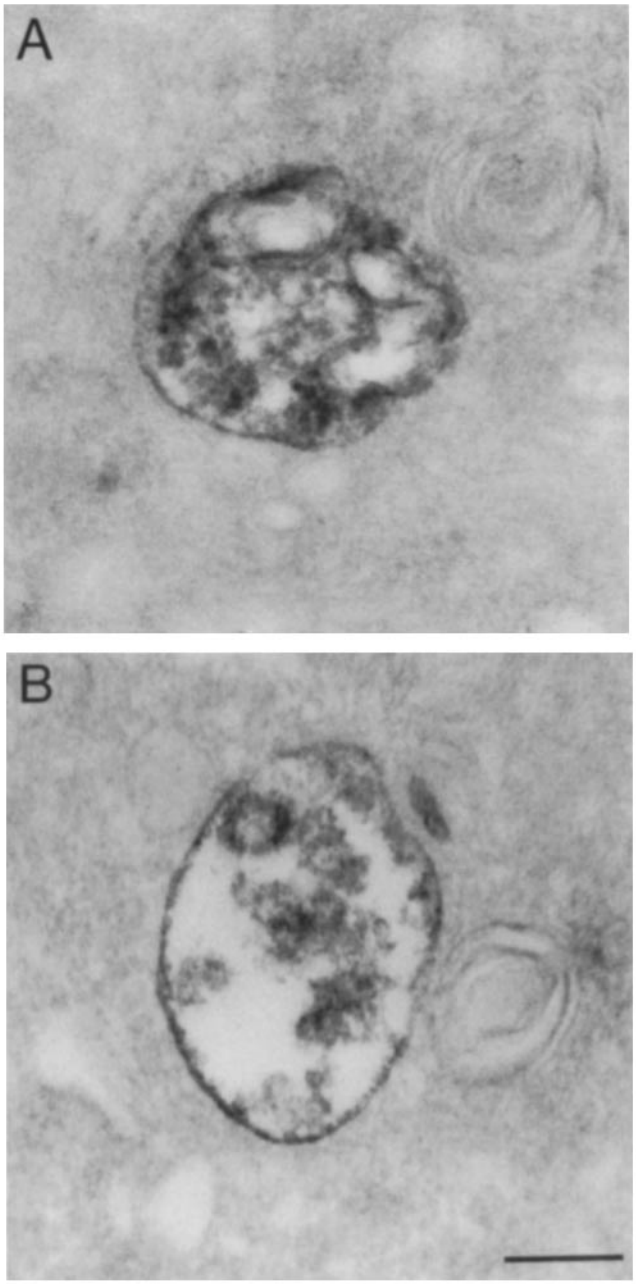

Figure 4. Immunoperoxidase labeling demonstrates the lysosomal localization of LEP100 and AC17 antigen in MDCK LEP-3F6 cells. Cells were fixed with formaldehyde in buffer containing 0.02% saponin and incubated with either the CV24 (*A*) or AC17 (*B*) mAb followed by HRP-conjugated anti-mouse F_{ab} fragments and visualized by electron microscopy. Bar = 0.2 μm.

with 0.1 mM chloroquine resulted in an approximately two-fold increase in the apical expression but had no effect on the basolateral expression of either protein (Fig. 6).

Surface Immunoprecipitation of Lysosomal Membrane Glycoproteins

Anti-LEP100 antibodies added to viable chicken fibroblasts are specifically taken up by surface-expressed LEP100 and transported to lysosomes (36, 37). Similarly, anti-lamp-1 antibodies added to h-lamp-1 transfected COS cells at 37°C are

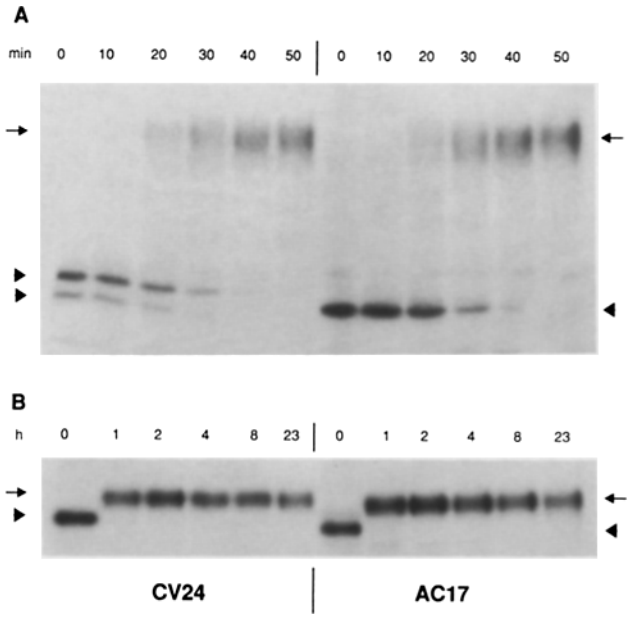

Figure 5. Biosynthetic processing and degradation of lysosomal glycoproteins in MDCK cells. MDCK cells were pulse-labeled with [^{35}S]methionine/cysteine for 20 min and chased for the indicated times before lysis and immunoprecipitation with the CV24 (*left-most lanes*) or AC17 (*right-most lanes*) mAb. (*A*) After 0, 10, 20, 30, 40, or 50 min of chase the precipitated proteins were treated with endo H. Unprocessed LEP100 (a doublet of 46/40 kD) and the AC17 antigen (40 kD) are indicated with arrowheads, while the processed forms are indicated with arrows. High mannose N-linked oligosaccharides on both proteins were processed with a $t_{1/2}$ of ~25 min (*B*). Cells were chased for 0, 1, 2, 4, 8, or 23 h before lysis. The precursor form of LEP100 (95 kD) and the AC17 antigen (85 kD) are indicated by arrowheads, while the mature forms are indicated by arrows to the left and right of the figure, respectively. Densitometric analysis and extrapolation reveals a $t_{1/2}$ for the two proteins of 120–150 h.

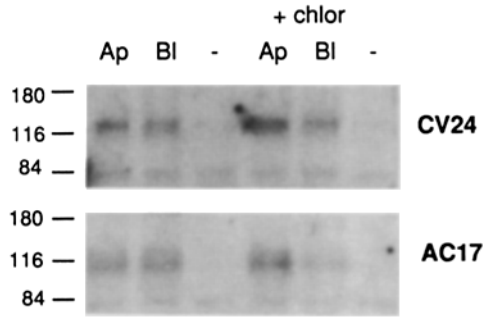

Figure 6. Steady-state surface expression of lysosomal glycoproteins in MDCK cells. Confluent monolayers of LEP100 transfected cells on filters were treated at 4°C with 10 mM periodate followed by biotin-X-hydrazide to label cell surface glycoproteins. LEP100 (CV24) and the AC17 antigen were immunoprecipitated from monolayers labeled from the apical (*Ap*) or basolateral (*Bl*) surfaces. Where indicated, the cells were treated with 0.1 mM chloroquine for 5 h before cell surface labeling. Control labeling in the absence of periodate treatment (−) revealed no labeling of either LEP100 or the AC17 antigen, indicating the specificity of the labeling for oligosaccharide residues, while other nonspecifically labeled bands were still present. The increased molecular weight of the two proteins probably reflects the presence of multiple biotins per molecule.

