

Bidirectional Transcytosis Determines the Steady State Distribution of the Transferrin Receptor at Opposite Plasma Membrane Domains of BeWo Cells

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Abstract. Trophoblast-like BeWo cells form well-polarized epithelial monolayers, when cultured on permeable supports. Contrary to other polarized cell systems, in which the transferrin receptor is found predominantly on the basolateral cell surface, BeWo cells express the transferrin receptor at both apical and basolateral cell surfaces (Cerneus, D.P., and A. van der Ende. 1991. *J. Cell Biol.* 114: 1149–1158). In the present study we have addressed the question whether BeWo cells use a different sorting mechanism to target transferrin receptors to the cell surface, by examining the biosynthetic and transcytotic pathways of the transferrin receptor in BeWo cells. Using trypsin and antibodies to detect transferrin receptors at the cell surface of filter-grown BeWo cells, we show that at least 80% of newly synthesized transferrin receptor follows a direct pathway to the basolateral surface, demonstrating that the transferrin receptor is efficiently intracellularly sorted. After surface arrival, pulse-labeled transferrin receptor equilibrates between apical and basolateral cell surfaces, due to ongoing transcytotic transport in both directions. The subsequent

redistribution takes over 120 min and results in a steady state distribution with 1.5–2.0 times more transferrin receptors at the basolateral surface than at the apical surface. By monitoring the fate of surface-bound ^{125}I -transferrin, internalized either from the apical or basolateral surface transcytosis of the transferrin receptor was studied. About 15% of ^{125}I -transferrin is transcytosed in the basolateral to apical direction, whereas 25% is transcytosed in the opposite direction, indicated that the fraction of receptors involved in transcytosis is roughly twofold higher for the apical receptor pool, as compared to the basolateral pool. Upon internalization, both apical and basolateral receptor pools become redistributed on both surfaces, resulting in a twofold higher number of transferrin receptors at the basolateral surface. Our results indicate that in BeWo cells bidirectional transcytosis is the main factor in surface distribution of transferrin receptors on apical and basolateral surfaces, which may represent a cell type-specific, post-endocytic, sorting mechanism.

IN epithelial cells the cell surface is divided into morphologically and biochemically distinct apical and basolateral domains. The apical domain faces the external milieu and is separated by tight junctions from the basolateral domain, which contacts the underlying connective tissue and faces the internal milieu (e.g., the blood supply). Epithelial cells use several strategies to establish and maintain this cell surface polarity. Among these are sorting and targeting of apical and basolateral proteins to their correct domain after they exit the Golgi complex, and retention and/or efficient recycling of proteins at their proper domain (Rodriguez-Boulant and Nelson, 1989).

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Recent findings indicate that apical proteins can follow a direct pathway from the TGN to the apical domain, as well as an indirect (*trans*-cytotic) pathway, depending on the cell type. In MDCK cells apical proteins are efficiently sorted from basolateral proteins to the TGN and targeted via distinct transport vesicles directly to their proper domain (reviewed by Simons and Wandinger-Ness, 1990). In rat hepatocytes, both apical and basolateral proteins are transported together from the TGN to the basolateral (sinusoidal) surface. Apical proteins are then sorted from basolateral proteins (either at the cell surface and/or in endosomes) and reach the apical surface via transcytosis (Bartles et al., 1987). In enterocyte-like Caco-2 cells, however, apical proteins can use both direct and/or transcytotic pathways (Matter et al., 1990; LeBivic et al., 1990). In contrast, basolateral proteins are sorted directly to the basolateral surface in all cell types, suggesting that the sorting machinery is well conserved among the different cell types.

The transferrin receptor (TfR)¹ is localized predominantly at the basolateral cell surface in a variety of epithelial cells (Simons and Fuller, 1985). This receptor constitutively enters coated pits and recycles rapidly between the cell surface and the endosomal system. Apparently, two separate sorting steps are responsible for its localization at the basolateral surface; direct transport of newly synthesized TfR from the TGN to the basolateral surface (LeBivic et al., 1990), and efficient recycling to its original surface after endocytosis at the basolateral surface (Fuller and Simons, 1986; Godefroy et al., 1990; Hughson and Hopkins, 1990).

Recently, we have studied the binding properties and recycling pathways of the TfR in the trophoblast-like cell line BeWo (Cerneus and van der Ende, 1991). When grown on filters, these cells form tight polarized monolayers having most integral membrane proteins asymmetrically distributed over apical and basolateral domains. The TfR is localized on both apical and basolateral cell surfaces in an apical to basolateral surface ratio of 1:2 (Cerneus and van der Ende, 1991). The low level of polarity of the TfR suggests that BeWo cells use a cell type-specific sorting mechanism to target TfR to the cell surface. Possibly, newly synthesized TfR follows direct pathways to both surfaces in the same apical to basolateral ratio as found at steady state at the cell surface. Alternatively, the TfR may efficiently be sorted in the TGN and targeted directly to the basolateral surface, followed by transcytosis into the apical direction.

