Primary rat hepatocytes and two hepatoma cell lines have been used to study whether high mannose-type N-glycans of plasma membrane glycoproteins may be modified by the removal of mannose residues even after transport to the cell surface. To examine glycan remodeling of cell surface glycoproteins, high mannose-type glycoforms were generated by adding the irreversible mannosidase I inhibitor deoxymannojirimycin during metabolic labeling with [3H]mannose, thereby preventing further processing of high mannose-type N-glycans to complex structures. Upon transport to the cell surface, glycoproteins were additionally labeled with sulfo-NHS-SS-biotin, sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate. This strategy allowed us to follow selectively the fate of cell surface glycoproteins. Postbiosynthetic demannosylation was monitored by determining the conversion of Manα1,6GlcNAc2 to smaller structures during reculture of cells in the absence of deoxymannojirimycin. The results show that high mannose-type N-glycans of selected cell surface glycoproteins are trimmed from Manα1,6GlcNAc2 to Manα1,GlcNAc2 with Manα1,GlcNAc2 and Manα1,GlcNAc2 formed as intermediates. It could be clearly shown in MH 7777 as well as in HepG2 cells that demannosylation affects plasma membrane glycoproteins after they are routed to the cell surface. As was determined for total cell surface glycoproteins in HepG2 cells, this process occurs with a half-time of 6.7 h. By analyzing the size of high mannose-type glycans of glycoproteins isolated from the cell surface at the end of the reculture period, i.e. after trimming had occurred, we were able to demonstrate that glycoproteins carrying trimmed high mannose glycans become exposed at the cell surface. From these data we conclude that cell surface glycoproteins can be trimmed by mannosidases at sites peripheral to N-acetylglucosaminyltransferase I without further processing of their glycans to the complex form. This glycan remodeling may occur at the cell surface or during endocytosis and recycling back to the cell surface.

During their maturation, N-linked glycans of secretory and membrane glycoproteins undergo extensive processing by specific glycosidases and glycosyltransferases in the ER, the Golgi complex, and the TGN (for reviews, see Refs. 1–4). The sequence of processing events includes trimming of the precursor oligosaccharide, Glc3Man9GlcNAc2, by glycosidases I and II and by distinct α1,2-mannosidases to form Manα1,GlcNAc2. Several of the processing mannosidases have been described (for reviews, see Refs. 4 and 5) such as ER mannosidases and Golgi mannosidase IA/IB. Following the action of N-acetylglucosaminyltransferase I and Golgi mannosidase II, the transfer of N-acetyl-d-glucosamine, d-galactose, l-fucose, and sialic acids by an array of glycosyltransferases generates the wide variety of oligosaccharide structures found on mature glycoproteins.

Several lines of evidence suggest that oligosaccharide processing of cell surface glycoproteins is not restricted to biosynthesis but may also occur after the initial passage through the compartments of the secretory pathway to the cell surface (for a review, see Ref. 6). First, measurements of the turnover rates of the different sugar residues of glycoproteins isolated from rat liver plasma membranes have shown that these turnover kinetics are distinctly influenced by the position of each sugar within the N-linked oligosaccharides (7–10). The half-lives of the terminal or penultimate sugars, l-fucose, sialic acid, and d-galactose, are only 1⁄6 to 1⁄3 as long as that of the protein backbone. From these studies it has been proposed that terminal sugar residues may be removed from the nonreducing end of the N-glycans of plasma membrane glycoproteins. In distinct plasma membrane glycoproteins, even mannose residues were lost from the glycoproteins (11). Second, studies designed to examine the return of surface receptors to compartments of the secretory pathway have demonstrated that selected cell surface glycoproteins may also acquire terminal sugars, l-fucose, and sialic acid when recycling to fucosyl- and sialyltransferases in the medial/trans-Golgi and in the TGN (12–19). In Chinese hamster ovary cells, the cation-independent mannose 6-phosphate/insulin-like growth factor-II receptor has been reported to recycle even to galactosyltransferases in the trans-Golgi region (19). It has been proposed that reglcosylation might serve as a repair mechanism for surface glycoproteins trimmed by glycosidases encountered on the cell surface or during endocytosis and recycling and that cell surface glycoproteins may pass several rounds of de- and reglcosylation (15, 18, 20). However, as compared with recycling to glycosyltransferases, far less is known about postbiosynthetic trimming of cell sur-

\[ ^1 \text{The abbreviations used are: ER, endoplasmic reticulum; ConA, concanavalin A; dMM, 1-deoxymannojirimycin; DMEM, Dulbecco’s modified Eagle’s medium; DPPIV, dipeptidyl peptidase IV; Endo H, endo-β-N-acetylglucosaminidase H; HPAE, high performance anion exchange; HPLC, high performance liquid chromatography; PBB, phosphate-buffered saline; NHE-SS-biotin, sulfo-NHS-biotin; sulfo-NHS-biotin; 2-biotinamidoethyl-1,3-dithiopropionate; PNGase F, peptide F-N-(N-acetyl-β-glucosaminyl) asparagine amidase F; TIR, transferrin receptor; TGN, trans-Golgi network; PAGE, polyacrylamide gel electrophoresis.} \]
face glycoproteins by glycosidases. In an important study, Snider and Rogers (21) demonstrated that TIR and glycoproteins from the total cellular protein pool may return to mannosidase I in the early Golgi region in K 562 cells. This was shown in that cells were metabolically labeled with [3H]mannose in the presence of the reversible mannosidase I inhibitor 1-deoxymannojirimycin (dMM). Glycoproteins synthesized under these conditions retained immature oligomannosidic N-glycans during their initial transport through the Golgi complex. A return to early Golgi mannosidase I and a subsequent passage through peripheral Golgi elements was noticed by trimming of the immature oligomannosidic N-glycans and conversion to complex-type structures during reculture of cells in the absence of dMM. Employing this experimental strategy, a return to early Golgi mannosidase I was also shown for the cation-dependent and the cation-independent mannose 6-phosphate receptor in BW 5147 mouse lymphoma cells (12). It was calculated that glycoproteins recycle to mannosidase I at very low rates with half-times of 12 h for the total glycoprotein pool in K562 cells (19) and ~20 h for both mannose 6-phosphate receptors in BW 5147 cells (12). In none of these studies, however, was a return to Golgi mannosidase I examined by a sample of glycoproteins that had been covalently labeled on the cell surface beforehand. Hence, it could not be distinguished whether glycoproteins trimmed by early Golgi mannosidase I recycled from the cell surface or from other post-Golgi locations such as the TGN, secretory vesicles, endosomes, or lysosomes or even represented, in the case of the total glycoprotein pool, at least partly glycoproteins resident in the Golgi complex. Moreover, it remained unknown whether glycoproteins return to the cell surface after reentering the early Golgi. A recent study designed to examine the transport of TIR and DPPIV from the cell surface to compartments of the secretory pathway in HepG2 cells showed that oligomannosidic N-glycans of these two glycoproteins were not converted to complex structures during recycling (18). In accordance with this finding, Neefjes et al. (22) failed to detect conversion of oligomannosidic to complex-type glycans on recycling glycoproteins including TIR and HLA class II antigens in different cell lines, indicating that these proteins do not encounter mannosidase I in the early Golgi (i.e. at sites proximal to N-acetylgalactosaminyltransferase I). This enzyme is located in medial Golgi elements (23) and initiates the further processing of the oligomannosidic trimming intermediate Manα,GlcNAcβ to complex-type oligosaccharides. In a recent immunohistochemical study, however, mannosidase I was found to be less compartmentalized than previously assumed and was also detected in the medial and the trans-Golgi and, in some cell types, even in the TGN, in secretory vesicles, and in the plasma membrane (24). Hence, it became conceivable that cell surface glycoproteins could return to mannosidase I also at sites peripheral to N-acetylgalactosaminyltransferase I, resulting in trimming of oligomannosidic glycans without further processing to complex N-glycans. In that case, membrane glycoproteins retaining trimmed oligomannosidic glycans might return to the plasma membrane and become exposed on the cell surface.