Table I. Steady-State Surface Expression of the AC17 Antigen and LEP100

	Apical	Basolateral
AC17 antigen	0.8 ± 0.6%	2.1 ±1.4%
LEP100	0.8 ± 0.6%	2.2 ± 2.1%

LEP-3F6 MDCK cells grown on 6-mm Transwell filters were incubated with ^{125}I-labeled AC17 or CV24 antibody on the apical or basolateral surfaces at 4°C. The values represent surface binding as a percent of antibody binding to saponin permeabilized cells. All values were corrected for nonspecific binding in the presence of cold antibody.

transported to lysosomes (65). We have used a similar approach to study the surface expression of lysosomal glycoproteins in MDCK cells. As seen in Fig. 7, incubation of viable MDCK LEP-3F6 cells at 37°C with monoclonal antibodies against either LEP100 or AC17 antigen for 30 min followed by a 60-min chase resulted in the accumulation of antibody in vesicular lysosomal structures. In untransfected MDCK cells, only antibodies against the endogenously expressed AC17 antigen are detectable in lysosomes. No staining was visible after addition of the CV24 anti-LEP100 antibody to untransfected MDCK cells (Fig. 7 *D*) or after addition of equivalent concentrations of nonimmune mouse IgG (not shown) to either transfected or untransfected MDCK cells, suggesting that antibody uptake under these experimental conditions is due to specific antibody–antigen interaction and not to nonspecific fluid phase uptake.

We used the ability to "catch" lysosomal glycoproteins by the addition of antibodies to the culture medium to study the domain-specific passage of newly synthesized MDCK lysosomal glycoproteins. To ensure that lysosomal glycoprotein surface expression was not due to the redirection of an overexpressed transfected protein to the cell surface, we studied the trafficking of the endogenous MDCK lysosomal membrane glycoprotein recognized by the AC17 mAb. Cells were pulse labeled with [^{35}S]methionine and cysteine for 20 min, chased in the absence of antibody for 10 min, and then incubated with the AC17 mAb in the apical or basolateral chase media for 45, 90, or 180 min. As seen in Fig. 8, AC17 antigen is detected basolaterally within 45 min. Extended incubation with antibody for 180 min on the basolateral surface results in a decreased detection of the antigen, perhaps reflecting arrival of the antigen–antibody complex to lysosomes were the acidic intraluminal pH may disrupt antigen–antibody interaction. No AC17 antigen was detected apically at any time point, indicating that newly synthesized AC17 antigen is targeted to the basolateral domain, encom-

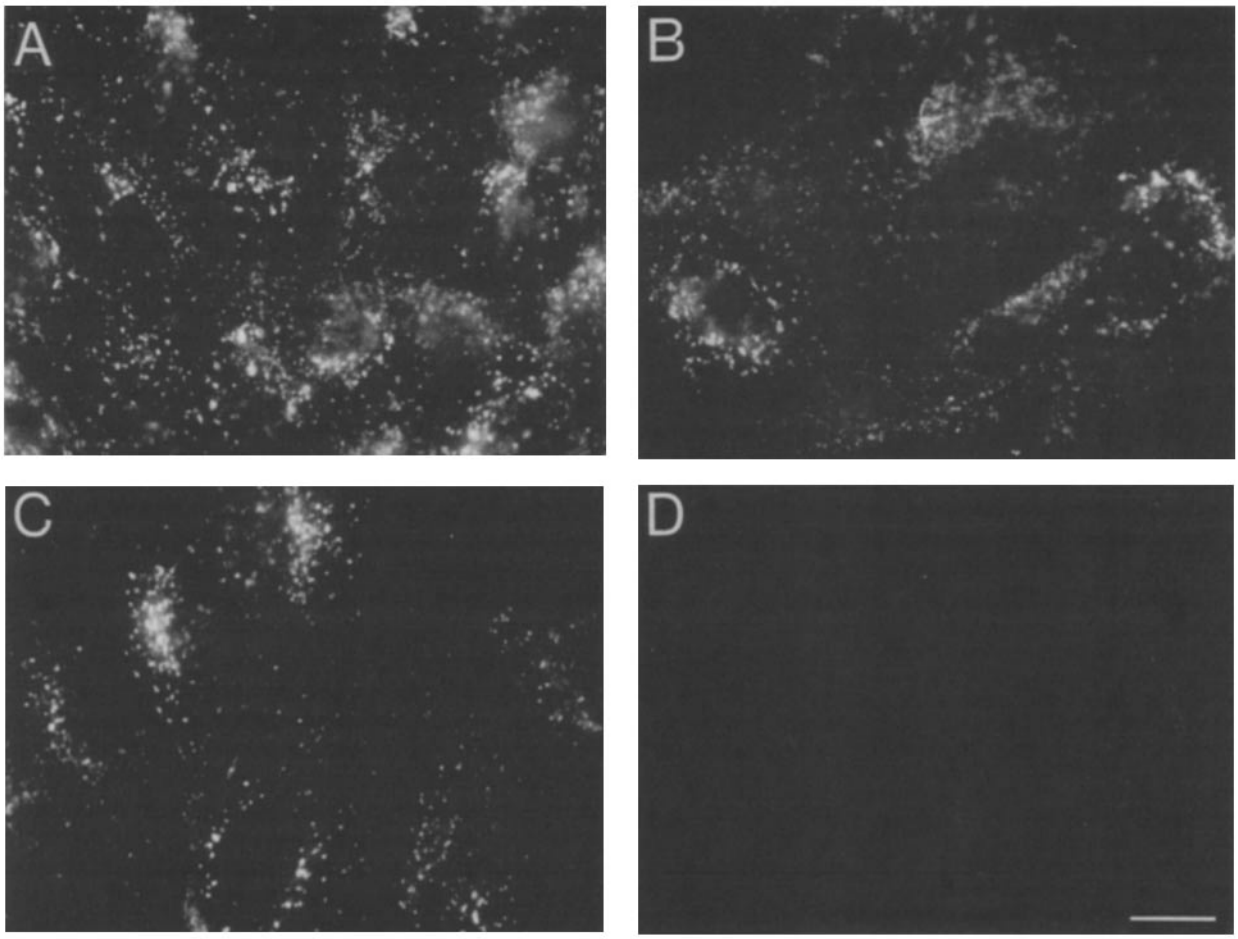

Figure 7. Uptake of the AC17 and CV24 mAbs by MDCK cells. Sparsely plated LEP100 transfected (*A* and *B*) or untransfected (*C* and *D*) MDCK cells on cover slides were incubated with the AC17 (*A* and *C*) or CV24 (*B* and *D*) mAb for 30 min in medium and then chased in antibody-free medium for 1 h at 37°C. The cells were then fixed, permeabilized, and incubated with rhodamine-conjugated anti-mouse secondary antibodies. No uptake of an equivalent concentration of nonimmune IgG was observed (identical image to *D*). Bar = 5 μm.

