Intracellular Movement of Cell Surface Receptors After Endocytosis: Resialylation of Asialo-Transferrin Receptor in Human Erythroleukemia Cells

MARTIN D. SNIDER and OPHELIA C. ROGERS
Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210

ABSTRACT The intracellular movement of cell surface transferrin receptor (TfR) after internalization was studied in K562 cultured human erythroleukemia cells. The sialic acid residues of the TfR glycoprotein were used to monitor transport to the Golgi complex, the site of sialyltransferases. Surface-labeled cells were treated with neuraminidase, and readdition of sialic acid residues, monitored by isoelectric focusing of immunoprecipitated TfR, was used to assess the movement of receptor to sialyltransferase-containing compartments. Asialo-TfR was resialylated by the cells with a half-time of 2–3 h. Resialylation occurred in an intracellular organelle, since it was inhibited by treatments that allow internalization of surface components but block transfer out of the endosomal compartment. Moreover, roughly half of the resialylated molecules were cleaved when cells were retreated with neuraminidase after culturing, indicating that this fraction of the molecules had returned to the cell surface. These results suggest that TfR is transported from the cell surface to the Golgi complex, the intracellular site of sialyltransferases, and then returns to the cell surface. This pathway, which has not been previously described for a cell surface receptor, may be different from the route followed by TfR in iron uptake, since reported rates of transferrin uptake and release are significantly more rapid than the resialylation of asialo-TfR.

In recent years, it has become clear that plasma membrane proteins move into and out of cells. Membrane proteins are exchanged between the cell surface and intracellular organelles during the uptake of membrane by pinocytosis and phagocytosis and during the addition of membrane by the fusion of intracellular vesicles with the plasma membrane (for reviews, see references 7, 17, and 49). These processes have been studied extensively by microscopic and cell fractionation techniques. The movement of surface proteins into coated vesicles and endosomes during pinocytosis and into phagosomes and secondary lysosomes during phagocytosis have been described in studies that have used specific antibodies and ligands as probes. However, these noncovalently bound probes may not report accurately on the intracellular transport of surface proteins, since they may dissociate from their original binding sites after endocytosis. In addition, these techniques yield information about only the distribution of the probe at the moment the cells are examined.

To overcome some of these problems, we are studying intracellular protein transport using covalent modifications of cell surface proteins as probes. This approach was inspired by the many studies that have followed the movement of newly made proteins through organelles of the secretory apparatus by analyzing the posttranslational modifications that occur in those organelles (24, 48). Our strategy involves making covalent alterations to cell surface components so that they become substrates for enzymes of known intracellular location. Movement of proteins to the enzyme-containing compartment can then be monitored by studying the covalent structure of the molecule.

In this paper, we describe the development of an assay of this type that uses the sialic acid residues of cell surface glycoproteins to monitor movement to the Golgi complex. Cells are treated with neuraminidase to remove sialic acid residues from surface glycoproteins. The movement of these asialoglycoproteins to the Golgi complex, the major intracellular site of sialyltransferases, is then followed by monitoring the addition of sialic acid.

We have followed the intracellular movement of the transferrin receptor (TfR) using this assay. It is one of many

Abbreviations used in this paper: A, acidic species; Met, methionine; NP-40, Nonidet P-40; PNE, 10 mM sodium phosphate (pH 7.2), 150 mM NaCl, 1 mM EDTA; TfR, transferrin receptor.
surface glycoprotein receptors that participate in the uptake of ligands from serum. TIR is a ubiquitous cell surface asialoglycoprotein ($M_r \sim 180,000$) composed of two identical disulfide-linked subunits ($M_r \sim 90,000$) (39, 47, 50). Its function is the uptake of iron from transferrin, the serum iron transport protein. Iron-transferrin binds to surface receptor and the complex is internalized through coated vesicles (5, 19, 29). The iron dissociates from the TIR-transferrin complex, probably in the acidic endosomal compartment, and the complex then returns to the cell surface, where apo-transferrin is released (9, 31).