In the present paper, this assumption was examined in two hepatoma cell lines and primary rat hepatocytes by a sample of cell surface glycoproteins of well defined function, i.e. the TIR, the serine peptidase DPPIV, the cell adhesion molecule gp110/cell-CAM105 (25) (a member of the Ig superfamily), and LIcadherin (26) (a member of the cadherin family of cell adhesion molecules).

Using a strategy based on the experimental design initially described by Snider and Rogers (21), in conjunction with covalent labeling of cell surface glycoproteins with NHS-SS-biotin, it was determined whether plasma membrane glycoproteins might be trimmed by mannosidases after transport to the cell surface. Moreover, to examine whether subsequently glycoproteins carrying trimmed oligomannosidic N-glycans are exposed on the cell surface, glycoproteins were allowed to encounter mannosidases and were, thereafter, isolated selectively from the cell surface. In this report, we present evidence that N-glycans of selected cell surface glycoproteins are postbiosynthetically trimmed from Manα,GlcNAcβ to Manα,GlcNAcβ. Following demannosylation, glycoproteins carrying trimmed oligomannosidic structures become exposed on the cell surface, indicating that demannosylation occurs at sites peripheral to N-acetylgalactosaminyltransferase I, either at the cell surface or during endocytosis and recycling back to the cell surface.

EXPERIMENTAL PROCEDURES

Materials—Materials were obtained from the following sources. Culture media were from Biochrom (Berlin, Germany), and other materials for tissue culture were from Falcon (Heidelberg, Germany) or Nunc (Wiesbaden, Germany). Tran35S-label containing l-[35S]methionine and l-[35S]cysteine (specific radioactivity 40.29 TBq/mmol) was from ICN (Meckenheim, Germany). Protein A-Sepharose and Sepharose-4B were from Pharmacia (Freiburg, Germany). dMM was a gift of Dr. Schüller (Bayer AG, Wuppertal, Germany). Decanoyl-N-methylglucamide was from Calbiochem (Frankfurt, Germany). All glycosidases were purchased from Boehringer Mannheim (Mannheim, Germany). Sulfosuccinimidyl-2-(biotinamido)ethyl-1,3-dithiopropionate (NHS-SS-biotin) and streptavidin were from Vector (Palo Alto, CA). The authentic oligosaccharides Manα,GlcNAcβ were from Oxford Glycosystems (Krefeld, Germany). The monoclonal antibodies De 13.4, directed against rat DPPIV, and Lo 47.2, directed against rat LI-cadherin, have been described previously (27, 28). The rabbit polyclonal antibody directed against rat LI-cadherin, have been described previously (27, 28). The monoclonal antibody 188 A2, recognizing rat TIR, was a gift from Dr. D. C. Hixon (Brown University). The rabbit polyclonal antibody directed against rat gp110/cell-CAM105 was that described previously (25). As-cites fluid containing monoclonal antibody HBB3/775 (29) directed against human DPPIV was kindly provided by Dr. H. P. Hauri (Bio-center of the University of Basel, Switzerland).

Cell Culture—The rat hepatoma cell line MH 7777 derived from Morris hepatoma 7777 was described previously (30). Primary rat hepatocytes were isolated according to the procedure of Seglen (31) at a minimum viability of 80–90% as determined by trypan blue exclusion. Cells were seeded on collagen I-coated dishes (80-mm diameter). Hepatocytes and hepatoma cells were maintained in DMEM supplemented with penicillin (50 units/ml), streptomycin (50 μg/ml), insulin (0.08 milliunits/ml), dexamethasone (1 μM), and 10% (v/v) complement-inactivated horse serum in a humidified atmosphere with 5% CO2 at 37 °C as described (32). HepG2 cells (33) obtained from ATCC (Rockville, MD) were cultured as described (18).

Metabolic Labeling of Cells—Confluent layers of cells were trypsinized and seeded on collagen I-coated dishes (60-mm diameter). Cells were allowed to adhere overnight. For labeling in the polypeptide moiety, the monolayers were washed and preincubated for 60 min in DMEM without l-methionine/l-cysteine. The cells were pulse-labeled for 4 h with l-[35S]methionine/l-[35S]cysteine (5.5 MBq/× 106 cells) and then chased for 3 h in DMEM with 1 mM unlabeled l-methionine/l-cysteine. When used, 3 mM dMM was present during the preincubation, pulse, and chase periods. For labeling of glycoproteins in the oligosaccharide moiety, cells were washed, preincubated for 60 min in the presence of 3 mM dMM, and then labeled for 8 h with l-[2,6-3H]mannose (14.8 MMBq/× 106 cells) in glucose-free DMEM supplemented with 3 mM dMM, 5 mM Na-galactose, and 10 mM pyruvate. Cells were then washed and chased for 3 h in the presence of 3 mM dMM.

Labeling of Cell Surface Glycoproteins with NHS-SS-biotin—Cell surface glycoproteins were labeled with NHS-SS-biotin essentially as described (18). After cooling on ice, cells were washed four times with ice-cold PBS/
Characterization of the N-glycosylation of LI-cadherin, DPPIV, gp110/cell-CAM105, and TFR by digestion with Endo H and PNGase F.

MH 7777 cells were metabolically labeled with L-[35S]methionine/L-[35S]cysteine for 4 h followed by a 3-h chase, either in the presence or absence of 3 mM dMM. Cells were detergent-extracted, and LI-cadherin, DPPIV, gp110/cell-CAM105, and TFR were immunoadsorbed as described under “Experimental Procedures.” Immunoadsorbed glycoproteins from untreated cells were divided into three aliquots and were either mock-incubated (lanes 4, 9, 14, and 19) or treated with Endo H (lanes 5, 10, 15, and 20).