To examine the contribution of direct and indirect pathways to the establishment and maintenance of the surface distribution of the TfR in BeWo cells, we have used trypsin and antibodies to dissect the intracellular pathways of the TfR to the cell surface. We demonstrate that 80% of newly synthesized TfR is targeted directly to the basolateral surface. We also show that the basolateral pool of TfR slowly equilibrates between both surfaces, due to the fact that transcytosis occurs from both surfaces, thereby generating an equilibrium distribution with twice as many receptors at the basolateral surface. Our results demonstrate that in BeWo cells the TfR is sorted at the level of the TGN and mainly targeted to the basolateral surface, probably in a similar manner as found in other epithelial cells. However, the apical expression of the TfR at steady state is generated and maintained by bidirectional transcytosis, and may reflect a cell type-specific, post-endocytic sorting mechanism.

Materials and Methods

Materials

TPCK-treated trypsin, soybean trypsin inhibitor (SBTI) and apo-Tf were obtained from Sigma Chemical Co. (St. Louis, MO). ApoTf was saturated with iron and labeled with ¹²⁵I as described previously (Cerneus and van der Ende, 1991). mAbs against the human TfR (van de Rijn et al., 1983) was kindly provided by Dr. J. Hilken (Netherlands Cancer Institute, Amsterdam).

Cell Culture

The b24 clone of the cell line BeWo was maintained in MEM, supplemented with 10% FCS, and antibiotics (MEM/FCS) in 5% CO₂/95% air (van der Ende et al., 1987). For all experiments, cells were cultured on 24 mm Transwell permeable polycarbonate filters (Costar Corp., Cambridge, MA) for 4–5 days as described previously (Cerneus and van der Ende, 1991).

Routinely, tightness of the monolayer was checked by adding medium to the upper chamber up to a slightly higher level than that in the lower chamber. Normally, with 4- or 5-d-old monolayers this difference in level was maintained even after an overnight incubation at 37°C. The monolayers showed then the optimal transepithelial resistance as reported earlier (Cerneus and van der Ende, 1991).

Cell Surface Immunoprecipitation

Cells on filters were washed twice with PBS containing Ca²⁺ and Mg²⁺ (PBS-CM) at room temperature, and then preincubated in methionine-free MEM for 30 min at 37°C. Cells were pulse-labeled for 20 min with [³⁵S]methionine from the basal side by adding 1.5 ml methionine-free MEM containing 100 μCi Trans³⁵S-label (ICN Radiochemicals, Irvine, CA) per filter culture. The apical compartment received 0.75 ml MEM minus methionine. Cells were then chased in MEM/FCS containing excess unlabeled methionine for the indicated time points at 37°C. After the chase, cells were washed twice with ice cold MEM containing 20 mM Hepes, pH 7.4, and 0.2% BSA (MEMH/BSA) and incubated for 90 min on ice in MEMH/BSA with antibodies (anti-TfR; 5 μl ascites/ml) added either into the apical (1 ml) or basolateral (2 ml) compartment. Thereafter, cells were washed four times with MEMH/BSA within 30 min and lysed in the presence of an excess of unlabeled cell extract to avoid binding of surface IgG to labeled intracellular antigen. Unlabeled cell extracts were prepared by solubilizing one confluent 150-cm² flask (Costar Corp.) of BeWo cells in 5 ml of 1% Triton X-100 in 20 mM Tris, 150 mM NaCl pH 8 (TBS) containing 1 mM PMSF and 4 mM EDTA. The lysate was centrifuged for 10 min in an Eppendorf centrifuge and from the resulting supernatant 1 ml per filter was used to lyse labeled cells. After a 60-min incubation on a rocker, nuclei and debris were pelleted by centrifugation for 10 min in an Eppendorf centrifuge. The resulting supernatant was transferred to a tube containing 50 μl of a 50% slurry of protein A-sepharose (Pharmacia, Uppsala, Sweden), and incubated for 60 min on an end-over-end rotation incubator. The immunoprecipitates were washed and analyzed by SDS-PAGE; fluorography and densitometry were performed as described before (Cerneus and van der Ende, 1991).

Trypsin Assays

To determine the efficiency of trypsin digestion of newly synthesized TfR at the cell surface, and to see whether under these conditions monolayer integrity was maintained, cells were pulse-labeled for 20 min and then chased for 90 min in MEMH at 37°C in a water bath. During the chase trypsin was added to the basolateral compartment at a concentration ranging from 0 to 100 μg/ml. A twofold higher concentration of SBTI was present in the apical compartment. Thereafter, cells were washed with ice-cold PBS-CM containing SBTI and incubated on ice in the same buffer with a ¹²⁵I-labeled protein (orosomuroid) added to the apical medium to test leakage through the monolayer. After 1.5 h, the basolateral medium was collected and the cells were washed and lysed as described. Less than 1% of the apically added ¹²⁵I-labeled protein was found in the basolateral medium at all trypsin concentrations tested. Analysis of immunoprecipitated TfR revealed that cleavage of pulse-chased TfR was maximal at trypsin concentrations of 25 μg/ml or higher. In further experiments 50–100 μg/ml trypsin was used.

Two different chase and trypsinization protocols were used to detect radio-labeled TfR at the cell surface. (a) Filter-grown cells were pulse-labeled with [³⁵S]methionine for 20 min as described above, and then chased in MEMH at 37°C in a water bath for the indicated time points. During the chase, trypsin was added either to the apical compartment (1 ml), or the basolateral compartment (2 ml). A twofold higher concentration of SBTI was added opposite to the compartment receiving trypsin. Control cells were incubated in MEMH only. After the chase, cells were rapidly chilled by immersing in ice cold PBS-CM containing 100 μg/ml SBTI, whereafter they were lysed in 1 ml lysis-buffer containing 100 μg/ml SBTI and the TfR was immunoprecipitated and analyzed as described above. (b) Cells labeled for 10 min were chased for 80 min in MEMH at 37°C with trypsin and SBTI added either to the apical or basal compartment as described above. After the chase, cells were chilled and washed with PBS-CM containing SBTI, and thereafter anti-TfR antibodies (5 μl ascites/ml) was added opposite to the trypsinized side of the monolayer. The cells were further processed as described under "Cell Surface Immunoprecipitation."

