Abstract. The enterocyte-like cell line Caco-2 forms a polarized epithelium when grown on filters. We have investigated the interaction of endocytic pathways from the apical and basolateral surfaces. The transferrin receptor was an appropriate marker for the basolateral route; uptake of radiolabeled transferrin was highly polarized, and recycling of this ligand back to the basolateral surface occurred with an efficiency of 95%, even after prolonged incubations with transferrin. Using a transferrin-peroxidase conjugate to delineate the morphological pathway, we have identified an early endocytic compartment in the basolateral cytoplasm of the cells. Longer incubations revealed a deeper endocytic compartment in the apical cytoplasm. Concanavalin A complexed to gold was used to simultaneously label the apical endocytic route. After 60 min, extensive mixing of the two labels was seen in endocytic elements throughout the apical cytoplasm, including in the Golgi area, but never in the basal cytoplasm. Using a second double labeling procedure in which antitransferrin receptor antibody complexed to gold was applied to the basolateral surface for up to 2 h and free peroxidase applied to the apical surface for shorter periods, we demonstrated that this apical marker rapidly (within 5 min) reached endosomes containing antibody-gold. Our results indicate that, in Caco-2 cells, the endocytic pathways from the apical and basolateral surfaces meet in an endosomal compartment from which transferrin can still be recycled.
We have begun to investigate the endocytic routes in the Caco-2 cell line. These cells, which are derived from a human colon carcinoma (Pinto et al., 1983), form a tight monolayer when grown on a permeable support. They have an enterocyte-like morphology, express brush border enzymes (Hauri et al., 1985) and exhibit other characteristics of the ileum (Dix et al., 1987; Hughes et al., 1987; Faust and Albers, 1988). By growing the cells on filters, we have a highly polarized system that gives relatively free access to both surfaces. This system has far more potential for experimental manipulation than the in vivo and explant models previously employed (Blok et al., 1981; Gonella and Neutra, 1984). We show that transferrin and its receptor are a good marker for the basolateral endocytic system in Caco-2 cells and demonstrate that at some points on this pathway they are found in the same compartment as markers introduced from the apical surface.

Materials and Methods

Cell Culture

Wild-type Caco-2 cells were cultured, and plated for experiments on to Transfilter (Costar, Cambridge, MA), as described elsewhere (Hughson et al., 1989). They were used for experiments between 12 and 19 d after plating. The transepithelial resistance, measured using a device similar to that described (Fuller et al., 1984), was at least 200 ohms-cm².

Transferrin-Peroxidase (Tr-HRP) Conjugation

4 mg transferrin (iron-free; Sigma Chemical Co., St. Louis, MO) was conjugated to 6 mg horse radish peroxidase (HRP) (type 2; Sigma Chemical) according to Benhamou and Oueilette (1986). Con A (Sigma Chemical) was dialyzed against distilled water overnight. The amount of protein needed to stabilize the gold was determined by adding different aliquots of protein to the gold colloid. NaCl was added to 1% (final concentration), and the tubes were spun in a microfuge. The gold was stable when the resulting pellets could be resuspended. 20-40 μg Con A/ml were needed. The gold complex was spun for 40 min, at 25,000 rpm in a rotor (Ti60; Beckman Instruments, Inc., Palo Alto, CA) in an ultracentrifuge, (I.,8-55, Beckman Instruments, Inc.). The pellets were resuspended in PBS with 0.05% Carbowax (BDH Chemicals Ltd.).

Fluorescence Microscopy Studies

Indirect immunofluorescence was performed as described (Hopkins et al., 1989) on filter-grown Caco-2 cells permeabilized with 0.05% saponin (Sigma Chemical Co.). The ATR antibody was used as the first antibody (1:1,000), and a sheep anti–mouse IgG antibody conjugated to FITC (Serumab) was used in the second step. Photographs were taken on a fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY; or Polvar microscope, Reichert Scientific Instruments, Buffalo, NY). To make FITC-transferrin, 0.5 mg FITC (Sigma Chemical Co.), dissolved in 100 μl ethanol, was added to 5 mg transferrin and 23 mg NaHCO₃. This was made up to 1 ml with water and then dialyzed overnight against PBS. The conjugate was applied (1:10) to the filters apically or basolaterally in DME plus 0.1% BSA, and the cells were incubated at 37°C, in 5% CO₂, for 60 min. The cells were fixed in 3% paraformaldehyde in PBS, quenched in 50 mM ammonium chloride and examined on a fluorescence microscope (Carl Zeiss, Inc.) or on a laser scanning confocal apparatus (500; Lasersharp MRC, Bio-Rad Microscopy Ltd., Hemel Hempstead, UK) in conjunction with an Optophot microscope (Nikon Inc., Garden City, NY).