Immunoaffinity Absorption and SDS-Polyacrylamide Gel Electrophoresis—The following steps were carried out at 4 °C. After washing with PBS/Ca2+/Mg2+ containing 0.1% (w/v) bovine serum albumin and 50 mM Tris/HCl, pH 7.8, 0.1% (w/v) SDS, 0.1% (w/v) sodium deoxycholate, 0.5% Nonidet P-40, and 0.5% Nonidet P-40, and finally with PBS. Immunoadsorption absorption of gp110/cell-CAM105 with a polyclonal antibody was performed as described previously (25). Immunocomplexes bound to protein A-Sepharose were pelleted by centrifugation and discarded. For immunoaffinity absorption, 20 µg of mouse monoclonal antibody (De 13.4, Lo 47.2, or 188 A2) coupled to 8 mg of protein was added to the supernatant and rotated end-over-end for 4 h. Immunocomplexes bound to protein A-Sepharose were pelleted by centrifugation and washed twice with washing buffer B (500 mM NaCl, 10 mM Tris/Cl, pH 8.0, 1 mM CaCl2, 1 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 1% (w/v) sodium deoxycholate, 0.5% Nonidet P-40), and streptavidin-agarose was added. Before the absorption of gp110/cell-CAM105 with a polyclonal antibody was performed as described previously (25). Immunocomplexes bound to protein A-Sepharose were pelleted by centrifugation and washed twice with washing buffer B (500 mM NaCl, 10 mM Tris/Cl, pH 8.0, 1 mM CaCl2, 1 mM MgCl2, 1 mM phenylmethylsulfonyl fluoride, 1% (w/v) sodium deoxycholate, 0.5% Nonidet P-40), and the mixture was shaken at 4 °C for 4 h. After washing five times in lysis buffer A, biotinylated proteins were eluted by boiling for 3 min in 100 µl of 0.4% SDS, 5% mercaptoethanol. Biotinylated DPPIV was isolated as described previously (18). Briefly, the protein was immunoadsorbed from detergent extracts and eluted from protein A-Sepharose with 3x KSCN, 0.5% Nonidet P-40, and streptavidin-agarose was added. The suspension was shaken at 4 °C for 4 h; washed four times in 50 mM Tris/Cl, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1% (w/v) sodium deoxycholate, 0.5% Nonidet P-40, and 0.5% Nonidet P-40, and treated with 10 milliunits of Endo H from Streptomyces pallidus (EC 3.2.1.96) in 50 mM sodium phosphate, pH 6.0, 0.1% Nonidet P-40 for 20 h at 37 °C. Treatment with 10 milliunits of Endo H from Streptomyces pallidus (EC 3.2.1.96) was performed in 50 mM sodium phosphate, pH 6.0, 0.1% Nonidet P-40 for 20 h at 37 °C.

Preparation of High Mannose-type Oligosaccharide Alditols—High mannose-type oligosaccharides were prepared from immunoadsorbed glycoproteins or from biotinylated proteins eluted from streptavidin-agarose as described previously (36, 37). Briefly, oligosaccharides were released from glycoproteins by incubation with Endo H and then separated from proteins by ultrafiltration through Centricon-10 microcentrators. The filtrates were desalted by mixed bed ion exchange chromatography on a column (0.6 × 10 cm) containing 500 µl of Dowex ylmannopyranoside in lysis buffer B. Biotinylated cell surface glycoproteins were then isolated from this cellular glycoprotein fraction by binding to streptavidin-agarose. Streptavidin-agarose (100 µg/ml of protein) was added, and the mixture was shaken at 4 °C for 4 h. After washing five times in lysis buffer A, biotinylated proteins were eluted by boiling for 3 min in 100 µl of 0.4% SDS, 5% mercaptoethanol. Biotinylated DPPIV was isolated as described previously (18). Briefly, the protein was immunoadsorbed from detergent extracts and eluted from protein A-Sepharose with 3x KSCN, 0.5% Nonidet P-40, and streptavidin-agarose was added. The suspension was shaken at 4 °C for 4 h; washed four times in 50 mM Tris/Cl, pH 8.0, 500 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1% (w/v) sodium deoxycholate, 0.5% Nonidet P-40, and 0.5% Nonidet P-40, and treated with 10 milliunits of Endo H from Streptomyces pallidus (EC 3.2.1.96) was performed in 50 mM sodium phosphate, pH 6.0, 0.1% Nonidet P-40 for 20 h at 37 °C.
Demannosylation of Cell Surface Glycoproteins

AG50W-X12 and 500 μl of Dowex AG3-X4. After washing the column with four bed volumes of water, the combined filtrates were dried by evaporation. The oligosaccharides were converted to their correspond-

oligosaccharide alditols by radiofluorography.

To characterize the N-glycosylation of LI-cadherin, DPPIV, gp110/cell-CAM105, and TfR—

FIG. 2. The molecular mass of DPPIV and gp110/cell-CAM105 decreases during reculture. MH 7777 cells were metabolically la-

beled with L-[35S]methionine/L-[35S]cysteine for 4 h and then chased, both in the presence of 3 mM dMM. Thereafter, cells were recultured for different times either in the absence (A) or in the presence of 3 mM dMM (B). DPPIV, gp110/cell-CAM105, LI-cadherin, and TfR were immuno-

adsorbed from detergent extracts, separated by SDS-PAGE, and visu-

alized by radiofluorography.

HPLC Separation of Oligosaccharide Alditols—HPLC separation of the oligosaccharide alditols was performed as described previously (37) using a Bio-Rad model 700 chromatography workstation equipped with two Bischoff (Leernburg, Germany) model 2200 pumps, a Knauer (Ber-

lin, Germany) dynamic mixing chamber, and a Shimadzu fluorescence HPLC monitor RF-535. Briefly, oligosaccharide alditols were separated on two Spherisorb-NH2 columns (4.6 × 250 mm, 5 μm; Bischoff) equilibrated with a mixture containing 65% acetonitrile and 35% 15 mM sodium dihydrogenphosphate, pH 5.2, at a flow rate of 1.5 ml/min and were eluted by decreasing the proportion of acetonitrile to 45% within 100 min. Fractions of 1 ml were collected and assayed for radioactivity by liquid scintillation counting using a Tri-Carb 1900 CA liquid scintil-

lation analyzer (Canberra Packard). A mixture of glucose oligomers (n = 1–20), fluorescence-labeled by reductive amination with 8-amino-

2-naphthol, was used as an internal standard. Columns were calibrated with authentic oligosaccharide alditols Man5–9GlcNAcOH, prepared from HA2 subunits of influenza virus hemagglutinin by Endo H treatment and NaBH4 reduction after metabolic labeling with [2–3H]mannose (38).

