

Transferrin Receptor Polarity and Recycling Accuracy in "Tight" and "Leaky" Strains of Madin–Darby Canine Kidney Cells

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Abstract. We have characterized the polarity of the transferrin receptor in the epithelial Madin–Darby canine kidney (MDCK) cell line. The receptor is present in $\sim 165,000$ copies per cell, migrates as a diffuse band upon SDS gel electrophoresis with M_r 90,000, displays a dissociation constant for diferritransferrin at neutral pH of ~ 2 nM, and is active in essentially all of the cells of the population.

Transferrin-mediated ^{55}Fe uptake was used to measure the polarity of active transferrin receptors in filter-grown MDCK cells. The ratio of basolateral to apical receptors was $\sim 800:1$ for the high resistance strain I MDCK cells (typically $>2,000$ ohm-cm 2) and $\sim 300:1$ for the lower resistance strain II cells (<350 ohm-cm 2). In combination with morphometric data

this shows that a difference in resistance between these two strains is not reflected in a significant difference in cell surface polarity.

We used the recycling of transferrin receptor in filter-grown MDCK cells to evaluate the accuracy of the sorting of a basolateral protein during endocytosis. Monitoring the amount of apically released ^{125}I -labeled transferrin after application of ^{55}Fe - and ^{125}I -labeled transferrin to the basolateral surface provided a sensitive assay of the accuracy of sorting during recycling of the receptor from endosomes to the plasma membrane. The accuracy of transferrin receptor sorting ($>99.88\%$) during a single cycle of transit between the endosome and the plasma membrane is sufficient to maintain the high level of polarity of the cell.

THE cells of a transporting epithelium accomplish their vectorial functions as a consequence of the asymmetric distribution of plasma membrane components (for a review see Simons and Fuller, 1985). The transporting function is also dependent on the selective permeability barrier of the tight junction which seals the epithelial cells into a continuous electrically resistant sheet that separates the luminal from the serosal space. The tight junction also defines and separates the two domains of the plasma membrane: the apical domain, which lines the luminal side of the epithelium, and the basolateral, which faces the basal lamina and the blood supply.

Relatively little is known concerning the mechanisms that allow the epithelial cell to generate and maintain the polarity of its plasma membrane components. Recent progress has involved the use of model systems that display the polar features of *in vivo* transporting epithelia as well as the convenience of *in vitro* systems. The most widely used of these systems is the Madin–Darby canine kidney (MDCK) cell system. This system has long been used for studies of epithelial transport by physiologists and has more recently been adapted to the study of the generation and maintenance of polarity by an increasing number of cell biologists (for reviews see Rodriguez-Boulan, 1983; Simons and Fuller, 1985).

Cell surface polarity is a quantitative property of the cell. In this paper we present a characterization of the transferrin

receptor in polarized MDCK cells and then use it to obtain quantitative answers to three questions concerning the polarity of the cell surface. First, we quantitate polarity of the receptor on the cell surface and ask how this polarity is affected by the growth conditions (growth on plastic versus growth on filters). Second, we ask how the polarity of this cell surface marker varies with the maximal electrical resistance of the cellular tight junction in two strains of the MDCK cell line: MDCK strain I cells which have a trans-epithelial resistance of $>2,000$ ohm-cm 2 , and strain II cells which typically exhibit a resistance of 100–200 ohm-cm 2 (Richardson et al., 1981; Balcarova-Ständer et al., 1984). Finally, we address the mechanism of the maintenance of polarity by measuring the accuracy of sorting during a single cycle of endocytosis to see whether this is sufficient to maintain the observed polarity of the cell.

Our assays of sorting accuracy utilize the pH dependence of the surface polarity and the iron–transferrin–transferrin receptor interaction. This interaction has been well characterized in human cells where the transferrin receptor is a disulfide-linked homodimer of 90-kD subunits (Schneider et al., 1982; Newman et al., 1982). The ligand, transferrin, is a soluble monomeric protein of 80 kD. The function of the transferrin–transferrin receptor system is to provide iron for the cell. Each transferrin binds two ferric irons with very high affinity ($>10^{22}$) at neutral pH and very much lower affinity at low pH (Aisen and Listowsky, 1980). Diferritrans-

ferrin binds with high affinity ($K_d \sim \text{nM}$) to cell surface transferrin receptor at neutral pH and is endocytosed. Upon reaching the low pH of the endosome, the transferrin releases its iron, which is then taken across the endosomal membrane and used or stored in ferritin. The affinity between apotransferrin and the transferrin receptor is high at acid pH so that the complex remains intact and is recycled to the cell surface. Upon the return of the transferrin receptor to the cell surface and to neutral pH, the apotransferrin is displaced by the tighter binding diferritransferrin and the cycle is repeated (Klausner et al., 1983b; Dautry-Varsat et al., 1983).

We show here that the transferrin receptor in MDCK cells resembles that in previously studied human cell systems with respect to apparent molecular weight and pH-dependent affinity for transferrin. We present a very sensitive assay for the polarity of the receptor in filter-grown cells and use this assay to demonstrate that the difference in tight junction resistance between strain I and strain II cells is not reflected in a difference in the degree of cell surface polarity of the transferrin receptor. We also measure the accuracy of transferrin receptor sorting during a single cycle of transit from the basolateral plasma membrane through endosomes and back to the cell surface to find out whether this is sufficient to maintain the measured surface polarity of the receptor.

Materials and Methods

Growth of Cells

MDCK strain I and strain II cells were grown on 0.45- μm pore size nitrocellulose-cellulose acetate mixed fiber filters (HATF, Millipore France S.A., Molsheim, France) in mini-Marbrook chambers (MMCs)¹ for 4 d, as described in Fuller et al. (1984), for most of the experiments in this paper. For the transcytosis experiments, strain I cells were grown on 0.22- μm pore size nitrocellulose-cellulose acetate mixed fiber filters (GSTF, Millipore France S.A.). Only monolayers that displayed transepithelial electrical resistances of $>4,000 \text{ ohm}\cdot\text{cm}^2$ were selected for use in transcytosis experiments. Plastic-grown cells were as described in Fuller et al. (1985a) and used 2 d after seeding when the monolayer was confluent but not blistering. A431 cells were a cloned line obtained from Dr. R. Bravo (European Molecular Biology Laboratory [EMBL]) and were grown in Dulbecco's modified Eagle's (DME)/F12 medium supplemented with 10% fetal calf serum, 10 mM Hepes, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin. A431 cells were seeded at a density of $5 \times 10^4 \text{ cells}/\text{cm}^2$ and used after 2 d of growth when they were still subconfluent.

Preparation of Labeled Transferrin

Before labeling with ^{125}I , transferrin (Sigma Chemical Co., St. Louis, MO) was loaded with iron by the nitriloacetate-catalyzed procedure described by Klausner et al. (1983a). Diferritransferrin was labeled with ^{125}I using Iodo-Beads (Pierce Chemical Co., Rockford, IL) by a modification of the method suggested by the supplier. 0.5–3 mCi of Na^{125}I (Amersham Buchler GmbH, Braunschweig, FRG) was incubated with one bead for 5 min in a volume of 0.4 ml of Dulbecco's phosphate-buffered saline lacking Ca^{++} and Mg^{++} (PBS⁻) on ice. 10 μl of 100 mg/ml diferritransferrin was then added to the solution, mixed, and allowed to stand on ice for 20 min. The reaction mixture was then removed from the bead, and 10 μl of 20 mg/ml KI as well as 10 μl of 0.1 mM tyrosine were added. The nonincorporated iodine was removed by chromatography on a $25 \times 0.5\text{-cm}$ Sephadex G50 column (Pharmacia Fine Chemicals, Piscataway, NJ)

1. *Abbreviations used in this paper:* BSS/BSA, Hanks' basal salt solution with 0.6% bovine serum albumin, 10 mM Hepes; EGF, epidermal growth factor; EMEM, minimum essential medium with 0.2% (wt/vol) bovine serum albumin, 10 mM Hepes (for the transcytosis experiments, 0.6% [wt/vol] was used); MMC, mini-Marbrook chamber; PBS⁺, PBS⁻, Dulbecco's phosphate-buffered saline with or without Ca^{++} and Mg^{++} , respectively; TX114, Triton X-114.

equilibrated with PBS⁻ containing 0.2% wt/vol bovine serum albumin (BSA). The labeled transferrin was stored at -80°C and used within 1 mo.

Transferrin was labeled with ^{55}Fe by a modification of the method of Klausner et al. (1983a). 2 mCi of $^{55}\text{Fe}(\text{III})$ chloride in 0.1 M HCl (IES Q 5853, Amersham Buchler GmbH) was lyophilized in a 5-ml plastic tube and resuspended in 0.25 ml of 0.1 M sodium nitriloacetate in H_2O . 20 mg apotransferrin in 0.25 ml 0.5 M TrisCl, 10 mM NaHCO_3 was added to this, and the mixture was incubated at room temperature for 4 h. Excess iron was separated from the transferrin by desalting on a Sephadex G-50 column pre-equilibrated with 20 mM Tris pH 7.6, 250 mM NaCl. Incorporation of iron in transferrin was $>80\%$ so that the specific activity of the product exceeded 100 cpm/ng. The ferritransferrin is stable when stored at -80°C for at least 6 mo.

Transferrin-binding Assays with Plastic-grown Cells

MDCK cell monolayers were grown in 35-mm diam dishes as described above. For binding to the apical surface, monolayers were washed twice with PBS containing Ca^{++} and Mg^{++} (PBS⁺) at 37°C and transferred to Hanks' balanced salt solution with 0.6% bovine serum albumin, 10 mM Hepes (BSS/BSA) containing Ca^{++} and Mg^{++} on ice. For binding to both the apical and basolateral surfaces, the tight junctions of the monolayers were opened by washing twice with PBS⁻ at 37°C followed by incubation with 1 mM EGTA in PBS⁻ for 3 min at 37°C (Pesonen and Simons, 1983; Martinez-Palomo et al., 1980). These monolayers were then transferred to BSS/BSA lacking Ca^{++} and Mg^{++} on ice. To begin the binding, this medium was removed from the cells and replaced with 0.52 ml of BSS/BSA (either lacking or containing Ca^{++} and Mg^{++} as appropriate) that contained 350,000 cpm ^{125}I -labeled transferrin and nonlabeled transferrin in concentrations between 0 and 10 μM . Binding was allowed to proceed for 1 h at 0°C with tilting. The binding was stopped by adding 2 ml of ice-cold BSS/BSA (+/-) to each monolayer and then rinsing the monolayers with six 5-min washes of 2 ml of BSS/BSA (+/-). All medium was then removed and the monolayers solubilized with 70°C 2% wt/vol SDS and counted in a gamma counter (Nuclear Enterprises Ltd., Sighthill, Edinburgh, UK).