Release of Surface-bound Tf into Apical and Basal Media

The release of surface-bound ¹²⁵I-Tf was monitored after a 2 h incubation

1. Abbreviation used in this paper: TfR, transferrin receptor.

at 37°C, essentially as described previously (Cerneus and van der Ende, 1991). Briefly, filter-grown BeWo cells were incubated in MEMH for 30 min at 37°C to deplete cells from serum Tf and subsequently with 5 µg/ml ¹²⁵I-Tf, with or without a 50 times excess of unlabeled Tf, in either the apical or basolateral compartment for 60 min at 4°C. After removal of unbound ligand, the incubation was continued for 2 h at 37°C in MEMH, while 20 µg/ml unlabeled Tf was present in both compartments to prevent reinternalization of released ¹²⁵I-Tf. Thereafter, apical and basal media were collected separately, and cells and media were assayed for radioactivity in a gammacounter.

Detection of Transcytosed ¹²⁵I-TfR Using Trypsin

Filter-grown BeWo cells were labeled with ¹²⁵I using the lactoperoxidase procedure exactly as described before (Cerneus and van der Ende, 1991). After labeling, cells were washed extensively with MEMH and incubated at 37°C in MEM/FCS for various periods of time. Thereafter, cells were washed twice with MEMH and incubated for an additional 10 min at 37°C in MEMH with 100 µg/ml trypsin added to the apical or basolateral compartment. SBTI (200 µg/ml) was present opposite to the side receiving trypsin. Control cells were incubated in the absence of trypsin. After the incubation, cells were rapidly chilled by washing in ice cold PBS-CM containing 100 µg/ml SBTI and lysed in Triton X-100 lysis-buffer. TfR was immunoprecipitated and analyzed as described above.

Results

Surface Immunoprecipitation Assay

Surface immunoprecipitation is often used to monitor the presence of radio-labeled proteins at either the apical or basolateral surface of epithelial cells when cultured on filters. However, detection of proteins on this domain is potentially less efficient as compared to the apical surface, because the basolateral surface is opposed to the filter, which may act as a diffusion barrier for antibodies. In polarized BeWo cells the TfR is expressed at both the apical and basolateral surface (Cerneus and van der Ende, 1991). To assure that the precipitation of TfR from either surface occurs with similar efficacy, filter-grown BeWo cells were iodinated at either the apical or basolateral surface, and thereafter incubated on ice for 90 min with anti-TfR antibodies added to either side of the monolayer. After removal of unbound IgG, cells were lysed in Triton X-100 extracts from unlabeled cells to prevent rebinding of surface-bound IgG with labeled anti-

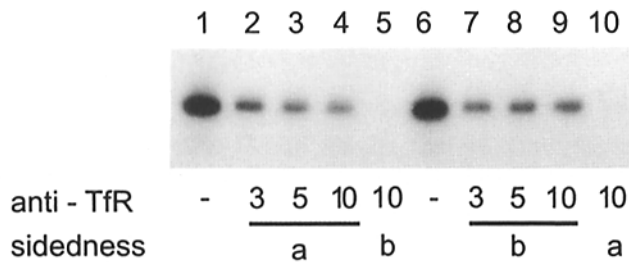


Figure 1. Immunoprecipitation of ¹²⁵I-TfR from apical and basolateral surfaces. BeWo cells were labeled with ¹²⁵I on ice either at the apical (lanes 1-5) or basolateral surface (lanes 6-10), and then incubated with anti-TfR mAbs (3, 5, or 10 µl ascites/ml) at the apical (a) or basolateral (b) side for 90 min. The cells were then solubilized in Triton X-100 lysates from nonlabeled cells, immunocomplexes were precipitated with protein A-sepharose, and analyzed by SDS-PAGE, autoradiography, and densitometry. The amount of surface immunoprecipitated TfR was compared to the total immunoprecipitable ¹²⁵I-TfR from cells which were extracted in Triton X-100 lysis buffer immediately after the iodination procedure (lanes 1 and 6).

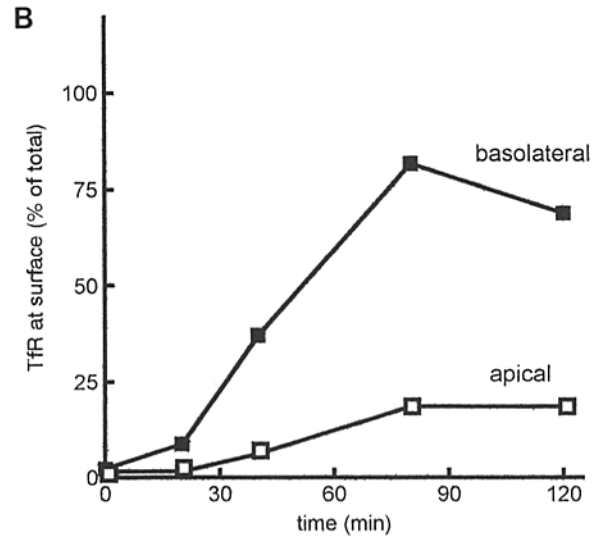
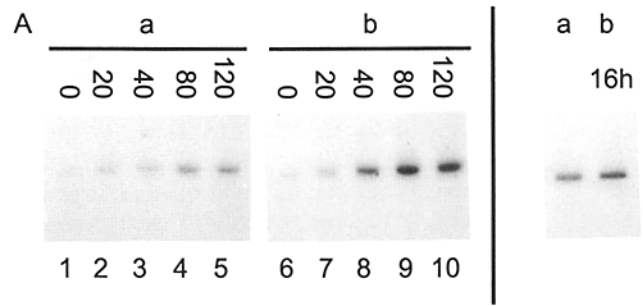