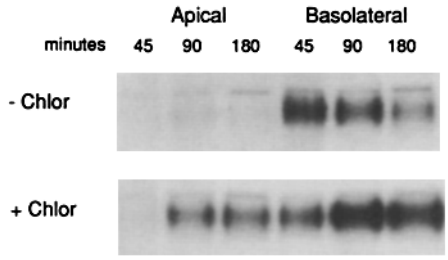

Figure 8. Surface appearance of ^{35}S-labeled AC17 antigen. Confluent monolayers of MDCK cells on filters were pulse-labeled with [^{35}S]met/cys for 20 min, chased for 10 min, and then chased for 45, 90, or 180 min in the presence of the AC17 mAb added to the apical or basolateral surface as indicated. Antigen–mAb complexes were recovered from detergent extracts of the cells by immune precipitation and the labeled AC17 antigen was detected by SDS-PAGE and fluorography. AC17 antigen could not be detected apically in the absence of chloroquine (*−Chlor*) but appeared apically, although retarded relative to its basolateral appearance, after treatment of the cells for 5 h with 0.1 mM chloroquine (*+Chlor*).

passing both the basolateral cell surface and basolateral early endosomes, before transport to lysosomes. The lack of ^{35}S-labeled AC17 antigen detected apically precludes the possibility that ^{35}S-labeled AC17 antigen was precipitated due to fluid phase uptake and transport of the antibody to prelysosomes or lysosomes or resulted from antibody exchange with labeled molecules during subsequent experimental manipulations.

Effect of Chloroquine on Targeting of the AC17 Antigen

After treatment of cells for 5 h with 0.1 mM chloroquine, expression of the AC17 antigen does not decrease with time in the basolateral domain and can be detected in significant amounts in the apical domain consistent with the enhanced surface expression of LEP100 in chloroquine-treated fibroblasts (Fig. 8) (37). The de novo appearance of AC17 antigen in the apical domain following chloroquine treatment corresponds to the increase in steady-state AC17 antigen levels detected on the apical surface by biotin hydrazide labeling (Fig. 6). Apical appearance is delayed relative to basolateral appearance and detection of the AC17 antigen after antibody incubation for 180 min is not decreased, consistent with the depletion of LAMPs from lysosomes in the presence of chloroquine (37).

Targeting of the AC17 Antigen

To follow the time course of basolateral expression of newly synthesized AC17 antigen, surface-capture by AC17 antigen was monitored both apically and basolaterally for discrete time periods after pulse labeling with [^{35}S]methionine/cysteine. The newly synthesized AC17 antigen appeared transiently basolaterally with a $t_{1/2}$ of appearance of 35 min, comparable to the surface delivery of basolateral surface proteins in MDCK cells (33). Expression peaked 60 min after the pulse and then diminished with a $t_{1/2}$ of 35 min, indicating the removal of the AC17 antigen from the surface and early endosomes and its redirection to lysosomes. Considering the time required for exit from the Golgi ($t_{1/2}$ = 25 min based on acquisition of endo H resistance), the rapid transit of the AC17 antigen to the basolateral domain is not consistent with its prior delivery to lysosomes (Fig. 11). No apical ^{35}S-labeled antigen was detected at any time point, suggesting that the AC17 antigen is routed from the Golgi apparatus exclusively to the basolateral surface or basolateral early endosomes. As seen in Fig. 8, in the presence of chloroquine newly synthesized AC17 antigen is targeted basolaterally and is then more stably expressed in both the apical and basolateral domains (Fig. 9 *B*), consistent with the nonpolar distribution of lysosomes in MDCK cells (Fig. 1).

74% of Newly Synthesized AC17 Antigen Passes through the Basolateral Domain

The efficient surface capture achieved in the short 15-min antibody incubations of the targeting experiments (Fig. 9) strongly suggests that precipitated AC17 antigen interacts with the antibody on the surface, or perhaps within basolateral early endosomes, but not within late endosomes (prelysosomes), since these are reached by fluid phase endocytosis in MDCK cells only after 15 min from both the apical and basolateral surfaces (2, 47). Furthermore, addition of antibody for even shorter periods, such as 5 or 10 min, results in the precipitation of AC17 antigen in amounts proportional to the time of incubation (Fig. 10 *A*), precluding interaction of the AC17 antibody with its antigen in prelysosomes.

To determine whether the basolateral expression of the AC17 antigen is due to mistargeting of lysosome-bound pro-

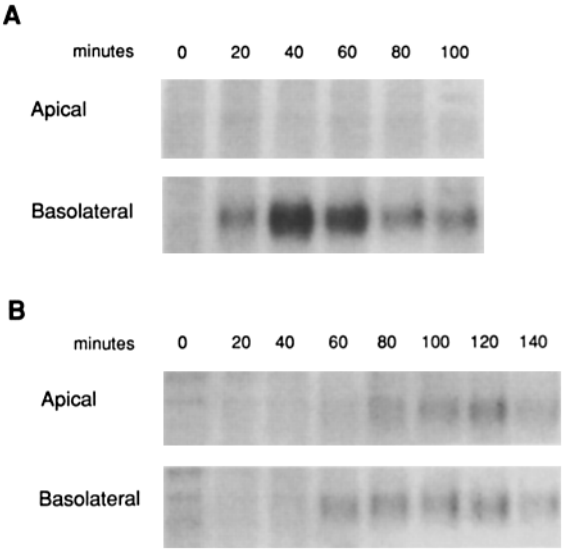

Figure 9. Surface targeting of the AC17 antigen. MDCK cells were pulse-labeled for 20 min with [^{35}S]methionine/cysteine and chased in the presence of the AC17 mAb as follows. (*A*) At 0, 20, 40, 60, 80, and 100 min after the pulse, the AC17 mAb was added in medium to the apical or basolateral surface for 15 min and captured AC17 antigen was analyzed as in Fig. 8. The AC17 antigen was not detected apically and arrived to and disappeared from the basolateral domain with a $t_{1/2}$ of ~35 min. In *B*, the cells were treated with 0.1 mM chloroquine for 5 h before surface immunoprecipitation and the AC17 mAb was added at two additional time points of 120 and 140 min after the pulse. Apical AC17 antigen could be detected after chloroquine treatment, but its delivery was retarded relative to basolateral delivery.

