Compartmentation of Asparagine-linked Oligosaccharide Processing in the Golgi Apparatus

WILLIAM G. DUNPHY and JAMES E. ROTHMAN
Department of Biochemistry, Stanford University, Stanford, California 94305

ABSTRACT Golgi-associated processing of complex-type oligosaccharides linked to asparagine involves the sequential action of at least six enzymes. By equilibrium sucrose density gradient centrifugation of membranes from Chinese hamster ovary cells, we have partially resolved the set of four initial enzymes in the pathway (Mannosidase I, *N*-acetylglucosamine (GlcNAc) Transferase I, Mannosidase II, and GlcNAc Transferase II) from two later-acting activities (galactosyltransferase and sialyltransferase). In view of the recent demonstration that galactosyltransferase is restricted to the *trans* face of the Golgi complex in HeLa cells (Roth, J., and E. G. Berger, 1982, *J. Cell Biol.*, 93:223–229), our results suggest that removal of mannose and attachment of peripheral *N*-acetylglucosamine may occur in some or all of the remaining cisternae on the *cis* side of the Golgi stack.

After the removal of glucose in the endoplasmic reticulum (ER),[1] asparagine-linked oligosaccharides destined to become complex chains are processed by a battery of Golgi complex-associated enzymes (21). The processing steps that yield a two-branched, complex-type sugar chain are summarized in Fig. 1. We recently reported a partial separation, by density gradient centrifugation, of Chinese hamster ovary (CHO) cell membranes containing the earliest enzyme in the pathway (Mannosidase I) from those possessing the two late-acting glycosyltransferases galactosyltransferase and sialyltransferase (6). On the basis of this and related findings, we proposed a spatial separation or compartmentation of these enzymes within the Golgi complex. Here we extend these studies to include all six enzymatic steps described in Fig. 1.

MATERIALS AND METHODS

MATERIALS: UDP-*N*-acetylglucosamine (UDP-GlcNAc), UDP-galactose, CMP-sialic acid, α-methylmannoside, Concanavalin A-Sepharose, and ovalbumin were obtained from Sigma Chemical Co. (St. Louis, MO). [2-^{3}H]-Mannose (15 Ci/mmol), [1-^{3}H]galactose (11.6 Ci/mmol), UDP-[6-^{3}H]*N*-acetylglucosamine (24 Ci/mmol), UDP-[1-^{3}H]galactose (11.6 Ci/mmol), and CMP-[9-^{3}H]sialic acid (18.9 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Fetuin was purchased from Gibco Laboratories (Grand Island, NY). AG1-X8 (formate from 200–400 mesh) was obtained from Bio-Rad Laboratories (Richmond, CA). Endo-β-*N*-acetylglucosaminidase H (Endo H) was purchased from Health Research, Inc. (Albany, NY). Purified rabbit liver GlcNAc Transferase I (sp act, 2.5 U/mg protein; see reference 30) was generously supplied by Dr. R. Hill (Duke University).

CELLS AND VIRUSES: Wild-type, clone 15B, and clone 1021 CHO cells and clone 6 of L cells (kindly provided by Dr. S. Kornfeld, Washington University) were cultivated as before (6). Vesicular stomatitis virus (VSV) infections were carried out as described (6).

MEMBRANE FRACTIONATION: In all fractionation experiments, membranes from postnuclear supernatants of CHO clone 1021 cells (4) were used. The membranes were washed as described before (6) and fractionated on a six-step sucrose gradient consisting of 4.5 ml of 55%; 1.5 ml of 40%; 2.5 ml of 35%, 30% and 25%; and 1 ml of 20% (wt/wt) sucrose containing 1 mM Tris-HCl/1 mM EDTA, pH 8.0. After centrifugation (20 h at 2°C in the Beckman SW 27.1 rotor), 14 fractions (~1 ml) were collected. The bottom two fractions (which routinely contained no protein or enzyme activity) were discarded. Membranes from the remaining fractions were harvested (6), resuspended in 0.1 ml of H_2O, and assayed for enzyme activity.

PREPARATION OF TRITIATED OLIGOSACCHARIDES: We isolated ([^{3}H]-Man_{8+9}) GlcNAc, a nearly equimolar mixture of ([^{3}H]Man_8)GlcNAc and ([^{3}H]Man_9)GlcNAc, and ([^{3}H]Man_5)GlcNAc from Endo H-treated glycopeptides prepared from VSV-infected clone 15B CHO cells labeled with [^{3}H] mannose (39, 40). ([^{3}H]Man_8)GlcNAc (40) was also isolated from VSV-infected clone 6 of mouse L cells as before (6, 40). GlcNAc([^{3}H]Man_5)GlcNAc, the product of GlcNAc Transferase I action, was prepared as described by Tabas and Kornfeld (39) except that 0.02 U of purified rabbit liver GlcNAc Transferase I was used and incubation time was lengthened to 12 h. ([^{3}H] Glc_3)Man_9GlcNAc was prepared from the lipid-linked oligosaccharide fraction (41) isolated from VSV-infected clone 15B cells labeled with [^{3}H]galactose (25). The oligosaccharide was released from the lipid carrier by mild acid treatment (37), digested with Endo H, and partially purified by passage through a 0.5 × 2.5-cm AG1-X8 column equilibrated in 0.15 M NH_4formate. All of the above oligosaccharides were purified by gel-filtration on columns (1 × 115-cm) of Bio-Gel P-4 (400- mesh) equilibrated with 0.1 M NH_4HCO_3 (pH 8.0).