Figure 2. Surface delivery of ³⁵S-labeled TfR. (A) BeWo cells were pulse-labeled with [³⁵S]methionine for 20 min, chased for the indicated times, and then incubated with monoclonal anti-TfR antibodies (5 µl/ml) at the apical (a) or basolateral (b) surface for 90 min on ice. Thereafter, cells were extracted in Triton X-100 lysates from nonlabeled cells, and immunocomplexes were recovered, precipitated with protein A-sepharose and the amount of TfR was analyzed by SDS-PAGE, fluorography and densitometry. A graphical representation of the data is shown in B, each point represents the mean of two filters and varies < 10%. Maximal surface expression was obtained after 80 min of chase and was taken as 100%.

gen. The recovery of surface immunoprecipitated ¹²⁵I-TfR was compared to the total immunoprecipitable signal of ¹²⁵I-TfR, which was obtained by extracting the iodinated cells directly in Triton X-100 lysis-buffer, followed by immunoprecipitation with saturating amounts of anti-TfR antibody (Fig. 1). Quantitation by densitometry revealed that 20-25% of total ¹²⁵I-labeled TfR was recovered after addition of antibodies (3-10 µl ascites/ml) to either the apically (lanes 2-4) or basally (lanes 7-9) iodinated surface. No ¹²⁵I-TfR was immunoprecipitated when antibodies in the highest concentration (10 µl ascites/ml) were added to the side opposite to the iodinated surface (lanes 5 and 10). These results demonstrate that recovery of TfR from both apical or basolateral surfaces occurs with similar efficacy at the antibody concentrations tested, and that our detection assay is domain-selective.

Surface Delivery of ³⁵S-TfR Measured by Surface Immunoprecipitation

To determine the kinetics of surface arrival of newly synthe-

sized TfR, pulse-chase experiments were combined with surface immunoprecipitation. Cells were pulse-labeled and then chased for the indicated times. Thereafter, TfR was precipitated from either the apical or basolateral surface and analyzed by SDS-PAGE and densitometry (Fig. 2). Newly synthesized TfR is first detectable at the basolateral surface after 20 min, although the appearance at both surfaces occurs with a similar half time of ~ 45 min (Fig. 2). After a chase of 80 min transport to both surfaces reached a maximum, resulting in fourfold higher amount of TfR at the basolateral side than at the apical surface. This ratio was maintained after a chase of 120 min, and indicates that the bulk (80%) of newly synthesized TfR is delivered directly from the TGN to the basolateral surface. In a previous study we showed that, at steady state, the basolateral surface contains twice as many Tf binding sites. To examine the distribution of newly synthesized TfR at equilibrium the chase time was extended to 16 h. In this case an apical to basolateral TfR ratio of 1:1.8 was obtained (Fig. 2), indicating that newly synthesized TfRs become redistributed on both cell surfaces, presumably as a consequence of transcytotic transport.

Surface Delivery of ^{35}S -TfR by Trypsin Assays

The above findings may suggest that transcytotic pathways play an important role in the redistribution of newly synthesized TfR to apical and basolateral cell surfaces. Because the TfR is rapidly endocytosed at both cell surfaces (Cerneus and van der Ende, 1991), we used trypsin to detect also newly synthesized TfR that appears only transiently on either cell surface. Pulse-labeled cells were chased up to 80 min in the continuous presence of trypsin added either to the apical or basolateral surface. During the chase SBTI was added opposite to the side receiving trypsin. Control cells were chased in the absence of trypsin. As seen in Fig. 3, after a chase of 40 min, the cleavage by basolaterally administered trypsin was \sim fourfold higher than with trypsin present in the apical compartment again demonstrating that the bulk of newly synthesized TfR is targeted directly to the basolateral surface. At 80 min, the ratio of TfR accessible for apical and basolateral administered trypsin had decreased to $\sim 1:2$ (Fig. 3) probably due to ongoing transcytosis. In the experiment with trypsin, all TfR, transiently present at the plasma membrane domain to which trypsin is administered, are detected. These TfRs transiently present at the cell surface consist of receptors, which were (a) directly transported from the TGN to the cell surface, (b) endocytosed and (c) transcytosed from the opposite domain.