Semi-thin Frozen Sections

Filter-grown Caco-2 cells were fixed with 3% paraformaldehyde in PBS for 30 min. The filter was excised and pieces were briefly immersed in 10% gelatin at 37°C, and then placed on ice for 10 min. They were then fixed for a further 30 min in paraformaldehyde. Small triangles were cut out of the gelatin “sandwich,” infiltrated with 2.3 M sucrose, mounted on copper stubs, and frozen in liquid nitrogen. 0.5 μm frozen sections were cut on a recryo-ultramicrotome (MT2-B; Sorvall Instruments, Du Pont Company).
Figure 1. Electron micrographs of a monolayer of Caco-2 cells. Cells were seeded onto Transwell filters and grown for 14 d. After fixing with Karnovsky fluid, cells were postfixed in 1% reduced osmium tetroxide and processed for electron microscopy. Sections were stained with uranyl acetate and lead citrate. (a) Low power magnification of the monolayer. (b) Higher magnification of a Caco-2 cell, showing position of the Golgi apparatus (arrows). Inset shows a tight junctional complex from a monolayer that was incubated with HRP basolaterally before fixation. DAB reaction product is seen in the lateral space up to the tight junction. f, filter. Bars, (a) 10 μm and (b) 2 μm; inset, 0.1 μm.
Figure 2. Transferrin recycles basolaterally in Caco-2 cells. Triplicate sets of filter-grown cells were incubated in serum-free medium for 1 h. \(^{125}\text{I}\)Transferrin was added basolaterally, and the cells were incubated at 37°C for (a) 10 min or (b) 120 min. The cells were placed on ice and rinsed with PBS until no counts remained in the washes. The filters were then either counted or given prewarmed chase medium containing 0.5 mg/ml cold transferrin and incubated at 37°C. At each time point, cells (Δ), apical ([]), and basolateral (■) media were collected separately and counted. Standard errors were <10%, except where counts were <1 × 10³ cpm, when standard errors were <20%.

Electron Microscopy Tracer Experiments

HRP (1–10 mg/ml) was applied to either side of the filter in DME with 0.1% BSA. Incubations were done at 37°C, with 5% CO₂, for 5–60 min. Tr-HRP (~30 μg transferrin/ml) was applied for 1 h at 4°C on a shaker in

Figure 3. Uptake of transferrin-FITC by Caco-2 cells. Filter-grown cells were incubated with transferrin-FITC present in the basolateral chamber for 60 min. Cells were placed on ice, washed in PBS and fixed with 3% paraformaldehyde. A series of optical sections, 2-μm apart, were taken through the monolayer using the confocal microscope, starting at the apical side of the monolayer. There is strong labeling in the apical cytoplasm (a and b) and lateral to the nucleus (c and d) but only small punctate dots in the basal cytoplasm (d). Bar, 10 μm.
Figure 4. The basolateral endocytic route identified by Tr-HRP. Caco-2 cells were incubated at 4°C with medium containing Tr-HRP in the basal part of the filter chamber for 1 h. The medium was then replaced with prewarmed medium containing Tr-HRP basolaterally, and the incubation was continued at 37°C for (a) 5 min, (b and c) 15 min, or (d) 60 min. The cells were then fixed with 1% glutaraldehyde, processed with DAB to reveal the presence of HRP, and prepared for electron microscopy. The sections were stained with lead citrate only, which does not stain glycogen; hence some areas of the cytoplasm have no contrast. Tr-HRP is first observed in coated pits on the basal and lateral surfaces (a). Inset shows the detail of a coated pit budding off the lateral membrane. Label then appears in smooth tubular elements in the basal cytoplasm (b). Reaction product is first seen in endocytic structures in the Golgi area, apical and lateral to the nucleus after 15 min (c). These elements become more elaborate with prolonged incubations (d). Endosomes labeled under the same conditions are also shown on Figs. 8 and 9. Arrows indicate labeled pits (a) or endosomal elements (c-d). Arrowhead shows lateral membrane; g, Golgi apparatus. Bars, (a-d), 0.2 μm; inset, 0.1 μm.
Table I. Tr-HRP Recycles Basolaterally in Caco-2 Cells*

| Time (min) | Cell-associated | Apical | Basolateral |
|-----------|----------------|--------|------------|
| 0         | 100            | 0      | 0          |
| 20        | 17             | 8      | 75         |