UNLABELED GLYCOSYLTRANSFERASE ACCEPTORS: $Man_5(GlcNAc_2)$-asparagine (ovalbumin glycopeptide V) was prepared from ovalbumin as described (20). Ahexosotransferrin (transferrin with an exposed three-mannose core generated by exhaustive treatment with neuraminidase, β-galactosidase, and *N*-acetylglucosaminidase; see reference 32) was kindly provided by Dr. R. Hill. To prepare the substrate for GlcNAc Transferase II assays, we treated ahexosotransferrin exhaustively with purified rabbit liver GlcNAc Transferase I (30). The incubation contained 0.1 M Mes (pH 6.3), 10 mM $MnCl_2$, 0.2% Triton X-100, 24 mM UDP-GlcNAc, 5 mg of ahexosotransferrin, and 0.007

[1] *Abbreviations used in this paper:* CHO, Chinese hamster ovary; ER, endoplasmic reticulum; Endo H, Endo-β-*N*-acetylglucosaminidase H; GlcNAc,*N*-acetylglucosamine; VSV, vesicular stomatitis virus; Mes, 2[*N*-Morpholino]ethanesulfonic acid.

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U GlcNAc Transferase I in a 0.75-ml vol. After a 48-h incubation at 37°C, the enzyme was inactivated by heating (90 min at 56°C). Two lines of evidence indicated that the reaction had gone to completion. A parallel incubation containing UDP-[^{3}H]GlcNAc demonstrated that 1.07 mol of GlcNAc had been incorporated per mol of transferrin oligosaccharide. Also, further treatment of the reaction product with GlcNAc Transferase I resulted in no additional GlcNAc transfer. Agalactofetuin was prepared from desialated fetuin (38) by digestion with jack bean β-galactosidase (Sigma Chemical Co.) as described in reference 26.

Enzyme Assays

MANNOSIDASE I: We measured this activity as described by Tabas and Kornfeld (40), with some modifications. Assays contained in a 0.03-ml vol: 0.1 M sodium acetate (pH 6.0), 0.3% Triton X-100, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5,000 cpm of ([^{3}H]Man_{8+9})GlcNAc. Assay with ([^{3}H]Man_8)GlcNAc as substrate gave the same results. After incubation (60 min at 37°C), released [^{3}H]mannose was quantitated as described (40).

GlcNAc TRANSFERASE I: For assay of GlcNAc Transferase I, we measured transfer of [^{3}H]GlcNAc to $Man_5(GlcNAc_2)$-asparagine (ovalbumin glycopeptide V). Incubation conditions were based on those described by Oppenheimer and Hill (30). The labeled glycopeptide was separated by binding to Concanavalin A-Sepharose. Assays contained 0.1 M Mes (pH 6.5), 10 mM $MnCl_2$, 0.2% Triton X-100, 2 mM ATP, 1 μCi UDP-[^{3}H]GlcNAc, 1 mM nonradioactive UDP-GlcNAc, and 10 nmol of ovalbumin glycopeptide V in a final volume of 0.05 ml. After incubation (30 min at 37°), samples were boiled for 5 min, mixed with 1 ml of phosphate-buffered saline (PBS) (see reference 6 for composition), and centrifuged for 2 min at 12,800 *g* in an Eppendorf microfuge. The supernatant was applied to a 0.5-ml (0.5 × 2.5-cm) Concanavalin A-Sepharose column (equilibrated with PBS). The column was first washed with 7 ml of PBS; bound glycopeptides were then eluted over a 5-min period with 2.5 ml of 0.1 M α-methylmannoside containing 10 mM Tris-HCl (pH 8.0). Greater than 90% of the added glycopeptide was routinely recovered in this eluate. Negligible [^{3}H]GlcNAc incorporation in the absence of glycopeptide acceptor was detected.

MANNOSIDASE II: We assayed this enzyme by measuring the release of [^{3}H]mannose from GlcNAc([^{3}H]Man_5)GlcNAc as described by Tabas and Kornfeld (39) except that the incubation buffer was that used by Tulsiani et al. (43). Assays contained 0.1 M sodium acetate (pH 6.0), 0.3% Triton X-100, and GlcNAc([^{3}H]Man_5)GlcNAc oligosaccharide (5,000 cpm) in a 0.03-ml vol. Incubations were carried out for 30 min at 37°C.

GlcNAc TRANSFERASE II: For this assay, we used GlcNAc Transferase I-treated ahexosotransferrin (prepared as described above) as the acceptor. Assays contained in a 0.05-ml vol: 0.1 M Mes (pH 6.5), 10 mM $MnCl_2$, 0.2% Triton X-100, 2 mM ATP, 1 μCi UDP-[^{3}H]GlcNAc, 1 mM nonradioactive UDP-GlcNAc, and 2 mg/ml of the glycoprotein acceptor. Incubations were terminated by the addition of 1 ml of ice-cold 1% phosphotungstic acid/0.5 N HCl. The precipitate was trapped on a glass fiber filter, and the filter washed with seven 5-ml aliquots of 1% phosphotungstic acid/0.5 N HCl. Radioactivity incorporated in the absence of exogenous acceptor was subtracted as the blank.