To see to what extent transcytosis contributes to the surface arrival of newly synthesized TfR, cells were pulse-labeled for 10 min, and then chased for 80 min in the presence of trypsin, which was added either to the apical or basal side of the cells. After the chase the cells were washed with ice cold PBS-CM containing SBTI and anti-TfR antibodies were subsequently applied to the side opposite to the trypsinized surface. TfR, once delivered to the trypsinized surface, is efficiently degraded, and thus, no longer detectable in our surface precipitation assay. In this way we were able to estimate the pool of TfR transiently present at either cell surface, which is involved in transcytosis. Control experiments were performed which showed that under the conditions of continuous trypsinization the integrity of the

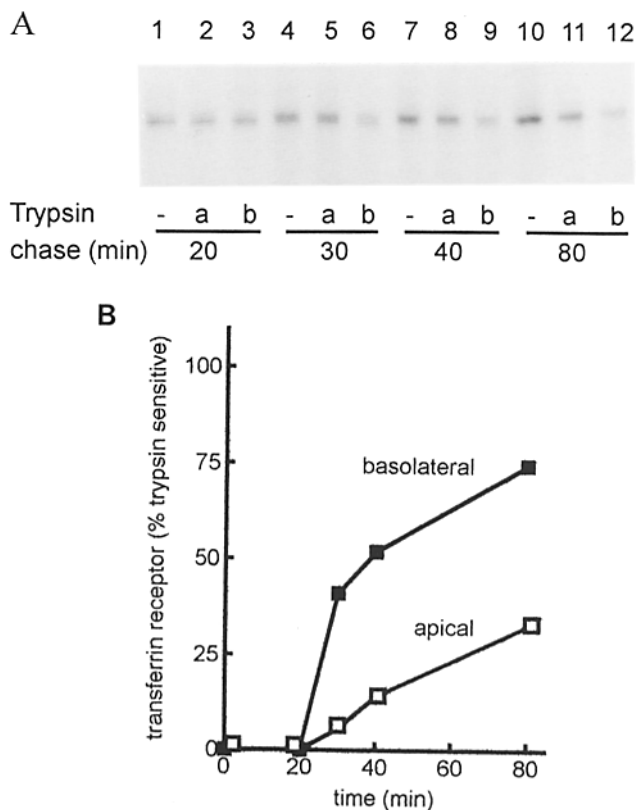


Figure 3. Trypsin assay to monitor surface arrival of TfR. (A) Cells were pulse labeled for 20 min with ^{35}S methionine and chased for the indicated time with trypsin ($100 \mu\text{g/ml}$) present at the apical (a) or basolateral (b) surface, to remove all TfR which arrived on the surface during the chase. Control cells (–) were chased in the absence of trypsin. After the chase the cells were lysed and TfR was immunoprecipitated, and analyzed by SDS-PAGE, fluorography, and densitometry. A graphical representation is shown in B.

monolayer was maintained, while the cleavage of pulse-labeled TfR was equally efficient at trypsin concentrations ranging 25 – $100 \mu\text{g/ml}$ (see Materials and Methods). The results show that, within 80 min of chase the majority (75%, compare lanes 5, 6 with lanes 7, 8) of surface detectable TfR was found on the basolateral surface, thus confirming our previous observations (Fig. 4, A and B). However, when trypsin was present at the apical surface the amount of TfR immunoprecipitated from the basolateral surface was decreased to 60%. This indicates that $\sim 20\%$ [$(75-60)/75 \times 100\%$] of the newly synthesized TfR which is targeted to the basolateral surface was initially inserted into the apical surface. Similarly, the amount of TfR immunoprecipitable from the apical surface was decreased from 25 to 17% when trypsin was added to the basolateral surface (Fig. 4, A and B), indicating that 35% [$(25-17)/25 \times 100\%$] of the TfR was targeted indirectly to the apical surface. These results demonstrate that transport of newly synthesized TfR to either the apical or basolateral surface involves both direct and transcytotic pathways. It should be noted that within 80 min the fraction of TfR delivered by transcytosis is higher for the apical domain than for the basolateral domain (35% vs 20%), indicating that intracellular sorting and direct targeting to the basolateral domain is even more efficient.