* The experiment was performed as described for Fig. 2, but using \([^{125}I]\)Tr-HRP instead of \([^{125}I]\)transferrin.

fore use. ATR-gold was resuspended in DME plus BSA and then applied basolaterally to the filters. Incubations were carried out at 37°C for 1-2 h. Con A-gold was resuspended in buffered medium with 0.1 mM magnesium chloride and no BSA. This was applied apically (1 ml/filter), and the cells were incubated at 4°C for 1 h on a shaker. After 1 h, the medium was removed and replaced with prewarmed DME with BSA and the cells were placed at 37°C for the desired time period.

After labeling with gold complexes, the cells were fixed with glutaraldehyde. All experimental samples were postfixed with 1% osmium tetroxide for 1 h and processed further for electron microscopy by standard techniques. For routine electron microscopy, cells were fixed with Karnovsky fluid (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.5), 1:1 with PBS for 2 h at room temperature, postfixed in reduced osmium tetroxide and processed further as before. Sections were examined on an electron microscope (CM12; Philips Electronic Instruments, Inc., Mahwah, NJ). Reynold’s lead citrate stain alone was used for peroxidase samples because when uranyl acetate was used, the glycogen in the cells stained strongly and it was difficult to distinguish the DAB reaction product. Hence, the contrast of the sections is sometimes weak.

Results

Characterization of the Polarized Monolayer

The Caco-2 cells grown in our laboratory on Transwell filters are highly polarized with respect to the expression of surface membrane proteins and secretion (Hughson et al., 1989), and they form tight monolayers with a transepithelial resistance of >200 ohms-cm². The morphology of the cells is also polarized (Fig. 1). Seen in section, the cells form a typical lining epithelium and, although they are not as tall as normal human enterocytes, they have a well-developed brush border and their nuclei are usually basally placed. Electron microscopy shows their lateral membranes to be closely opposed (unlike cells grown on plastic; Pinto et al., 1983), highly interdigitated and connected by junctional complexes. Their Golgi stacks are usually found in the apical cytoplasm above the nuclei but may lie more laterally between the nucleus and basolateral border. The cells often contain substantial amounts of glycogen.

The Polarized Expression of Transferrin and EGF Receptors

Preliminary studies using antibodies specific for the transferrin and EGF receptors or their ligands conjugated to FITC demonstrated that these receptor populations were located predominantly on the basolateral surface of the polarized monolayers. Analyses using radiolabeled ligands confirmed these observations (as reported elsewhere for EGF; Hughson et al., 1989). Thus, for example, when monolayers were incubated with \([^{125}I]\)transferrin in the basolateral chamber, there was a significant uptake of label that was inhibited by cold transferrin. Only 0.5% of these counts appeared in the apical chamber after 4 h, indicating a very low level of leakage (or transcytosis). No degraded counts could be detected. This was in contrast to the results obtained with EGF. After 4 h of incubation with basolaterally applied \([^{125}I]\)EGF, 3% of

Figure 5. Localization of the transferrin receptor in Caco-2 cells. Filter-grown cells were fixed with 3% paraformaldehyde and (a) labeled for the transferrin receptor by indirect immunofluorescence. In b, fixed cells were embedded in gelatin (see Materials and Methods), prepared for cryomicrotomy and semi-thin frozen sections were cut. The sections were labeled by indirect immunofluorescence. Concentrations of fluorescent label are seen mainly lateral and apical to the nucleus. (c) Nomarski image of monolayer shown in b. Bars, (a) 5 μm; (b) 10 μm.
Endosomes labeled by a fluid-phase marker applied at the basolateral surface. Filter-grown cells were incubated with free HRP in the basal chamber for 1 h at 37°C, fixed, and processed to develop the HRP reaction. HRP is present in the lateral space (b, arrowhead) and in endosomal elements (arrows) in the lateral cytoplasm (b) and underneath the brush border (a). n, nucleus. Bars, 0.2 μm.

The total counts appeared in the apical chamber. As only 40% of this label migrated with intact EGF on a Biogel column, it was concluded that much of the label resulted from degradation of internalized peptide.