GALACTOSYLTRANSFERASE: This enzyme was measured as before (6) except that agalactofetuin (2 mg/ml) was the acceptor and 2 mM ATP was included. No [^{3}H]galactose incorporation was observed in the gradient fractions in the absence of this exogenous glycoprotein acceptor.

SIALYLTRANSFERASE: Assays contained 50 mM Tris-HCl (pH 7.0), 0.5% Triton X-100, 0.1 μCi CMP-[^{3}H]sialic acid, 0.05 mM nonradioactive CMP-sialic acid, and desialated fetuin (4 mg/ml) in a final volume of 0.03 ml. After incubation (60 min at 37°C), acid-precipitable radioactivity was measured as before (6). Negligible incorporation was observed in the absence of desialated fetuin.

GLUCOSIDASE I: This enzyme was measured under the conditions of Grinna and Robbins (16). The substrate, ([^{3}H]Glc_3)Man_9GlcNAc (25), was prepared as described above. Assays contained in a final volume of 0.03 ml: 50 mM sodium phosphate (pH 6.8), 0.1% Triton X-100, and 2,500 cpm ([^{3}H]-Glc_3)Man_9GlcNAc. After incubation (30 min at 37°C), released [^{3}H]glucose was measured as for [^{3}H]mannose (40).

RESULTS

Membranes from CHO clone 1021 cells were washed, centrifuged in a sucrose density gradient, and the distributions of the enzymes catalyzing the six enzymatic steps depicted in Fig. 1 were determined. As before (6), we observed that Mannosidase I resided in somewhat denser membranes than galactosyltransferase (Fig. 2*A*). Qualitatively, the same separations were observed in wild-type and clone 15B CHO cells, but clone 1021 cells routinely gave the best results. Prolonged centrifugation (20 h at 85,000 *g*) to apparent equilibrium was required to effect this separation. After a 4-h centrifugation, both activities were diffusely distributed throughout the gradient. EDTA (1 mM) could be removed from the procedure with little effect. We previously demonstrated that the glycoprotein products newly processed in vivo by Mannosidase I and galactosyltransferase could also be separated in this manner (6). [^{3}H]Palmitate-labeled (36) VSV G protein with five mannose-containing oligosaccharides followed the distribution of Mannosidase I, whereas G protein pulse-labeled in vivo with [^{3}H]galactose distributed with galactosyltransferase.

The enzymes catalyzing steps 2–4 (Fig. 1) (GlcNAc Transferase I, Mannosidase II, and GlcNAc Transferase II) all codistributed with Mannosidase I in the gradient. Sialyltransferase, the last enzyme in the pathway, closely paralleled galactosyltransferase activity (Fig. 2*E*). These results suggest that removal of mannose and attachment of at least two terminal GlcNAc residues takes place in a Golgi complex region(s) distinct from the site of galactose and sialic acid transfer. Our data do not, however, exclude the possibility that further compartmentation might exist.

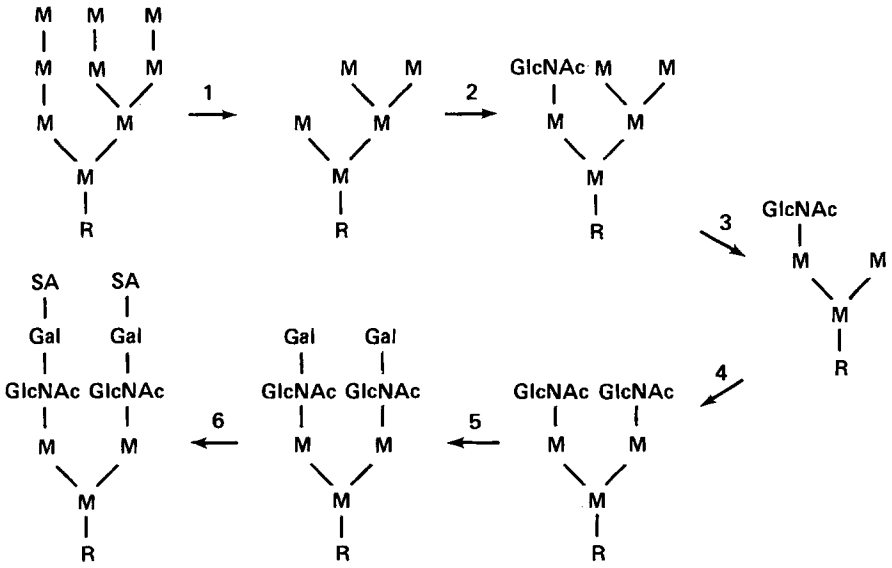

FIGURE 1 Stages in construction of a biantennary, asparagine-linked oligosaccharide (21). Enzymes are: (*1*) Mannosidase I; (2) GlcNAc Transferase I; (3) Mannosidase II; (4) GlcNAc Transferase II; (5) Galactosyltransferase; and (6) Sialyltransferase. *M*, mannose; *Gal*, galactose; *SA*, sialic acid; *R*, $GlcNAc_2$-asparagine (polypeptide chain).