The movement of surface TIR to the Golgi complex was studied in K562 human erythroleukemia cells. When surface-labeled cells were treated with neuraminidase and returned to culture, nearly all the labeled asialo-TfR was resialylated, indicating that these molecules had been transported to the Golgi complex. In addition, some of the resialylated molecules were found on the cell surface, showing that there is a cycle of transport to the Golgi complex and return to the cell surface.

**MATERIALS AND METHODS**

**Cells:** K562 cells, a human erythroleukemia line (obtained from Dr. Alan Schwartz, Children’s Hospital, Boston) were grown in $\alpha$- minimal essential medium, supplemented with penicillin (50 U/ml), streptomycin (50 $\mu$g/ml), and 10% fetal calf serum (all from Gibco Laboratories, Grand Island, NY). The cells were maintained in suspension culture at 37°C in a humidified 5% CO$_2$ atmosphere.

**Labeling of Cells:** Surface iodination of cells was performed using the method of Hynes (27). Cultures were chilled, and centrifuged for 5 min at 500 g. The cell pellet was washed twice in cold phosphate-buffered saline (PBS) and resuspended in the same buffer at $1 \times 10^7$ cells/ml. Iodination was carried out in 5 mM KI, 400 $\mu$Ci/ml Na$^{125}$I (carrier-free) (Amersham Corp., Arlington Heights, IL), 0.1 U/ml glucose oxidase (Calbiochem-Behring Corp., San Diego, CA), and 2 U/ml lactoperoxidase (Calbiochem-Behring Corp.). After incubating for 20 min on ice, the mixture was made 1 ml in unlabeled NaI, and centrifuged for 5 min at 500 g. The supernatant was decanted and the cells were washed three times with PBS containing 1 mg/ml bovine serum albumin (BSA), 1 mg/ml Glc (PBS/BSA).

Cells were metabolically labeled with $^{125}$I-methionine (I$^{125}$I)Met in Met-free minimal essential medium containing nonessential amino acids and 5% dialyzed fetal calf serum. Cells were centrifuged and the pellet washed twice with 1 ml of labeling medium/5 x 10$^8$ cells. The cells were then resuspended in the same medium containing 160 $\mu$Ci/ml I$^{125}$I (1,200 Ci/mmol, Amer- sham Corp.) at a density of 1.33 x 10$^7$/ml and incubated at 37°C. The label was chased by adding 1 ml of growth medium/5 x 10$^8$ cells, centrifuging, resuspending the cells in medium, and incubating them in a 5% CO$_2$ atmosphere.

**Neuraminidase Treatment of Cells:** Iodinated cells were used immediately after labeling. Cells were resuspended in PBS/BSA at 5 x 10$^7$/ml and *Vibrio cholerae* neuraminidase was added (6 µl/10$^7$ cells) (Calbiochem-Behring Corp.). After 60 min on ice, the cells were centrifuged and the supernatant was decanted. The cells were washed twice by resuspension and centrifugation in 10 mM sodium phosphate (pH 7.2), 150 mM NaCl, 1 mM EDTA (PNE) containing 1 mg/ml BSA, 1 mg/ml Glc followed by one wash in PBS/BSA. Control cells were treated identically except that they were incubated without neuraminidase. In some experiments, unlabelled cells were similarly treated with neuraminidase. Cells were centrifuged from growth medium, washed twice with PBS/BSA, and treated as described for I$^{125}$I-labeled cells.

**Reculture of Cells:** After neuraminidase treatment, cells were resus- pended at a density of 2 x 10$^7$/ml in growth medium. They were then incubated for the times and at the temperatures indicated in a 5% CO$_2$ atmosphere. Human transferrin (0.3 mg/ml, Calbiochem-Behring Corp.) that had been saturated with iron (8) was added where noted.