Transferrin-mediated ^{55}Fe Uptake by Filter-grown Cells

After measurement of transepithelial electrical resistance, filter-grown MDCK monolayers were rinsed in two changes of 37°C PBS⁺, removed from their MMCs, and incubated in three changes of 10 ml of minimum essential medium with 0.2% (wt/vol) BSA, 10 mM Hepes (EMEM) for 45 min, each at 37°C in a 5% CO_2 incubator. The monolayers were then rinsed with 37°C BSS/BSA and laid atop a BSS/BSA soaked 3MM Whatman paper filter to which 50 μl of newborn calf serum had been added in a 12-well plexiglass filter holder (Fuller et al., 1985b). This holder is formed from two pieces, one a solid piece of plexiglass with 12 1-mm deep, 25-mm diam indentations, and the other containing 12 corresponding 2-cm deep, 18-mm diam holes terminated with Neoprene O-rings. When clamped together one side of each MDCK monolayer forms the bottom of each well and can be accessed while the other is held against the serum and BSS/BSA containing paper filter. The O-rings prevent leakage between wells.

Uptake was begun by rinsing each well with 1 ml of BSS/BSA and then adding ^{55}Fe -labeled transferrin (typically 9×10^5 cpm) in 0.3 ml BSS/BSA. Controls for nonspecific uptake were performed by adding 25 μl of 100 mg/ml transferrin to the well before the addition of labeled transferrin. After addition of label, the plexiglass holder was sealed with Parafilm (American Can Co., Greenwich, CN) and placed on a tilting shaker for 90 min at 37°C . The plexiglass holder was then placed on ice in a 4°C room where all subsequent washing steps were performed. 1 ml of ice-cold BSS/BSA containing 10% vol/vol newborn calf serum was added to each well to stop incorporation. The medium was aspirated from the wells, the holder unclamped, and the cell monolayers transferred to separate dishes containing 5 ml each of PBS⁻ at pH 5.0 containing 10% vol/vol newborn calf serum. After 15 min of vigorous shaking the pH 5.0 medium was aspirated and replaced with 5 ml of PBS⁻ at pH 8.0 containing 10% vol/vol newborn calf serum. The filters were shaken in this pH 8.0 medium for 15 min. After a further four cycles of pH 5.0–8.0 washing, the filters were given a final 15-min pH 5.0 wash, blotted from the basal surface to remove excess fluid, and counted with 10 ml of Rotiszint 22 (Carl Roth, Karlsruhe, FRG) in a Searle Mark III, 6800 Liquid Scintillation counter (Searle Analytic Systems, Highwycombe, UK).

Purification of Transferrin Receptor from MDCK Cells

The procedure used was based on the Triton X-114 (TX114) phase partitioning method of Bordier (1981) and the transferrin affinity column method of receptor purification described by Enns and Sussman (1981).

Four 175-cm² tissue culture flasks containing a total of 10⁸ plastic-grown MDCK strain II cells were incubated with 5 ml each of 200 µCi/ml [³⁵S]methionine in growth medium containing 1/10th the normal amount of methionine for 24 h to label cellular protein. The cells were rinsed three times for 10 min each with PBS⁻ and then removed from the plastic by an incubation for 1 h with PBS⁻ containing 1 mM EDTA at 37°C followed by scraping. Unless specified, all further operations were performed on ice or at 4°C. The cells were spun down at low speed, and the cell pellet was lysed with 10 ml of 2% wt/vol TX114 in PBS⁻ containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and 100,000 cpm of ¹²⁵I-labeled transferrin. The lysate was centrifuged at 8,000 g to remove nuclei, and the supernatant was subjected to three cycles of phase partitioning. The hydrophobic lower phase was retained and adjusted to the initial volume with PBS⁻ for each cycle. The final hydrophobic phase was resuspended in 10 ml of 0.1 M sodium acetate, 0.5 M sodium chloride, 1% wt/vol TX114, pH 5.5 and mixed with 2 ml of transferrin Sepharose (10 mg transferrin/ml coupled to CNBr Sepharose 2B; Pharmacia) and left mixing overnight. The transferrin Sepharose-lysate mixture was poured into a column (0.7-cm diam) and, after settling, washed with 100 ml of 0.1 M sodium acetate, 0.5 M sodium chloride, pH 5.5 over a period of 6 h. The column was then eluted with 0.1 M glycine, 1.0% wt/vol TX114, 0.5 M sodium chloride, pH 10.0. 25-µl aliquots of each fraction were TCA precipitated and analyzed by electrophoresis.

Assay of Transferrin Binding to Solubilized Receptor

An assay for the binding of transferrin to solubilized receptor was developed by modifying the method of Fernandez-Pol and Klos (1980) to include the use of glass fiber filters as used by Dunn and Hubbard (1984) for the assay of epidermal growth factor receptor. Cells were solubilized in 2% wt/vol TX114 in PBS⁻ containing 1 mM PMSF (0.5 ml per 10⁶ cells) by several passes through a 1-ml Eppendorf pipette and then left on ice for 10 min with shaking. The nuclei were spun out at low speed at 4°C and the supernatant subjected to two cycles of phase partitioning. The hydrophobic phase was then resuspended to the original volume with PBS⁻ and stored on ice for up to 24 h. For the binding assay, 50–100 µl of TX114 lysate was brought to 0.5 ml with 0.2% wt/vol Triton X-100, 0.1% BSA in 50 mM TrisCl, pH 7.4. A mixture of 10⁵ cpm of ¹²⁵I-labeled transferrin and the appropriate amount of cold diferritranferrin to give the desired final concentration was added and the sample incubated at 25°C for 30 min. After the incubation 0.5 ml of 0.1% wt/vol rabbit IgG (Sigma Chemical Co.) in 100 mM sodium phosphate pH 7.4 was added to each sample and the samples mixed and placed on ice. The receptor was precipitated by addition of 1 ml of 25% wt/vol polyethylene glycol (PEG) 6000 (Sigma Chemical Co.) in 100 mM sodium phosphate pH 7.4. Then the samples were mixed vigorously and set on ice for 30 min. The precipitate was collected by filtration through Whatman GFC filters that had been prewashed with 0.1% wt/vol rabbit IgG under vacuum. The sample tubes and filters were washed three times with ice-cold 12.5% wt/vol PEG 6000 in 100 mM sodium phosphate pH 7.4, and the filters were dried and counted in a gamma counter.

⁵⁵Fe Autoradiography of Filter-grown Cells

4-d-old filter-grown monolayers were removed from their MMCs and incubated with three changes of 10 ml of EMEM for 45 min each at 37°C in a 5% CO₂ incubator. The monolayers were then rinsed twice more with EMEM and then each was incubated for 6 h with 1 × 10⁶ cpm of ⁵⁵Fe transferrin in 2 ml of EMEM at 37°C in a 5% CO₂ incubator. A control for nonspecific labeling was performed by including 1 mg/ml diferritranferrin in the incubation medium. After the incubation the monolayers were washed with alternating pH 5.0 and 8.0 solutions as described above and then washed for 1 h on ice with PBS⁻. The monolayers were then fixed for 20 min in 2% wt/vol glutaraldehyde in Hanks' basal salt solution on ice and then for a further 10 min at room temperature with fresh fixative. The monolayers were then washed with PBS⁻ on ice and the fixation quenched with 50 mM ammonium chloride in PBS⁻ on ice for 1 h. The filters were then quartered with a scalpel and dried under a heat lamp for 2 h. The pieces of filter were affixed to glass slides by laying them on a 25-µl drop of propylene oxide, cell side up, and storing the slides and filters overnight in an atmosphere of propylene oxide. The slides were then processed for autoradiography using Kodak stripping film following the suppliers instructions and

using an exposure of 4 d. After development the samples were stained with Farbelösung II Blau (Merz and Dade, Dedingen, Schweiz) and photographed.

Cross-reactivity of the MDCK Transferrin Receptor with Anti-human Transferrin Receptor Antibodies

Four anti-human transferrin receptor antibodies were tested: a rabbit polyclonal antibody raised against native human transferrin receptor obtained from Drs. J. D. Bleil and M. S. Bretscher (Medical Research Council Laboratory of Molecular Biology, Cambridge, England), a rabbit polyclonal antibody raised against SDS-denatured human transferrin receptor obtained from Dr. C. Schneider (EMBL), a goat polyclonal antibody raised against denatured human transferrin receptor obtained from Dr. R. Klausner (National Institutes of Health, Bethesda, MD), and a mouse monoclonal against human transferrin receptor obtained from Dr. I. Trowbridge (Department for Cancer Biology, Salk Institute for Biological Studies, San Diego, CA) by both immunofluorescence and immunoblotting against plastic-grown A431 cells and MDCK cells. All four were positive for the human receptor in A431 cells and negative for the canine receptor in MDCK cells.

Polarity of Recycling of Transferrin Receptor

¹²⁵I-labeled transferrin (typically 1,400 cpm/ng) and ⁵⁵Fe-labeled transferrin (160 cpm/ng) were prepared as described above. The binding activity of ¹²⁵I-labeled transferrin was assayed by the PEG 6000 precipitation method using TX114 lysates of plastic-grown MDCK cells. More than 85% of the ¹²⁵I-labeled transferrin was active in this assay.