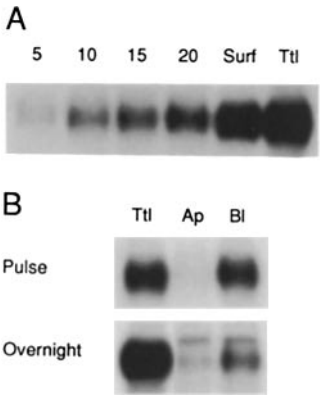

Figure 10. Quantitation of surface appearance of the AC17 antigen. (*A*) After 50 min of chase following a 20-min pulse of [^{35}S]methionine/cysteine, the AC17 mAb was added to the basolateral medium for 5, 10, 15, or 20 min. The amount of AC17 antigen captured in these short time periods is compared with that captured by addition of antibody to the basolateral surface from 10 to 90 min after the pulse (*Surf*) and to the total AC17 ^{35}S-labeled antigen precipitated from detergent extracts with AC17 immunobeads (*Ttl*). (*B*) After a 20-min pulse or overnight labeling, the AC17 mAb was added to either the apical (*Ap*) or basolateral (*Bl*) surfaces from 10 to 90 min after the end of the labeling period. An identical filter chased in the absence of antibody was lysed and the AC17 antigen immunoprecipitated by addition of AC17 immunobeads to the lysate (*Ttl*). After a pulse labeling, 74 ± 12% (n = 5) of the total immunoprecipitable AC17 antigen could be recovered by surface immunoprecipitation.

tein to the cell surface or whether basolateral passage is the constitutive route for the AC17 antigen to the lysosome, the amount of AC17 antigen that could be detected by the addition of antibody to the basolateral medium was quantified. After a 20-min pulse, cells were chased in the presence of AC17 antibody on the apical or basolateral surface from 10 to 90 min, the time of basolateral delivery determined from Fig. 9. A parallel culture was chased in the absence of mAb, lysed, and then immunoprecipitated to determine the total amount of labeled AC17 antigen in the sample (Fig. 10 *B*). While no AC17 antigen was detected apically, as expected, 74 ± 12% (n = 5) of the total immunoprecipitable protein was recovered by basolateral surface immunoprecipitation.

In contrast, after overnight labeling to generate a steady-state pool of labeled molecules, a small proportion of total labeled AC17 antigen was detected both apically and basolaterally (Fig. 10 *B*). While the basolateral signal is clearly greater than the apical signal, the extended exposure required to visualize the surface immunoprecipitated protein, resulting in overexposure of the total protein band and appearance of a background band just above the AC17 antigen, made quantification of the apically and basolaterally expressed AC17 antigen difficult. The detection of small amounts of the AC17 antigen both apically and basolaterally by surface immunoprecipitation after overnight labeling corroborates our finding by ^{125}I-labeled mAb binding and by biotin hydrazide surface labeling that the AC17 antigen is found on both surfaces at steady state. The greater basolateral accumulation of AC17 antigen and LEP100 may be due in part to the continual targeting of newly synthesized molecules to that domain. The discrepancy in degree and polarity of the distribution of surface immunoprecipitated ^{35}S-labeled AC17 antigen after pulse and overnight metabolic labeling is best explained by the polar delivery of the AC17 antigen via the basolateral surface and early endosomes to prelysosomes and lysosomes from which it shuttles in small amounts, as reported for LEP100 in chick fibroblasts (37), to both the apical and basolateral surface domains in a nonpolar fashion (Fig. 11).

Discussion

To characterize the pathway to lysosomes in epithelial cells, we have studied the expression of an endogenous lysosomal membrane glycoprotein in MDCK cells. A monoclonal antibody, AC17, recognizes distinct vesicular structures by immunofluorescent labeling. These structures were identified

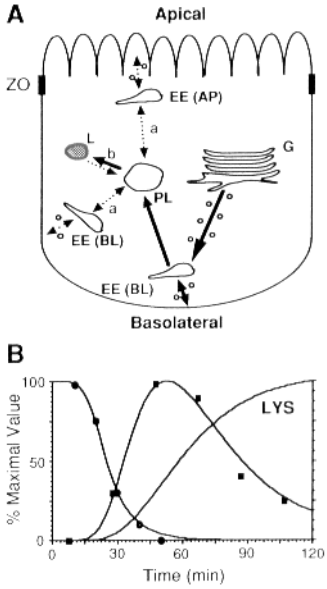

Figure 11. Schematic representation of the trafficking of the AC17 antigen. (*A*) After biosynthesis, newly synthesized AC17 antigen is targeted from the Golgi apparatus (*G*) to the basolateral plasma membrane and/or early endosomes and from there transferred to lysosomes (*L*) via basolateral early endosomes (*EE(BL)*) and prelysosomes (*PL*; *heavy arrows*). The AC17 antigen recycles at low levels from lysosomes to prelysosomes and the apical and basolateral domains (*dashed arrows*). In the presence of chloroquine, the movement of the AC17 antigen from the prelysosome to lysosome is blocked (*b*) and recycling with both the apical and basolateral domains accentuated (*a*). The diagram represents the fusion of vesicles budding off the TGN with basolateral early endosomes and cell surface recycling from the prelysosome. However, our results cannot distinguish between targeting from the TGN directly to the basolateral surface or through an early endosomal compartment nor determine from which intracellular compartment recycling occurs. (*B*) Using the Stella modeling program on a Macintosh computer, a model was generated incorporating the various steps of AC17 trafficking outlined in *A*. Using the known parameters of acquisition of endo H resistance in the Golgi apparatus (*circles*) and basolateral appearance (*squares*) and assuming similar kinetic parameters for AC17 endocytosis and recycling as described for LEP100 in chick fibroblasts (37), the Stella program generated a model consistent with the rapid passage of the AC17 antigen to lysosomes (*LYS*). The model incorporated the experimental data of passage through the Golgi (Fig. 5 *A*), basolateral expression (Fig. 9), and lysosomal degradation (Fig. 5 *B*) of the AC17 antigen. Rate constants for the distribution and shuttling of LEP100 in chick fibroblasts (37) were used to describe the endocytosis (k_1 = 0.33 min^{-1}), exocytosis (k_{-1} = 0.16 min^{-1}), and shuttling from endosome to lysosome (k_2 = 0.067 min^{-1}). A fivefold decreased rate of recycling from lysosome to endosome (k_{-2} = 0.00083 min^{-1}) of the AC17 antigen, relative to LEP100, was used due to the minimal effect of chloroquine on steady-state AC17 antigen surface expression in MDCK cells (Fig. 6) compared with that found for LEP100 in chick fibroblasts (37). Not unexpectedly, this change was not found to significantly affect the rate of arrival of the AC17 antigen to lysosomes. To simplify the model a single endosomal compartment was used corresponding to the early endosome. Altering of the model to incorporate targeting of vesicles from the Golgi to either the plasma membrane or the early endosome did not significantly affect the rate of arrival of the AC17 antigen to the lysosome.

