GOLGI AND SECRETED GALACTOSYLTRANSFERASE

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I. INTRODUCTION

Galactosyltransferase (GT), as reviewed in this paper, refers to a specific glycosyltransferase which catalyzes the formation of a galactose beta 1,4 acetylglucosamine and galactose beta 1,4 glucose linkage (E.C. 2.4.22). The enzyme has attracted special attention from cell biologists for a number of reasons:

1. GT is involved in galactosylation of glycoproteins or glycolipids at nonreducing *N*-acetylglucosamine or, in some cases, *N*-acetylgalactosamine residues. Almost all eukaryotic proteins destined for secretion or for residence in either an intracellular membrane system or in the plasma membrane are glycoproteins, having galactose at the penultimate or ultimate position of their *N*-linked oligosaccharide chains.
2. In the presence of alpha-lactalbumin, the enzyme is able to synthesize lactose, an unique example of enzyme modification.
3. The enzyme is present in the same location as an integral membrane protein in all eukaryotic cells thus far studied, i.e., the trans cisternae of the Golgi complex. Thus, GT is not only an ideal marker enzyme for the Golgi, but also serves as a valuable probe for studies of the function and turnover of this organelle.
4. Evidence suggests that during cellular interactions cell surface GT may participate in adhesion by binding to specific carbohydrate substrates on adjacent cells or in the extracellular matrix.

A major development during the past few years has been the gradual realization among cell biologists of the magnitude of intracellular membrane traffic in relation to the related processes of secretion of soluble proteins, endocytosis of macromolecules, biogenesis of lysosomes, and transepithelial transport of nutrients and immunoglobulins. Moreover, it has become evident that the biogenesis of all intracellular and cell membrane domains requires an immense sorting system for the transport of (glyco)proteins from their site of synthesis (generally the rough endoplasmic reticulum [RER]) to the different membrane-limited intracellular compartments or to the extracellular space.

This review deals with biological aspects of GT enzymology and will emphasize the importance of study of a typical Golgi enzyme for understanding the functions and sorting capability of the Golgi complex. The reader should refer to reviews by Schachter and Roseman,[1] Ram and Munjal,[2] and Berger et al.[3] for more detailed discussions on the role of GT in glycosylation in general and to recent reviews by Shur[4] and Pierce et al.[5] for the role of GT in cell-cell or cell-matrix interactions.

II. PHYSICAL AND CHEMICAL CHARACTERIZATION OF GT

Many of the physical and chemical properties of GT have been studied in considerable detail. Different species and organs have been utilized as the source of the enzyme. On a cellular level, GT, derived from Golgi fractions or tissue fluids and to a lesser degree from plasma membranes, has been used for biochemical characterization.

A. Purification

GT has been purified to homogeneity from human milk, bovine colostrum, and rat serum either by classical methods[6] or by affinity chromatography using either alpha-lactalbumin-Sepharose, uridine diphosphate (UDP)-hexanolamine-Sepharose, or *N*-acetylglucosamine-Sepharose columns.[7-14] The purification procedure for soluble human milk GT is a typical example of a standard purification procedure; the milk is defatted initially and casein is removed by acid precipitation. Two affinity steps are needed to purify the enzyme to homogeneity: the first step involves use of acetylglucosamine-Sepharose; binding occurs in the presence of Mn^{2+} and UDP, and the enzyme is specifically eluted by omitting the manganese ions. In a second purification step using alpha-lactalbumin-Sepharose, GT binds to the column in the presence of either glucose or *N*-acetylglucosamine and can be eluted from the column by the omission of the glucose or *N*-acetylglucosamine.

Antiserum raised in rabbits to GT purified from human milk according to this procedure also contained antibodies against human IgG. However, this apparent contamination was not detected by polyacrylamide gel electrophoresis in sodium dodecylsulfate (SDS).[15] Purification of GT from other sources, such as malignant effusions obtained according to the same purification procedure, often resulted in as much as 50% of total protein as IgG. This phenomenon of copurification of IgG and GT cannot be reasonably explained at present. The ultimate step in the purification was a third affinity column consisting of protein A-Sepharose, which removed IgG efficiently.[16] Powell and Brew[12] found that bovine, ovine, or porcine colostrum was a richer source of GT than milk. From bovine colostrum, they isolated a single GT polypeptide of molecular weight 51,000 daltons. Soluble GT was also isolated from fetal calf serum by Turco and Heath.[17] Comparison with the enzyme from bovine milk revealed identity in many aspects. The enzyme was purified 286,000-fold from human serum by Fujita-Yamaguchi and Yoshida.[14] They calculated that human serum contains 182 $\mu g/l$ of the enzyme. GT isolated from several body fluids are all soluble proteins. On the other hand, intracellular GT is present as an integral (Golgi) membrane protein. The isolation of the membrane-bound enzyme became feasible by application of the same affinity chromatographic techniques as applied for the soluble form, but in the presence of Triton® X-100. Using 1% Triton® X-100 to solubilize the membrane-bound enzyme, Smith and Brew[18] purified the enzyme from Golgi membranes obtained from lactating sheep mammary gland. Purification was accomplished by a combination of gel filtration and affinity chromatography, resulting in two diffuse bands on a SDS-polyacrylamide gel (apparent molecular weights of 67,000 and 54,000 daltons). An enzyme similar to the mammary gland enzyme has also been purified from fat globule membranes isolated from bovine colostrum and milk.[19] The enzyme from swine mesentary lymph nodes has been purified on a column of *p*-aminophenyl-beta-acetylglucosamine-Sepharose.[20] Fraser and Mookerjea[21] purified GT 680-fold from liver microsomes in the presence of 1% Triton® X-100 using an alpha-lactalbumin-Sepharose column. The molecular weight of the membrane enzyme was 65,000 to 70,000 daltons. Recently, Furukawa and Roth[22] purified two GTs with nearly identical M_r values (68,000 daltons) from microsomal membranes of chick-embryo livers. Both enzymes bound specifically to UDP-hexanolamine-, alpha-lactalbumin-, and acetylglu-