Internalization from the Basolateral Surface

Uptake and Recycling of Transferrin. The presence of the filter at the base of the monolayer reduces the efficiency with which conventional pulse-chase procedures can be carried out. Nevertheless, by preincubating at 37°C to preload the cells with [125I]transferrin, rinsing thoroughly at 5°C and then chasing with excess cold transferrin (to reduce internalization of any recycled [125I]transferrin) the release of internalized transferrin provides a direct indication of recycling. As shown in Fig. 2a and Table I, after being preloaded for 10 and 20 min with radiolabel, the cells rapidly recycle >80% of their transferrin to the basal chamber. If, however, the monolayer is preloaded for 120 min, while release remains predominantly (>95%) basal, the kinetics of release are different. The initial rapid phase of 30 min is followed by a slower, more prolonged phase. Eventually after 2 h, 95% of the label can be chased out into the basolateral medium (Fig. 2b). The release observed after the 10–20-min load, and the initial rapid phase of release following the 120-min load, both indicate that transferrin initially enters a compartment from which recycling is rapid and almost 100% efficient. More prolonged loading, however, involves a larger capacity compartment from which recycling is much slower. Passage over a P60 Biogel column showed that the released counts were because of recycled intact transferrin, as negligible amounts of label migrated with the free iodide peak.

The absence of radiolabel in the apical chamber and the data suggesting that the internalized [125I]transferrin remains undegraded (even 4 h) indicates that the pathways involved in this intracellular processing do not include lysosomal elements.

Morphology of the Transferrin Processing Pathways

Light microscopy using transferrin-FITC as a tracer applied basolaterally showed that after incubation at 37°C for 60 min, the internalized transferrin penetrates compartments throughout the cell. Optical sectioning in the confocal microscope revealed that small punctate label is seen in the basal cytoplasm (Fig. 3d). Larger concentrations are observed lateral to the nucleus (Fig. 3c) and substantial labeling is present within the apical cytoplasm (Figs. 3a and b). For electron microscopy, Tr-HRP was used as a tracer. By radiolabeling the conjugate it could be shown that the uptake was inhibited by excess cold transferrin and that it had the same short term kinetics for recycling as [125I]transferrin (Table I).

When monolayers were incubated with Tr-HRP in the basal chamber for 1 h at 4°C, DAB reaction product was diffusely distributed within the intercellular spaces and concentrated in coated pits on the lower part of the lateral membrane and occasionally on the basal membrane (Fig. 4a). When the temperature was raised to 37°C after this labeling up period and the incubation continued with Tr-HRP in the medium, peripheral endosomal structures in the form of smooth, sometimes branching tubules in the basolateral...
cytoplasm became labeled within 5 min (Fig. 4 b). After 10 min of incubation at 37°C, small vacuolar elements adjacent to the nucleus also labeled. These structures were in the basal cytoplasm; no label was evident in the apical region of the cell at this time. After 15 min chase at 37°C, conjugate reached elements in the Golgi area (Fig. 4 c). These were predominantly vacuolar elements with extensive branching tubules. At this time, a few small endosomal tubular elements could also be found in the apical cytoplasm just below the brush border. After a 60-min incubation at 37°C, the most prominently labeled endosomal elements were in the Golgi area above and lateral to the nucleus (Fig. 4 d) and in the apical cytoplasm below the brush border. The elements in the Golgi area now included tubular elements and prominent multivesicular bodies.

Incubating the basolateral surface of the monolayer with gold particles conjugated to ATR antibody, for 60 min at 37°C, labeled intracellular elements with the same form and distribution as those seen with Tr-HRP. Experiments similar to those described above for [125I]transferrin, but using [125I]ATR antibody, demonstrated that this label also recycled in a polarized fashion to the basolateral surface (data not shown).

The Distribution of Transferrin Receptors
Receptors were localized by light microscopy using the ATR antibody on permeabilized cells and on cryosections. As shown in Fig. 5, the transferrin receptor in these cells is localized in a variety of elements throughout the cell cytoplasm. The largest and most strongly labeled structures are in the perinuclear area although positive structures are also present in both basal and apical regions of the cell. The overall pattern of fluorescence thus closely resembles that seen in cells labeled with transferrin-FITC (Fig. 3).