as lysosomes by their identical colocalization with the major protein component of the lysosomal membrane, the highly glycosylated membrane proteins referred to as lysosomal associated membrane proteins (LAMP) or lysosomal glycoproteins (lgp) (28). In MDCK cells, the AC17 antigen colocalizes with transfected LEP100, the avian LAMP (14, 36, 37), and an endogenous MDCK antigen recognized by a polyclonal antibody raised against the highly conserved cytoplasmic tail of LEP100 (Fig. 3); the labeled structures exhibited a typical lysosomal morphology by immunoelectron microscopy (Fig. 4). LAMPs typically have a molecular size between 100 and 120 kD with a core protein of ~40 kD. They are synthesized as precursor molecules of 90 kD containing 17–20 N-linked high mannose oligosaccharides that are processed to complex oligosaccharides containing a large amount of polylactosamines (28). The endogenous MDCK lysosomal glycoprotein recognized by the AC17 monoclonal antibody partitions to the detergent phase following Triton X-114 extraction, is synthesized as a precursor molecule of 85 kD, has a molecular size of 95 kD, and has a core polypeptide of 40 kD. The time course of acquisition of endo H resistance and of protein degradation for the AC17 antigen is identical to that of LEP100 expressed in the same transfected MDCK clonal cell line. These characteristics strongly indicate that the AC17 antigen is an endogenous MDCK LAMP.

In chick fibroblasts, LEP100 has been shown to be localized primarily to lysosomes (90%) but is also present in the plasma membrane (3%) and in endosomes (7%) (36, 37). Other LAMPs are barely detectable at the cell surface (28). By three different methods, we demonstrated low levels of surface expression of both transfected LEP100 and the endogenous AC17 antigen on the apical (0.8%) and basolateral (2.1%) membranes of MDCK cells. LEP100 mAb added to chick fibroblasts at 37°C is specifically endocytosed to lysosomes, as are LAMP antibodies added to h-lamp-1 transfected cells (37, 65). Similarly, both AC17 and CV24 mAbs were taken up by LEP-3F6 MDCK cells and delivered to lysosomes (Fig. 7). The inability of untransfected MDCK cells to take up anti-LEP100 demonstrates the specificity of this delivery from the cell surface to lysosomes.

Interestingly, in contrast to its nonpolar steady-state surface distribution, newly synthesized AC17 antigen was targeted in a highly polar fashion. After a [^{35}S]methionine/cysteine metabolic pulse, the AC17 antigen was surface immunoprecipitated by the continuous addition of mAb at 37°C to the apical or basolateral medium; antibody–antigen complexes formed were collected by precipitation after cell lysis. Newly synthesized AC17 antigen could only be detected after addition of antibody basolaterally; extended incubation (180 min) resulted in a decreased signal, probably due to the arrival of antigen–antibody complexes to the lysosome. Addition of antibody for discrete (15 min) time periods indicate a $t_{1/2}$ of delivery to and removal from the basolateral domain of ~35 min, in agreement with kinetic studies of surface expression of LEP100 in chick fibroblasts, which revealed an extremely short residence time at the plasma membrane ($t_{1/2}$ = 2 min). In contrast, continuous presence of antibody in the apical medium during the chase detected no AC17 antigen. Apical and basolateral fluid phase markers have been shown to mix 15 min after addition of fluid phase marker in a late endosomal (prelysosomal) compartment (2, 47). The absence of apical detection and our ability to detect AC17 antigen after addition of basolateral antibody for only 5 min (Fig. 10) indicates that we are detecting AC17 antigen at the basolateral cell surface or within basolateral early endosomes and not within prelysosomes reached by fluid phase uptake of antibody.

These results contrast with a previous report (21), which suggested that two integral membrane lysosomal glycoproteins, lgp110 and lgp120, are not targeted to lysosomes in fibroblasts via the cell surface but rather through an acidic prelysosomal compartment, the route followed by M6P containing lysosomal hydrolases (62). This conclusion was based on the inability to detect lgp's at the cell surface and on the rapid delivery of these proteins to lysosomes ($t_{1/2}$ = 45 min), which, when compared with the rates of plasma membrane protein delivery to the cell surface and of membrane bound endocytic markers to lysosomes, was judged to preclude a passage of lysosomal glycoproteins through the cell surface. The low levels of surface expression of LAMPs could be ascribed to reequilibration of the proteins between lysosomes and the cell surface or to missorting in the Golgi due to oversaturation of sorting receptors, and would not reflect the major pathway of LAMPS out of the Golgi (28). Our ability to detect the transient surface expression of a LAMP during biogenesis may be attributed to the approach utilized (antibody "catch") and to the possession of a high affinity monoclonal antibody against an endogenous MDCK LAMP ($K_d \approx 5$ nM). Addition of the AC17 mAb to the basolateral medium from 10 to 90 min after pulse labeling, encompassing the peak of AC17 antigen basolateral appearance, resulted in the recovery of 74 ± 12% (n = 5) of the total precipitable AC17 antigen. Considering the loss inherent to any immunoprecipitation procedure and the possible disruption of some antigen–antibody complexes upon arrival to lysosomes, the detection of 74% of the total protein pulse by surface immunoprecipitation indicates that newly synthesized AC17 antigen is targeted basolaterally before delivery to lysosomes.

The time course of AC17 antigen basolateral delivery is similar to that described for the vectorial targeting of basolateral proteins in MDCK cells (33) and is consistent with the rapid delivery of LAMPs to lysosomes described previously (21). The AC17 antigen acquires endo H resistance with a $t_{1/2}$ of 25 min and is detected basolaterally with a $t_{1/2}$ of 35 min such that transport from the Golgi apparatus to the basolateral domain requires ~10 min. Considering the short residence time of LEP100 on the cell surface and its rapid transport to lysosomes (37), these values support the delivery of the AC17 antigen to lysosomes via the cell surface and early endosomes with a $t_{1/2}$ of 45–60 min. Computer modeling of the pathway of the AC17 antigen to lysosomes incorporating the known parameters of acquisition of endo H resistance in the Golgi apparatus and passage of the AC17 antigen through the basolateral domain confirm that the basolateral targeting of the AC17 antigen is consistent with its rapid delivery to lysosomes (Fig. 11). Similar rates of arrival of the AC17 antigen to lysosomes were predicted from models incorporating either direct vesicular fusion with the basolateral surface or fusion with early endosomes and rapid recycling between the early endosome and the basolateral

surface. It is therefore possible that some AC17 antigen may be transported to the lysosome by way of basolateral early endosomes without appearing on the cell surface.