Table 1
SIZE OF GALACTOSYLTRANSFERASE

Species	Origin	Method	Molecular weight in kdaltons (minor species)	Ref.
Human	Milk	Sedimentation equilibrium	43—54	9
	Milk	SDS-PAGE	55	16
	Serum	Gel filtration	70—75	6
	Serum	SDS-PAGE	49	14
	Serum	SDS-PAGE	50	16
	Pleural effusion	SDS-PAGE	55	16
	Amniotic fluid	SDS-PAGE	55	29
	Skin fibroblasts	SDS-PAGE*	54	Figure 4A
(HeLa)	Ovary carcinoma	SDS-PAGE*	54	24
	Medium	SDS-PAGE*	52	24
(HepG2)	Liver carcinoma	SDS-PAGE*	54	Figure 4A
(MCF-7)	Breast carcinoma	SDS-PAGE*	55	Figure 4A
Rat	Liver	SDS-PAGE	67	21
	Liver	Sedimentation equilibrium	48.2	30
	Parotid	SDS-PAGE	60	31
	Serum	SDS-PAGE	43	13
	Serum	Gel filtration	63	21
Bovine	Serum	Sedimentation equilibrium	47.8	17
	Milk	SDS-PAGE	55—59 (42—44)	32
	Colostrum	Gel filtration	51	12
	Milk fat	Gel filtration	63	19
Sheep	Mammary	SDS-PAGE	65—69 (53—55)	18
Swine	Lymph node	SDS-PAGE	57	20
Chicken	Liver	SDS-PAGE	68	22

* Assayed after biosynthetical labeling and immunoprecipitation using a specific polyclonal antibody raised in rabbit against galactosyltransferase from human milk. (The antiserum was kindly provided by Dr. E. G. Berger.)

cosamine-Sepharose, but catalyzed galactose to different acceptor molecules and with different linkages. Binding of a GT, involved in the formation of beta 1,3 linkages, to lactalbumin indicates a very close relationship between the beta 1,4 acetylglucosamine GT and this enzyme. Thus far, a comparable similarity in specificities is not yet known for mammalian GTs.

GT purified from rat microsomal membrane fractions as well as from human serum and dissolved in buffers with pH ranging from 5 to 9 containing 0.1% bovine serum albumin (BSA) and 5 m*M* of acetylglucosamine is stable at 4°C for at least 2 days. At room temperature, in dilute solution, the enzymatic activity is almost totally lost within 10 min in the absence of albumin.[14,21]

B. Size and Structure of GT

As GT occurs both in a soluble and a membrane-bound form, it is not surprising that the enzyme demonstrates various molecular weights and physical dimensions. Table 1 summarizes molecular weights of a number of enzymes, isolated either from body fluids or as membrane-bound species. The molecular weight of GT is generally between 35,000 and 55,000 daltons (M_r), with a highest value of 50,000 daltons for soluble forms. Gel filtration gave a single activity peak with a molecular weight of about 80,000.[14] Khatra and co-workers[10] and Powell and Brew[12] reported that preparations of human and bovine milk enzymes contained a minor component of 100,000 M_r in addition to the major component of 40,000 to 50,000 M_r. The minor component dis-

appeared after treatment with 2-mercaptoethanol in the presence of SDS. In comparable cases (compare milk vs. mammary Golgi, serum vs. liver, and HeLa cells vs. culture medium), the molecular weight of the soluble form is smaller than the corresponding membrane-associated enzyme. This observation led to the presumption that the soluble form is derived from the membrane-bound component by proteolysis.[18,19,23,24] Evidence in support of this theory is extended by observations that the enzymatic properties of soluble and membrane-bound GT are essentially identical; the soluble milk enzyme and the detergent-solubilized Golgi enzyme have similar kinetic properties, although the Golgi enzyme is larger than the milk enzyme by about 13,000 M_r.[18] Most recently, Paquet and Moscarello[25] compared rat liver Golgi and rat serum GT with the commercially available enzyme from bovine milk and showed that all three enzymes were very similar in their kinetic properties. Moreover, treatment of colostrum GT with trypsin generated three components having the same molecular masses as those found in milk enzyme, again suggesting that the heterogeneity was due to proteolytic degradation.[12,26]

Most laboratories identified more than one species of GT by molecular weight. Studies on the biosynthesis of GT showed two finely resolved species of 54,000 daltons after biosynthetic labeling of HeLa cells, human hepatoma HepG2 cells, and MCF-7 mammary tumor cells. One-dimensional peptide maps suggest that all three species contain closely related, if not identical, polypeptides (see also Section IV).[27] The soluble forms, released into the culture medium of these cell lines, consist initially of one species (52,000 M_r as judged from mobility on SDS-PAGE), but are gradually degraded into several species of apparent molecular weights of about 50,000 M_r.[28] This further supports the notion that the soluble species of GT is derived from the Golgi-associated enzyme.