**Cell Lysis and Immunoprecipitation of TIR:** Cells were centri- fuged from suspension, and the pellets were lysed in ice cold 1% Nonidet P-40 (NP-40) in PNE (10$^7$ cells/ml). After 20 min on ice, lysates were centrifuged 2 min at 13,000 g, supernatants decanted, and 1/10 vol of 10% NP-40, 10% sodium deoxycholate, 1% SDS in PNE was added. TIR was then immunoprecipi-
FIGURE 1 Removal of sialic acid from cell surface 125I-TfRe by neuraminidase. Cells were iodinated and samples of 10⁶ cells were incubated with the indicated amounts of V. cholerae neuraminidase. The cells were lysed and TfR was immunoprecipitated and analyzed by isoelectric focusing. An autoradiograph of the dried gel is shown, with the acidic end at the top. The major TfR bands are numbered 1–10 from the most basic to the most acidic.

K562 Cells Resialylate Asialo-TfR

The fate of surface asialo-TfR was studied in labeled cells that were treated with neuraminidase at 0°C and then incubated in growth medium at 37°C. Acidic TfR species began to reappear within 30 min, with a corresponding decrease in the basic species (Fig. 2). Most notable are the increases in the acidic forms 7 and 8 and the decreases in the species 3 and 4. After 20 h, TfR from neuraminidase-treated cells resembled receptor from control cells. The relative amounts of TfR species did not change when control cells were recultured for a similar period.

To prove that the acidic TfR species in recultured neuraminidase-treated cells had been resialylated, we treated lysates from recultured cells with neuraminidase. Labeled neuraminidase-treated and control cells were recultured and lysates were prepared. The lysates were then incubated with or without neuraminidase, and the TfR species were analyzed (Fig. 3). The acidic species that appeared during reculture of neuraminidase-treated cells were destroyed by this second treatment, with a corresponding increase in basic species. Thus the reappearance of acidic species is due to the addition of sialic acid residues to asialo-TfR. Moreover, because treated lysates from recultured control and neuraminidase-treated cells contained the same TfR species, no other modifications are made to asialo-TfR in recultured cells.

The resialylation of asialo-TfR in neuraminidase-treated cells was quantified by measuring the radioactivity in bands cut from the gel shown in Fig. 2. The extent of sialylation, which ranges from 0% in neuraminidase-treated cells that have not been cultured to 100% in control cells, was calculated as described in Materials and Methods (Fig. 4a). In recultured neuraminidase-treated cells, resialylation had a half-time of 2–3 h. Moreover, the recovery was nearly complete, since sialylation reached 85% by 20 h. The sialylation value in control cells remained close to 100% during the same time in culture.

The degradation rates of labeled TfR and asialo-TfR were also quantified in this experiment. A semi-log plot of the total radioactivity in TfR (Fig. 4b) showed that labeled TfR was lost from control and neuraminidase-treated cells with identical kinetics. A small fraction of the 125I-TfR was rapidly degraded, while the remainder disappeared with first order kinetics. The half-lives of the slow phase of turnover were 15.3 and 14.7 h in control and treated cells, respectively. This finding, combined with the fact that the amounts of the labeled acidic TfR species increased during the reculture of neuraminidase-treated cells (Fig. 2), demonstrates that sialic acid residues were added to the surface of these cells.
acid residues are added to asialo-TfR. Preferential degradation of less sialylated TfR species cannot account for the increase in the relative proportion of acidic TfR species. The lifetime of iodinated receptor is comparable to the value of 16 h obtained for [35S]Met-labeled receptor in K562 cells (not shown).

**Neuraminidase-Treated**

**Control**

**FIGURE 2** Time course of resialylation of asialo-TfR. Cells were iodinated and treated with neuraminidase at 0°C and then cultured for the indicated times at 37°C in growth medium. Control cells were treated identically except that the neuraminidase treatment was omitted. TfR was then isolated and analyzed by isoelectric focusing. An autoradiograph of the dried gel is shown, with the acidic end at the top. Bands are numbered as in Fig. 1.

**FIGURE 3** Removal of sialic acid residues from resialylated TfR in cell lysates. Iodinated neuraminidase-treated and control cells were cultured for 0 or 3 h in growth medium and lysates were prepared. The indicated lysates were then treated with neuraminidase, and TfR was isolated and analyzed by isoelectric focusing. An autoradiograph of the dried gel is shown, with the acidic end at the top. Bands are numbered as in Fig. 1.