The detection of AC17 antigen surface expression by addition of antibody at 37°C does not rule out the possibility of fluid phase endocytosis of antibody and its interaction with antigen in basolateral early endosomes. Endocytosed alpha$_2$-macroglobulin, which is targeted to lysosomes after release from its receptor within the early endosome, reaches a compartment inaccessible to fluid phase uptake (fusion-inaccessible) with a $t_{1/2}$ of 8 min (57). AC17 antigen endocytosis may be better represented by studies of lysosome targeted transmembrane VSV G protein implanted into the plasma membrane and then crosslinked by antibody which reaches a fusion-inaccessible compartment with a $t_{1/2}$ of <5 min (23). AC17 antigen that transited only the early endosome en route to lysosomes would therefore reside in a compartment accessible to fluid phase uptake of antibody for a very short period of time and we question whether our results can be explained by AC17 antibody–antigen interaction within intracellular compartments. The significantly greater rate of endosome to surface recycling (0.138 min^{-1}) relative to the rate of endosome to lysosome transport (0.076 min^{-1}) measured for LEP100 in chick fibroblasts (37) suggests that any LAMP molecules that reach the early endosome must necessarily also be expressed on the cell surface, if only very transiently. Whatever the route taken, it is clear that the AC17 antigen transits a compartment rapidly accessible to antibodies within the basolateral but not the apical medium, either the plasma membrane or the early endosome, and therefore does not follow the direct TGN–prelysosome route to lysosomes which has been described for lysosomal hydrolases (13, 62).

In the presence of chloroquine, the expression of the AC17 antigen in MDCK cells was dramatically altered. Newly synthesized protein was still targeted first basolaterally, although with slower kinetics, and could then be detected for extended times in both the apical and basolateral domains. Chloroquine has been shown to selectively block the pathway of LEP100 to lysosomes, resulting in its depletion from lysosomes and increased presence in endosomes and the plasma membrane (37). The increased surface residence time and the apical appearance of the AC17 antigen probably reflect its arrival to an intracellular compartment from which the protein can recycle to both the apical and basolateral domains. This compartment is probably the prelysosome (late endosome) where fluid phase markers endocytosed from the apical and basolateral surfaces have been shown to meet (47). This suggests that chloroquine specifically blocks the path of the AC17 antigen from the prelysosome to the lysosome and that recycling with early endosomes and the plasma membrane occurs from the prelysosome. Newly synthesized AC17 antigen is therefore targeted to the basolateral surface and/or early endosomes, endocytosed, and then transferred via the endocytic pathway to lysosomes from which it reequilibrates with prelysosomes and both apical and basolateral early endosomes and plasma membranes (Fig. 11).

The basolateral appearance of the AC17 antigen en route to lysosomes is consistent with the finding that the pathway of LAP, a transmembrane lysosomal enzyme lacking M6P, to lysosomes is via the cell surface (4). LAP is rapidly transported from the Golgi apparatus to the cell surface (in 10 min) and recycles between the cell surface and endosomes more than 15 times before transport to lysosomes with a $t_{1/2}$ of 6–7 h (4). Deletion of the cytoplasmic domain of LAP or replacement of the tyrosine within the cytoplasmic domain with another amino acid results in accumulation of the protein at the cell surface (48). Similarly, a tyrosine residue in the cytoplasmic domain is critical for the targeting of h-lamp-1 to lysosomes (65). The cytoplasmic tyrosine-containing motif, while clearly an endocytic signal, is not in itself sufficient to target proteins to lysosomes since it is present in a number of receptors that recycle from endosomes to the plasma membrane (12, 27). Additional unknown features may be required to target proteins to lysosomes. Ongoing studies of AC17 antigen expression in MDCK cells will help define the mechanisms involved in this novel biogenetic pathway in polarized epithelia.

We thank Ms. Lee Cohen-Gould for her excellent technical assistance with the electron microscopy and Ms. Lori van Houten and Ms. Joy Hornung for their photographic help.

This work was supported by National Institutes of Health grants RO1-GM34107 to E. Rodriguez-Boulan and RO1-NS23241 to D. M. Fambrough, as well as a New York Heart Association grant in aid. I. R. Nabi was supported by a Medical Research Council of Canada postdoctoral fellowship and A. Le Bivic was supported by a fellowship from L'Association pour la Recherche sur le Cancer (France) and UA179 CNRS (France).

Received for publication 17 July 1991 and in revised form 20 August 1991.

Note Added in Proof. Sequencing by Edman degradation of 20 NH_2-terminal amino acids of AC17 antigen immunopurified from dog kidney has revealed significant sequence identity to human and mouse LAMP-2. 15 of 18 identifiable amino acids are identical to human LAMP-2 and two of the nonidentical amino acids are present in the mouse LAMP-2 sequence. The high degree of sequence identity indicates that the AC17 antigen is the dog homologue of the LAMP-2 family of lysosomal membrane glycoproteins.

References

1. Bartles, J. R., H. M. Feracci, B. Stieger, and A. L. Hubbard. 1987. Biogenesis of the rat hepatocyte plasma membrane in vivo. Comparison of the pathways taken by apical and basolateral proteins using subcellular fractionation. *J. Cell Biol.* 105:1241–1251.
2. Bomsel, M., K. Prydz, R. G. Parton, J. Gruenberg, and K. Simons. 1989. Endocytosis in filter grown Madin-Darby Canine kidney cells. *J. Cell Biol.* 109:3243–3258.
3. Bordier, C. 1981. Phase separation of integral membrane proteins in Triton X-114 solution. *J. Biol. Chem.* 256:1604–1607.
4. Braun, M., A. Waheed, and K. von Figura. 1989. Lysosomal acid phosphatase is transported to lysosomes via the cell surface. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:3633–3640.
5. Brewer, C. B., and M. G. Roth. 1991. A single amino acid change in the cytoplasmic domain alters the polarized delivery of influenza virus hemagglutinin. *J. Cell Biol.* 114:413–421.
6. Brown, D. A., B. Crise, and J. K. Rose. 1989. Mechanism of membrane anchoring affects polarized expression of two proteins in MDCK cells. *Science (Wash. DC).* 245:1499–1501.
7. Casanova, J. E., G. Apodaca, and K. E. Mostov. 1991. An autonomous signal for basolateral sorting in the cytoplasmic domain of the polymeric immunoglobulin receptor. *Cell.* 66:65–75.
8. Cha, Y., S. M. Holland, and J. T. August. 1990. The cDNA sequence of mouse LAMP-2. *J. Biol. Chem.* 265:5008–5013.
9. Chamberlain, J. P. 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water soluble fluor, sodium salicylate. *Anal. Biochem.* 98:132–135.
10. Chen, J. W., Y. Cha, K. U. Yuksel, R. W. Gracy, and J. T. August. 1988. Isolation and sequencing of a cDNA clone encoding lysosomal membrane glycoprotein mouse LAMP-1. *J. Biol. Chem.* 263:8754–8758.
11. Compton, T., I. E. Ivanov, T. Gottlieb, M. Rindler, M. Adesnik, and D. D. Sabatini. 1989. A sorting signal for the basolateral delivery of the vesicular stomatitis virus (VSV) G protein lies in its luminal domain: analysis of the targeting of VSV G-influenza hemagglutinin chimeras. *Proc. Natl. Acad. Sci. USA.* 86:4112–4116.