After resistance measurement, filter-grown MDCK cell monolayers in MMCs were rinsed in three changes of EMEM (with 0.6% wt/vol BSA), and placed into 13.5-cm diam petri dishes containing 100 ml of 37°C EMEM. The MMC was always inserted into the liquid edge first and then turned right side up to minimize hydrostatic pressure on the monolayer. Six monolayers were placed around the circumference of each dish which was then centered on a magnetic stirring motor in a 37°C 5% CO₂ incubator. 10 ml of EMEM (bringing the total volume to 110 ml) was added to each dish and the levels of liquid inside each MMC were adjusted to be roughly 2 mm above the reservoir level. This resulted in an apical volume between 2.0 and 1.6 ml. A 1.5-cm Teflon-coated stirring bar was placed in the center of the dish and the reservoir gently stirred for 10 min. The transcytosis experiment was begun by adding labeled transferrin to the reservoir in a 0.5-ml vol of EMEM. The additions were as follows (see Fig. 6): (A) ⁵⁵Fe-labeled transferrin; (B) ⁵⁵Fe transferrin and ¹²⁵I-labeled transferrin; (C) first 100 mg of unlabeled transferrin and, after this was dispersed, ⁵⁵Fe transferrin and ¹²⁵I-labeled transferrin; (D) 20 µl of 100 mg/ml nonlabeled transferrin into the apical volume and then ⁵⁵Fe transferrin and ¹²⁵I-labeled transferrin into the reservoir. The amount of ⁵⁵Fe added to each dish was ~5 × 10⁶ cpm and the amount of ¹²⁵I varied between 1 × 10⁷ cpm and 1 × 10⁹ cpm in different experiments. After all additions the dishes were covered and left stirring for times between 4 and 6 h. At the end of this time, 50 ml of the reservoir was removed with a pipette, the dish was taken from the incubator, and then the apical solution was removed from each MMC. The filters and MMCs were then rinsed in PBS⁺ and the transepithelial resistance determined. The monolayer-bearing filters were removed from the MMCs and transferred to PBS⁺ on ice. When all filters had been removed they were washed with alternating pH 5.0 and pH 8.0 PBS⁻ solutions containing 10% fetal calf serum as described above, blotted, and then counted. The apical volume for each sample was determined gravimetrically.

Double Isotope Counting

The overlap of the ⁵⁵Fe and ¹²⁵I spectra is extensive and so particular care was taken to equalize quenching in all samples. Counts of ⁵⁵Fe and ¹²⁵I-labeled samples were done using a Searle Mark III 6800 liquid scintillation counter (Searle Analytic Systems, Highwycombe, UK) in double isotope mode. The amount of each isotope was determined from a linear least squares fit to the overlap of standard samples and checked by separate counting of the ¹²⁵I samples with a gamma counter. These independent determinations agreed to within 5% for all samples. Counts of medium were performed on 1.5-ml samples mixed with 20 ml of Rotiscint 22 (Carl Roth, Karlsruhe, FRG) and all samples were brought to 1.5-ml volume with EMEM before mixing with scintillation cocktail. Counts of cell incorporated iron were performed on the whole filter and cells in 20 ml of Rotiscint 22 using standards that contained a filter with cells that had been passed

through the same washing protocol as the samples. Counting was continued for up to 20 min on all samples so that the largest counting error for any sample including the backgrounds was <2%.

Other Methods

Resistance measurements and immunofluorescence were performed as described previously (Fuller et al., 1984; Perkins and Handler, 1981). PAGE and immunoblotting were performed as described in Fuller et al. (1985a). TX114 was purified as described in Bordier (1981) and stored as a 10% solution in PBS⁻ at 4°C.

Results

¹²⁵I Transferrin Binding to Plastic-grown MDCK Cells

Four antibodies against the human transferrin receptor showed no cross-reactivity with the canine receptor in MDCK cells (see Materials and Methods). Hence, we used the binding of ¹²⁵I-labeled transferrin to plastic-grown MDCK cells to assay the cell surface expression of the receptor. Confluent MDCK monolayers were incubated in several changes of serum-free medium (EMEM) for several hours to deplete the cells of transferrin. The monolayers were incubated with various concentrations of ¹²⁵I-labeled transferrin for 2 h on ice, and the number of counts bound as a function of total transferrin concentration was determined (Fig. 1). The control monolayers in which only the apical surface of the cells were exposed showed 32,000 low-affinity binding sites per cell ($K_d \sim 0.3 \mu\text{M}$). The monolayers that had been treated with EGTA to open the tight junctions and expose both the apical and the basolateral surfaces showed this low-affinity binding as well as 35,000 high-affinity ($K_d = 2 \text{ nM}$)

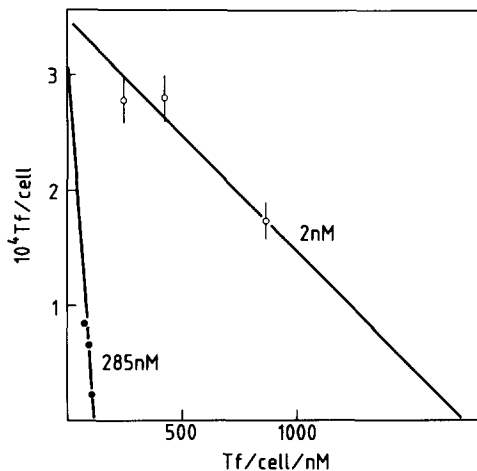


Figure 1. Binding of ¹²⁵I transferrin to plastic-grown MDCK cells. A Scatchard plot of transferrin binding to plastic-grown MDCK strain II cells at 4°C. ●, binding to the apical surface of the cells; ○, binding to the apical and basolateral surfaces of the cells that have been treated with EGTA to open tight junctions. Each point is the mean of three measurements, and the standard deviation of the values is shown when this is larger than the size of the symbol. Nonspecific background binding was determined from the number of counts bound in the presence of 10 μM diferritransferrin and has been subtracted from the values shown. The apparent dissociation constant (K_d) for the binding is shown above each line. The low-affinity binding has been subtracted from the basolateral curve. The high-affinity binding is clearly present primarily on the basal surface.

binding sites per cell. We interpreted the high-affinity binding to the cells as binding of transferrin to its normal cell surface receptor, since transferrin-transferrin receptor binding displays a similar affinity in other cells. The fact that the majority of the high-affinity sites is observed only when the cell junctions are opened to expose the basolateral surface indicates that the receptor is predominantly basolateral. The ratio of the basolateral to apical binding sites is probably an underestimate, since EGTA treatment may not have exposed the entire cell surface to the ligand.

Purification and Properties of the MDCK Transferrin Receptor

We compared the biochemical properties of the MDCK transferrin receptor to those of the human transferrin receptor as expressed in A431 cells. A useful property of the receptor was its behavior during TX114 phase partitioning. Fig. 2 shows an immunoblot of TX114-extracted A431 cells. The receptor is seen as a 90-kD immunoreactive band in the whole cell lysate. After phase partitioning the immunoreactive band is found exclusively in the detergent-rich lower phase and not in the detergent-poor supernatant. ¹²⁵I Transferrin, which is bound to the receptor before lysis, is not concentrated in the hydrophobic phase so that >90% of the ¹²⁵I counts remain in the supernatant. Several cycles of TX114 partitioning effect the complete removal of the labeled transferrin from the hydrophobic phase while retaining the transferrin receptor. Since the receptor binds transferrin in the presence of TX114 (see below), we presume that the transferrin receptor-transferrin interaction is broken when the complex enters the hydrophobic TX114 phase. Whatever the mechanism, TX114 partitioning provides a convenient means of concentrating the transferrin receptor and simultaneously stripping it of endogenous transferrin.

We used a combination of TX114 phase partitioning and transferrin-Sepharose chromatography to determine the characteristics of the MDCK transferrin receptor from [³⁵S]methionine-labeled plastic grown MDCK cells (see Materials and Methods). Elution of the transferrin-Sepharose column at high pH released one major protein species that migrated on a polyacrylamide SDS gel as a diffuse band with M_r 90,000 under reducing conditions (Fig. 3). These results show that the MDCK transferrin receptor has approximately the same molecular weight as the human receptor and behaves similarly with respect to pH-dependent transferrin affinity and TX114 phase partitioning.

We also used TX114 phase partitioning to strip endogenous transferrin from the receptor so that we could compare the binding affinity for transferrin of the MDCK transferrin receptor with that of the human receptor. The concentration dependence of ¹²⁵I transferrin binding to TX100-solubilized receptor was assayed by PEG 6000 precipitation of the receptor-ligand complex. Scatchard analysis of the results showed the presence of a single class of high affinity binding sites in each case. Lysate from plastic-grown strain I cells contains 165,000 transferrin binding sites per cell with an affinity of 2.5 nM. A431 cell lysate was assayed in parallel and gave 468,000 binding sites per cell with an affinity of 2 nM. Hence, the binding characteristics of the MDCK receptors match those of the A431 receptor although the number of receptors is fewer. Treatment with deferoxamine or with picolinic acid has been reported to induce the expression of

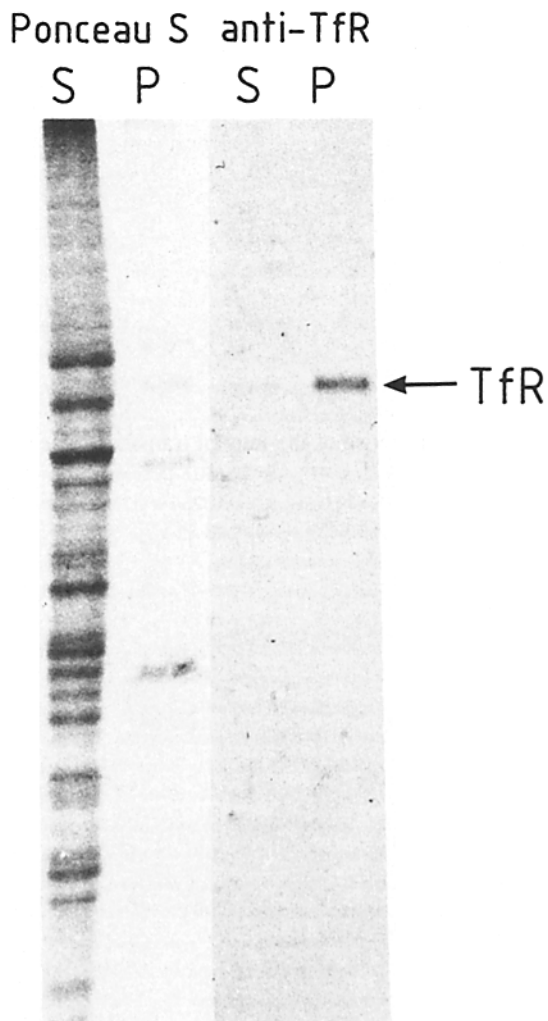


Figure 2. Immunoblot of A431 transferrin receptor after TX114 partitioning. The TX114 lysate from 10^5 A431 cells was phase partitioned, subjected to electrophoresis on a SDS 10% polyacrylamide gel, and transferred electrophoretically to nitrocellulose. On the left is shown the Ponceau S staining of a strip from the nitrocellulose indicating the distribution of protein. On the right is the immunoperoxidase staining of the same strip with the monoclonal antibody B3/25 obtained from Dr. I. Trowbridge and a rabbit anti-mouse peroxidase conjugate. The 90-kD band corresponding to the human transferrin receptor is found exclusively in the hydrophobic phase (*P*), although the majority of the cellular protein is found in the detergent-poor supernatant (*S*). In the same experiment >90% of 10,000 cpm of ^{125}I -labeled transferrin, which was included in the original lysate, partitioned into the supernatant.

transferrin receptors in other cells (Mattia et al., 1984; Fernandez-Pol, 1979) but caused only a small increase to 230,000 receptors per cell when applied to confluent plastic-grown MDCK monolayers. Assays of TX114 lysates of filter-grown strain I cells showed essentially the same total number of receptors (170,000 per cell) with the same binding constant as plastic-grown strain I cells.