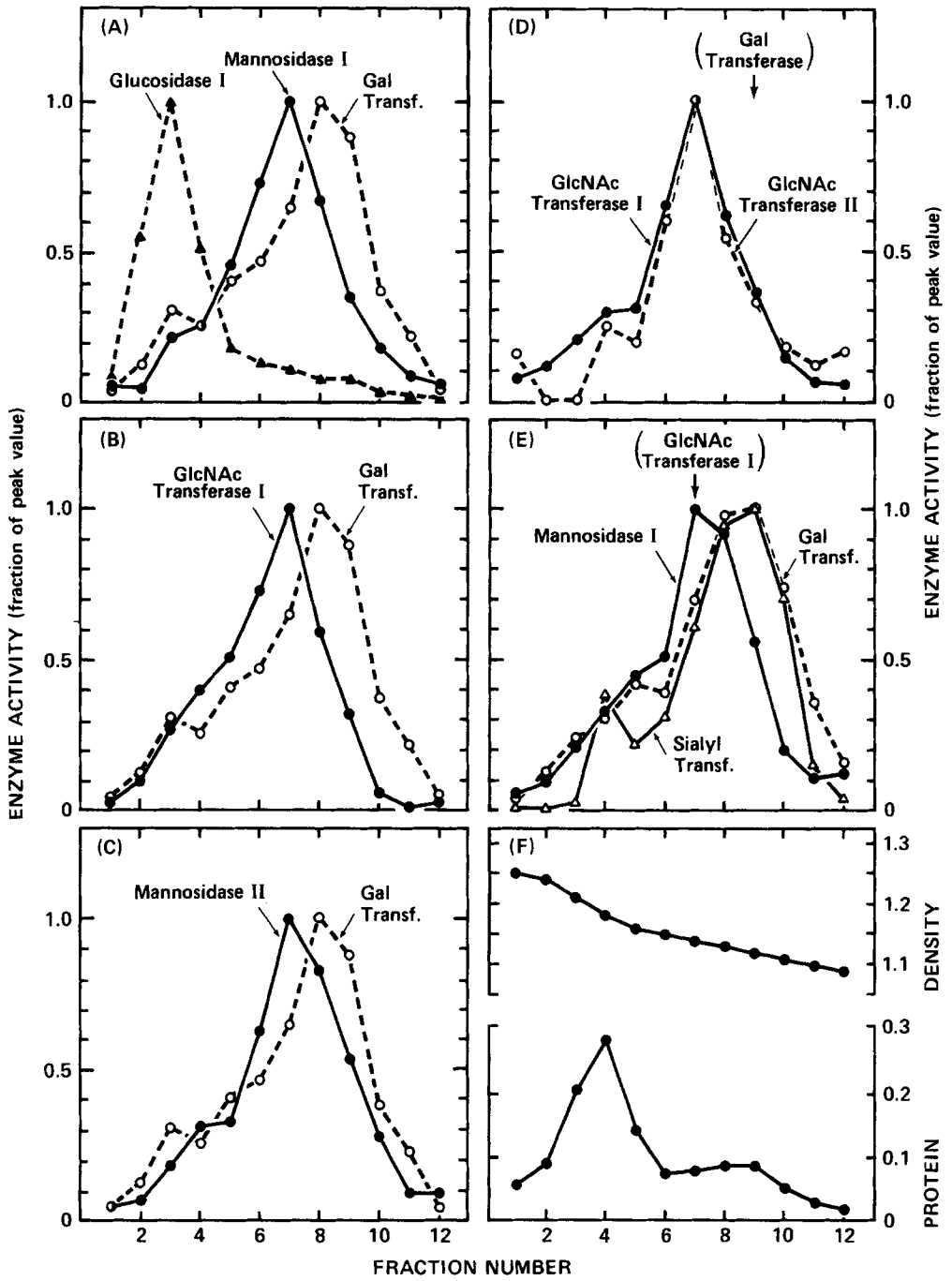

FIGURE 2 Distributions of oligosaccharide-processing enzymes in membranes from CHO clone 1021 cells. For each gradient, membranes from 4 ml of postnuclear supernatant were fractionated as described in Materials and Methods. Results from three different gradients are depicted in panels *A-C*, *D*, and *E-F*, respectively. (*A-C*) Distribution of Glucosidase I, Mannosidase I, GlcNAc Transferase I, Mannosidase II, and galactosyltransferase measured in the same gradient. For reference, the distribution for galactosyltransferase is presented in each panel. Recoveries of enzyme activity (relative to levels in the washed membranes applied to the gradient) and the levels of enzyme activity in peak fractions (activity per total fraction) were respectively: Glucosidase I (40%; 4.8×10^2 U); Mannosidase I (43%; 0.39 U); GlcNAc Transferase I (65%; 2.8 nmol of GlcNAc transferred/h); Mannosidase II (54%; 1.03 U); and galactosyltransferase (98%; 1.29 nmol of galactose transferred/h). The definitions for units of Glucosidase I and Mannosidases I and II are in references 16 and 40, respectively. (*D*) Codistribution of GlcNAc Transferase I and II activities. Galactosyltransferase (peak in activity indicated by arrow) was measured in this gradient as an internal control. Recovery of GlcNAc Transferase II was 81%; total enzyme activity in the peak fraction was 2.4 nmol of GlcNAc transferred/h. (*E*) Codistribution of galactosyltransferase and sialyltransferase. Mannosidase I and GlcNAc Transferase I (peak in activity indicated by arrow) were measured in the same gradient as internal controls. Recovery of sialyltransferase was 26%; total enzyme activity in the peak fraction was 0.19 nmol of sialic acid transferred/h. (*F*) Protein content (mg/fraction) (measured by the Lowry et al. (27) method) and sucrose densities (g/ml) of the fractions from the same gradient shown in *E*.