12. Davis, C. G., I. R. van Driel, D. W. Russel, M. S. Brown, and J. L. Goldstein. 1987. The low density lipoprotein receptor: identification of amino acids in cytoplasmic domain required for rapid endocytosis. *J. Biol. Chem.* 262:4075–4082.
13. Duncan, J. R., and S. Kornfeld. 1988. Intracellular movement of two mannose 6-phosphate receptors. Return to the Golgi apparatus. *J. Cell Biol.* 106:617–628.
14. Fambrough, D. M., K. Takeyasu, J. Lippincott-Schwarz, and N. R. Siegel. 1988. Structure of LEP100, a glycoprotein that shuttles between lysosomes and the plasma membrane, deduced from the nucleotide sequence of the encoding cDNA. *J. Cell Biol.* 106:61–67.
15. Fukuda, M., J. Viitala, J. Matteson, and S. R. Carlson. 1988. Cloning of cDNAs encoding human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2: comparison of their deduced amino acid sequences. *J. Biol. Chem.* 263:18920–18928.
16. Fuller, S. D., and K. Simons. 1986. Transferrin receptor polarity and recycling accuracy in "tight" and "leaky" strains of Madin-Darby canine kidney cells. *J. Cell Biol.* 103:1767–1779.
17. Graeve, L., K. Drickamer, and E. Rodriguez-Boulan. 1989. Functional expression of the chicken liver asialoglycoprotein receptor in the basolateral surface of MDCK cells. *J. Cell Biol.* 109:2809–2816.
18. Graeve, L., A. Patzak, K. Drickamer, and E. Rodriguez-Boulan. 1990. Polarized expression of functional rat liver asialoglycoprotein receptor in transfected Madin-Darby canine kidney cells. *J. Biol. Chem.* 265:1216–1224.
19. Graham, F., and A. Van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology.* 52:456–467.
20. Granger, B. L., S. A. Green, C. A. Gabel, C. L. Howe, I. Mellman, and A. Helenius. 1990. Characterization and cloning of lgp110, a lysosomal membrane glycoprotein from mouse and rat cells. *J. Biol. Chem.* 265:12036–12043.
21. Green, S. A., K. P. Zimmer, G. Griffiths, and I. Mellman. 1987. Kinetics of intracellular transport and sorting of lysosomal membrane and plasma membrane proteins. *J. Cell Biol.* 105:1227–1240.
22. Griffiths, G., B. Hoflack, K. Simons, I. Mellman, and S. Kornfeld. 1988. The mannose 6-phosphate receptor and the biogenesis of lysosomes. *Cell.* 52:329–341.
23. Gruenberg, J., and K. E. Howell. 1987. An internalized transmembrane protein resides in a fusion-competent endosome for less than five minutes. *Proc. Natl. Acad. Sci. USA.* 84:5758–5762.
24. Howe, C. L., B. L. Granger, M. Hull, S. A. Green, C. A. Gabel, A. Helenius, and I. Mellman. 1988. Derived protein sequence, oligosaccharides, and membrane insertion of the 120-kDa lysosomal membrane glycoprotein (lgp 120): identification of a highly conserved family of lysosomal membrane glycoproteins. *Proc. Natl. Acad. Sci. USA.* 85:7577–7581.
25. Hubbard, A. L., and B. Stieger. 1989. Biogenesis of endogenous plasma membrane proteins in epithelial cells. *Annu. Rev. Physiol.* 51:755–770.
26. Hunziker, W., and I. Mellman. 1989. Expression of macrophage-lymphocyte Fc Receptors in Madin-Darby Canine Kidney cells. Polarity and transcytosis differ for isoforms with or without coated pit localization domains. *J. Cell Biol.* 109:3291–3302.
27. Jing, S., T. Spencer, K. Miller, C. Hopkins, and I. S. Trowbridge. 1990. Role of the human transferrin receptor cytoplasmic domain in endocytosis. Localization of a specific signal sequence for internalization. *J. Cell Biol.* 110:283–294.
28. Kornfeld, S., and I. Mellman. 1989. The biogenesis of lysosomes. *Annu. Rev. Cell Biol.* 5:483–525.
29. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
30. Lazarovits, J., and M. Roth. 1988. A single amino acid change in the cytoplasmic domain allows the influenza virus hemagglutinin to be endocytosed through coated pits. *Cell.* 53:743–752.
31. Le Bivic, A., M. Hirn, and H. Reggio. 1987. Apical membrane marker is expressed early in colonic epithelial cells. *Biol. Cell.* 60:209–216.
32. Le Bivic, A., F. X. Real, and E. Rodriguez-Boulan. 1989. Vectorial targeting of apical and basolateral plasma membrane proteins in a human adenocarcinoma epithelial cell line. *Proc. Natl. Acad. Sci. USA.* 86:9313–9317.
33. Le Bivic, A., Y. Sambuy, K. Mostov, and E. Rodriguez-Boulan. 1990. Vectorial targeting of an endogenous apical membrane sialoglycoprotein and uvomorulin in MDCK cells. *J. Cell Biol.* 110:1533–1539.
34. Le Bivic, A., A. Quaroni, B. Nichols, and E. Rodriguez-Boulan. 1990. Biogenetic pathways of plasma membrane proteins in Caco-2, a human intestinal epithelial cell line. *J. Cell Biol.* 111:1351–1361.
35. Le Bivic, A., Y. Sambuy, A. Patzak, N. Patil, M. Chao, and E. Rodriguez-Boulan. 1991. An internal deletion in the cytoplasmic tail reverses the apical localization of human NGF receptor in transfected MDCK cells. *J. Cell Biol.* 115:607–618.
36. Lippincott-Schwartz, J., and D. M. Fambrough. 1986. Lysosomal membrane dynamics. Structure and interorganellar movement of a major lysosomal membrane glycoprotein. *J. Cell Biol.* 102:1593–1605.
37. Lippincott-Schwartz, J., and D. M. Fambrough. 1987. Cycling of the integral membrane glycoprotein, LEP100, between plasma membrane and lysosomes: kinetic and morphological analysis. *Cell.* 49:669–677.
38. Lisanti, M., M. Sargiacomo, L. Graeve, A. Saltiel, and E. Rodriguez-Boulan. 1988. Polarized apical distribution of glycosyl phosphatidylinositol anchored proteins in a renal epithelial line. *Proc. Natl. Acad. Sci. USA.* 85:9557–9561.