**FIGURE 4** Quantitation of resialylation of asialo-TfR and degradation of 125I-TfR. Data from neuraminidase-treated (○) and control (●) cells is shown. (a) Extent of sialylation, calculated as described in Materials and Methods. In neuraminidase-treated and control cells before reculture, acidic species represented 47.3 and 90% of total TfR, respectively. (b) Degradation of 125I-TfR. A semi-log plot of the total radioactivity in TfR is shown. The linear portion of the decay of labeled TfR had half-times of 14.7 h (r = 0.98) in neuraminidase-treated cells and 15.3 h (r = 0.99) in control cells, as determined by least squares analysis.
Internalization of Asialo-TfR and Transport to a Post-endosomal Compartment Are Required for Resialylation

We performed experiments to test whether asialo-TfR is resialylated on the cell surface or in an intracellular organelle. First, we examined the temperature dependence of the process. Temperatures below 20°C block many intracellular transport processes. These include the transfer of receptor-bound ligands from endocytic vesicles to lysosomes (11, 34, 45), phagocytosis by macrophages (42), and the movement of newly made glycoproteins from the Golgi to the cell surface (35). Table I shows that resialylation was observed at 37, 31, and, to a lesser extent, 25°C, but not at 18 or 10°C. No change in receptor composition was seen in control cells at any temperature. This result suggests that asialo-TfR must be transported to an intracellular compartment to be resialylated.

To exclude the possibility that inhibition of sialyltransferase activity caused the reduction of resialylation at low temperature, we examined the sialylation of newly made TfR at low temperature. Cells were pulse-labeled with [35S]Met at 37°C, chased briefly at 37°C, and the chase was continued at various temperatures and TfR analyzed by isoelectric focusing (Fig. 5). During chase at 18, 25, 31, and 37°C, TfR was shifted from species 1–5 to species 6–10, indicating that sialylation was occurring, although smaller amounts of the acidic species were made at the lower temperatures. Of greatest significance is the fact that newly made TfR is sialylated at 18°C, indicating that sialyltransferases are active at this temperature. Thus, the absence of surface asialo-TfR resialylation at 18°C is due to the inhibition of transport to the intracellular organelle where the reaction occurs.

As a further test of the location of asialo-TfR resialylation, we examined the effects of chemical inhibitors of endocytosis. Monensin, a monovalent cation ionophore that interferes with both endocytosis and exocytosis (2, 52, 53) was used (Table II). This compound completely blocked resialylation at 25 μM, whereas 70% inhibition was seen with 10 μM monensin. In addition, ammonium chloride, a compound that blocks transport of cell surface ligands from endocytic vesicles to lysosomes (18, 20, 54–56), was studied. This compound caused 25 and 55% inhibition at 10 and 20 mM, respectively. None of these treatments had any effect on 125I-TfR in control cells.

The blockade of resialylation by reduced temperature, monensin, and ammonium chloride all support the idea that cell surface asialo-TfR must be transported to an intracellular organelle to be resialylated. Specifically, these treatments allow uptake of cell surface molecules into endosomes (7, 49, 57), smooth-surfaced vesicles found in the peripheral cytoplasm, but block transfer out of this compartment. Thus, resialylation does not occur at the cell surface or in endosomes, but must take place in another organelle.

Location of Resialylated TiR

Cell surface asialo-TfR is resialylated after transport to an intracellular compartment. To learn whether resialylated mol-

---

**TABLE I**

| Temperature | No incubation | 10°C | 18°C | 25°C | 31°C | 37°C |
|-------------|---------------|------|------|------|------|------|
| Acidic TfR | 34.6% | 33.8% | 35.7% | 39.0% | 44.9% | 45.0% |
| Sialylation | 91.1% | 90.1% | 90.6% | 89.4% | 89.8% | 89.5% |

Cells were iodinated, treated with neuraminidase, and recultured in growth medium for 3.5 h at the indicated temperatures. Transferrin receptor was then isolated and analyzed, as described in Materials and Methods.