The Mannosidase I and II activities we have measured had the expected properties (40, 43). Mannosidase I was strongly inhibited (Table I) by 5 mM EDTA or 50 mM Tris-maleate (pH 6.0); Mannosidase II was unaffected by these treatments (Table II). In both assays, hydrolysis of $Man_5GlcNAc$, a substrate for neither enzyme, was only slightly above background levels (Tables I and II). To be certain that the two *N*-acetylglucosaminyltransferase assays indeed measure distinct enzymes, we compared levels of the two activities in wild-type CHO cells and the mutant clone 15B line that selectively lacks GlcNAc Transferase I (13). Membranes from wild-type but not clone 15B CHO cells possessed GlcNAc Transferase I (Table III), as expected (13). GlcNAc Transferase II levels in both cells lines were comparable (13, 28).

All the Golgi complex activities floated to densities much lighter than Glucosidase I (Fig. 2*A*), the enzyme that removes the outermost glucose (15) from the glucosylated precursor oligosaccharide transferred from dolichol. Kinetic (22, 24) and cell fractionation (15) studies have indicated that this enzyme resides in the ER. The distribution of Glucosidase I is quite similar to that reported for glucose-containing VSV G protein pulse-labeled in vivo with [^{35}S]methionine (6).

Pohlmann et al. (31), using a variation of our procedure (6), recently reported an apparent separation of Mannosidase I and galactosyltransferase in rat liver Golgi complex fractions. However, using this procedure (Dunphy, W., and J. Rothman, unpublished data) we have not observed a separation of Mannosidases I and II from galactosyltransferase in rat liver Golgi complex subfractions prepared by three separate methods (described in references 3, 19, and 40). In measurement of galactosyltransferase in these experiments, we had to take care to inhibit a pyrophosphatase (by including 2 mM ATP in the assays; see reference 3) present in rat liver in order to avoid the erroneous underestimation of galactosyltransferase in certain pyrophosphatase-rich gradient fractions. This difficulty was not encountered with CHO membrane fractions. ATP (2 mM) had no effect on galactosyltransferase levels in the gradient fractions of CHO membranes. Also, mixing experiments ruled out the presence of an inhibitor (or activator) of either galactosyltransferase or Mannosidase I in the CHO membrane fractions. Upon mixing peak fractions, we observed that levels of both galactosyltransferase and Mannosidase I were linearly additive (not shown). In a separate experiment, we ruled out a potential inhibition of GlcNAc Transferase I in fractions containing high galactosyltransferase levels by mixing the fractions with a known amount of purified rabbit liver GlcNAc Transferase I (Table IV).

TABLE I

Properties of Mannosidase I in CHO Membranes

Condition	Cleavage of ([^{3}H]-Man_{8+9})GlcNAc	Cleavage of ([^{3}H]Man_5)GlcNAc
	*(U/mg protein)**	
Control	2.03 (1.00)‡	0.22 (0.11)
+5 mM EDTA	0.15 (0.07)	0.10 (0.05)
0.05 M Tris-maleate (pH 6.0)	0.15 (0.07)	0.13 (0.06)

Membranes from clone 1021 CHO cells were washed as for gradient fractionation (see Materials and Methods) and assayed for hydrolysis of ([^{3}H]Man_{8+9})GlcNAc or ([^{3}H]Man_5)GlcNAc under Mannosidase I assay conditions (see Materials and Methods). Both substrates (5,000 cpm per assay) were obtained from the same glycopeptide preparation (see Materials and Methods). Control incubations contained 0.1 M sodium acetate (pH 6.0), and all incubations contained 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.3% Triton X-100. Values are the average of determinations at two separate protein concentrations (45 and 90 μg per assay) within the linear assay range. Analysis of the pooled peak fractions from gradients such as those depicted in Fig. 2 gave identical results.

* A unit of activity was defined by Tabas and Kornfeld (40).

‡ Values in parentheses indicate activity as a fraction of that observed for hydrolysis of ([^{3}H]Man_{8+9})GlcNAc under control conditions.

TABLE II

Properties of Mannosidase II in CHO Membranes

Condition	Cleavage of GlcNAc([^{3}H]-Man_5)GlcNAc	Cleavage of ([^{3}H]Man_5)GlcNAc
	*(U/mg protein)**	
Control	12.39 (1.00)‡	0.30 (0.02)
+5 mM EDTA	12.10 (0.98)	0.43 (0.03)
0.05 M Tris-maleate (pH 6.0)	10.80 (0.87)	0.24 (0.02)

Washed membranes (60 μg of protein) from the same membrane preparation used for the experiments in Table I were assayed for hydrolysis of GlcNAc([^{3}H]Man_5)GlcNAc and ([^{3}H]Man_5)GlcNAc under Mannosidase II assay conditions (see Materials and Methods). GlcNAc([^{3}H]Man_5)GlcNAc was prepared from the batch of ([^{3}H]Man_5)GlcNAc used in this experiment, and is thus of identical specific radioactivity. Control incubations contained 0.1 M sodium acetate (pH 6.0), and all incubations contained 0.3% Triton X-100 and 5,000 cpm of the appropriate tritiated oligosaccharide.

* A unit of activity was defined as for Mannosidase I (Table I).

‡ Values in parentheses indicate activity as a fraction of that observed for hydrolysis of GlcNAc([^{3}H]Man_5)GlcNAc under control conditions.