In addition to the heterogeneity originating from differences in the cellular site (i.e., intra- or extracellular), differences in charge may also result in species heterogeneity. The enzyme purified from human milk, which migrates on SDS-PAGE as a single band of about M_r = 55,000, resolves into 7 to 13 different forms by isoelectric focusing with isoelectric points between 4.9 and 6.44.[29] This phenomenon cannot be explained simply by differences in terminal neuraminic acid residues present in oligosaccharide side chains, since cleavage of neuraminic acid by neuraminidase shifts the isoelectric points towards the cathode and only reduces the heterogeneity of the native enzyme to approximately six species. Biosynthetically labeled GT isolated by immunoprecipitation from HeLa cells was separated into a large number of different species appearing as a complex smear by two-dimensional gel analysis with isoelectric points ranging between 4 and 7.[28] Davey et al.[33] reported a comparable heterogeneity for GT from human serum (i.e., at least 12 different isoproteins having isoelectric points between 4.33 and 5.23). Upon neuraminidase treatment, a shift towards the range of values between 5.41 and 5.97 was observed. Treatment with neuraminidase also appeared to abolish charge differences between GT purified from milk and other sources such as ascitic and amniotic fluid.[29] Strous et al.[28] demonstrated that sialic acid residues on *N*-linked oligosaccharides contribute little if any to the total negative charge of HeLa cell GT polypeptides. Therefore, a partial explanation for charge heterogeneity can be a various number of sialic acid residues on *O*-linked oligosaccharides, but the experiments with neuraminidase indicate that sialic acid is probably not the only reason for this phenomenon. Furthermore, Whitehead et al.[34] reported that the GT isoenzyme pattern for whole tissue is more complex than the patterns for individual cell lines established from that tissue. They also showed each cell line has its own unique isoenzyme pattern. This suggests there may be cell- and tissue-specific GT isoenzymes and that the complexity seen in serum and ascitic fluid may result from a combination of cell- and tissue-specific isoenzymes. This is particularly important since alpha-lactalbumin affinity pu-

rification has been used in some of the aforementioned isofocusing experiments and since alpha-lactalbumin preferentially binds some isoenzymes.[33] Thus, selective binding must be considered when interpreting the results of such purifications.

Qian et al.[35] observed that serum GT from patients suffering from liver neoplasms showed a different isofocusing pattern than GT from patients with nonneoplastic liver disease or from healthy subjects. This result supports the hepatocyte as the (major) cell of origin of serum GT. Since Kim et al.[36] investigated the relationship between GT serum levels and liver disease, evidence has accumulated that suggests certain tumors cause elevated serum levels of GT. Closer examination using isoelectric focusing and electrophoresis under nonreducing conditions revealed that in patients with carcinomas from breast, colon, ovary, liver, gastrointestinal tract, and lung certain of the 12 or more isoenzymes ranging in isoelectric points between 4.5 and 7 were considerably elevated.[35,37-39] This elevation is more or less tumor specific.[40] The isoenzyme present in the serum is believed to originate from the tumor tissue for two reasons: (1) the serum GT enzyme activity closely relates to the tumor mass and (2) isoenzyme patterns are tumor related. Whether the differences in GT from control serum and serum obtained from cancer patients are only due to differences in post-translational modifications or whether they are caused by differences at the transcriptional level is unclear.

Biosynthetic labeling of cells in tissue culture offers the possibility of comparing the molecular weights and the process of post-translational maturation among cell lines derived from cancer tissue and cells under more normal growth control such as fibroblasts. The molecular weights of GT immunoprecipitated from several (cancer-derived) cell lines is exactly the same as GT isolated from human fibroblasts (Table 1). These experiments indicate that charge heterogeneities most probably arise from differences in glycosylation or other post-translational modifications.

The exact relationship between different species isolated from different cells and fluids remains unclear. However, it is reasonable to assume that all soluble GT present in fluids originates from the Golgi membrane-bound enzyme and that upon release from the cells the enzyme loses its hydrophobicity. The apparent molecular weight as judged from mobility on SDS-PAGE of the Golgi enzyme is lowest (54,000 M_r) for the species studied in human cell lines such as HeLa, hepatoma, and breast cancer cells and highest (69,000 M_r) in rat liver and sheep mammary gland (Table 1). This most probably arises from proteolysis, which occurs upon release of the polypeptide into body fluids or tissue culture medium. Little is known about the molecular structure of the enzyme aside from its molecular weight and what can be inferred from binding studies in relation to the enzymatic properties (see Section III). Studies using biosynthetic labeling in combination with immunoprecipitation have shed some light on the molecular structure of GT.[24,27,28] GT appears to be composed of two different polypeptides (54,000 to 56,000 M_r), differing slightly in molecular weight. There is no evidence for a di- or polymeric appearance of GT in the Golgi membranes.

Amino acid analysis has been performed on the enzyme from bovine milk,[7] rat liver Golgi,[21] rat serum,[21] and human serum.[14] The amino acid composition of human serum GT is similar to that of the enzyme from bovine milk and is somewhat different from the two rat species. The amino acid composition of the rat serum enzyme is nearly identical to that from rat liver.