**TABLE II**

| Temperature | Acidic TfR | Sialylation | Acidic TfR | Sialylation |
|-------------|------------|-------------|------------|-------------|
| Not recultured | 35.3% | 91.1% | 89.7% | 100% |
| Recultured | 46.7% | 21% | 89.1% | 99% |
| No additions | 39.1% | 7% | 86.3% | 94% |
| + 10 μM monensin | 33.7% | -3% | 88.1% | 97% |
| + 25 μM monensin | 44.3% | 16% | 86.6% | 94% |
| + 10 mM NH₄Cl | 40.6% | 10% | 88.0% | 97% |

Cells were iodinated, treated with neuraminidase, and recultured in growth medium with the indicated additions for 3.5 h at 37°C. Monensin was added as a 120-mM stock in ethanol. Transferrin receptor was then isolated and analyzed, as described in Materials and Methods.
cules remain in this compartment or return to the cell surface, labeled neuraminidase-treated or control cells were cultured at 0 or 37°C for 3.5 h and then retreated with neuraminidase at 0°C (Fig. 6). Roughly half of the resialylated TfR (species 6-10) in neuraminidase-treated cells cultured at 37°C was digested by the second treatment. This suggests that this fraction of the resialylated molecules had returned to the cell surface. A similar fraction of the 125I-TfR was found on the cell surface when control cells were cultured at 37°C and then treated with neuraminidase. A second neuraminidase treatment of treated cells cultured at 0°C had no effect, because no resialylation had occurred. In control cells cultured at 0°C, neuraminidase treatment completely desialylated 125I-TfR, since all the 125I-TfR had remained on the cell surface.

**Effect of Transferrin on Resialylation of Asialo-TfR**

Similar amounts of resialylation occurred when labeled neuraminidase-treated cells were cultured in serum-free medium or growth medium, indicating that transferrin is not required for this process (Table III). Surprisingly, addition of human transferrin to serum-free medium caused a reduction in the extent of resialylation. Although human transferrin decreased resialylation, the bovine transferrin in the fetal calf serum present in the growth medium had no effect. This can be explained by the finding that bovine transferrin binds poorly to TfR on K562 cells (32).

There are two possible explanations for the inhibition of asialo-TfR resialylation by human transferrin. First, the ligand could alter the intracellular transport of TfR, with the unoccupied receptor having a better chance of reaching the site where resialylation occurs. Alternatively, if TfR oligosaccharides are sterically hindered by bound transferrin, then TfR-transferrin complexes might not be resialylated, even though transport to the correct intracellular compartment had occurred. Though it is not possible to decide among these alternatives, the idea that transferrin reduces the accessibility of TfR oligosaccharides is supported by evidence that bound ligands interfere with the action of neuraminidase on TfR. Removal of sialic acid residues from TfR in cell lysates was inhibited by human transferrin (not shown). In addition, sialic acid residues were not removed when TfR-anti-TfR monoclonal antibody complexes were treated with neuraminidase (46).

**TfR Function Is Not Affected by Neuraminidase Treatment**

The resialylation of asialo-TfR is a valid assay for the intracellular transport of receptor only if the absence of sialic acid does not affect the behavior of TfR. Several experiments have been performed to show that this is the case. First, as discussed above, the half-lives of cellular 125I-TfR and 125I-asialo-TfR are the same, indicating that removal of sialic acid residues does not affect receptor stability. In addition, TfR and asialo-TfR bind transferrin equally well. Extracts from 125I-labeled cells were incubated with transferrin-Sepharose and the bound proteins eluted and analyzed on an SDS polyacrylamide gel (Fig. 7). In samples from control and neuraminidase-treated cells, a single polypeptide was bound (lanes c and f) which migrated with immunoprecipitated TfR (lanes a and h). The specificity of this binding was established by the fact that TfR and asialo-TfR did not bind to Sepharose (lanes a and c) or to transferrin-Sepharose in the presence of excess-free transferrin (lanes d and g). Because equal amounts of TfR and asialo-TfR were recovered from transferrin-Sepharose, neuraminidase treatment did not affect the transferrin binding activity of TfR. Moreover, iodination did not greatly alter the transferrin-binding properties of TfR since the amount of immunoprecipitated receptor was the same as the amount bound to transferrin-Sepharose.