Transferrin-mediated ^{55}Fe Uptake in Filter-grown MDCK Cells

We used transferrin-mediated ^{55}Fe uptake as a sensitive measure of the polarity of transferrin receptor in filter-grown

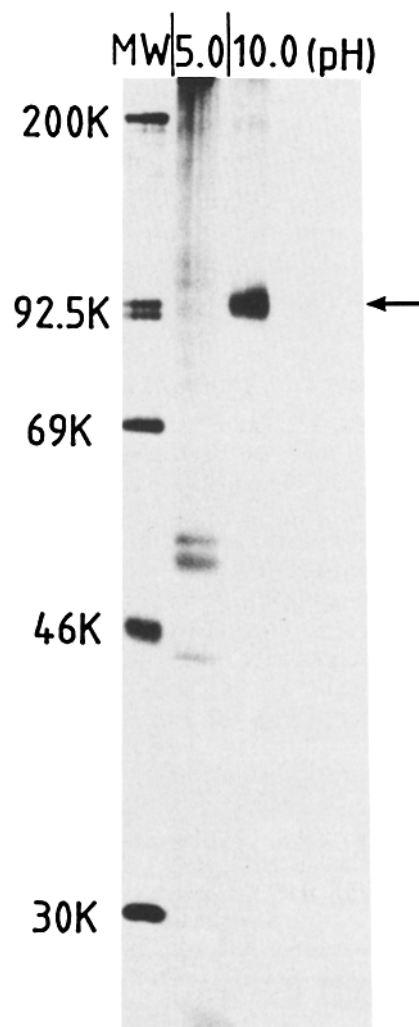


Figure 3. Purification of the MDCK transferrin receptor. TX114 lysate from [^{35}S]methionine-labeled plastic-grown MDCK cells was subjected to four rounds of phase partitioning to concentrate the receptor and to strip it of endogenous transferrin. The detergent phase was then brought to low pH and applied to a transferrin-Sepharose column which was then washed extensively with low pH buffer. SDS PAGE of fractions from transferrin affinity chromatography of TX114 lysate of MDCK cells is shown. The lanes in the autoradiograph show: *MW*, molecular weight standards; *5.0*, material released by a pH 5.0 wash of the column; and *10.0*, the material released by a subsequent pH 10.0 wash of the column. The dominant species released by the pH 10.0 wash is ~90 kD, similar to that of the human transferrin receptor.

MDCK cells. ^{55}Fe -labeled transferrin was incubated with the cells for various periods at 37°C , the incorporation stopped by transferring the cells to 0°C , the surface of the cells washed with a series of pH 5.0 and pH 8.0 buffers and the cells counted. The uptake in the presence of a high concentration of nonlabeled diferritransferrin was subtracted from the total uptake to yield the receptor-dependent uptake. This assay of receptor distribution offers two primary advantages over binding studies. First, the ability to measure the total incorporation of iron over a period of hours allows an amplification of the signal while binding can only determine the number of receptors on the cell surface at a single time. Second, although the sensitivity of either method is limited

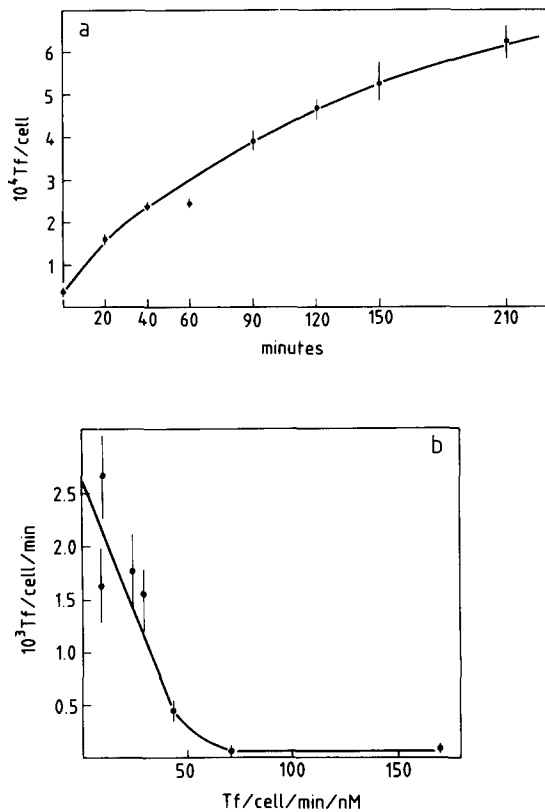


Figure 4. Transferrin uptake with filter-grown cells. The time course of transferrin mediated ^{55}Fe uptake by filter-grown MDCK strain I monolayers at a transferrin concentration of 5 nM is shown in *a*. Each point is the mean of two values and the range is shown when it is greater than the size of the symbol. A background corresponding to the ^{55}Fe counts found associated with the cells at the end of the same time period in the presence of 12.5 μM unlabeled transferrin has been subtracted. An Eadie-Scatchard plot of the concentration dependence of the ^{55}Fe uptake by filter-grown MDCK strain I cells is shown in *b*. Each point represents the mean of three measurements and the standard error of the mean is shown. Several experiments have been combined to give these data. Incorporation was for 90 min at 37°C. The nonlinear nature of the plot probably reflects the effect of the filter in reducing the effective transferrin concentration at the basolateral surface of the cells.

by nonspecific binding of labeled transferrin particularly by the filter, the uptake method measures only the intracellular ^{55}Fe . Rinsing the filter and cells with low pH releases the ^{55}Fe from the extracellular transferrin leaving only the intracellular ^{55}Fe to be counted. The apotransferrin itself remains bound to the filter. It is this background of filter-bound transferrin that makes direct transferrin binding experiments on filter-grown cells impractical.

Fig. 4 shows the kinetics and concentration dependence of transferrin-mediated ^{55}Fe uptake through the basal surface

of filter-grown MDCK strain I cells. Uptake was essentially linear with time before 60 min and displayed a rate of 100 transferrins/min per cell at a transferrin concentration of 5 nM (Fig. 4 *a*). The specific uptake, although relatively small, can be very precisely determined because the background of ^{55}Fe uptake increases with increasing transferrin concentration even at concentrations significantly above the K_d . The Eadie-Scatchard plot of this data (Fig. 4 *b*) is curvilinear, which indicates that this behavior is not explained by simple, single site binding kinetics. Such behavior has not been seen in previous studies of transferrin uptake (Klausner et al., 1983a; Karin and Mintz, 1981) and was also not observed in our studies of transferrin uptake by plastic-grown cells (see below). We believe that it reflects the influence of either transferrin binding to the filter or of the gradual release of unlabeled transferrin from the filter during the uptake. Either of these phenomena would decrease the effective transferrin concentration at the basal surface of the cell and could result in the concentration dependence observed. The maximal uptake rate reached by the cells was $\sim 2,600$ transferrins/min per cell.

The polarity of transferrin-mediated ^{55}Fe uptake is shown in Table I. Application of label to the basolateral surface resulted in uptake that was more than two orders of magnitude greater than that through the apical surface. The mean ratio in five experiments was 770:1 (basolateral to apical) with a range from 260:1 to 2,012:1. The variation between the lowest observed value and the highest is more apparent than real since a high value of the polarity is dramatically affected by the apical background level, which is a relatively small number. Under the conditions of the assay the basolateral uptake corresponded to ~ 100 transferrins/min per cell. When higher concentrations of labeled transferrin were used, the uptake rate increased although the measured polarity of uptake was somewhat lower due to leakage and an increased basolateral background. The monolayers used in the assay all had resistances between 2,000 ohm-cm² and 4,000 ohm-cm². Increases in resistance above the 2,000 ohm-cm² level caused no significant change in the measured polarity of uptake. Monolayers with significantly lower resistances often displayed a lower polarity of uptake.

Transferrin and transferrin-mediated ^{55}Fe uptake was also studied in plastic-grown MDCK cells (not shown). Application of labeled transferrin to the apical surface resulted in an uptake that was $\sim 20\%$ of that seen through the basolateral surface of filter-grown cells. However, the opening of cells with EGTA to examine uptake through the basolateral surface caused a dramatic decrease in the total uptake by the cells. Although it is known that transferrin binding to its receptor is not dependent on the presence of Ca^{++} (Anderson and Kaplan, 1983), it appears that EGTA treatment of the cells is somewhat toxic since the total iron uptake decreased.

Table I. Transferrin Uptake Polarity for Filter-grown Strain I MDCK Cells

Apical	Apical + cold	Basal	Basal + cold	Ratio
Highest 26.2 (6.1)	24.5 (6.5)	4,250 (364)	225.8 (27.9)	$(4,250 - 225.8)/(26.2 - 24.5) = 2,012:1$
Lowest 39.5 (9.0)	24.0 (6.1)	4,293 (128)	270.2 (29.4)	$(4,293 - 270.2)/(39.5 - 24.0) = 260:1$

The average polarity found in five experiments was 770:1 (basolateral/apical). The results for the two experiments which gave the highest and lowest polarities for transferrin receptor observed in filter-grown Strain I cells are shown in the table. Each value represents the mean of at least three samples and is given in cpm. The standard error of the mean is shown in parentheses.