GT from each species or tissue is a glycoprotein. The carbohydrate composition was determined for GT from bovine milk and human serum.[14,41] The human serum species contains an estimated 11% of carbohydrate and unlike GT from human milk no acetylgalactosamine is present. This fact suggests that the enzyme has only asparagine-linked carbohydrate chains. The values of mannose, galactose, acetylglucosamine, and sialic acid are 5, 5, 9, and 7 residues per polypeptide, respectively. They are very close to the expected values of 6, 5, 9, and 5, which are the sums of the carbohydrate com-

ponents in each of the combined triantennary and biantennary chains commonly found in serum glycoproteins.[42] The data are most consistent, with serum GT containing one triantennary "complex" type and one biantennary "complex" type *N*-linked oligosaccharide. Strous and Berger[24] have studied the oligosaccharides on GT from HeLa and human hepatoma cells by biosynthetic labeling studies in the presence of tunicamycin, an inhibitor of the synthesis of the *N*-linked oligosaccharide precursor dolichol pyrophosphate *N*-acetylglucosamine, or by digestion of the biosynthetic intermediates with beta-endoglucosaminidase H. Comparison of GT biosynthetically labeled in the presence of tunicamycin (52,000 M_r) and GT from control cells revealed that the radioactivity pattern did not change upon isofocusing. This result shows that either Golgi GT does not bear terminal neuraminic acid residues at the nonreducing end of *N*-linked oligosaccharides or that neuraminic acid residues are present without contributing significantly to the total charge heterogeneity of GT. In contrast to human serum GT, the enzyme isolated from human and bovine milk contains a considerable amount of acetylgalactosamine residue. This is evidence for the presence of *O*-linked oligosaccharides, as galactosamine does not occur in *N*-linked oligosaccharides. *O*-linked sugars are linked through acetylgalactosamine to serine or threonine residues of the "backbone" polypeptide chain. In HeLa cells, Strous et al.[27,28] showed that GT is provided with several *O*-linked oligosaccharides. This was established after biosynthetic labeling in the presence of (^{3}H)galactose and beta-elimination. Treatment with mild alkali cleaves oligosaccharides, specifically in *O*-glycosidic linkage, to serine or threonine. The presence of 1 *M* of sodium borohydride during the cleavage converts the linking *N*-acetylgalactosamine to *N*-acetylgalactosaminitol. Analyses of beta-eliminated (^{3}H)galactose-containing oligosaccharides derived from GT revealed a number of oligosaccharides upon gel filtration on Biogel® P-2, ranging from more than 1800 to 100 M_r. Calculations from incorporation studies with (^{3}H)galactose and (^{3}H)mannose in the presence and absence of tunicamycin indicate that when mannose incorporation is completely inhibited galactose incorporation into GT is still 70% of that found in the absence of tunicamycin. This is consistent with a substantial number (5 to 10) of galactose-containing *O*-linked oligosaccharides.[27] Further analysis following neuraminidase treatment indicated that the majority of *O*-linked oligosaccharides consists of galactose-*N*-acetylgalactosamine linked to serine or threonine and contains one or two sialic acid residues. The same oligosaccharides have been found on the low density lipoprotein receptor, an intrinsic membrane protein recycling between the plasma membrane and intracellular organelles,[43] and on glycophorin,[44] an erythrocyte membrane protein. A minor portion of the labeled oligosaccharides was of a more complex nature, as they eluted in the void volume of the column, even after neuraminidase treatment.

III. ENZYMATIC PROPERTIES

The physiological functions associated with GT are dependent upon its ability to recognize a donor substrate and an acceptor molecule. The donor substrate is UDP-alpha-D-galactose (UDP-Gal), and the acceptor is either *N*-acetylglucosamine or glucose. Acetylglucosamine may be the free sugar molecule or the nonreducing terminus of *N*-linked oligosaccharide of the sequence GlcNAc-beta-Man-alpha-Man-beta-GlcNAc-beta-GlcNAc-asparagine, as they occur in eukaryotic glycoproteins (Figure 1). Also, (Gal-beta 1,4-GlcNAc)-repeats on *N*- and *O*-linked oligosaccharides and glycolipids can be galactosylated by the enzyme.[45,46] Transfer to glucose only occurs in the presence of alpha-lactalbumin. As alpha-lactalbumin is only synthesized in the mammary gland at parturition and during lactation, lactose synthesis is confined to this organ. The combination of Golgi-localized GT and alpha-lactalbumin is also called

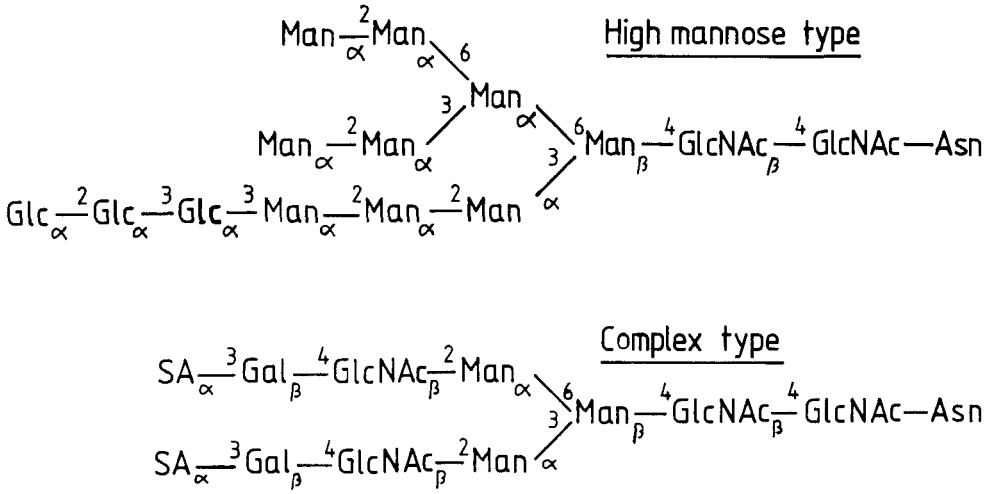

FIGURE 1. The "high mannose" oligosaccharide as it is attached to asparagine residues of the growing peptide in the lumen of the rough endoplasmic reticulum contains two acetylglucosamine, nine mannose, and three glucose residues. The biantennary "complex" configuration as commonly found in glycoproteins is shown in the lower part of the figure. Gal = galactose, GlcNAc = acetylglucosamine, and Man = mannose.