Uptake of a Fluid-Phase Marker
Free HRP was used as a fluid-phase endocytic marker from the basolateral surface to see if this pathway differed from the basolateral recycling route delineated by Tr-HRP. After 60 min of incubation, essentially similar endocytic elements to those seen with Tr-HRP contained peroxidase (Fig. 6). However, labeling of coated pits at the basolateral membrane and in the tubular endosome elements of the Golgi area was weaker.

Uptake from the Apical Surface
Coated pits are frequently observed on the apical membrane at the base of the microvilli in polarized monolayers, and it has been demonstrated with cobalamin-intrinsic factor complexes (Dix et al., 1987) that receptor mediated endocytosis can occur from this boundary in Caco-2 cells. However, this receptor is not expressed in sufficient amounts for morphological studies and no other mobile receptor populations in high copy number have yet been identified on the apical membrane of Caco-2 cells. Morphological studies of endocytosis must therefore use either fluid-phase tracers (HRP) or ligands of broad specificity such as plant lectins.

When Caco-2 monolayers were incubated with HRP in the apical chamber, within 5 min DAB reaction product was present in coated pits and also in very fine tubules and small tubular endosomes immediately below the brush border. After 15 min, larger endosomes became labeled, and, by 30 min, vacuolar endosomes including multivesicular bodies were present deeper in the cell. After 60 min, labeled elements were widespread in the apical cytoplasm (Fig. 7) but were infrequently seen in the Golgi area. No labeled elements occurred in the basal regions below the nucleus.

Incubation with Con A-gold at 4°C (60 min) followed by rinsing and then incubation at 37°C for 60 min introduced the gold conjugate into the endocytic pathway. As expected for a label with such broad specificity (terminal D-mannose and D-glucose on glycoproteins and glycolipids), large amounts remained on the surface even after 60 min at 37°C. There was, however, significant uptake into apical endo-
Apical and basolateral endocytic pathways meet in the apical part of the cell. Monolayers of cells were labeled for 60 min with Tr-HRP in the basal chamber of the filter and Con A-gold in the apical chamber, as described in Materials and Methods. Although some endosomal elements contain only peroxidase (small arrow) or gold particles (arrowheads), a substantial number of these deep apical endosomes are labeled with both peroxidase and gold particles (large arrows). All the gold particles are within membrane-bound structures. The micrograph has been printed at a light density so that the double label can be clearly seen. BB, brush border. Bar, 0.2 µm.

Figure 8. Apical and basolateral endocytic pathways meet in the apical part of the cell. Monolayers of cells were labeled for 60 min with Tr-HRP in the basal chamber of the filter and Con A-gold in the apical chamber, as described in Materials and Methods. Although some endosomal elements contain only peroxidase (small arrow) or gold particles (arrowheads), a substantial number of these deep apical endosomes are labeled with both peroxidase and gold particles (large arrows). All the gold particles are within membrane-bound structures. The micrograph has been printed at a light density so that the double label can be clearly seen. BB, brush border. Bar, 0.2 µm.

somess, and, in general, the elements labeled with gold were similar to those identified with free HRP (Fig. 8) although larger numbers were seen in the Golgi region (Fig. 9). Only rarely were particles found in endosomal elements in the basal cytoplasm.

Accessibility of Common Endosome Elements from Apical and Basolateral Surfaces

Our data showing that transferrin/transferrin receptors internalized at the basolateral border remain undergraded and become distributed within endosomal elements in the apical cytoplasm suggested that these ligand-receptor complexes may be able to penetrate compartments that are also accessible to ligands endocytosed from the apical border. This possibility was explored with two kinds of double label procedures.

In the first procedure, monolayers were incubated with their basolateral border exposed to Tr-HRP and the apical surface exposed to Con A-gold. At all time points examined, the majority of the labeled endosomes contained only one tracer. However, mixing of the two elements was observed after 15 min. After 60 min at 37°C, 25% of the gold-labeled elements also contained Tr-HRP. All of these double labeled elements were in the apical cytoplasm; some were distrib-
Discussion