High Performance Anion Exchange (HPAE) Separation of Oligosaccharides—In some experiments, oligosaccharides were released from glycoproteins with PNGase F according to Anumula and Taylor (39) with some modifications and separated by HPAE chromatography. Briefly, biotinylated proteins were eluted from streptavidin-agarose with 0.4% SDS, 5% mercaptoethanol by boiling for 3 min. Eluted pro-

teins were concentrated in a Centricon-10 microconcentrator and sus-
pended in 0.5% NH4HCO3, pH 8.0. Trypsin (2% by mass) was added to the samples from a fresh stock solution of 20 μg/ml in 0.5% NH4HCO3 and incubated at 37 °C for 16 h. Trypsin was inactivated by heating at 100 °C for 5 min. After cooling, the pH was readjusted to 8.5, and PNGase F (100 units/ml) was added and incubated at 37 °C for 16 h. Oligosaccharides were purified by passing the samples through a col-

umn containing AG3-X4 in the bottom layer and AG50W-X12 in the top layer. After washing with two bed volumes of water, the filtrates were dried by evaporation. Oligosaccharides were separated using a Dionex (Sunnyvale, CA) DX-300 system and a CarboPac PA-100 (4 × 250 mm) in series with a CarboPac PA-100 guard column as described (18). Columns were calibrated with authentic oligosaccharides Man5–9GlcNAcOH-

RESULTS

To examine whether cell surface glycoproteins lose mannose residues from their oligomannosidic N-glycans during their life span, the following protocol was employed. Cells were metabolically labeled with either L-[35S]methionine/L-[35S]cysteine or D-[2-3H]mannose in the presence of the Golgi-mannosidase I inhibitor dMM. As a consequence, the N-glycans of newly syn-

thesized glycoproteins normally processed to the complex type retain high mannose-type structures. After a chase period suf-

ficient to allow the passage of the newly synthesized glycopro-

teins through the Golgi region to the cell surface, the inhibition of mannosidase I was reversed by washout of the inhibitor. Cells were then recultured for different times, and the plasma membrane glycoproteins DPPIV, LI-cadherin, TfR, and gp110/cell-

CAM105 were isolated and analyzed for trimming of their oligomannosidic glycans.

Characterization of the N-Glycosylation of LI-cadherin, DP-

PIV, gp110/cell-CAM105, and TfR—To characterize the N-

glycans of LI-cadherin, DPPIV, gp110/cell-CAM105, and TfR generated either in the absence or in the presence of dMM, MH 7777 cells were pulse-labeled with L-[35S]methionine/L-[35S]cysteine or D-[2,6-3H]mannose and NaBH4 reduction after metabolic labeling with D-[2-3H]man-

nose (40). The doublet observed for the TfR has been reported for a wide variety of cell types and most likely represents the phosphorylated and the nonphos-

phorylated form of the receptor (41). Glycoproteins synthesized

on two Spherisorb-NH2 columns (4.6 × 250 mm, 5 μm; Bischoff) equilibrated with a mixture containing 65% acetonitrile and 35% 15 mM sodium dihydrogenphosphate, pH 5.2, at a flow rate of 1.5 ml/min and were eluted by decreasing the proportion of acetonitrile to 45% within 100 min. Fractions of 1 ml were collected and assayed for radioactivity by liquid scintillation counting using a Tri-Carb 1900 CA liquid scintillation analyzer (Canberra Packard). A mixture of glucose oligomers (n = 1–20), fluorescence-labeled by reductive amination with 8-amino-

2-naphthol, was used as an internal standard. Columns were calibrated with authentic oligosaccharide alditols Man5–9GlcNAcOH, prepared from HA2 subunits of influenza virus hemagglutinin by Endo H treatment and NaBH4 reduction after metabolic labeling with [2–3H]mannose (38).
in the absence of dMM had a molecular mass of approximately 120 kDa (LI-cadherin, lane 1), 110 kDa (DPP IV, lane 6), 110 kDa (gp 110/cell-CAM105, lane 11), and 94/90 kDa (TfR, lane 16), in accordance with previous reports (25, 26, 32). For characterization of their N-linked glycans, the immunoadsorbed glycoproteins were digested with Endo H or PNGase F. Digestion of the glycoproteins synthesized in the absence of dMM with Endo H either did not reduce the molecular mass or reduced it only slightly (LI-cadherin (lane 2), DPP IV (lane 7), gp110/cell-CAM105 (lane 12), TIR (lane 17)), indicating that the majority of the N-linked glycans of these glycoproteins is of the complex type. By contrast, digestion with Endo H reduced the molecular mass of the four glycoproteins obtained from dMM-treated cells from approximately 110 to 100 kDa (LI-cadherin, lane 5), from 100 to 88 kDa (DPP IV, lane 10), from 84/78 to 58/48 kDa (gp110/cell-CAM105, lane 15), and from 92/88 to 84/80 kDa (TIR, lane 20), respectively. Polypeptides of the same size were obtained when the glycoproteins generated in the absence of dMM were digested with PNGase F (lanes 3, 8, 13, and 18). These results show that the N-linked glycans of the four glycoproteins when synthesized in the presence of dMM are at least predominantly of the complex type.

The Molecular Mass of the High Mannose-type Glycoforms of DPP IV and gp110/cell-CAM105 Decreases during Reculture—Loss of mannose residues from the nonreducing end of oligomannosidic N-glycans during the life span of a glycoprotein should result in a decrease in the molecular mass. This was studied by monitoring the molecular mass of DPP IV and gp110/cell-CAM105 during a reculture period of up to 100 h (DPP IV, L1-cadherin, and gp110/cell-CAM105) or 72 h (TIR). MH 7777 cells were radiolabeled for 4 h with L-[35S]methionine/L-[35S]cysteine in the presence of dMM and were further chased for 3 h in the presence of the inhibitor to generate the high mannose glycoforms of the four glycoproteins.
Demannosylation of Cell Surface Glycoproteins

Fig. 4. High mannose-type N-glycans of DPPIV are postbiosynthetically trimmed from Man$_{8-9}$GlcNAc$_2$ to Man$_{6}$GlcNAc$_2$. MH 7777 cells were metabolically labeled with $\text{D-}[2,6-3\text{H}]$mannose for 8 h followed by a 3-h chase, both in the presence of 3 mM dMM. Cells were then recultured either in the absence or presence of 3 mM dMM. DPPIV and TIR were immunoadsorbed from the detergent extracts, and oligosaccharides were released from the glycoproteins by digestion with Endo H. Oligosaccharides were converted to their corresponding oligosaccharide alditols by reduction with NaBH$_4$, and were analyzed by HPLC as detailed under “Experimental Procedures.” Separation of the high mannose oligosaccharide alditols of DPPIV (A–C) and TIR (D and E), isolated immediately after the chase (0 h; A and D), after 70 h (E), or after 85 h (B and C) of reculture is shown. Reculture was performed either in the absence (A, B, D, and E) or in the presence of dMM (C). Open arrowheads indicate the elution positions of the authentic oligosaccharide alditols, Man$_8$GlcNAcOH (5), Man$_9$GlcNAcOH (6), Man$_{10}$GlcNAcOH (7), Man$_{11}$GlcNAcOH (8), and Man$_{12}$GlcNAcOH (9). For comparison, the eluting positions of glucose-oligoriter units, fluorescence-labeled with 8-amino-2-naphthol, are indicated by black arrowheads.