The rapid internalization of cell surface asialo-TfR was also demonstrated as a further check on receptor function. Neuraminidase-treated and control cells were incubated with transferrin at 0°C to saturate surface binding sites. One set of samples was kept at 0°C, whereas a second set was warmed to 37°C briefly to allow internalization of receptor-ligand complexes. Surface TfR was then labeled by iodination at 0°C (Fig. 8). While only asialo-TfR was found on the surface of

| Experiment | Treatment | Acidic Tf | Sialylation | Acidic Tf | Sialylation |
|------------|-----------|-----------|-------------|-----------|-------------|
| 1         | No reculture | 40.3 | 0 | 80.5 | 100 |
|           | Growth medium | 49.6 | 20 | 82.3 | 105 |
|           | Serum-free medium | 49.1 | 19 | 77.9 | 94 |
|           | + BSA | 43.0 | 6 | 81.9 | 104 |
|           | Serum-free medium | 43.0 | 6 | 81.9 | 104 |

Cells were iodinated, treated with neuraminidase, and recultured in the indicated media for 3.5 h at 37°C. BSA (1 mg/ml) and iron-saturated human transferrin (0.3 mg/ml) were added where indicated. Transferrin receptor was then isolated and analyzed, as described in Materials and Methods.
neuraminidase-treated cells kept at 0°C, 60% of the labeled TfR in warmed cells was sialylated. Thus, during the brief incubation at 37°C, much of the surface asialo-TfR was internalized and replaced by TfR from internal pools that had not been exposed to neuraminidase. This rate of internalization of asialo-TfR is comparable to that measured for TfR, both directly (5), and by monitoring the internalization of transferrin (8, 28, 32, 37). In control cells that were not exposed to neuraminidase, the 37°C incubation did not affect the composition or amount of surface TfR; the expected internalization of TfR and its replacement by identical molecules cannot be detected by this experiment.

Finally, we examined the effect of neuraminidase and iodination on cell growth and viability. When neuraminidase-treated and control cells were cultured for 20 h, the two cultures contained the same number of cells and >98% of the cells in both samples excluded trypan blue. The rates of DNA and protein synthesis, measured by the incorporation of [3H]thymidine and [35S]Met into acid-precipitable material, were also the same in neuraminidase-treated and control cultures (not shown). In addition, after iodination, >95% of the cells excluded trypan blue.

DISCUSSION

We have taken advantage of a new approach to examine the intracellular movement of cell surface proteins. The method involves alteration of surface molecules to make them substrates for enzymes of known intracellular location. Transport to the intracellular compartment that contains the enzymes is then monitored by analysis of isolated proteins. This strategy has several advantages over previously used techniques. First, our approach offers a significant increase in sensitivity, since it provides an estimate of all the molecules that have passed through a compartment, rather than the number in that compartment when the cells are examined. For this reason, assays of this type should be able to detect the movement of cell surface molecules through organelles that contain a small fraction of the total pool at any moment. A second advantage is the use of covalent modifications to surface proteins as a probe, allowing molecules to be followed directly. In contrast, many other studies have used antibody and ligand probes, which may dissociate from their binding sites during the experiment. Nonspecific tracers, whose relation to individual membrane proteins is unclear, have also been used. A further advantage of covalent modifications as probes is that the modified surface molecules are stably altered when they reach the compartment under study, so that they can be followed after leaving the intracellular site of modification.

Our assay uses sialyltransferases to study the transport of
cell surface glycoproteins to the Golgi complex. It is well established that these enzymes are found in the Golgi, most likely in the trans region of the organelle. Sialic acid is incorporated into glycoproteins and glycolipids in the Golgi complex in vivo in a number of tissues, as demonstrated by electron microscope autoradiography (3, 4). Moreover, in cell fractionation experiments, the specific activity of sialyltransferase is highest in Golgi-derived membranes, and these membranes have most of the enzyme activity in the cell (16, 36). The placement of sialyltransferases in the trans Golgi compartment is based on indirect evidence. Because galactosyltransferase and sialyltransferases are found in the same membranes during cell fractionation (6, 10, 12) and because morphological studies have localized galactosyltransferase to the trans Golgi (44), it is likely that sialyltransferase is also found in this part of the Golgi complex.