lactose synthetase; in that respect, GT is called the lactose synthetase A protein, while alpha-lactalbumin is called the lactose synthetase B protein.

In glycoproteins, galactose may be the terminal nonreducing sugar or may be penultimate to a sialic acid residue (Figure 1). It is usually linked to *N*-acetylglucosamine by a beta 1,4 linkage, but galactose beta 1,6-acetylglucosamine and beta 1,3-acetylglucosamine sequences have also been described. A scheme for the transfer reaction is:

$$\text{UDP-alpha-D-Gal} + \text{GlcNAc-beta-R} \rightarrow \text{Gal-beta-GlcNAc-beta-R} + \text{UDP}$$

R can be a hydrogen atom, an oligosaccharide, a glycopeptide, a glycoprotein, or a glycolipid.

The interaction of milk GT and alpha-lactalbumin is a unique example of enzyme modification. Many kinetic studies have been reported on purified human and bovine milk GT. The pure enzyme requires Mn^{2+} for activity. Detailed studies have been carried out on the milk enzyme in the presence and absence of alpha-lactalbumin.[10,47-50] Furthermore, the binding of monosaccharide, UDP-Gal, UDP, Mn^{2+}, alpha-lactalbumin, and disaccharide products to the enzyme has been extensively studied. The interaction between GT and alpha-lactalbumin requires Mn^{2+} and either UDP-Gal or an acceptor sugar such as *N*-acetylglucosamine or glucose. In the absence of alpha-lactalbumin, the interaction of GT with Mn^{2+} ions, UDP-Gal, and acceptor molecules has been studied in detail.[10,48,51-53] Manganese ions always bind first to the enzyme, followed by the binding of UDP-Gal; it is also possible that the complex Mn^{2+}-UDP-Gal binds as a second ligand to the Mn^{2+}-enzyme complex. The acceptor then binds to this complex to form an enzyme-Mn^{2+}-UDP-Gal-acceptor complex, which dissociates to generate the product and UDP. The presence of alpha-lactalbumin lowers the K_m for glucose about 1000-fold. Bell et al.[49] have reported that alpha-lactalbumin can either bind to an enzyme-Mn^{2+}-UDP-Gal complex or to an enzyme-Mn^{2+}-acceptor complex.

The kinetic studies on the mechanism of lactose synthetase have contributed considerably to an understanding of the behavior of GT (the lactose synthetase A protein) on various affinity absorbents which have been used in its purification. GT requires a sulfhydryl group.[54] Its interaction with UDP-Gal has been studied by circular dichro-

ism.[52] The membrane-bound GT species can bind an amount of the nonionic detergent Triton® X-100 equivalent to its own molecular mass.[30] Bretz and Staübli[55] observed that the enzyme activity of rat liver GT is not only dependent on sufficient detergent to dissolve the Golgi membranes, but also that the activation of enzyme is correlated to the Triton®/phospholipid ratio, irrespective of the amount or the purity of the subcellular fraction. In addition, a stimulatory effect of lipids on GT activity has been reported by Mitranic et al.,[56] who observed that lecithins such as phosphatidylcholine enhanced the transferase reaction 600% for the purified milk enzyme. Although GT from milk is not a membrane-bound enzyme, certain lipids can presumably mimic the membrane environment needed for optimal enzyme activity.

GTs show a very high degree of specificity. The earliest indications of the specificity prompted the "one linkage-one enzyme" hypothesis, which predicts a different enzyme for each glycosidic linkage found in oligosaccharides of glycoproteins.[57,58] In general, the observed specificities of purified GTs have supported this hypothesis.[1,59] However, a glycosidic linkage is not just defined by its anomeric configuration (alpha or beta) and its position of linkage to the underlying sugar residue. In addition, the sequence of sugars in the oligosaccharides can influence the specificity of glycosyltransfer reactions; Rao and Mendicino,[60] working with mouse lymph node GT, showed that a desialylated and degalactosylated glycopeptide, derived from IgG, served as a very good acceptor for the first galactose residue, but that the transfer rate of the next galactose moiety to another free ultimate acetylglucosamine residue on the same (branched) oligosaccharide was considerably reduced. Studies on the synthesis of Ii antigenic determinants have shown that bovine colostrum beta 1,4-GT transfers galactose to the beta-6-linked acetylglucosamine residue of the trisaccharide GlcNAc beta 1,6-(GlcNAc beta 1,3-)Gal at a rate 20 times faster than the beta-3-linked GlcNAc residue.[61] Most recently, Paquet et al.[62] have extended these studies and found that the addition of galactose residues onto biantennary asparagine-linked oligosaccharides is accomplished sequentially and that the galactose located on the alpha 1,3-linked branch is transferred first, whereas the galactose found on the alpha 1,6-linked branch is transferred second. It is not clear whether different isoenzymes are responsible for this specificity. Strous and Berger,[63] studying the biosynthesis of GT, found that both in vitro, using HeLa mRNA, and in vivo, using tunicamycin-treated HeLa cells, GT is synthesized as two different polypeptides (Figure 4A, Section V). These results suggest that the penultimate galactose residues may be transferred by different, but closely related, enzymes.