TABLE III

Distinction between GlcNAc Transferase I and II Activities

Cell line	Relative enzyme levels: GlcNAc Transferase I	Relative enzyme levels: GlcNAc Transferase II
Wild-type CHO	1	1
Clone 15B CHO	0.02	1.25

Washed membranes (150 μg of protein) from wild-type or clone 15B CHO cells were assayed for GlcNAc Transferase I and II activities as described in Materials and Methods. Values (average of duplicate determinations) are expressed relative to enzyme levels in wild-type CHO cells (6.2 and 2.0 nmol GlcNAc transferred/mg protein per 30 min for GlcNAc Transferase I and II, respectively).

TABLE IV

GlcNAc Transferase I is not Inhibited in the Presence of CHO Membrane Fractions

Gradient fraction	[^{3}H]GlcNAc transferred: −Enzyme	+Enzyme	Difference
		(cpm/30 min)	
None	0	3,199	3,199
9	2,582	6,177	3,595
10	1,798	5,510	3,712
11	1,143	4,763	3,620
12	519	4,181	3,662

Fractions 9–12 from the gradient depicted in Fig. 1 *D* were assayed for GlcNAc Transferase I activity (see Materials and Methods) in the absence and presence of added pure rabbit liver GlcNAc Transferase I (18 ng, an amount resulting in 3,199 cpm [^{3}H]GlcNAc incorporated in the assay). 10% (10 μl) of resuspended membranes from each gradient fraction was used for each determination.

DISCUSSION

A large body of electron microscopic evidence has established that the stacked cisternae of the Golgi complex are heterogeneous in composition (see references 8, 34, and 42 for recent reviews). But a few examples are selective osmium deposition in *cis* Golgi cisternae (10); restriction of thiamine pyrophosphatase activity to *trans* saccules (5); and a graded increase in cholesterol concentration from *cis* to *trans* Golgi membranes (29).

We have described the resolution of membranes containing the late-acting sugar transferases galactosyltransferase and sialyltransferase from those housing four earlier-acting oligosaccharide-processing enzymes (Mannosidase I, GlcNAc Transferase I, Mannosidase II, and GlcNAc Transferase II). All of these enzymes have been shown to be enriched greatly in total Golgi fractions (9, 17, 28, 35, 40, 44). Using a variation of our procedure (6), Goldberg and Kornfeld (12) recently observed a separation of GlcNAc Transferase I and Mannosidase II (as well as fucosyltransferase and GlcNAc Transferase IV) from galactosyltransferase in murine macrophage and lymphoma cell lines.

To what morphologically defined elements of the Golgi complex might the sequentially-acting membrane fractions that we have identified correspond? Two recent reports have furnished compelling evidence supporting the conclusion (6, 34) that galactose transfer occurs in *trans* Golgi cisternae. By immunocytochemistry, Roth and Berger (33) have directly localized galactosyltransferase to the cisternae at the *trans* side of the Golgi stack in HeLa cells. Also, Griffiths et al. (14) have shown that the galactose-specific lectin ricin binds to *trans* saccules that would be expected to contain the products of galactosyltransferase action. The distribution defined by galactosyltransferase (and sialyltransferase) in our fractionation experiments therefore most likely corresponds to remnants of these *trans* cisternae. We have shown here that four earlier-acting enzymes (the two mannosidases and *N*-acetylglucosaminyltransferases) reside, at least in part, in a different region of the Golgi complex. An ample and tenable hypothesis, therefore, is that removal of mannose and attachment of peripheral GlcNAc takes place in some or all of the remaining *cis* saccules, while galactose and sialic acid transfer occurs in the *trans* Golgi. A direct test of this model awaits immunoelectron microscopic localization of early-acting enzymes in the processing pathway.

Numerous investigators have reported methods for subfractionation of rat liver Golgi apparatus. Among the techniques described have been immunoaffinity adsorption (23), countercurrent separation in aqueous polymer two-phase systems (19), and sucrose density gradient separation based upon density shifts imparted to a subset of Golgi elements by digitonin (2) or enclosed lipoprotein particles (1, 3, 7). In most of these studies, galactosyltransferase was the only oligosaccharide-processing enzyme measured.

One exception was the study of Bretz et al. (3), who found that N-acetylglucosaminyltransferase, galactosyltransferase, and sialyltransferase fractionated in parallel in GF_1, GF_2, and GF_3 Golgi subfractions separated according to lipoprotein content by the method of Ehrenreich et al. (7). Fractions GF_1 and GF_2 consisted of very low density lipoprotein-containing vesicles, intact cisternae, and detached cisternal rims, whereas the GF_3 fraction contained lipoprotein-free vesicles, cisternae, and central plates of fragmented cisternae. Following this procedure closely, we have similarly observed no separation of Mannosidase I and GlcNAc Transferase I from galactosyltransferase (Dunphy, W., and J. Rothman, unpublished data). Conceivably, the Ehrenreich et al. (7) resolution of lipoprotein-containing Golgi complex elements from those lacking lipoproteins does not reflect a *cis-trans* separation but, instead, may largely represent a separation of peripheral from central elements.

The detailed route traversed by newly made proteins through the Golgi apparatus is unknown. Although a preponderance of indirect evidence has pointed to an overall *cis* to *trans* movement, in situ visualization of oligosaccharide-processing enzymes that act at distinct temporal stages in the maturation of glycoproteins is needed to delineate the intra-Golgi complex transport pathway with precision. The initial step in this task, localization of galactosyltransferase to the *trans* Golgi complex cisternae (33), has corroborated the notion that glycoproteins exit at the *trans* face. Since precursors destined for the plasma membrane, lysosomes, and secretion granules all can contain terminal galactose (11, 18, 21), these classes of protein must all have passed through the *trans* cisternae. The implication, as we pointed out earlier (6, 34), is that the sorting of these three types of protein most likely occurs within or during departure from the *trans* Golgi cisternae.