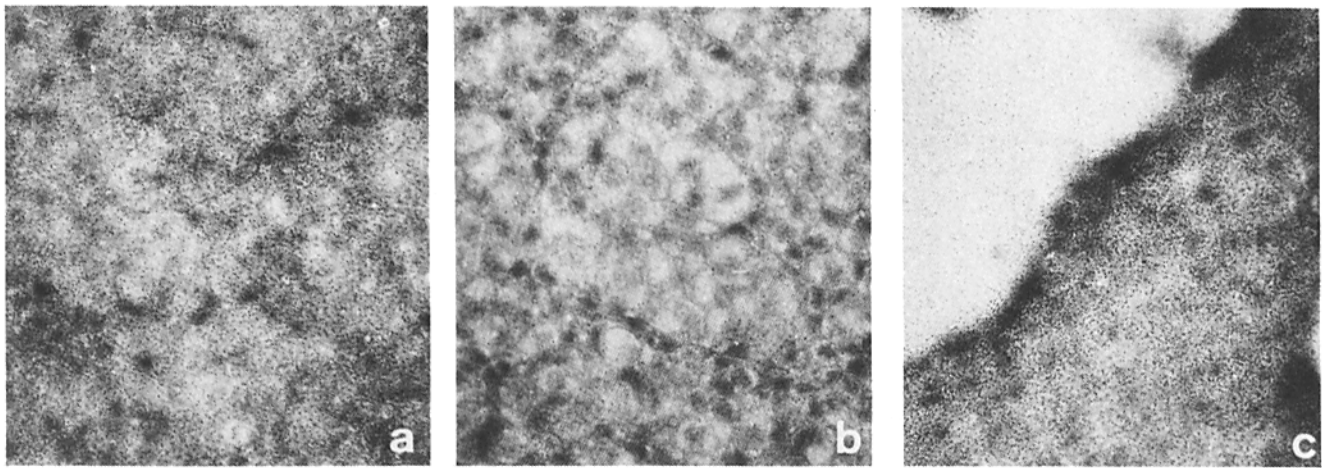


Figure 5. Autoradiographs of transferrin-mediated ^{55}Fe uptake by filter-grown MDCK monolayers. *a* shows the total incorporation in the monolayer, while *b* shows the nonspecific incorporation of ^{55}Fe in the presence of excess unlabeled diferritransferrin. The autoradiograph of the edge of a cell monolayer (*c*) shows that the only significant contribution to the ^{55}Fe uptake is from cell-associated label.

Table II. Transferrin Uptake Polarity for Filter-grown Strain II MDCK Cells

ohm-cm ²	Apical	Apical + cold	Basal	Basal + cold	Ratio
300–350	58.3 (20.5)	28.9 (8.2)	9171.7 (2168)	1066 (77)	276:1
200–100	331.0 (197.2)	63.7 (30.0)	9076 (239.4)	249 (28.9)	33:1

Each value represents the mean of at least three samples and is given in cpm. The standard error of the mean is shown in parentheses. The values for higher resistance and lower resistance samples are tabulated separately.

We did not find transferrin-mediated ^{55}Fe uptake a useful indicator of the polarity of plastic-grown monolayers for this reason.

Transferrin Uptake Is Not Restricted to a Subpopulation of Filter-grown Cells

An autoradiographic approach was used to investigate whether all the cells of the population internalized transferrin. Filter-grown strain I MDCK monolayers were incubated with ^{55}Fe -labeled transferrin for several hours, rinsed as described for the uptake polarity experiments, dehydrated, and covered with stripping film for autoradiography. After several days exposure the autoradiograph was developed and the cells stained with methylene blue. Fig. 5 shows autoradiographs of cells incubated in labeled transferrin (Fig. 5, *a* and *c*) and of cells incubated in the presence of a high concentration of unlabeled transferrin (Fig. 5 *b*). The transferrin uptake is distributed throughout the population and not confined to a few cells (Fig. 5 *a*). The average grain density is 14.5 ± 3.65 grains/cell for the incubations in labeled transferrin and 2.52 ± 0.15 grains/cell for the incubations in excess unlabeled transferrin. Fig. 5 *c* shows a detail of a field from a labeled incubation in which an edge of the filter is exposed. All of the label in the field is clearly confined to the cells showing that nonspecific binding of label to the filter does not affect our results.

Transferrin Uptake Polarity in "Leaky" Strain II Cells

We repeated the uptake experiments with filter-grown strain II cells (Table II). Using only the highest resistance monolayers (between 300 and 350 ohm-cm²), an uptake polarity of more than 250:1 was observed. These results show that

the ~ 10 -fold increase in the resistance of the tight junctions of the strain I cells is not accompanied by a corresponding increase in the polarity of the transferrin receptor. Two observations made during these experiments are of importance in interpreting this result and applying it to other work. First, the high polarity was only obtained when the highest resistance monolayers were used. When somewhat lower resistance monolayers were used the polarity of uptake was significantly worse (Table II). Second, the monolayer to monolayer variability was much greater with the strain II cells than in the strain I cells. These two observations almost certainly reflect the fact that a hole in a low resistance monolayer changes its resistance much less than a similar sized hole in a high resistance monolayer.²

2. This is a simple consequence of the addition formula for resistances in parallel. A monolayer with holes covering a fraction f of its area will have a net resistance $R = 1/(f/RO + (1-f)/RC)$, where RC is the resistance of a completely closed monolayer and RO is the resistance of a completely open monolayer. For our measuring system RO corresponds to 10–15 ohms, essentially the resistance of the solution between the ends of the salt bridges. RC is <100 ohms for a 3.14-cm² monolayer of strain II cells and more than 1,000 ohms for the same sized monolayer of strain I cells. The dependence of the net resistance on hole size is then

Hole area	Strain I	Strain II
none	1,000 ohms	100 ohms
0.5%	753 ohms	97 ohms
1.0%	603 ohms	95 ohms
5.0%	233 ohms	78 ohms
10.0%	132 ohms	63 ohms.

Resistance is only a sensitive measure of leakiness for a high resistance monolayer. This calculation is only valid for relatively large holes where the flow of current through the hole can be considered separately from the flow through the monolayer. This concept has recently been used to validate the relationship proposed by Claude (1978) between strand number and resistance in epithelial T₈₄ cells by Madara and Dharmasathaphorn (1985).

Principle of the Receptor Recycling Accuracy Assay

Our assay for accuracy of receptor recycling is based on the characteristics of the transferrin receptor which we have established in the earlier portion of this paper. We measure missorting during membrane recycling as the fraction of the transferrin that has passed through the low pH of the endosome and is then routed to the apical surface rather than being returned to the basolateral surface. To evaluate this fraction we follow the fate of ^{55}Fe and ^{125}I -labeled transferrin which has been applied to the basal surface. Exposure to the low pH of the endosome causes loss of iron so that the total amount of iron taken up by the cell reflects the number of transferrins that have passed through the endosome. The amount of apotransferrin, detectable by its ^{125}I label found on the apical surface, is the total number of missorted transferrins. The pH dependence of the binding of the ligand ensures that apotransferrin will be found on the side at which the receptor–apotransferrin complex first emerged and encountered neutral pH. The disposition of the ligand reflects the accuracy of the sorting of the receptor.

Accuracy of Transferrin Receptor Recycling

Fig. 6 presents a schematic of the implementation of our scheme for the measurement of transferrin recycling accuracy using filter-grown MDCK cells. The filter grown cells were incubated in several changes of EMEM to deplete the internal stores of the cell as well as to remove transferrin loosely bound to the filter and holder and then the electrical resistance of the monolayer was measured. Each monolayer in its MMC was gently placed in a dish and, after equilibration, labeled transferrin was added to the basal medium. The whole dish was then placed in a 37°C 5% CO_2 incubator for several hours. The basal medium was kept well mixed by gentle stirring with a magnetic stirring bar. At the end of the experiment, samples of the upper (apical) and lower (basal) reservoirs were taken, the resistance of the filters was measured, and the filters themselves washed with a series of high and low pH solutions to remove extracellular transferrin-bound iron. All samples were counted so that the amount of ^{55}Fe incorporated by the cells and the amount of ^{125}I -labeled transferrin that had crossed to the apical surface were determined.

Three factors needed to be controlled to ensure the validity

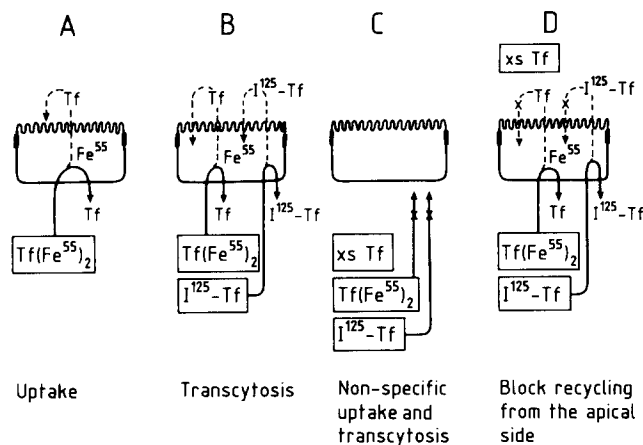


Figure 6. Schematic diagram of the transferrin recycling polarity experiment.

of the measurements: the integrity of the monolayer during the experiment, the activity of the ^{125}I -labeled transferrin, and the accuracy of the double-labeled counting. Several factors were found to be important in keeping the monolayers intact during the several hour course of the experiment. These included the use of 0.6% BSA in the medium and 0.22- μm pore size filters. This smaller pore size enabled us to grow monolayers routinely with resistances $>4,000$ ohm-cm 2 . The resistance provided a useful check for leakage, and conditions were optimized so that the decrease in resistance during the experiment was <100 ohms. Values from monolayers with greater losses in resistance were not included in the calculations. The activity of the ^{125}I -labeled transferrin is particularly important because the native ^{55}Fe transferrin would compete with denatured ^{125}I transferrin. The number of internalized ^{55}Fe counts would then represent a much larger amount of ^{125}I transferrin than was actually endocytosed. This would yield an overestimate of the accuracy of the recycling. Using the soluble binding assay (see Material and Methods) we could show that $>85\%$ of the ^{125}I -labeled transferrin was bound to solubilized receptor with an ~ 2 nM dissociation constant. Finally, the reliability of the double label counting was controlled by recounting the samples with a gamma counter. After correction for efficiency, the accuracy of the ^{125}I scintillation counts was seen to be $>95\%$.