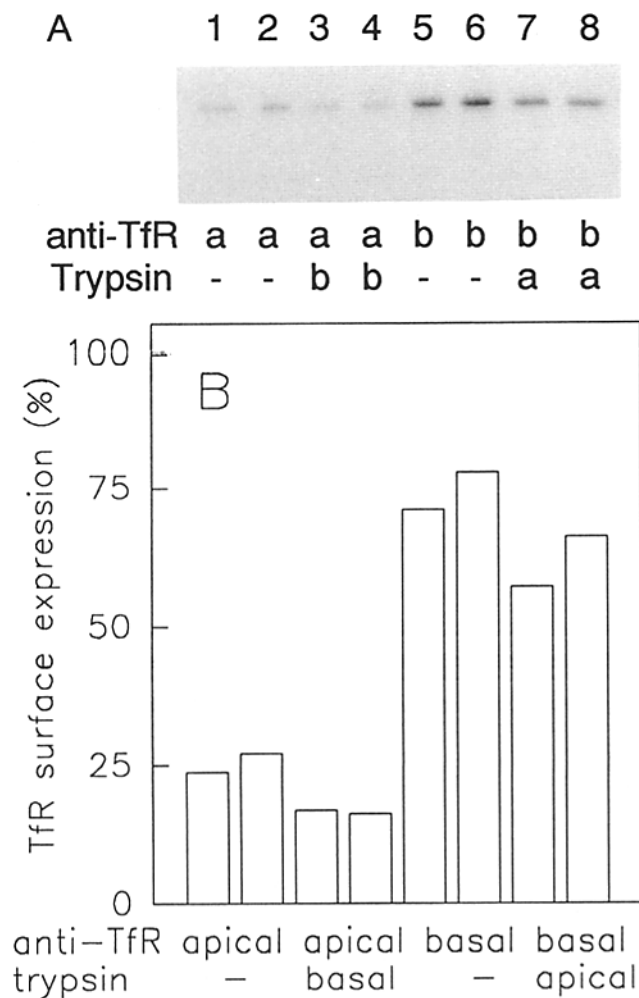


Figure 4. TfR is targeted to apical and basolateral surfaces by both direct and indirect pathways. (A) Filter-grown BeWo cells were pulse-labeled for 10 min with [³⁵S]methionine and chased for 80 min at 37°C in the absence (–) or presence of trypsin (50 μg/ml) added either at the apical (a) or basolateral (b) surface. After the chase, cells were rapidly chilled and the action of trypsin was blocked with SBTI. To detect TfR at the cell surface anti-TfR antibodies were added either to the apical (a) or basolateral (b) surface. Immunocomplexes were precipitated and analyzed as in Fig. 2. (B) Quantitation of the data obtained from A. Values are expressed as percentage of total surface expression on apical and basolateral surfaces obtained from cells chased in the absence of trypsin. The experiment was carried out in duplicate.

Transcytosis of the TfR

The results described above suggest that transcytosis in both directions contributes to the steady state distribution of the TfR at the apical and basolateral surface in BeWo cells. To extend and substantiate this further we used two assays to study the transcytosis of the total receptor pools at either cell surface. First, because the TfR and its ligand follow the same itinerary upon internalization, we determined the fate of ¹²⁵I-Tf prebound at either the apical or basolateral surface after an incubation of 2 h at 37°C. Although most of the internalized ¹²⁵I-Tf is recycled to the original surface, part of it is transcytosed to the opposite surface (Table I). Furthermore, it appears that the basolateral surface binds 1.5 times more radioactive ligand than the apical surface, which is

close to earlier observations (Cerneus and van der Ende, 1991). However, equal amounts of prebound ligand are transcytosed from either surface. This means that the proportion of TfR involved in transcytosis at the apical surface is 1.5 times higher than vice versa (24 vs 16%, respectively). Interestingly, this implicates that recycling at the apical surface is less efficient as compared to the basolateral surface.

We next examined the time course of redistribution of either the apical or basolateral pool of TfR between both surfaces (Fig. 5). TfRs at the cell surface were labeled by iodination either at the apical or basolateral side of the monolayer and then allowed to endocytose at 37°C for the indicated time. Thereafter, cells were washed and incubated for an additional 10 min at 37°C with trypsin (100 μg/ml) present at either the apical or basal side of the cells. During the trypsin incubation SBTI (200 μg/ml) was added opposite to the side receiving trypsin. Cells were then rapidly chilled by extensive washing in ice cold PBS containing SBTI, and the amount of cleavage of ¹²⁵I-TfR was analyzed as described above. As seen in Fig. 5, A and B, both apically and basolaterally ¹²⁵I-TfR were efficiently cleaved when trypsin was added directly to the iodinated surface prior to preincubation at 37°C (lanes 1–3). In this case, no cleavage was observed when trypsin was added opposite to the iodinated surface, indicating that no TfR is transcytosed in either direction within 10 min of trypsin incubation (lanes 1–3). Following incubation at 37°C, the amount of apically iodinated TfR that became accessible for basolateral trypsin increased, while cleavage by apical trypsin decreased with time (Fig. 5 C). After a chase of 4 h, an equilibrium was reached at which ~20 and 45% of the apically labeled TfR pool was cleaved by apical and basolateral trypsin, respectively (Fig. 5 C). These patterns were essentially the same after an overnight incubation (Fig. 5 C). A similar time course was observed for the redistribution of the basolaterally labeled TfR pool between both surfaces (Fig. 5 D). This indicates that at equilibrium the TfR pool from either side was distributed over apical and basolateral surfaces in a ratio of 1:2, which is in agreement with previous results obtained by ligand binding (Cerneus and van der Ende, 1991). It should be noted, that following a chase of 1 h or longer, cleavage of both apical and basolateral TfR pools by basolateral trypsin was always greater than by apical trypsin (Fig. 5, C and D). This further indicates that the fraction of the apical TfR pool transcytosed is significantly higher than that of the basolateral TfR pool and ultimately determines the steady state distribution of TfR on both surfaces.

In summary, our results show that in BeWo cells at least 80% of newly synthesized TfR is efficiently sorted at an intracellular site, presumably in the TGN, and targeted directly to the basolateral surface. In addition, due to bidirectional transcytosis newly synthesized TfR subsequently equilibrates between both surfaces, resulting in a 1.5–2.0 higher number of TfR at the basolateral surface at steady state.