As shown in Fig. 1. After washout of the inhibitor, cells were recultured in the absence of dMM. DPPIV, gp110/cell-CAM105, LI-cadherin, and TIR were immunoadsorbed at different times and were analyzed by SDS-PAGE and radiofluorography (Fig. 2A). A distinct decrease in the molecular mass was noted for DPPIV and gp110/cell-CAM105. DPPIV obtained immediately after the chase (0 h) had a molecular mass of approximately 100 kDa, which shifted to 95 kDa during reculture. The decrease in the molecular mass of gp110/cell-CAM105 was more prominent, probably due to its high carbohydrate content of approximately 50% (25). No decrease in the molecular mass was detectable for the high mannose-type glycoforms of LI-cadherin and TIR. Immunoprecipitates of DPPIV obtained during reculture contained an additional faint polypeptide band with a molecular mass of approximately 110 kDa that corresponded to the molecular mass of mature complex-type DPPIV and might represent some DPPIV processed to the complex-type glycoform. For comparison, the behavior of the complex-type glycoforms of the four glycoproteins, labeled and chased in the absence of dMM, was analyzed. In contrast to the high mannose-type glycoforms of DPPIV and gp110/cell-CAM105, none of the four glycoproteins with complex-type N-glycans exhibited a decrease in the molecular mass during reculture (shown for DPPIV and gp110/cell-CAM105 in Fig. 3, lanes 9 and 10). To exclude the possibility that the observed reduction in the molecular mass of the high mannose-type glycoforms of DPPIV and gp110/cell-CAM105 is a particular feature of transformed MH 7777 cells, the same experiments were performed with primary cultured rat hepatocytes. In accordance with the results obtained in MH 7777 cells, the molecular mass of the high mannose glycoforms of DPPIV and gp110/cell-CAM105 decreased after 70 h of reculture (Fig. 3, lanes 5 and 6), and no decrease in the molecular mass was observed for the complex-type glycoforms of the two glycoproteins (Fig. 3, lanes 13 and 14). Whereas in MH 7777 cells the two isoforms of the oligomannosidic gp110/cell-CAM105 were labeled to a similar extent, in hepatocytes the 78-kDa isoform prevailed (Fig. 3, gp110, lanes 1 and 5). The 84-kDa isoform was only faintly detectable and could not be assessed. In accord with the results shown in Fig. 1 (lanes 11 and 12) the complex-type glycoforms of the two isoforms of gp110/cell-CAM105 generated in the absence of dMM migrated as a very broad band and could not be discriminated (Fig. 3, gp 110, lanes 9–16). Taken together, these results indicate that the high mannose glycoforms of DPPIV and of gp110/cell-CAM105 undergo a trimming process most likely by loss of mannose residues from the nonreducing end of the oligomannosidic N-glycans.

The Decrease in the Molecular Mass of the High Mannose Glycoforms of DPPIV and gp110/cell-CAM105 Is Due to Glycer trimm inig and Not to Limited Proteolysis—To rule out that the decrease in the molecular mass of the high mannose-type glycoforms of DPPIV and gp110/cell-CAM105 during reculture is caused by limited proteolysis of the polypeptide backbone, the sizes of the deglycosylated polypeptides were compared immediately after the chase and after 70 h of reculture. MH 7777 cells and hepatocytes were radiolabeled with $\text{L-}[35\text{S}]$methionine/$\text{L-}[35\text{S}]$cysteine and chased, both in the presence of dMM, and were recultured after washout of the inhibitor. DPPIV and gp110/cell-CAM105 immunoadsorbed immediately after the chase (Fig. 3, lanes 1 and 5), and after 70 h of reculture (Fig. 3, lanes 2 and 6) they were digested with Endo H. Digestion with the endoglycosidase converted both forms of the glycoproteins (DPPIV: 0 h, 100 kDa; 70 h, 95 kDa; gp110/cell-CAM105: 0 h, 84/78 kDa; 70 h, 78/72 kDa) to polypeptides exhibiting the same molecular mass of approximately 88 kDa as for DPPIV and 58/48 kDa as for gp110/cell-CAM105 (Fig. 3, lanes 3 and 4 (MH 7777 cells) and lanes 7 and 8 (hepatocytes)). The additional polypeptide band of 110 kDa present in immunoprecipitates of DPPIV obtained from MH 7777 cells after reculture (Fig. 3, DPPIV, lanes 2 and 4) was resistant to Endo H and hence most likely reflects reprocessing of some DPPIV to the complex-type glycoform as mentioned above. These experi-
ments demonstrate that the decrease in the molecular mass does not result from limited proteolysis but reflects trimming of the oligomannosidic N-glycans.

High Mannose-type N-Glycans of Surface Glycoproteins Are Trimmerd from Man$_{8-9}$GlcNAc$_2$ to Man$_5$GlcNAc$_2$ after Transport to the Cell Surface—To characterize the mannone trimming of the high mannose N-glycans of DPPIV and gp110/cell-CAM105, in particular to determine the end product of the trimming, oligosaccharides were analyzed immediately after washout of dMM, i.e. after the chase period, and after reculture using the following protocol. MH 7777 cells were radiolabeled with 2,6-$^3$H]mannose and then chased, both in the presence of the inhibitor (Fig. 2). To examine whether trimmed glycoproteins are exposed at the cell surface at the end of the reculture period, i.e. after demannosylation occurred, glycoproteins were labeled with NHS-SS-biotin at the cell surface immediately after the reculture period (B). Biotinylated proteins were isolated selectively and analyzed for mannone trimming.

Demannosylation of Cell Surface Glycoproteins

Fig. 5. Schematic representation of the experimental strategy for analyzing postbiosynthetic mannone trimming of biotin-labeled cell surface glycoproteins. To determine whether the oligomannosidic glycans of glycoproteins are trimmed after their transport to the cell surface, glycoproteins were additionally labeled with NHS-SS-biotin at the cell surface prior to the reculture period (A). To examine whether trimmed glycoproteins are exposed at the cell surface at the end of the reculture period, i.e. after demannosylation occurred, glycoproteins were labeled with NHS-SS-biotin at the cell surface immediately after the reculture period (B). Biotinylated proteins were isolated selectively and analyzed for mannone trimming.

The inhibited effect of dMM on the high mannose-type N-glycans was detectable for the TIR during reculture of cells (Fig. 4E). To examine whether DPPIV being trimmed from Man$_{8-9}$GlcNAc$_2$ to Man$_5$GlcNAc$_2$ derives in fact from the plasma membrane, demannosylation of DPPIV was examined after covalent labeling with NHS-SS-biotin at the cell surface, as schematized in Fig. 5A. This approach allows us to unambiguously discriminate between cell surface proteins and proteins localized in intracellular compartments. Cells were radiolabeled and chased as above and then surface-labeled at 4°C with NHS-SS-biotin prior to reculture. Biotin-labeled surface DPPIV was isolated by immunoadsorption in conjunction with affinity chromatography on streptavidin-agarose. HPAE-separation of the high mannose-type oligosaccharides released from biotinylated DPPIV by PNGase F revealed the same shift from Man$_{8-9}$GlcNAc$_2$ to Man$_5$GlcNAc$_2$ structures (Fig. 6, A and B) that was observed for total cellular DPPIV (Fig. 4, A and B). This clearly demonstrates that trimming of high mannose-type N-glycans affects DPPIV molecules that have exited the secretory pathway and were exposed at the cell surface. Demannosylation was also observed for the bulk of cell surface glycoproteins in MH7777 cells (Fig. 6, C and D), showing that this process is not restricted to DPPIV. In an attempt to find out whether a class I or class II mannosidase is involved that is known to be inhibited by dMM or swainsonine, respectively (for reviews, see Refs. 4 and 5), reculture in the presence of either one of the inhibitors. When cells were recultured in the presence of dMM, trimming of DPPIV was completely blocked (Fig. 4C), which is in agreement with previous reports (12, 21). The inhibitory effect of dMM on demannosylation could also be demonstrated by the finding that the molecular mass of DPPIV and gp110/cell-CAM105 as analyzed by SDS-PAGE did not decrease during reculture in the presence of the inhibitor (Fig. 2B). In contrast, in the presence of swainsonine (3 μg/ml) the high mannose-type N-glycans of DPPIV were trimmed to the same extent as in the absence of the inhibitor (not shown). In summary, these results show that high mannose-type glycans of cell surface glycoproteins...
Demannosylation of Cell Surface Glycoproteins