The aim of the experiment was the determination of the fraction of the transferrin, internalized by the cell, which was released at the apical surface rather than returned to the basolateral. This missorting of endocytosed transferrin must be distinguished from leakage of labeled transferrin across the monolayer. The amount of this leakage and the reliability with which it can be determined set the experimental limit for the detection of missorting. Table III shows numbers from one experiment. Four sets of six filters were used for each experiment. Set A received only ^{55}Fe -labeled transferrin from the basal side. Set B received both ^{55}Fe -labeled transferrin and ^{125}I -labeled transferrin. Set C received both ^{55}Fe -labeled and ^{125}I -labeled transferrin as well as 12.5 μM unlabeled transferrin in the basal medium. Set D was identical to B but contained 12.5 μM unlabeled transferrin in the apical medium. Since iron is released from the transferrin by the low endosomal pH and stored by the cell, transferrin-dependent iron uptake measures the total transferrin endocytosis. Hence, the difference between the monolayer associated ^{55}Fe counts in A (12,356 cpm) and those in C (3,286 cpm) represents the total specific (i.e., transferrin receptor-mediated) transferrin uptake by the cell (9,070 cpm or 210,000 transferrins/cell during a 4-h period). This number of ^{55}Fe -labeled transferrins is equivalent to a certain number of ^{125}I -labeled transferrin counts (5.7 ^{125}I cpm/ ^{55}Fe cpm in Table III but similar results were obtained using different ratios in other experiments), and hence the number of ^{125}I counts found apically can be expressed as a fraction of the total transferrins internalized. The number of ^{125}I counts found apically in C represent transferrins that have passed to the apical surface in a non-transferrin receptor-dependent manner, i.e., via a hole in the monolayer (undetectable in the experiment described in Table III; highest value obtained in five experiments was 0.20%). This control also provides a measure of the effect of fluid phase transcytosis on the result. A calculation based on our previous measurements of the amount of fluid phase transcytosis in

Table III. Transferrin Recycling Accuracy

	A	B	C	D
Reservoir				
⁵⁵ Fe	234,143 cpm/ml	198,271 cpm/ml	191,034 cpm/ml	214,120 cpm/ml
¹²⁵ I	—	1,129,625 cpm/ml	1,209,207 cpm/ml	1,156,238 cpm/ml
Apical				
⁵⁵ Fe	42 (17) cpm/ml	48 (14) cpm/ml	14 (6) cpm/ml	40 (16) cpm/ml
¹²⁵ I	—	90 (20) cpm/ml	45 (15) cpm/ml	85 (21) cpm/ml
Monolayer				
⁵⁵ Fe	12,356 (2,808) cpm	12,431 (2,119) cpm	3,286 (569) cpm	10,464 (1,703) cpm
¹²⁵ I	—	1,272 (197) cpm	924 (147) cpm	1,077 (81) cpm
Specific incorporated				
⁵⁵ Fe	9,070 cpm	9,145 cpm	—	7,178 cpm
¹²⁵ I equiv.	—	52,102 cpm	—	39,296 cpm
Apical as % of specific incorporated				
⁵⁵ Fe	0.31%	0.37%	—	0.36%
¹²⁵ I	—	0.086%	—	0.10%

Each point is the mean of measurements from at least five filter-grown monolayers and is accompanied by its standard error in parentheses.

MDCK cell (von Bonsdorff et al., 1985) indicates that its contribution will be negligible. The difference between the ¹²⁵I counts found apically in B and in C represents the transferrin receptor-dependent passage to the apical surface or the missorting after endocytosis (mean 0.12%; range 0.16–0.08%). A final control (D) was needed to rule out the possibility that transferrin was being carried from the basal surface to the apical surface and picking up iron so rapidly from the medium that it could remain bound to the receptor and be re-internalized and directed to the basal surface. The high excess of apical unlabeled transferrin would compete for the receptor with the bound apotransferrin and block re-internalization. The amount of apical ¹²⁵I label in D was virtually identical to that seen in B, showing that re-internalization from the apical surface was not a significant factor in the experiment.

The ratio of apical apotransferrin to incorporated iron is the fraction of the receptor that was endocytosed from the basolateral surface and routed to the incorrect surface, the apical. The conditions of the experiment are such that the ratio represents the rate of missorting of the receptor during a single cycle of endocytosis and return to the plasma membrane. This is true even though many such cycles occur during the several hour course of the measurement. The accumulation of transferrin within the cell is very small so that essentially all of it can be assumed to be returned to the plasma membrane. This has been shown in a variety of human cell lines (Klausner et al., 1983a, b; Dautry-Varsat et al., 1983; Hopkins and Trowbridge, 1983) as well as in MDCK cells (see above) and validates our assumption that the amount of iron incorporated during the experiment reflects the total number of cycles between the plasma membrane and the endosome. The large volume of the basal reservoir makes it unlikely that a single transferrin was internalized more than once from the basal surface. Each missorting event during the course of the experiment must have produced an apically localized apotransferrin that remained apical. Since both the apical apotransferrin and the incorporated iron arise from events that occur during one cycle between the plasma membrane and the endosome, their ratio repre-

sents the probability of an error, the error rate, during a single cycle.

One seemingly paradoxical observation is that more ⁵⁵Fe was found in the apical medium in A and B than in C. This cannot represent increased leakage of transferrin under low transferrin conditions since it is not accompanied by a corresponding number of ¹²⁵I counts. Tests suggested that this apical ⁵⁵Fe was no longer transferrin associated and was completely dependent on the amount of ⁵⁵Fe taken up by the cell. It presumably represents a small leakage of the internalized iron from the cell. A release of this magnitude would not have been observed in previous studies (Klausner et al., 1983a; Karin and Mintz, 1981). Precipitation of the protein from the apical medium with 20% TCA resulted in recovery of more than half of the ¹²⁵I counts, showing that the majority of these counts remain bound to protein.

Our results showed that the amount of apically localized transferrin under conditions where it represents missorting during the recycling of its receptor ranged between 0.16 and 0.08% in six experiments. We believe that this level of error is a real phenomenon that reflects the precision of sorting by the cell since our limit of detection for missorted transferrin is less than a quarter of this under our experimental conditions.

Discussion

This paper presents the first quantitative study of the polarity of the transferrin receptor in a polarized epithelial cell. We showed that the MDCK transferrin receptor is very similar to the receptor in nonpolarized cells in terms of its molecular weight and pH-dependent affinity for transferrin. Our best determined K_d at neutral pH for diferritransferrin was 2.5 nM, a value that lies well within the range of K_d 6.7 nM reported for mouse teratocarcinoma cells (Karin and Mintz, 1981), 4.4 nM for HepG2 cells (Ciechanover et al., 1983), and 0.9 nM for erythroleukemia K562 cells (Klausner et al., 1983a), although it is significantly less than the 27 nM reported for HeLa cells (Ward et al., 1982).

One difference between the MDCK cell and the other sys-

tems in which the transferrin receptor has been previously described is the total number of receptors per cell. Filter-grown MDCK cells express fewer total receptors and, judging from the rate of transferrin uptake, a smaller fraction of these receptors appears to be on the cell surface. The soluble receptor assay showed 170,000 transferrin receptors/cell. Although binding studies to determine the surface receptor number can only be performed on plastic-grown cells, the maximal rate of ^{55}Fe uptake (2,600 transferrins/min/cell) can be combined with data from other systems to give an estimate of this number in filter-grown cells. ^{59}Fe uptake has been used to measure the receptor activity in two other systems. In the human erythroleukemia cell line, K562, Klausner et al. (1983a) determined the total receptor number of 500,000/cell of which 160,000 are on the cell surface, and these internalize iron with a rate constant of 0.13/min or 20,800 transferrins/cell per min. Karin and Mintz (1981) showed that cultured mouse teratocarcinoma cells have only 5,700 cell surface receptors but take up iron with a rate corresponding to 1,800 transferrins/cell per min. Ciechanover et al. (1983) measured the internalization rate of ^{125}I -labeled transferrin in HepG2 hepatoma cells which contain 51,000 surface receptors and found a rate constant of 0.7/6 min or 5,950 transferrin/min per cell. Bleil and Bretscher (1982) report a half time for internalization of transferrin receptor in HeLa cells of ~ 5 min or a rate constant of 0.1/min. In summary, the rate constant for transferrin receptor internalization lies between 0.1/min and 0.3/min in several systems whose levels of receptor are very different. If this holds true for our system our uptake rates would yield a value between 26,000 and 8,000 for the number of receptors on the MDCK basolateral surface. Our measured number of high affinity transferrin binding sites on plastic-grown strain II cells lies within this range. These estimates for the number of receptors expressed on the cell surface probably represent lower limits because in both the plastic-grown and filter-grown systems the substrate may interfere with access to the receptor.

This low number of surface receptors in the filter-grown cells raises the question of whether the receptor is only active in a small fraction of our population. We used an autoradiographic approach to show that the transferrin was taken up by essentially all the cells in a filter-grown monolayer. This is true even though the presence of a significant number of cell surface receptors has been described as a characteristic of rapidly proliferating or transformed cells (Gatter et al., 1983; Omary et al., 1980; Trowbridge and Omary, 1981). Only the number of active receptors in MDCK cells distinguishes this terminally differentiated and static population from the more active cells previously described.

Our primary interest in these studies is cell surface polarity. The transferrin receptor is a basolateral protein in MDCK cells and its polarity is as high as that of any of the other markers reported for any system (see Simons and Fuller, 1985). The basolateral localization of the receptor was expected since this surface corresponds to the one that faces the blood *in vivo* and hence should be involved in the uptake of plasma proteins such as transferrin. This consideration suggested to us that the growth of MDCK cells on a permeable support would not only make the study of the polarity of the cell more convenient but might also result in an increase in the polarity of the cell. A plastic-grown monolayer that kept its transferrin receptors exclusively confined to the

basolateral surface would not be able to incorporate any iron. In fact, plastic-grown strain II monolayers do incorporate transferrin when it is applied to the apical surface, showing that such monolayers are less polar or at least more leaky than their filter-grown counterparts. The fact that the polarity of the receptor is so high is an important constraint for any model of the mechanism of the generation and maintenance of polarity. It also shows that the high polarities seen for virus receptors (Fuller et al., 1984; Fuller et al., 1985b) are not artificially high due to special characteristics of those systems. They reflect the sorting ability of the cell.