Discussion

In all epithelial cell types studied thus far, the TfR shows a high level of polarity; i.e., it is predominantly expressed at the basolateral surface. In contrast, in BeWo cells, the steady

Table I. Release of Surface-bound ¹²⁵I-Tf into Apical and Basal Media

Prebound ¹²⁵ I-Tf	Apical Surface		Basolateral Surface	
	cpm	%	cpm	%
Apical medium	11434	68	3906	16
Basal medium	4068	24	19581	79
Cellular	1295	8	1280	5
Total	16797	100	24767	100

BeWo monolayers were allowed to bind ¹²⁵I-Tf added either to the apical or basolateral surface for 1 h at 4°C. Unbound ligand was removed by washing, and the cells were incubated at 37°C for 2 h in MEM containing a 50-fold excess of unlabeled transferrin to prevent re-entrance of ¹²⁵I-Tf. Thereafter, apical and basolateral media were collected separately and the radioactivity associated with the filters and media was monitored. Each value represents the mean of two filters, and varies <5%.

state ratio of the TfR on apical and basolateral surfaces is 1:2 (Cerneus and van der Ende, 1991). Evidence presented in this paper indicates that at least 80% of newly synthesized TfR is directly sorted to the basolateral surface with a half time of 45 min. Similar results were reported for CaCo-2 cells (LeBivic et al., 1990), and may suggest that the sorting information required for direct targeting from the TGN to the basolateral surface is well preserved among different epithelial cell types. However, the sorting determinant on the TfR for basolateral targeting is still unknown. Interestingly, recent findings in MDCK cells suggest a strong correlation between the capability for endocytosis and basolateral sorting (Brewer and Roth, 1991; Hunziker et al., 1991; LeBivic et al., 1991). The signal for endocytosis of the TfR is located in the cytoplasmic tail and contains a four amino acid motif, including a critical tyrosine residue, which is responsible for clustering into coated pits and subsequent endocytosis (Collawn et al., 1990). Similar motifs have been identified in other receptors involved in endocytosis such as the low density lipoprotein receptor (LDL-R) and poly-Ig receptor (pIg-R) (Collawn et al., 1990, 1991). However, mutations in the signal for endocytosis of the LDL-R and pIg-R had no effect on the basolateral targeting of these receptors when transfected in MDCK cells (Hunziker et al., 1991; Casanova et al., 1991; Yokode et al., 1992). A similar finding has been reported for the TfR (Hunziker et al., 1991; Dargemont et al., 1993), suggesting that separate sorting signals may operate in basolateral targeting and endocytosis in these proteins. Indeed, within the cytoplasmic tail of both the LDL-R and the pIg-R basolateral sorting sequences were identified, which appeared not to function in endocytosis (Casanova et al., 1991; Yokode et al., 1992). Our results support the general notion that different polarized cell systems use direct pathways to target basolateral proteins to their correct domain.

After surface arrival, newly synthesized TfRs subsequently equilibrate between both surfaces, due to bidirectional transcytosis, reaching a steady state level with a two-fold higher number of receptors on the basolateral surface. A similar surface ratio was obtained after redistribution of surface-iodinated apical and basolateral receptor pools upon endocytosis. If we used ¹²⁵I-Tf we found that upon endocytosis at the basolateral surface, 16% of the internalized Tf is transcytosed to the apical surface, whereas the remainder is efficiently recycled to the original surface. Similarly, at the

apical surface the majority of internalized Tf returns to the apical surface, although a higher fraction (26%) is transcytosed to the opposite surface. Thus, the percentage of apical TfRs involved in transcytosis is 1.5–2.0 times higher at the apical surface than at the basolateral surface, indicating that recycling of TfRs at the apical surface is less efficient than at the basolateral surface. Different post-endocytic sorting at apical and basolateral surfaces has also been described for the pIg-R in MDCK cells (Breitfeld et al., 1989). This receptor is directly targeted from the TGN basolaterally followed by unidirectional transcytosis to the apical cell surface. Once the apical surface is reached, this receptor either recycles at this domain or the extracellular domain of the receptor is cleaved and subsequently released into the apical medium (Breitfeld et al., 1989).

Our results resemble the observations made by Godefroy et al. (1990), using the human enterocyte-like cell line HT29, in which the uptake of Tf is 10 times higher at the basolateral surface than at the apical surface. These authors showed that only 10% of basolaterally internalized Tf was transcytosed to the apical domain, while 40% of apically internalized Tf transported in the apical-to-basal direction (Godefroy et al., 1990). Inefficient recycling of TfRs at the apical surface may serve to redirect missorted TfR from the apical surface to the basolateral surface. In MDCK cells and Caco-2 cells both binding and recycling of Tf appears to be entirely restricted to the basolateral surface (Fuller and Simons, 1986; Hughson and Hopkins, 1990). However, in Caco-2 cells 20% of the newly synthesized TfR is transported to the apical surface (LeBivic et al., 1990). It is possible that in Caco-2 cells and perhaps also in MDCK cells the fraction of apical TfRs transcytosed to the basolateral surface is significantly higher as compared to BeWo and HT-29 cells, resulting in a low apical TfR expression at steady state.

The difference in post-endocytotic sorting of the TfR in BeWo cells, as compared to other epithelial cell types, may reflect a cell type/tissue-specific phenomenon in order to retain a substantial number of TfR at the apical surface. The BeWo cell line shares many morphological and biochemical properties with placental trophoblasts *in vivo*, of which a functional correlation between apical TfRs and the uptake of Tf from the maternal blood circulation has been suggested (van Dijk, 1988; Cerneus and van der Ende, 1991). Transport of iron across the trophoblast layer is essential for the developing fetus and is initiated by the binding of iron-loaded Tf to its receptor at the apical surfaces, which is in direct contact with the maternal blood supply (van Dijk, 1988). Presumably, apical recycling of the TfR reflects the fact that BeWo cells are especially adapted to endocytose iron-loaded Tf from the maternal blood via this receptor. This is the more striking as these cells have a “normal” distribution of placental alkaline phosphatase (at the apical plasma membrane, Cerneus et al., 1993, and unpublished) as well as of the Na/K-ATPase β -chain (at the basal plasma membrane, not shown).