The assay for GT activity is generally based on the incorporation of radioactively labeled galactose from UDP-Gal into an acceptor protein, which has only acetylglucosamine exposed as the acceptor site. Thereafter, the acceptor protein is precipitated by acid and the incorporated galactose determined by liquid scintillation counting. It is imperative, however, that products are well identified whenever a crude enzyme preparation is used. In serum, for example, two different GT activities have been distinguished by several criteria including analysis of the linkage type catalyzed. One enzyme (A) produced a galactose beta 1,3-acetylgalactosamine-protein linkage using asialo-ovine submaxillary mucin, while the other (B) catalyzed a galactose beta 1,4-acetylglucosamine using free acetylglucosamine as acceptor. Upon separation of the two on a Sepharose-acetylglucosamine affinity column, the B-enzyme still transferred galactose to asialo-mucin. Careful analysis of the products revealed hitherto undetected amounts of terminal acetylglucosamine residues in desialylated submaxillary mucin.[46]

Another complication in enzyme assays using crude cellular fractions is the presence of nucleotide pyrophosphatase, which catalyzes the hydrolysis of sugar nucleotides.

Inactivation of nucleotide pyrophosphatase without affecting GTs can be achieved by removal of Zn^{2+} ions in the presence of EDTA.[64]

Thus, study of the enzymatic properties of GT has contributed significantly to the understanding of enzyme specificity in glycoprotein biosynthesis. Moreover, it has revealed a unique illustration of enzyme modification by alpha-lactalbumin.

IV. DISTRIBUTION OF GT

The Golgi complex is the main locus for GT which serves to catalyze galactose transfer to ultimate acetylglucosamine residues of glycoproteins passing through this organelle. Fractionation studies, autoradiographic studies, and immunohistochemical and immunocytochemical evidence have demonstrated the association of this enzyme with the Golgi complex. As a consequence, GT activity measurements have been widely used as a specific marker for the Golgi membranes in fractionation studies. In addition, there is a considerable body of evidence to show that in some specialized cells the same enzyme may also be found at the cell surface. In this position, the enzyme is thought to take part in cell migration, tissue interactions, neuronal specificity, immune recognition, and tumor metastasis. A third important localization is in body fluids such as blood, milk, saliva, cerebral spinal fluid, and effusions.

A. The Golgi Complex

A number of polypeptide-modifying enzymatic activities have been ascribed to the Golgi complex including trimming and rebuilding of *N*-linked oligosaccharides,[1] proteolytic cleavage of secretory proteins,[65-67] terminal sugar addition to mucins,[68,69] and phosphorylation of putative lysosomal enzymes.[70,71] All these enzymatic activities and functions are considered Golgi-specific. Growing insight on the mechanism of these functions has made it clear that different Golgi cisternae contain their own specialized sets of enzymes, which can thus be regarded as "marker" enzymes for subfractions of the Golgi system.[72-74]

On the basis of cell fractionation and autoradiographic studies, it was discovered that GT activity is enriched in putative Golgi fractions.[75-84] Autoradiographic studies have shown that radioactive fucose and galactose are initially incorporated in the Golgi complexes of all tissues when examined after very short pulse-labeling. Poort[85] showed that thin tissue slices from gastric epithelial cells incorporated radioactive galactose from UDP-(^{3}H)Gal in the Golgi region. All these findings led to the classical use of GT activity measurements to define Golgi membranes in fractionation studies. A relatively trans-sided location of terminal sugar addition was initially indicated by autoradiographic data[69] and thereafter by studies employing carboxylic ionophores to interrupt intracellular transport.[86] A definitive answer to the precise localization of GT came with the use of immunocytochemistry at the electron microscopic level. Roth and Berger[87] used affinity-purified antibodies raised against the human milk enzyme to demonstrate that GT is present in the two or three trans-cisternae of the Golgi complex. They applied antibodies to ultrathin sections of Lowicryl-embedded HeLa cells and combined the immunocytochemistry with cytochemistry of thiamin pyrophosphatase. The experiments clearly show that GT co-localizes with the pyrophosphatase activity, an enzyme activity cytochemically localized in the trans-cisternae of the Golgi complex.[88] GT not only adds galactose to growing oligosaccharide chains, it also liberates UDP, a strong feedback inhibitor of the transfer reaction.[10] In order to remove this inhibitory effect, trans-cisternae of the Golgi complex contain UDP-ase activity. This enzyme is probably the same as thiamin pyrophosphatase.[89,90] The recognition that GT activity is functionally associated with the presence of UDP-ase was first made by Kühn and White.[89] The model, as modified by Capasso and Hirschberg,[92] describes