The second portion of the paper addresses the relationship between cell surface polarity and the electrical resistance of the cellular tight junction. The MDCK cell lines provide a good system for testing this relationship since the two strains have very different resistances but are similar by most other criteria. It is unlikely that transcellular ion fluxes cause the low electrical resistance of monolayers of MDCK strain II cells. The paracellular route seems to determine the "leakiness" of the monolayers (Cereijido et al., 1981). The observation that epithelia *in vivo* have widely varying transepithelial resistances but all fulfill vectorial functions suggests that the polarity of the cell and the resistance of the junctions are independent. This could remain true even if the tight junction acts as a lateral diffusion barrier for plasma membrane components. A single junctional strand may serve as a complete barrier to large, relatively slowly moving surface components and yet be an inefficient barrier to rapidly diffusing small ions (Ü et al., 1979). Indeed, in proposing a logarithmic relationship between resistance and number of junctional strands, Claude (1978) suggested that the strands were dynamic. Rapid rearrangements of strands would result in this discrimination between rapidly moving and slowly moving components. Our results with the two lines of MDCK cells support this view. If diffusion through the tight junction limited the polarity of the cell this would be reflected in the gradient of receptor concentration between the apical and basolateral surfaces. A model in which the electrical resistance of the tight junctions is directly reflected in the polarity of the cell, would predict that the difference in receptor concentration between the apical and the basolateral surfaces would be ~ 10 -fold lower in strain II cells than in strain I cells. Alternatively, if the number of strands were the factor limiting polarity, a roughly threefold difference in the gradient of concentration would be predicted by the logarithmic relationship of Claude (1978).

One of the advantages of working with the well characterized MDCK system is that we can use the morphometric results for filter-grown cells (von Bonsdorff et al., 1985) to express the roughly twofold difference in the polarity of the total number of receptors in terms of the surface concentration of receptors. Filter-grown strain I cells have a ratio of apical to basolateral surface area of 1:7.6 so that a receptor polarity of $\sim 1:800$ results in a ratio of concentrations of 1:99, while strain II cells with a surface area ratio of 1:4 and a receptor polarity of $\sim 1:300$ yields a surface concentration ratio of 1:75. Although we cannot say that the polarity of the strain I cells is identical to that of the strain II cells, we can exclude the possibility that the difference in polarity is as great as 10-fold as would be expected from the resistance or even threefold as would be suggested from the number of strands.

The third portion of the paper addresses the mechanism by which polarity is maintained in the face of continual endocytosis. If the receptor is being internalized with a rate constant of 0.1/min, very precise sorting must occur to maintain the high polarity of total receptors described above. We examined the recycling of the transferrin receptor to evaluate the error rate during sorting after endocytosis. The special nature of the interaction between the transferrin receptor and transferrin made this possible and allowed us to monitor the sorting of the receptor by following the ligand. Application of a mixture of ^{55}Fe transferrin and ^{125}I -labeled transferrin to the basolateral surface of the cell gave two complementary pieces of information. The amount of ^{55}Fe incorporated by the cell measures the amount of transferrin that had been internalized by the cell and exposed to the low pH of the endosome. The amount of ^{125}I -labeled transferrin released at the apical surface is a direct measure of the error rate in sorting of the receptor after endocytosis. We found that the error rate averaged 0.12%.

Two fundamentally different models have been advanced for the sorting mechanisms that establish and maintain the surface polarity of an epithelial cell (for a review see Simons and Fuller, 1985). The first is that sorting is accomplished intracellularly so that newly synthesized proteins are sent directly to the appropriate cell surface and that endocytosed proteins are returned directly to that surface. In this model the pathways of transport to the two surfaces diverge internally and have equal priority. The second model differs in giving one surface priority over the other. All newly synthesized proteins would emerge first at one plasma membrane domain and are then sorted by further transport of the proteins which should be localized on the other surface. The transport pathways in this model diverge after the proteins have arrived at the cell surface. One of the attractions of this model is that a single mechanism would be responsible for the establishment of polarity and its maintenance. All components are sorted at the cell surface by recognizing and relocalizing improperly localized components. Implantation experiments have shown that such correction of improperly localized components does occur (Pesonen and Simons, 1983) and cell fractionation studies suggested that microvillar proteins appear first on the basolateral surface before they appear on the apical (Hauri et al., 1979; Quaroni et al., 1979).

Several groups have invested a great deal of effort over the last several years in attempting to demonstrate one of these types of mechanisms for newly synthesized proteins in epithelial cells. The clearest results have been obtained for the transport of newly synthesized viral proteins in the filter-grown MDCK cells. Most workers now agree that apically directed proteins and basolaterally directed ones are transported through the Golgi complex together (Fuller et al., 1985a; Rindler et al., 1983) and are then separated and transported directly to the appropriate surface domain (Matlin and Simons, 1984; Misek et al., 1984; Pfeiffer et al., 1985). Newly synthesized apical proteins emerge first at the apical surface, whereas newly synthesized basolateral ones appear first on the basolateral. Relatively little work has been done on the question of the maintenance of polarity. Recent evidence suggests that there is relatively little overlap between the endocytotic pathway and that followed by newly synthesized proteins to the cell surface in non-epithelial cells (Snider and Rogers, 1985; Griffiths et al., 1985). A separate,

correction type mechanism could be responsible for maintaining the polarity of the cell surface.

Measurements of the accuracy of recycling of receptors after endocytosis address this question. If the accuracy of a single cycle of endocytosis and return to the plasma membrane is sufficiently high to maintain the polarity of a surface component this obviates the need for a special correction mechanism to remove improperly localized protein. Of course such a correction mechanism could still exist in epithelial cells but it would not be the primary mechanism for maintaining the polarity of the cell. The work with perfused liver systems has provided a measure of the accuracy of sorting of internalized ligands for the asialoglycoprotein receptor (Schiff et al., 1984) and for the epidermal growth factor (EGF) receptor (Burwen et al., 1984). Both of these studies show relatively high levels of error (up to 4%) in the sorting of these components. The observation that the missorting of epidermal growth factor is not affected by chloroquine treatment (Burwen et al., 1984) indicates that this error rate also applies to the receptor. If the mechanism involved is the same for endocytosis from the apical and the basolateral surfaces, the maximum polarity that these receptors could achieve would be 25:1 (or 3.7:1 in terms of concentration using the morphometric results of Weibel, 1976). Estimates of the receptor polarity in the hepatocyte are significantly higher than this (Hubbard et al., 1983; Matsuura et al., 1982) so that a further sorting step would be needed to correct the localization of the receptor.

Our studies on the recycling of the transferrin receptor in polarized MDCK strain I cells reveal a very different picture. The error rate lies between 0.16 and 0.08% and would allow the cell to maintain a transferrin receptor polarity of greater than the 770:1 which is observed. These results show that no continuous correction mechanism is needed for the maintenance of transferrin receptor polarity in this system.

The discrepancy between the high error rate in the liver system and the low rate observed in MDCK cells is puzzling. We do not believe that it reflects the absence of transcellular traffic in MDCK cells such as exists for the IgA receptor and the haptoglobin-hemoglobin receptor in the hepatocyte (Mullock and Hinton, 1981). Previous work has shown that transcytosis of fluid phase markers (von Bonsdorff et al., 1985) and of membrane proteins (Pesonen and Simons, 1983) occurs in MDCK cells, proving that some connection between the apical and basolateral endocytotic pathways exists. Another explanation of the discrepancy may lie in the difference between the proteins studied rather than in a difference between the two systems. The recycling of transferrin receptor and its ligand may be quite different from the asialoglycoprotein receptor and EGF receptor which carry ligands to the lysosomes. One possibility is that asialoglycoproteins and EGF might bind nonspecifically to the membrane and be transcytosed as a result. Transcytosis of horseradish peroxidase was observed to occur via this mechanism in the hepatocyte system (Lowe et al., 1985). This hypothesis also accommodates the observation that EGF and asialoglycoprotein missorting could be blocked by excess ligand since a limited number of the membrane-binding sites would make this a saturable route, although not one mediated by the specific physiological receptor for these ligands.

Our conclusion that a single cycle of endocytosis can sort membrane proteins with very high accuracy has implications

for an understanding of membrane protein sorting in general. If this degree of accuracy is typical of other sorting events in the cell, then the specificity of the recognition event involved rather than the organization of those events is responsible for the precision with which the cell organizes its components. This issue has arisen previously in the context of the organization of the stack of Golgi cisternae. It was proposed (Rothman, 1981) that the separation of Golgi proteins from endoplasmic reticulum proteins could not occur in a single step but rather occurred in multiple steps. The multicisternal organization of the Golgi complex, likened to the plates of a distillation device, was seen as a consequence of this. Later work showed that the quantitative arguments used to motivate this hypothesis did not reflect the situation in the cell (Griffiths et al., 1984; Quinn et al., 1984). Further, examination of the carbohydrates of endoplasmic reticulum proteins shows that they have not undergone Golgi processing and makes it unlikely that they ever entered the Golgi complex (Lewis et al., 1985; Rosenfeld et al., 1984; Yamamoto et al., 1985). This aspect of the model has recently been deemphasized (Brands et al., 1985). The high accuracy of single step sorting of membrane proteins presents a telling argument against such mechanisms. High accuracy of protein localization can be achieved without them.