We have found that asialo-TfR generated on the cell surface is resialylated. Because sialyltransferases are located in the Golgi and because resialylation is blocked by treatments that interfere with the movement of molecules out of the endosomal compartment, it is likely that TfR is transported from the cell surface to the trans region of the Golgi complex. Moreover, since nearly all asialo-TfR molecules are resialylated, it is likely that they all pass through this compartment.

Though asialo-TfR molecules are resialylated in neuraminidase-treated cells, they do not become as highly sialylated as receptor in control cells. This could mean that surface asialo-TfR and newly made TfR are not sialylated in the same compartment. However, a more likely explanation is that surface asialo-TfR is not identical in structure to newly made molecules, with the latter being better substrates for sialyltransferases. If asialo-TfR is a poor substrate for resialylation, then each molecule may make several trips through the Golgi complex before it is resialylated. Thus, transport through this compartment could be more rapid than the 2–3 h half-time of resialylation. This idea is supported by the possibility that bound transferrin interferes with asialo-TfR resialylation. Moreover, we have recently found that newly made TfR molecules on the cell surface have fewer sialic acid residues than older receptor molecules, with sialic acid residues being added to the TfR molecules over many hours (unpublished observations). This suggests that newly made TfR molecules pass through the Golgi without being fully sialylated and then mature as they return from the cell surface to the Golgi complex.

TfR does not accumulate at the site of resialylation, since roughly half of the resialylated TfR was found on the cell surface. Moreover, a similar fraction of receptor was found on the cell surface in studies with 125I-TfR in recultured K562 cells, with [35S]Met-labeled TfR in K562 cells (unpublished observations), and with TfR in HeLa cells (5). This suggests that resialylated TfR mixes rapidly with surface and internal receptor pools. It is not known whether resialylated TfR molecules return to the surface from the Golgi complex along the route used by newly made proteins, or whether some other pathway is used.

Several previous studies are consistent with the transport of TfR from the cell surface to the juxtanuclear region that contains the Golgi complex. In electron microscopic studies, anti-TfR bound to surface receptor was transported to this juxtanuclear region in cultured cells (26, 60). In addition, Hopkins (25) and Yamashiro et al. (61) have found that transferrin bound to surface TfR moves into vesicles in the Golgi region. Moreover, when cells are incubated with transferrin for long periods, material is accumulated in intracellular compartments that exchange slowly with the cell surface (37). However, none of these studies have directly demonstrated the movement of transferrin-TfR complexes into Golgi cisternae.

Previous reports also support the existence of a transport pathway that carries other cell surface molecules through the Golgi complex. Electron microscopic studies have found that nonspecific tracers can be transported from the plasma membrane to the Golgi complex in many cell types (13, 21–23, 40). Of particular interest is the fact that the tracers are usually found in greatest concentration in the rims of trans Golgi cisternae, which may be the sialyltransferase-containing elements. Willingham and Pastan (59) have also reported the transport of epidermal growth factor–horseradish peroxidase conjugates to the Golgi region. Moreover, Regoeczi et al. (43) found that asialotransferrin injected into rats was taken up by the liver and resialylated, presumably in the Golgi complex. This transport to the Golgi complex was probably mediated by the hepatic asialoglycoprotein receptor. Finally, surface 5′-nucleotidase in rat embryo fibroblasts was found to exchange with an internal pool with a half-time of 6 h, and electron microscopic evidence suggested that the internal pool is found in the Golgi region (58).