39. Lisanti, M., A. Le Bivic, M. Sargiacomo, and E. Rodriguez-Boulan. 1989. Steady state distribution and biogenesis of endogenous MDCK glycoproteins. Evidence for intracellular sorting and polarized cell surface delivery. *J. Cell Biol.* 109:2117–2128.
40. Lisanti, M., I. P. Caras, M. A. Davitz, and E. Rodriguez-Boulan. 1989. A glycolipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. *J. Cell Biol.* 109:2145–2156.
41. Maddon, P. J., D. R. Littman, M. Godfrey, D. E. Maddon, L. Chess, and R. Axel. 1985. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. *Cell.* 42:93–104.
42. Matlin, K. S., and K. Simons. 1984. Sorting of an apical plasma membrane glycoprotein occurs before it reaches the cell surface in cultured epithelial cells. *J. Cell Biol.* 99;2131–2139.
43. Matter, K., M. Brauchbar, K. Bucher, and H. P. Hauri. 1990. Sorting of endogenous plasma membrane proteins occurs from two sites in cultured human intestinal epithelial cells (Caco-2). *Cell.* 60:429–437.
44. McQueen, N. L., D. P. Nayak, E. B. Stephens, and R. W. Compans. 1986. Polarized expression of a chimeric protein in which the transmembrane and cytoplasmic domains of the influenza virus hemagglutinin have been replaced by those of the vesicular stomatitis virus G protein. *Proc. Natl. Acad. Sci. USA.* 83:9318–9322.
45. Misek, D. E., E. Bard, and E. Rodriguez-Boulan. 1984. Biogenesis of epithelial cell polarity: intracellular sorting and vectorial exocytosis of an apical plasma membrane glycoprotein. *Cell.* 39:537–546.
46. Mostov, K. E., and D. L. Deitcher. 1986. Polymeric immunoglobulin receptor expressed in MDCK cells transcytoses IgA. *Cell.* 46:613–621.
47. Parton, R. G., K. Prydz, M. Bomsel, K. Simons, and G. Griffiths. 1989. Meeting of the apical and basolateral endocytic pathways of the Madin-Darby canine kidney cell in late endosomes. *J. Cell Biol.* 109:3259–3272.
48. Peters, C., M. Braun, B. Weber, M. Wenland, B. Schmidt, R. Pohlmann, A. Waheed, and K. von Figura. 1990. Targeting of a lysosomal membrane protein: a tyrosine-containing endocytosis signal in the cytoplasmic tail of lysosomal acid phosphatase is necessary and sufficient for targeting to lysosomes. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3497–3506.
49. Pfeiffer, S., S. D. Fuller, and K. Simons. 1985. Intracellular sorting and basolateral appearance of the G protein of vesicular stomatitis virus in Madin-Darby canine kidney cells. *J. Cell Biol.* 101:470–476.
50. Prydz, K., A. W. Brandli, M. Bomsel, and K. Simons. 1990. Surface distribution of the mannose 6-phosphate receptors in epithelial Madin-Darby canine kidney cells. *J. Biol. Chem.* 265:12629–12635.
51. Rindler, M. J., I. E. Ivanov, H. Plesken, and D. D. Sabatini. 1985. Polarized delivery of viral glycoproteins to the apical and basolateral plasma membranes of Madin-Darby canine kidney cells infected with temperature-sensitive viruses. *J. Cell Biol.* 100:136–151.
52. Rodriguez-Boulan, E. 1983. Polarized assembly of enveloped viruses from cultured epithelial cells. *Methods Enzymol.* 98:486–501.
53. Rodriguez-Boulan, E., and W. J. Nelson. 1989. Morphogenesis of the polarized epithelial cell phenotype. *Science (Wash. DC).* 245:718–725.
54. Rodriguez-Boulan, E., K. T. Paskiet, P. J. Salas, and E. Bard. 1984. Intracellular transport of influenza virus hemagglutinin to the apical surface of Madin-Darby canine kidney cells. *J. Cell Biol.* 98:308–319.
55. Roth, M. G., D. Gundersen, N. Patil, and E. Rodriguez-Boulan. 1987. The large external domain is sufficient for the correct sorting of secreted or chimeric influenza virus hemagglutinins in polarized monkey kidney cells. *J. Cell Biol.* 104:769–782.
56. Salicinski, P. R. P., C. McClean, J. E. Sykes, V. V. Clement-Jones, and P. J. Lowry. 1981. Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3,6-diphenyl glycoluril (Iodogen). *Anal. Biochem.* 117:136–146.
57. Salzman, N. H., and F. R. Maxfield. 1989. Fusion accessibility of endocytic compartments along the recycling and lysosomal endocytic pathways in intact cells. *J. Cell Biol.* 109:2097–2104.
58. Simons, K., and A. Wandinger-Ness. 1990. Polarized sorting in epithelia. *Cell.* 62:207–210.
59. Tokuyasu, K. T. 1973. A technique for ultracryotomy of cell suspensions and tissues. *J. Cell Biol.* 57:551–565.
60. Towbin, H. T., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.
61. Viitala, J., S. R. Carlsson, P. D. Siebert, and M. Fukuda. 1988. Molecular cloning of cDNAs encoding lamp A, a human lysosomal membrane glycoprotein with apparent Mr = 120,000. *Proc. Natl. Acad. Sci. USA.* 85:3743–3747.
62. von Figura, K., and A. Hasilik. 1986. Lysosomal enzymes and their receptors. *Annu. Rev. Biochem.* 55:167–193.
63. Waheed, A., S. Gottschalk, A. Hille, C. Krentler, R. Pohlmann, T. Braulke, H. Hauser, H. Geuze, and K. von Figura. 1988. Human lysosomal acid phosphatase is transported as a transmembrane protein to lysosomes in transfected baby hamster kidney cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:2351–2358.

64. Wandinger-Ness, A., M. K. Bennett, C. Antony, and K. Simons. 1990. Distinct transport vesicles mediate the delivery of plasma membrane proteins to the apical and basolateral domains of MDCK cells. *J. Cell Biol.* 111:987–1000.
65. Williams, M. A., and M. Fukuda. 1990. Accumulation of membrane glycoproteins in lysosomes requires a tyrosine residue at a particular position in the cytoplasmic tail. *J. Cell Biol.* 111:955–966.
66. Wilson, J. M., N. Fasel, and J.-P. Kraehenbuhl. 1990. Polarity of endogenous and exogenous glycosyl-phosphatidylinositol-anchored membrane proteins in Madin Darby canine kidney cells. *J. Cell Sci.* 96:143–149.