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References

- Aisen, P., and I. Listowsky. 1980. Iron transport and storage proteins. *Annu. Rev. Biochem.* 49:357-393.
- Anderson, R. G., and J. Kaplan. 1983. Receptor mediated endocytosis. *Mod. Cell Biol.* 1:1-52.
- Balcarova-Ständer, J., S. E. Pfeiffer, S. D. Fuller, and K. Simons. 1984. Development of cell surface polarity in the epithelial Madin-Darby canine kidney (MDCK) cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2687-2694.
- Bleil, J. D., and M. S. Bretscher. 1982. Transferrin receptor and its recycling in HeLa cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:351-355.
- Bordier, C. 1981. Phase separation of integral membrane proteins in Triton X-114 solution. *J. Biol. Chem.* 256:1604-1607.
- Brands, R., M. D. Snider, Y. Hino, S. S. Park, H. V. Gelboin, and J. E. Rothman. 1985. Retention of membrane proteins by the endoplasmic reticulum. *J. Cell Biol.* 101:1724-1732.
- Burwen, S. J., M. E. Baker, I. S. Goldman, G. T. Hradek, S. E. Raper, and A. L. Jones. 1984. Transport of epidermal growth factor by rat liver: evidence for a nonlysosomal pathway. *J. Cell Biol.* 99:1259-1265.
- Cerejido, M., I. Meza, and A. I. Martinez-Palomo. 1981. Occluding junctions in cultured epithelial monolayers. *Am. J. Physiol.* 240:C96-C102.
- Ciechanover, A., A. L. Schwartz, and H. F. Lodish. 1983. The asialoglycoprotein receptor internalizes and recycles independently of the transferrin and insulin receptors. *Cell.* 32:267-275.
- Claude, P. 1978. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. *J. Membr. Biol.* 39:219-232.
- Dautry-Varsat, A., A. Ciechanover, and H. F. Lodish. 1983. pH and the recycling of transferrin during receptor-mediated endocytosis. *Proc. Natl. Acad. Sci. USA.* 80:2258-2262.
- Dunn, W., and A. Hubbard. 1984. Receptor-mediated endocytosis of epidermal growth factor by hepatocytes in the perfused rat liver: ligand and receptor dynamics. *J. Cell Biol.* 98:2148-2159.
- Enns, C. A., and H. H. Sussman. 1981. Physical characterization of the transferrin receptor in human placenta. *J. Biol. Chem.* 256:9820-9823.
- Fernandez-Pol, J. A. 1979. Isolation and immunological characterization of an iron-regulated transformation-sensitive cell surface protein of normal rat kidney cells. *J. Supramol. Struct.* 11:371-390.
- Fernandez-Pol, J. A., and D. J. Klos. 1980. Isolation and characterization of normal rat kidney cell membrane proteins with affinity for transferrin. *Biochemistry.* 19:3409-3912.
- Fuller, S. D., C.-H. von Bonsdorff, and K. Simons. 1984. Vesicular stomatitis virus infects and matures only through the basolateral surface of the polarized epithelial cell line, MDCK. *Cell.* 38:65-77.
- Fuller, S. D., R. Bravo, and K. Simons. 1985a. An enzymatic assay reveals that proteins destined for the apical or basolateral domains of an epithelial cell share the same late Golgi compartments. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:297-307.
- Fuller, S. D., C.-H. von Bonsdorff, and K. Simons. 1985b. Cell surface influenza haemagglutinin can mediate infection by other animal viruses. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2475-2485.
- Gatter, K. C., G. Brown, I. S. Trowbridge, R. E. Woolston, and D. Y. Mason. 1983. Transferrin receptors in human tissues: their distribution and possible clinical relevance. *J. Clin. Pathol.* 36:539-545.
- Griffiths, G., G. Warren, P. Quinn, Mathieu-Costello, and H. Hoppeler. 1984. Density of newly synthesized plasma membrane proteins in intracellular membranes I - Morphometric studies. *J. Cell Biol.* 98:2133-2141.
- Griffiths, G., S. F. Pfeiffer, K. Simons, and K. Matlin. 1985. Exit of newly synthesized membrane proteins from the trans cisterna of the Golgi complex to the plasma membrane. *J. Cell Biol.* 101:949-964.
- Hauri, H.-P., A. Quaroni, K. J. Isselbacher. 1979. Biosynthesis of intestinal plasma membrane: posttranslational route and cleavage of sucrose-isomaltase. *Proc. Natl. Acad. Sci. USA.* 76:5183-5186.
- Hopkins, C. R., and I. S. Trowbridge. 1983. Internalization and processing of transferrin and the transferrin receptor in human carcinoma A431 cells. *J. Cell Biol.* 97:508-521.
- Hubbard, A. L., D. A. Wall, and A. Ma. 1983. Isolation of rat hepatocyte plasma membranes. I. Presence of three major domains. *J. Cell Biol.* 96:217-229.
- Karin, M., and B. Mintz. 1981. Receptor-mediated endocytosis of transferrin in developmentally totipotent mouse teratocarcinoma stem cells. *J. Biol. Chem.* 256:3245-3253.
- Klausner, R. D., J. van Renswoude, G. Ashwell, C. Kempf, A. N. Schechter, A. Dean, and K. R. Bridges. 1983a. Receptor-mediated endocytosis of transferrin in K562 cells. *J. Biol. Chem.* 258:4715-4724.
- Klausner, R. D., G. Ashwell, J. van Renswoude, J. B. Hanford, and K. R. Bridges. 1983b. Binding of apotransferrin to K562 cells: explanation of the transferrin cycle. *Proc. Natl. Acad. Sci. USA.* 80:2263-2266.
- Lewis, M. J., S. J. Turco, and M. Green. 1985. Structure and assembly of the endoplasmic reticulum: biosynthetic sortine of endoplasmic reticulum proteins. *J. Biol. Chem.* 260:6926-6931.
- Lowe, P. J., K. S. Kan, S. G. Barnwell, R. K. Sharma, and R. Coleman. 1985. Transcytosis and paracellular movements of horseradish peroxidase across liver parenchymal tissue from blood to bile: effects of alpha-naphthylisothiocyanate and colchicine. *Biochem. J.* 229:529-537.
- Madara, J. L., and K. Dharmasathaphorn. 1985. Occluding junction structure-function relationships in a cultured epithelial monolayer. *J. Cell Biol.* 101:2124-2133.
- Martinez-Palomo, A., I. Meza, G. Beaty, and M. Cerejido. 1980. Experimental modulation of occluding junctions in a cultured transporting epithelium. *J. Cell Biol.* 87:736-745.
- Matlin, K. S., and K. Simons. 1984. Sorting of a plasma membrane glycoprotein occurs before it reaches the cell surface in cultured epithelial cells. *J. Cell Biol.* 99:2131-2139.
- Matsuura, S., H. Nakada, T. Sawamura, and Y. Tashiro. 1982. Distribution of an asialoglycoprotein receptor on rat hepatocyte cell surface. *J. Cell Biol.* 95:864-875.
- Mattia, E., K. Rao, D. S. Shapiro, H. H. Sussman, and R. D. Klausner. 1984. Biosynthetic regulation of the human transferrin receptor by desferrioxamine in K562 cells. *J. Biol. Chem.* 259:2689-2692.
- Misek, D. E., E. Bard, and E. Rodriguez-Boulan. 1984. Biogenesis of epithelial cell polarity in intracellular sorting and vectorial endocytosis of an apical plasma membrane glycoprotein. *Cell.* 39:537-546.
- Mullock, B. A., and R. H. Hinton. 1981. Transport of proteins from blood to bile. *Trends Biochem. Sci.* 188-191.
- Newman, R., C. Schneider, R. Sutherland, L. Vodinelich, and M. Greaves. 1982. The transferrin receptor. *Trends Biochem. Sci.* 397-400.
- Omary, M. B., I. S. Trowbridge, and J. Minavada. 1980. Human cell-surface glycoprotein with unusual properties. *Nature (Lond.)* 286:888-891.
- Perkins, F. M., and J. S. Handler. 1981. Transport properties of toad kidney epithelia in culture. *Am. J. Physiol.* 241:C154-159.
- Pesonen, M., and K. Simons. 1983. Transepithelial transport of a viral membrane glycoprotein implanted into the apical plasma membrane of Madin-Darby canine kidney cells. *J. Cell Biol.* 97:638-643.

- Pfeiffer, S. E., 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.
- Quaroni, A., K. Kirsch, and M. M. Weiser. 1979. Synthesis of membrane glycoproteins in rat small intestinal villus cells. Redistribution of L-[1,5,6-³H] glucose-labeled membrane glycoproteins among Golgi, lateral, basal and microvillus membranes. *Biochem. J.* 182:203-212.
- Quinn, P., G. Griffiths, and G. Warren. 1984. Density of newly synthesized plasma membrane proteins in intracellular membranes II - Biochemical studies. *J. Cell Biol.* 98:2142-2147.
- Richardson, J. C. W., V. Scalera, and N. L. Simmons. 1981. Identification of two strains of MDCK cells which resemble separate nephron tubule segments. *Biochim. Biophys. Acta.* 673:26-37.
- Rindler, M. J., I. E. Ivanov, H. Pleskin, 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.
- Rodriguez-Boulan, E. 1983. Membrane biogenesis, enveloped RNA viruses and epithelial polarity. *Mod. Cell Biol.* 1:119-170.
- Rosenfeld, M. G., E. E. Marcantonio, J. Hakimi, V. M. Ort, P. H. Atkinson, D. Sabatini, and G. Kreibich. 1984. Biosynthesis and processing of ribophorins in the endoplasmic reticulum. *J. Cell Biol.* 99:1076-1082.
- Rothman, J. E. 1981. The Golgi apparatus: two organelles in tandem. *Science (Wash. DC).* 213:1212-1219.
- Schiff, J. M., M. M. Fisher, and B. J. Underdown. 1984. Receptor-mediated biliary transport of immunoglobulin A and asialoglycoprotein: sorting and mis-sorting of ligands revealed by two radiolabeling methods. *J. Cell Biol.* 98:79-89.
- Schneider, C., R. Sutherland, R. Newman, and M. Greaves. 1982. Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9. *J. Biol. Chem.* 257:8516-8522.
- Simons, K., and S. D. Fuller. 1985. Cell surface polarity in epithelia. *Annu. Rev. Cell Biol.* 1:243-288.
- Snider, M. D., and D. C. Rogers. 1985. Intracellular movement of cell surface receptors after endocytosis: resialylation of a sialotransferrin receptor in human erythroleukemia cells. *J. Cell Biol.* 100:826-834.
- Trowbridge, I. S., and M. B. Omary. 1981. Human cell surface glycoprotein related to cell proliferation is the receptor for transferrin. *Proc. Natl. Acad. Sci. USA.* 78:3039-3043.
- Ü, H. S., M. H. Saier, and M. H. Ellisman. 1979. Tight junction formation is closely linked to the polar redistribution of intramembranous particles in aggregating MDCK cells. *Exp. Cell Res.* 122:384-391.
- von Bonsdorff, C.-H., S. D. Fuller, and K. Simons. 1985. Apical and basolateral endocytosis in Madin-Darby canine kidney (MDCK) cells grown on nitrocellulose filters. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2781-2792.
- Ward, J. H., J. P. Kushner, and J. Kaplan. 1982. Regulation of HeLa cell transferrin receptors. *J. Biol. Chem.* 257:10317-10323.
- Weibel, E. R. 1976. Stereological approach to the study of cell surface morphology. 6th Eur. Congress Electron Microsc. Jerusalem. 6-9.
- Yamamoto, A., R. Masaki, and Y. Tashiro. 1985. Is cytochrome P-450 transported from the endoplasmic reticulum to the Golgi apparatus in rat hepatocytes? *J. Cell Biol.* 101:1733-1740.