In two previous studies on the recycling of cell surface glycoproteins in HepG2 cells, no reconversion of oligomannosidic to complex-type glycans could be detected for DPPIV, TIR, and HLA class I antigens (18, 22), indicating that these proteins do not recycle to the cis-Golgi in this cell line. To find out whether, nevertheless, plasma membrane glycoproteins might be subject to mannoside trimming, demannosylation was also examined in HepG2 cells. As is shown in Fig. 7, N-glycans of cell surface DPPIV were trimmed from Man$_{8-9}$GlcNAc$_2$ to Man$_{5-7}$GlcNAc$_2$ essentially as found for surface DPPIV in MH 7777 cells. In conjunction with previous reports (18, 22), these data show that high mannoside-type glycans of DPPIV can be trimmed without further processing to complex structures.

**Kinetics of Postbiosynthetic Mannose Trimming**—A quantitative analysis of the kinetics of postbiosynthetic demannosylation was performed for total cell surface proteins in HepG2 cells. In these experiments, high mannoside-type glycans of cell surface-labeled glycoproteins were analyzed after different times of reculture. Data from HPAE fractionations (shown in Fig. 8A) were quantitated by totaling the radioactivity in each peak and by correcting for the number of mannose residues. In Fig. 8B, the radioactivity of each oligosaccharide species, expressed as a percentage of the total oligosaccharides recovered, is plotted versus time of reculture. As can be seen from the hydrolysis curves, Man$_{8-9}$GlcNAc$_2$ is converted to Man$_{6}$GlcNAc$_2$, Man$_{7}$GlcNAc$_2$, and Man$_{8}$GlcNAc$_2$ as trimming intermediates. The extent of demannosylation at each time point, defined as the conversion of Man$_{8-9}$GlcNAc$_2$ to Man$_{6}$GlcNAc$_2$, was calculated based on the amount of Man$_{8-9}$GlcNAc$_2$ obtained immediately after the chase and plotted versus time. As can be seen in Fig. 8C, the extent of demannosylation increased during the first 24 h and then reached a plateau. With the assumption of first order kinetics, demannosylation followed the equation $D_t = D_0 e^{-kt}$, where $D_t$ is the extent of demannosylation at the time $t = x$. The constant $k$ can be determined from this equation as the negative slope of a plot of ln($D_t$) over the time (Fig. 8D). For total cell surface glycoproteins of HepG2 cells, a half-time of 6.7 h was calculated for demannosylation according to the equation $t_{1/2} = \ln 2/k$.

**Trimmed Glycoproteins Can Be Isolated from the Cell Surface after Postbiosynthetic Demannosylation**—Trimming of plasma membrane glycoproteins after their transport to the cell surface could occur either at the cell surface or after endocytosis in intracellular compartments. In the latter case, it could not be distinguished on the basis of the previous experiments whether trimmed glycoproteins remain in intracellular compartments or return to the cell surface. To examine whether trimmed glycoproteins are exposed at the cell surface, mannoside trimming was analyzed for glycoproteins isolated selectively from the cell surface at the end of the reculture period. To do so, MH 7777 cells were radiolabeled and recultured as in

![Image](https://via.placeholder.com/150)
Man8–9GlcNAc2 species to Man5–7GlcNAc2 with Man5GlcNAc2. The radioactivity of each oligosaccharide species was expressed as a percentage of the total oligosaccharides recovered and plotted versus time. A, Man9GlcNAc2; B, Man8GlcNAc2; C, Man7GlcNAc2; D, Man6GlcNAc2. The extent of demannosylation at each time point, defined as the conversion of Man8–9GlcNAc2 to Man5–7GlcNAc2, was calculated based on the amount of Man8–9GlcNAc2 obtained immediately after the chase. D, plot of ln(A – A0) versus time. A linear regression leads to k = 0.1028 (R² = 0.969).

The experiments shown in Fig. 4. After reculture, proteins exposed at the cell surface were labeled with biotin immediately prior to harvesting and solubilization of cells and were then analyzed for mannose trimming, as schematized in Fig. 5B. HPLC separation of the high mannose-type glycans released from the biotinylated glycoproteins revealed that surface glycoproteins isolated after reculture carried mainly Man6GlcNAc2 and minor amounts of Man6,GlcNAc2 (Fig. 9B). In accordance with the results shown in Figs. 4 and 6, glycoproteins isolated immediately after the chase carried mainly Man6,GlcNAc2 (Fig. 9A). These results demonstrate that after demannosylation had occurred, trimmed glycoproteins were exposed at the cell surface. This provides additional evidence that demannosylation can occur without further processing to complex structures.

DISCUSSION

Two major conclusions can be drawn from the results of the present study. First, after exit from the secretory pathway and transport to the cell surface, selected cell surface glycoproteins undergo trimming of their oligomannosidic N-glycans by α-mannosidase(s) sensitive to dMM. The observed demannosylation of cell surface glycoproteins results in the conversion of Man6,GlcNAc2 species to Man6,GlcNAc2 with Man6,GlcNAc2 being the major product. Second, subsequent to demannosylation, trimmed glycoproteins are exposed at least partly at the cell surface. Hence, it is likely that demannosylation of glycoproteins occurs at sites peripheral to N-acetylgalactosaminyltransferase I either at the cell surface or during endocytosis and recycling back to the cell surface. The data clearly demonstrate that modification of plasma membrane glycoproteins by trimming of their oligomannosidic N-glycans is not restricted to biosynthesis. These conclusions are based on the following evidence.