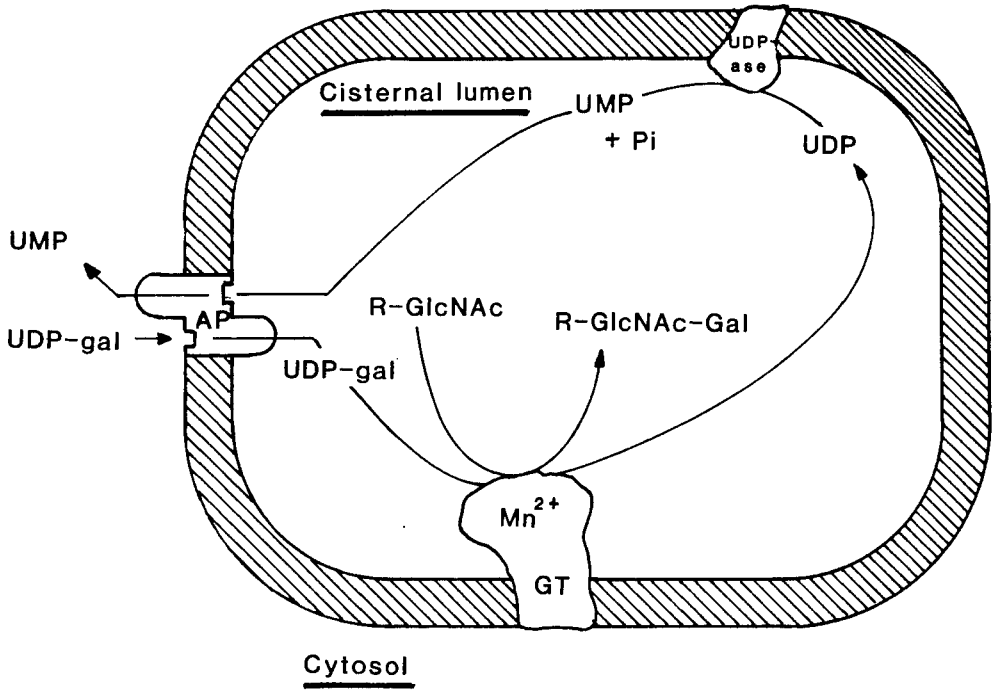

FIGURE 2. Proposed mechanism for the cooperative action of three membrane proteins involved in galactosylation of glycoproteins. GT binds UDP-galactose (UDP-Gal) and transfers the galactose residue onto terminal acetylglucosamine and at the same time delivers UDP to the luminal space. UDP, if not removed, interacts with GT and inhibits the transferase. Neighboring UDP-ase binds UDP and releases UMP and phosphate. UMP binds then to an antiport protein (AP) at the luminal side, while UDP-Gal is present at the cytosolic side of the Golgi membrane. The AP protein transports UDP-Gal into the Golgi cisterna and at the same time UMP to the cytosol.

the interaction of three proteins: GT, UDP-ase, and an antiport protein, which transports UDP-Gal to the lumen of the Golgi cisterna and uridine monophosphate (UMP) in the opposite direction on an equimolar basis (Figure 2).[91] In addition to the localization of GT in the Golgi complex of HeLa cells, the enzyme is also immunocytochemically localized in the Golgi complex of human fibroblasts, stomach, jejunum, liver, and pancreas.[93,94] In intestinal epithelial cells, the membranes facing the intestinal lumen were also heavily labeled, whereas basolateral membranes exhibited only faint staining in light microscopic immunocytochemistry; heavy labeling was also found adjacent to the brush border in the region of the terminal web. It is of interest that the distribution of GT appears to mimic that of thiamin pyrophosphatase in enterocytes as well.[95]

Geuze et al.[96] applied immunocytochemistry on ultrathin frozen sections of human hepatoma cells (HepG2) and observed that GT is also present in a trans-Golgi reticulum of smooth-surfaced tubules with coated areas and vesicles (Figure 3). They have concluded that this trans-Golgi reticulum comprises the same organelle as the anastomosing network of acid phosphatase-containing tubules designated by Novikoff as Golgi-endoplasmic reticulum-lysosome (GERL).[97] Several laboratories have cytochemically localized UDP-ase (or thiamin pyrophosphatase) to this organelle.[98-100] These results support the hypothesis of an obligatory co-localization of GT and UDP-ase.

GT is a membrane-bound enzyme that can be solubilized by use of Triton® X-100. When treated in this manner, GT forms a complex with Triton® X-100 consisting of 52% detergent.[30] Such high detergent-binding capability is a criterion of an intrinsic