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REFERENCES

1. Bergeron, J. J. M., J. H. Ehrenreich, P. Siekevitz, and G. E. Palade. 1973. Golgi fractions prepared from rat liver homogenates. II. Biochemical characterization. *J. Cell Biol.* 59:73–88.
2. Borgese, N., and J. Meldolesi. 1980. Localization and biosynthesis of NADH-cytochrome b_5 reductase, an integral membrane protein, in rat liver cells. I. Distribution of the enzyme activity in microsomes, mitochondria, and Golgi complex. *J. Cell Biol.* 85:501–515.
3. Bretz, R., H. Bretz, and G. E. Palade. 1980. Distribution of terminal glycosyltransferases in hepatic Golgi fractions. *J. Cell Biol.* 84:87–101.
4. Briles, E. B., E. Li, and S. Kornfeld. 1977. Isolation of wheat germ agglutinin-resistant clones of Chinese hamster ovary cells deficient in membrane sialic acid and galactose. *J. Biol. Chem.* 252:1107–1116.
5. Cheetham, R. D., D. J. Morre, C. Pannek, and D. S. Friend. 1971. Isolation of a Golgi apparatus-rich fraction from rat liver. IV. Thiamine pyrophosphatase. *J. Cell Biol.* 49:899–905.
6. Dunphy, W. G., E. Fries, L. J. Urbani, and J. E. Rothman. 1981. Early and late functions associated with the Golgi apparatus reside in distinct compartments. *Proc. Natl. Acad. Sci. USA.* 78:7453–7457.
7. Ehrenreich, J. H., J. J. M. Bergeron, P. Siekevitz, and G. E. Palade. 1973. Golgi fractions prepared from rat liver homogenates. I. Isolation procedure and morphological characterization. *J. Cell Biol.* 59:45–72.
8. Farquhar, M. G., and G. E. Palade. 1981. The Golgi apparatus (complex)—(1954–1981)—from artifact to center stage. *J. Cell Biol.* 91:(3, Pt 2):77s–103s.
9. Fleischer, B., S. Fleischer, and H. Ozawa. 1969. Isolation and characterization of Golgi membranes from bovine liver. *J. Cell Biol.* 43:59–79.
10. Friend, D. S., and M. H. Murray. 1965. Osmium impregnation of the Golgi apparatus. *Am. J. Anat.* 117:135–150.
11. Goldberg, D. E., and S. Kornfeld. 1981. The phosphorylation of β-glucuronidase oligosaccharides in mouse $P388D_1$ cells. *J. Biol. Chem.* 256:13060–13067.
12. Goldberg, D. E., and S. Kornfeld. 1983. Evidence for extensive subcellular compartmentalization of asparagine-linked oligosaccharide processing and lysosomal enzyme phosphorylation. *J. Biol. Chem.* 258:3159–3165.
13. Gottlieb, C., J. Baenziger, and S. Kornfeld. 1975. Deficient uridine diphosphate-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosaminyltransferase activity in a clone of Chinese hamster ovary cells with altered surface glycoproteins. *J. Biol. Chem.* 250:3303–3309.
14. Griffiths, G., R. Brands, B. Burke, D. Louvard, and G. Warren. 1982. Viral membrane proteins acquire galactose in *trans* Golgi cisternae during intracellular transport. *J. Cell Biol.* 95:781–792.
15. Grinna, L. S., and P. W. Robbins. 1979. Glycoprotein biosynthesis. Rat liver microsomal glucosidases which process oligosaccharides. *J. Biol. Chem.* 254:8814–8818.
16. Grinna, L. S., and P. W. Robbins. 1980. Substrate specificities of rat liver microsomal glucosidases which process glycoproteins. *J. Biol. Chem.* 255:2255–2258.
17. Harpaz, N., and H. Schachter. 1980. Control of glycoprotein synthesis. Processing of