Analysis of the size of high mannose-type N-glycans of cell surface glycoproteins demonstrated that during reculture of MFI 7777 cells and HepG2 cells Man6–9GlcNAc2 species were trimmed to Man6–9GlcNAc2 with Man6–9GlcNAc2 and Man6–9GlcNAc2 formed as trimming intermediates. Mannose trimming has also been observed for the cation-dependent and the cation-independent mannose 6-phosphate receptors in BW 5147 mouse lymphoma cells (12) and for TIR and for the total cellular glycoprotein pool in K562 cells (21). However, in both of these studies, mannose trimming was not examined by a sample of glycoproteins after previous labeling on the cell surface. Therefore, it remained unknown whether glycoproteins modified by mannosidase I were derived from the plasma membrane or from intracellular compartments. In comparison, by analyzing membrane glycoproteins that had been labeled with biotin at the cell surface prior to reculture of cells (schematized in Fig. 5A), we were able to examine selectively the fate of cell surface glycoproteins. The results of these experiments unequivocally demonstrate that demannosylation of oligomannosidic N-glycans affects glycoproteins after they have been delivered to the cell surface. Moreover, previous studies did not address the question of whether trimmed glycoproteins become exposed at the cell surface. By analyzing glycoproteins that were allowed to encounter mannosidases and were thereafter isolated selectively from the cell surface (schematized in Fig. 5B), it is clearly shown in the present study that subsequent to demannosylation trimmed glycoproteins are present at the cell surface.

Which α-mannosidase is involved in the postbiosynthetic mannose trimming remains to be established. Based on the finding that postbiosynthetic demannosylation could be inhib-
Many N-linked oligosaccharides are trimmed during maturation of cell surface glycoproteins, a process known as postbiosynthetic trimming or recycling. This phenomenon is crucial for the proper function of glycoproteins, as it allows for the adjustment of glycosylation depending on cellular needs. Three enzymes, Golgi alpha-mannosidase I, class I N-acetylglucosaminyltransferase I, and class II N-acetylglucosaminyltransferase I, are involved in this process. These enzymes can trim from 1 to 4 mannose residues from a glycoprotein's N-linked oligosaccharide.

Golgi alpha-mannosidase I is known to cleave Man4–9GlcNAc substrates to Man3GlcNAc and is inhibited by dithiothreitol (DTT), a reducing agent. The enzyme is also sensitive to dithioerythritol (DTE), another reducing agent. However, other reducing agents such as dithiooxam (DIO), dithiothreitol (DTT), and dithiothreitol (DTE) are not effective at inhibiting the enzyme.

Class I N-acetylglucosaminyltransferase I provides a high-mannose oligosaccharide with 9 mannose residues as a substrate for Golgi alpha-mannosidase I. This enzyme is also sensitive to reducing agents such as DTT and DTE.

Class II N-acetylglucosaminyltransferase I is involved in the trimming process. This enzyme is known to be inhibited by reducing agents such as DTT and DTE.

The trimming process is thought to occur in the medial Golgi or trans-Golgi network, where the enzyme is known to be localized. The trimming process is also thought to occur in endocytic compartments, such as in endosomes and lysosomes.

Demannosylation of cell surface glycoproteins could occur at the cell surface, during passage through endocytic compartments, or after return to the Golgi complex. Trimming of high mannose-type N-glycans at the cell surface seems feasible, since in a recent immunohistochemical study Golgi alpha-mannosidase I has been detected at the cell surface of enterocytes, pancreatic acinar cells, and goblet cells. However, it is unknown whether the mannosidase is enzymatically active at this location. Moreover, a rat sperm alpha/1,2,3,6-mannosidase has been reported to be an intrinsic plasma membrane component that is enzymatically active when assayed in sperm plasma membranes and intact spermatozoa, respectively.

The subcellular site of postbiosynthetic mannose trimming is unknown. Demannosylation of glycoproteins could occur at the cell surface, during passage through endocytic compartments, or even after return to compartments of the secretory pathway. Trimming of high mannose-type N-glycans at the cell surface seems feasible, since in a recent immunohistochemical study Golgi alpha-mannosidase I has been detected at the cell surface of enterocytes, pancreatic acinar cells, and goblet cells. However, it is unknown whether the mannosidase is enzymatically active at this location. Moreover, a rat sperm alpha/1,2,3,6-mannosidase has been reported to be an intrinsic plasma membrane component that is enzymatically active when assayed in sperm plasma membranes and intact spermatozoa, respectively.

As a second possibility, postbiosynthetic mannose trimming could occur in endocytic compartments during endocytosis and recycling of glycoproteins back to the cell surface. It has been shown that rat liver alpha/1,2,3,6-mannosidase activity is enriched in endosomal fractions (47). Although, as discussed above, this enzyme is probably not involved with respect to its substrate specificity, other mannosidases might also be present in endosomes. Third, since several plasma membrane glycoproteins have been shown to return to the Golgi complex and the TGN (12–21), demannosylation could also occur after the return of cell surface glycoproteins to these compartments. From our data, the possibility cannot be excluded that some glycoproteins return to the processing mannosidases in the cis-Golgi, since a small fraction of the oligomannosidic glycoform of DPPV was processed to the complex form during recycling in MH 7777 cells (Fig. 2A), whereas no reversion to the complex glycoform could be detected for surface DPPV in HepG2 cells (18). From these observations, combined with the fact that surface DPPV is trimmed from Man4-9GlcNAc2 species to Man3-6GlcNAc in MH 7777 cells as well as in HepG2 cells, we conclude that most of the DPPV is trimmed without further processing to the complex glycoform. This explanation is supported by the observation that trimmed glycoproteins can be found on the cell surface and is consistent with the recent finding of Velasco et al. (24) that Golgi mannosidase I previously assumed to reside specifically in the cis-Golgi (48) is less compartmentalized. In this study, the enzyme was primarily detected in the medial- and trans-Golgi cisternae, and in some cell types it was also localized in the TGN and even in secretory vesicles. Therefore, in case cell surface glycoproteins return to the Golgi at sites peripheral to N-acetylglucosaminyltransferase I, an enzyme that is localized in medial Golgi elements (23) and initiates the synthesis of hybrid and complex oligosaccharides, it seems feasible that high mannose-type N-glycans of recycling glycoproteins might be trimmed by Golgi mannosidase I without being further processed to complex structures.

Comparison of DPPV, TFR, gp110/cell-CAM105, and LI-cadherin showed that postbiosynthetic trimming did not affect each of the four glycoproteins. This may be due to differences in the kinetics or routes of internalization and recycling or due to a different susceptibility of the oligomannosidic glycans to trimming mannosidases. Which of these different mechanisms is responsible remains to be established. Although distinct proteins escape demannosylation, this process seems to affect a large number of cell surface glycoproteins, since it could be demonstrated for the bulk of plasma membrane glycoproteins.
in MH 7777 cells as well as in HepG2 cells. A quantitative analysis of the time course of demannosylation revealed that this process obeyed first order kinetics with a calculated half-time of 6.7 h as determined for total cell surface glycoproteins in HepG2 cells. The process of demannosylation occurs distinctly faster than degradation of [35S]methionine-labeled total in HepG2 cells. The process of demannosylation occurs distinctly faster than degradation of [35S]methionine-labeled total cell surface glycoproteins in HepG2 cells (t_{1/2} = 65 h). This indicates that cell surface glycoproteins may encounter trimming mannosidase(s) several times during their life span. The physiological role of postbiosynthetic demannosylation is unknown. Trimming of oligomannosidic N-glycans could reflect the occasional removal of mannose residues from surface glycoproteins by a mannosidase present at the cell surface or encountered during endocytosis and recycling. Alternatively, postbiosynthetic processing could provide a means by which cells can modify N-glycans of cell surface glycoproteins. With respect to the role of cell surface glycoproteins in cell-substratum and cell-cell recognition processes, this remodeling of cell surface glycoproteins may be of relevance for cell surface functions.