The uptake and intracellular processing of transferrin and EGF from the basolateral border of Caco-2 cells are, in most respects, similar to that observed in unpolarized epitheloid and fibroblastic cell lines. For transferrin there is a rapid short term recycling pathway (which returns the internalized ligand back to the surface where it was endocytosed within minutes) (Dautry-Varsat et al., 1983; Klausner et al., 1983; Hopkins and Trowbridge, 1983) and a longer larger capacity pathway from which recycling is much less rapid (Hopkins, 1983). The transferrin is efficiently recycled back to the basolateral surface, as demonstrated indirectly in MDCK cells (Fuller and Simons, 1986). There is no evidence (from this and other studies: Stoorvogel et al., 1988; Futter and Hopkins, 1989) that the transferrin-receptor complexes are degraded during the intracellular processing even when they are conjugated to HRP or gold-antibody complexes. Significant amounts of EGF, on the other hand, appear to be delivered to a degradative compartment (presumably the lysosome). No evidence was obtained in our experiments for an uptake pathway arising at the basolateral border that specifically delivered either of these ligands to the apical surface. This is in contrast to the results of a previous study using EGF in the MDCK cell line (Maratos-Flier et al., 1987).

Morphological analyses identify the intracellular pathways that process transferrin as consisting of peripheral or "early" elements below the basolateral border and deeper, later elements in the Golgi area. This arrangement is also similar to that which has been observed in "unpolarized" tissue culture cells (Hopkins and Trowbridge, 1983). The time taken for the tracer to reach this compartment is not significantly different from that recorded for unpolarized cells (10-30 min) (Hopkins, 1983; Yamashiro et al., 1984; Willingham et al., 1984; Stoorvogel et al., 1988). In Caco-2 cells, there is also a late, more apical compartment underneath the brush border where Tr-HRP is seen from 15 min onwards. Together our data suggest that the deeper, late endosome identified by the \[^{125}\text{I}]\text{transferrin studies lies in the endosomal elements of the apical cytoplasm and Golgi area. The absence of degradation of \[^{125}\text{I}]\text{transferrin demonstrates that these elements are endosomal rather than lysosomal. It is of interest that HRP introduced at the basolateral border also shows a distribution within apical elements similar to transferrin. A similar distribution has been reported in both intact epithelial tissue (Hugon and Borgers, 1967) and other polarized epithelial cell systems (von Bonsdorff et al., 1985). Presumably, as with EGF, some fluid-phase HRP is also delivered to lysosomes.}

Uptake at the apical membrane in enterocytes has been studied extensively. In the neonate, the apical endocytic system is elaborately developed and is capable of delivering endocytosed ligands either to an extensive lysosomal compartment in the apical cytoplasm or via the basolateral border, to the intercellular space (Gonella and Neutra, 1984; Abraham and Rodewald, 1981). Both routes include endosomal elements in the apical cytoplasm. In the enterocyte of the adult mucosa, no evidence has been found for a receptor-mediated transcytotic pathway from the apical surface to the basolateral border. Similarly, with the probes that we have at present, no apical to basolateral transcytotic pathway could be identified in the Caco-2 system (Hughson and Hopkins, 1983).
unpublished observations). Uptake studies in several mammalian systems have shown fluid-phase markers penetrating endosomal elements throughout the apical cytoplasm (Cornell et al., 1971; Blok et al., 1981). Our observations with HRP on polarized Caco-2 cells identify a similar system. They also indicate that the peripheral endosome system identified in the basolateral cytoplasm with transferrin tracers is inaccessible to apically applied fluid-phase tracer.
However, the double label experiments in which tracers are applied from apical and basolateral borders demonstrate that endocytic routes from these two boundaries can reach common endosomal elements. These elements, which lie in the apical cytoplasm and Golgi area, probably correspond to the late endosome identified after prolonged incubations with basolaterally applied radiolabeled transferrin. The more apical elements of this compartment are accessible within minutes to tracers applied at the apical boundary.

A previous study has shown that tracers applied to apical and basolateral surfaces of polarized epithelial cells can reach a common compartment. This, however, represents rather special circumstances since it described the recycling of apical membrane (poststimulation) in secretory cells (Oliver, 1982). In this system, the common compartment was identified as a lysosome or prelysosomal compartment. It is therefore different from the endosome identified in the present study, as our data suggests that transferrin recycles from this compartment to the basolateral surface.

The observations made on Caco-2 cells in the present study raise many questions on membrane trafficking in polarized epithelial cells. For example, what signals and mechanisms operate in the late endosome to allow efficient sorting of the transferrin receptor back to the basolateral membrane? Our results are also of significance for research into drug delivery across the intestinal epithelium. Even though a di-rect transcytotic pathway (apical to basolateral) may not exist in adult enterocytes, it may be possible to exploit the finding that apically applied ligands reach the same compartment as membrane proteins recycling to the basolateral membrane.

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