What is the role of TfR transport to the Golgi complex in iron uptake? The rate transferrin uptake and release both in K562 cells and other cultured cells is very rapid; ligand bound to surface receptors is internalized, returns to the cell surface, and is released into the medium within 10–20 min (8, 30, 32, 37). Our finding that surface asialo-TfR is resialylated with a half-time of 2–3 h suggests that not every transferrin-TfR complex moves through the Golgi complex during iron uptake. Alternatively, it is possible that only unoccupied receptors move through the Golgi complex. This could explain the inhibition of TfR resialylation by transferrin. The conclusion that transport of ligand-receptor complexes through the Golgi is not an obligatory part of ligand uptake was also reached for the intracellular transport of the hepatic asialoglycoprotein receptor based on electron microscopic observations (14), although a substantial fraction of the internal pool of this receptor has recently been found in the Golgi complex (15). However, microscopic studies on the uptake of epidermal growth factor suggest that transport to the Golgi complex may precede the movement of the hormone to lysosomes (59).

The extent to which other surface proteins follow the transport pathway we have found for TfR is unknown. Transport to the Golgi complex could be unique to TfR, could be a property common to receptors involved in endocytosis, or could occur for all plasma membrane proteins. The internalization of 5′-nucleotidase and its return to the cell surface (58) suggest that proteins other than surface receptors move between the Golgi complex and the cell surface. However, a recent study on the major histocompatibility antigen suggested that this molecule does not follow the same pathway as TfR, since the oligosaccharide structures of this surface glycoprotein were stable throughout the life of the molecule (51). To understand the importance of surface protein movement through the Golgi complex, we are currently using the resialylation assay to examine the intracellular movement of other surface glycoproteins, including ones involved in endocytosis and others that are not; we are also studying the transport of TfR in relation to cellular iron metabolism.
This page is dedicated to Professor Eugene P. Kennedy on the occasion of his sixty-fifth birthday.

We would like to thank Drs. D. Fambrough, N. Lipsky, R. Pagano, and R. Sleight for helpful discussions, and Dr. F. Maxfield for communicating unpublished results.

This work was supported by grant GM-31375 from the National Institutes of Health.

Received for publication 23 July 1984, and in revised form 7 November 1984.

REFERENCES

1. Ada, G. L., E. L. French, and P. E. Lind. 1961. Purification and properties of neumaminidase from Vibriio cholerae. J. Gen. Microbiol. 24:409-421.

2. Basu, S. K., J. L. Goldstein, R. G. W. Anderson, and M. S. Brown. 1981. Membrane retrieval of the recycling of low density lipoprotein receptors in human fibroblasts. Cell. 24:493-502.

3. Bennett, G., F. W. K. Kan, and D. O'Shangnessy. 1981. The site of incorporation of sialic acid residues into glycoproteins and the subsequent fates of these molecules in various rat and mouse cell types as shown by autoradiography after injection of [3H]N-acetylmannosamine. I. Observations in hepatocytes. J. Cell Biol. 88:1-15.

4. Bleil, J. D., and M. S. Bretscher. 1982. Transferrin receptor and its recycling in HeLa cells. J. Biol. Chem. 257:15511-15516.

5. Brown, M. S., R. G. W. Anderson, and J. L. Goldstein. 1983. Recycling receptors: the round-trip itinerary of migrant membrane proteins. J. Cell Biol. 97:223-229.

6. Cell. Biol. 97:270-275.

7. Cell. Biol. 97:299-329.

8. Cell. Biol. 97:508-521.

9. Cell. Biol. 97:607-617.

10. Cell. Biol. 97:5971-5978.

11. Cell. Biol. 97:351-355.

12. Cell. Biol. 97:597-607.

13. Cell. Biol. 97:694-707.

14. Cell. Biol. 97:241-247.

15. Cell. Biol. 97:315-330.

16. Cell. Biol. 97:789-798.

17. Cell. Biol. 97:152-163.

18. Cell. Biol. 97:175-179.

19. Cell. Biol. 97:191-197.

20. Cell. Biol. 97:255-263.

21. Cell. Biol. 97:307-315.

22. Cell. Biol. 97:319-329.

23. Cell. Biol. 97:336-344.

24. Cell. Biol. 97:336-344.

25. Cell. Biol. 97:336-344.