Taken together, our results indicate that the apical expression of the TfR in BeWo cells is not caused by an inefficient sorting in the TGN, but rather reflects a cell type-specific difference in the post-endocytotic sorting of the TfR in BeWo cells as compared to other epithelial cell types.

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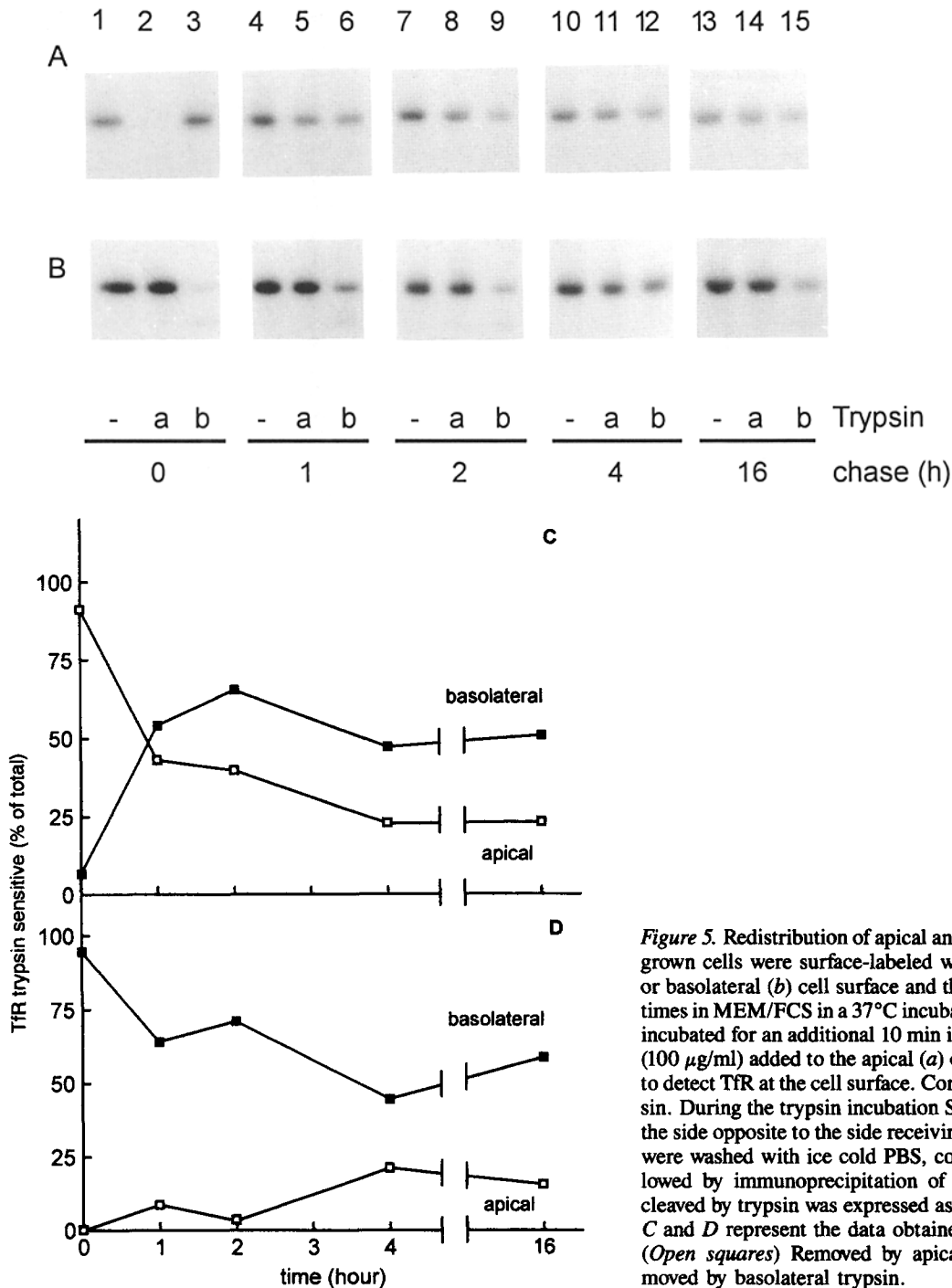


Figure 5. Redistribution of apical and basolateral TfR pools. Filter-grown cells were surface-labeled with ^{125}I at either the apical (a) or basolateral (b) cell surface and then incubated for the indicated times in MEM/FCS in a 37°C incubator. After the chase, cells were incubated for an additional 10 min in MEMH at 37°C with trypsin ($100\ \mu\text{g/ml}$) added to the apical (a) or basolateral (b) compartment to detect TfR at the cell surface. Control cells (-) received no trypsin. During the trypsin incubation SBTI ($200\ \mu\text{g/ml}$) was added to the side opposite to the side receiving trypsin. Thereafter, the cells were washed with ice cold PBS, containing SBTI, and lysed, followed by immunoprecipitation of TfR. The amount of ^{125}I -TfR cleaved by trypsin was expressed as percentage of control. Graphs C and D represent the data obtained from A and B, respectively. (Open squares) Removed by apical trypsin; (solid squares) removed by basolateral trypsin.

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