Acknowledgments—We are indebted to Prof. Dr. R. Geyer (Biochemisches Institut, Universität Gießen, Germany) for the generous gift of the authentic glycan standards and to Dr. D. Hixon (Brown University) and Dr. H. P. Hauri (Biocenter of the University of Basel, Switzerland) for the gift of monoclonal antibodies.

REFERENCES
1. Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
2. Schachter, H. (1986) Biochim. Biophys. Acta 84, 163–181
3. Cummings, R. D. (1992) Glycoconjugates (Allen, H. J., and Kisailus, E. C., eds) pp. 353–360, Marcel Dekker, New York
4. Moremen, K. W., Trimble, R. B., and Herscovics, A. (1994) Glycobiology 4, 113–125
5. Daniel, P. F., Winchester, B., and Warren, C. D. (1994) Glycobiology 4, 551–566
6. Tauber, R., Kreisel, W., and Reutter, W. (1992) in Endocytosis (Courtoy, P. J., ed.) pp. 187–194, Springer, Heidelberg
7. Kreisel, W., Volk, B. A., Buchsel, E., and Reutter, W. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1829–1831
8. Volk, B. A., Kreisel, W., Kittgen, E., Gerok, W., and Reutter, W. (1983) FEBS Lett. 163, 150–152
9. Tauber, R., Park, C. S., and Reutter, W. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 4026–4029
10. Baumann, H., Hou, E., and Jahreis, G. P. (1983) J. Cell Biol. 96, 139–150
11. Tauber, R., Park, C. S., Becker, A., Geyer, R., and Reutter, W. (1989) Eur. J. Biochem. 186, 55–62
12. Duncan, J. R., and Kornfeld, S. (1988) J. Cell. Biol. 106, 617–628
13. Snider, M. D., and Rogers, O. C. (1985) J. Cell. Biol. 106, 826–834
14. Reichner, J. S., Whiteheart, S. W., and Hart, G. W. (1988) J. Biol. Chem. 263, 16316–16326
15. Krügel, S., Hanski, C., Tran-Thi, T. A., Katz, N., Decker, K., Reutter, W., and Gerok, W. (1988) J. Biol. Chem. 263, 11736–11742
16. Brandli, A. W., and Simons, K. (1989) EMBO J. 8, 3207–3213
17. Green, S. A., and Kelly, R. B. (1990) J. Biol. Chem. 265, 21209–21278
18. Volz, B., Orberger, G., Porwoll, S., Hauri, H. P., and Tauber, R. (1995) J. Cell. Biol. 130, 537–551
19. Huang, K. M., and Snider, M. D. (1993) J. Biol. Chem. 268, 9302–9310
20. Snider, M. D. (1991) in Intracellular Trafficking of Proteins (Steer, C. J., and Hanover, J. A., eds) pp. 361–386, Cambridge University Press, New York
21. Snider, M. D., and Rogers, O. C. (1986) J. Cell. Biol. 106, 265–275
22. Neefjes, J. J., Verkerk, J. M., Bruxterman, H. J., van, D. M. G., van, B. J., and Plaegh, H. L. (1988) J. Cell. Biol. 107, 79–87
23. Dunphy, W. G., Brands, R., and Rothman, J. E. (1985) Cell 40, 463–472
24. Velasco, A., Hendricks, L., Moremen, K. W., Touster, O., and Farquhar, M. G. (1993) J. Cell Biol. 122, 39–51
25. Becker, A., Lucka, L., Kilian, C., Kammich, C., and Reutter, W. (1993) Eur. J. Biochem. 214, 539–548
26. Berndorf, D., Gessner, R., Kreft, B., Schnoy, N., Lajous, P. A., Loch, N., Reutter, W., Hortsch, M., and Tauber, R. (1994) J. Cell. Biol. 125, 1353–1369
27. Becker, A., Neumeier, R., Heidrich, C., Loch, N., Hartel, S., and Reutter, W. (1986) Biol. Chem. Hoppe Seyler 367, 681–688
28. Loch, N., Geilen, C. C., Sporndle, I., Oberdorfer, F., Kerpller, D., Tauber, R., and Reutter, W. (1991) FEBS Lett. 294, 217–220
29. Hauri, H. P., Sterchi, E. J., Brien, D., Fransen, J. A., and Marxer, A. (1985) J. Cell. Biol. 101, 838–851
30. Neumeier, R., Dethlefs, U., and Reutter, W. (1984) FEBS Lett. 168, 241–244
31. Seglen, P. O. (1976) Methods Cell Biol. 13, 29–83
32. Loch, N., Tauber, R., Becker, A., Hartel, S. S., and Reutter, W. (1992) Eur. J. Biochem. 210, 161–168
33. Knowles, B. B., Howe, C. C., and Aden, D. P. (1980) Science 209, 497–499
34. Laemmli, U. K. (1970) Nature 227, 680–685
35. Bonner, W. M., and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83–88
36. Tauber, R., Nuck, R., Gerok, W., Buchsel, R., Kittgen, E., Lohle, W., Karasiewicz, C., and Reutter, W. (1992) Glycoconjug. J. 9, 132–140
37. Nuck, R., Paul, C., Wieland, B., Heidrich, C., Geilen, C. C., and Reutter, W. (1993) Eur. J. Biochem. 216, 215–221
38. Geyer, R., Diabate, S., Geyer, H., Klenk, H. D., Niemann, H., and Stirm, S. (1987) Glycoconjug. J. 4, 17–32
39. Anumula, K. R., and Taylor, P. B. (1991) Eur. J. Biochem. 195, 269–280
40. Lucka, L., Cichocka, I., Baumler, K., Bechler, K., and Reutter, W. (1995) Eur. J. Biochem. 234, 527–535
41. Schneider, C., Sutherland, R., Newman, R., and Greaves, M. (1982) J. Biol. Chem. 257, 8516–8522
42. Tulsiani, D. R., and Touster, O. (1985) J. Biol. Chem. 260, 13081–13087
43. Tulsiani, D. R., Skudlarek, M. D., and Orgebin, C. M. (1989) J. Cell. Biol. 109, 1257–1267
44. Bonay, P., and Hughes, R. C. (1991) Eur. J. Biochem. 197, 229–238
45. Bause, E., Breuer, W., Schweden, J., Roeseer, R., and Geyer, R. (1992) Eur. J. Biochem. 208, 451–457
46. Ohn, H., and Hase, S. (1991) J. Biochem. 110, 982–989
47. Bonay, P., Roth, J., and Hughes, R. C. (1992) Eur. J. Biochem. 205, 399–407
48. Balch, W. E., Elliott, M. M., and Keller, D. S. (1986) J. Biol. Chem. 261, 14681–14689