asparagine-linked oligosaccharides by one or more rat liver Golgi α-D-mannosidases dependent on the prior action of UDP-*N*-acetylglucosamine: α-D-mannoside β-2-*N*-acetylglucosaminyltransferase I. *J. Biol. Chem.* 255:4894–4902.
18. Hasilik, A., and K. von Figura. 1981. Oligosaccharides in lysosomal enzymes. Distribution of high-mannose and complex oligosaccharides in cathepsin D and β-hexosaminidase. *Eur. J. Biochem.* 121:125–129.
19. Hino, Y., A. Asano, and R. Sato. 1978. Biochemical studies on rat liver Golgi apparatus. III. Subfractionation of fragmented Golgi apparatus by counter-current distribution. *J. Biochem.* 83:935–942.
20. Huang, C.-C., H. E. Mayer, Jr., and R. Montgomery. 1970. Microheterogeneity and paucidispersity of glycoproteins. Part 1. The carbohydrate of chicken ovalbumin. *Carbohydr. Res.* 13:127–137.
21. Hubbard, S. C., and R. J. Ivatt. 1981. Synthesis and processing of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 50:555–583.
22. Hubbard, S. C., and P. W. Robbins. 1979. Synthesis and processing of protein-linked oligosaccharides *in vivo*. *J. Biol. Chem.* 254:4568–4576.
23. Ito, A., and G. E. Palade. 1978. Presence of NADPH-cytochrome P-450 reductase in rat liver Golgi membranes. Evidence obtained by immunoadsorption method. *J. Cell Biol.* 79:590–597.
24. Kornfeld, S., E. Li, and I. Tabas. 1978. The synthesis of complex-type oligosaccharides. II. Characterization of the processing intermediates in the synthesis of the complex oligosaccharide units of the vesicular stomatitis virus G protein. *J. Biol. Chem.* 253:7771–7778.
25. Li, E., I. Tabas, and S. Kornfeld. 1978. The synthesis of complex-type oligosaccharides. I. Structure of the lipid-linked oligosaccharide precursor of the complex-type oligosaccharides of the vesicular stomatitis virus G protein. *J. Biol. Chem.* 253:7762–7770.
26. Li, S.-C., M. Y. Mazzotta, S.-F. Chien, and Y.-T. Li. 1975. Isolation and characterization of jack bean β-galactosidase. *J. Biol. Chem.* 250:6786–6791.
27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
28. Narasimhan, S., P. Stanley, and H. Schachter. 1977. Control of glycoprotein synthesis. Lectin resistant mutant containing only one of two distinct *N*-acetylglucosaminyltransferase activities present in wild-type Chinese hamster ovary cells. *J. Biol. Chem.* 252:3926–3933.
29. Orci, L., R. Montesano, P. Meda, F. Malaisse-Lagae, D. Brown, A. Perrelet, and P. Vassalli. 1981. Heterogeneous distribution of filipin-cholesterol complexes across the cisternae of the Golgi apparatus. *Proc. Natl. Acad. Sci. USA.* 78:293–297.
30. Oppenheimer, C. L., and R. L. Hill. 1981. Purification and characterization of a rabbit liver α-1,3 mannoside β-1,2 *N*-acetylglucosaminyltransferase. *J. Biol. Chem.* 256:799–804.
31. Pohlmann, R., A. Waheed, A. Hasilik, and K. von Figura. 1982. Synthesis of phosphorylated recognition marker in lysosomal enzymes is located in the *cis* part of Golgi apparatus. *J. Biol. Chem.* 257:5323–5325.
32. Prieels, J. P., S. V. Pizzo, L. R. Glasgow, J. C. Paulson, and R. L. Hill. 1978. Hepatic receptor that specifically binds oligosaccharides containing fucosyl α-1,3-*N*-acetylglucosamine linkages. *Proc. Natl. Acad. Sci. USA.* 75:2215–2219.
33. Roth, J., and E. G. Berger. 1982. Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in *trans* Golgi cisternae. *J. Cell. Biol.* 93:223–229.
34. Rothman, J. E. 1981. The Golgi apparatus: two organelles in tandem. *Science (Wash. DC)* 213:1212–1219.
35. Schachter, H., I. Jabbal, R. L. Hudgin, L. Pinteric, E. J. McGuire, and S. Roseman. 1970. Intracellular localization of liver sugar nucleotide glycoprotein glycosyltransferases in a Golgi-rich fraction. *J. Biol. Chem.* 245:1090–1100.
36. Schmidt, M. F. G., and M. J. Schlesinger. 1979. Fatty acid binding to vesicular stomatitis virus glycoprotein: a new type of post-translational modification of the viral glycoprotein. *Cell.* 17:813–819.
37. Sefton, B. 1977. Immediate glycosylation of Sindbis virus membrane proteins. *Cell.* 10:659–668.
38. Spiro, R. 1960. Studies on fetuin, a glycoprotein of fetal serum. I. Isolation, chemical composition, and physiochemical properties. *J. Biol. Chem.* 235:2860–2869.
39. Tabas, I., and S. Kornfeld. 1978. The synthesis of complex-type oligosaccharides. III. Identification of an α-D-mannosidase activity involved in a late stage of processing of complex-type oligosaccharides. *J. Biol. Chem.* 253:7779–7786.
40. Tabas, I., and S. Kornfeld. 1979. Purification and characterization of a rat liver Golgi α-mannosidase capable of processing asparagine-linked oligosaccharides. *J. Biol. Chem.* 254:11655–11663.
41. Tabas, I., S. Schlesinger, and S. Kornfeld. 1978. Processing of high mannose oligosaccharides to form complex type oligosaccharides on the newly synthesized polypeptides of the vesicular stomatitis virus G protein and the IgG heavy chain. *J. Biol. Chem.* 253:716–722.
42. Tartakoff, A. 1980. The Golgi complex: crossroads for vesicular traffic. *Int. Rev. Exp. Pathol.* 22:228–251.
43. Tulsiani, D. R. P., S. C. Hubbard, P. W. Robbins, and O. Touster. 1982. α-D-Mannosidases of rat liver Golgi membranes. Mannosidase II is the $GlcNAcMAN_5$-cleaving enzyme in glycoprotein biosynthesis and Mannosidases IA and IB are the enzymes converting MAN_9 precursors to MAN_5 intermediates. *J. Biol. Chem.* 257:3660–3668.
44. Tulsiani, D. R. P., D. J. Opheim, and O. Touster. 1977. Purification and characterization of α-D-mannosidase from rat liver Golgi membranes. *J. Biol. Chem.* 252:3227–3233.