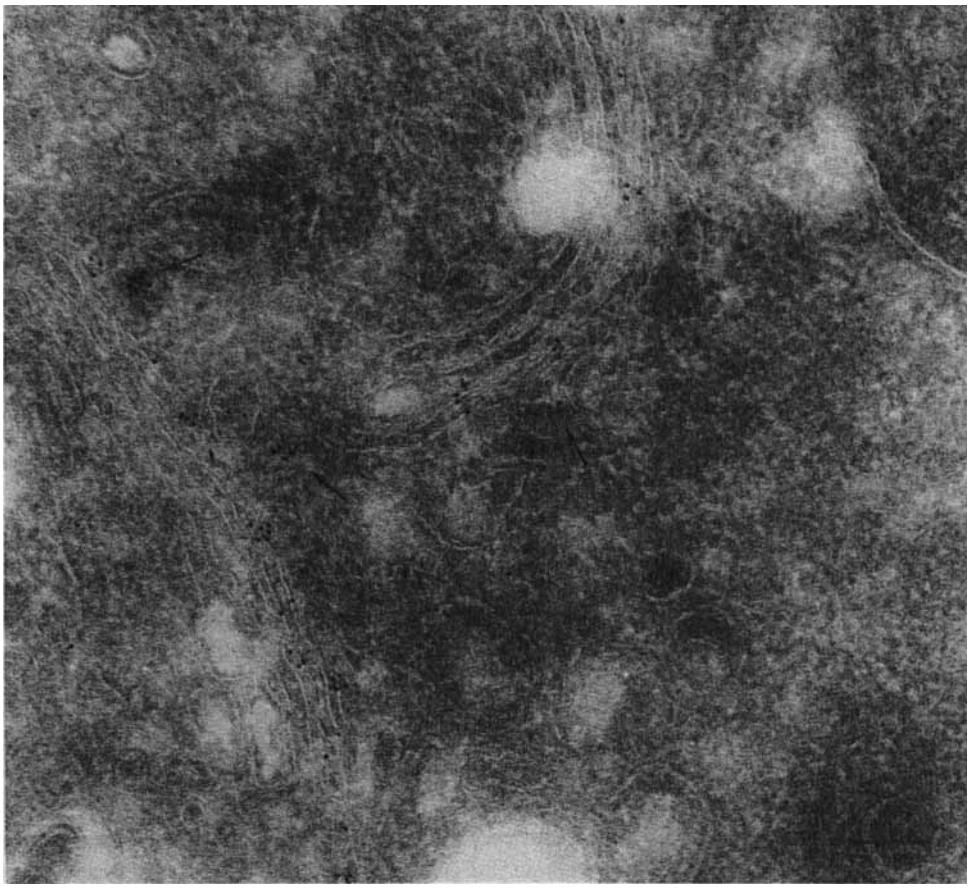

FIGURE 3. Immunoelectron microscopic localization of GT in the Golgi complex and the trans-Golgi reticulum of HepG2 cells. Ultrathin cryosection from HepG2 cell was fixed with 0.2% glutaraldehyde and 1% acrolein. The section was first incubated with affinity pure polyclonal antibodies raised in rabbit against human soluble milk GT and then with swine anti-rabbit IgG. Thereafter, 4 nm of colloidal gold-protein A was applied. Label can be seen in the two trans-most cisternae and in the trans-Golgi reticulum, indicated with arrows. Bar = 0.15 μm. (Micrograph courtesy of Prof. H. J. Geuze, Laboratory of Cell Biology, University of Utrecht, Utrecht, The Netherlands.)

membrane protein.[101,102] Since GT is involved in the processing of glycoproteins, it is expected to be oriented with its enzymatic site towards the lumen of the Golgi cisterna. Kühn and White[104] showed that newly synthesized lactose, a product of GT and alpha-lactalbumin, was formed within vesicles derived from rat lactating mammary gland.[103] GT activity present in Golgi-derived vesicles can be enhanced 26-fold in the presence of Triton® X-100.[105] Fleischer[106] showed that in a well-characterized Golgi fraction from rat liver GT is largely inaccessible to large molecular weight substrates or to inactivation by trypsin. The vesicles are disrupted either with a nonspecific detergent such as Triton® X-100 or with a more selective agent such as filipin to fully expose the enzymatic activity. Additional evidence related to the orientation of GT in the Golgi membrane has been provided by Strous et al.[27] Using an isolated membrane fraction and biosynthetic labeling, they showed that both biosynthetic precursors, present in the RER fraction, and the mature GT, present in Golgi-derived microsomes, are present intramembraneously and at the luminal side in both membrane preparations; no protease-cleavable polypeptide is detected at the outside of microsomal vesicles.

Thus, GT is an integral membrane glycoprotein. It requires detergent for removal from the membrane, it is not accessible to proteolytic enzymes from the cytosolic face of the endomembranes, and its active site faces the cisternal lumen. It is localized in the two or three trans-most cisternae of the Golgi stack and in the trans-Golgi reticulum. In both locations, it co-localizes with thiamin pyrophosphatase.

B. The Cell Surface

GTs have been localized to the cell surface of many cell types. Immunocytochemically, GT has been localized in the brush border membranes of enterocytes, at the cell surface of human stomach, jejunum, liver, and pancreas, and at the cell surface of mouse embryos in the preimplantation stage.[93,107,108] As will be explained in Section V, GT can be released from cells in a soluble form; biochemical evidence for the existence of cell surface or "ecto-GT" has been summarized in a review by Pierce et al.[5] The presence of "ecto-GTs" has stimulated hypotheses involving these enzymes in cell-cell recognition,[109] malignant transformation and growth control,[110] morphogenesis, and sperm-egg recognition.[4] The extent and possible implications of these findings make it important to study the enzymes in great detail. Unfortunately, in only a few instances have the enzymes been purified and well characterized.[111-114] In these processes, GT is thought to function as a type of lectin: anchored in the cell membrane, it binds to an acceptor molecule (an acetylglucosamine-terminal macromolecule) either present in the cell membrane of an adjacent cell or part of the extracellular matrix. In this concept, the cell-cell or cell-matrix interaction will terminate if UDP-Gal is present, since, in the presence of UDP-Gal, the transferase reaction will proceed and GT will lose its affinity for its former substrate. In addition, UDP-dialdehyde and alpha-lactalbumin should also be expected to specifically perturb interactions between cells or between cells and matrix since the former competes with UDP-Gal for binding places on the enzyme and the latter changes the specificity of the enzyme. Evidence for ecto-GT is mainly based upon measurements using intact cells as the enzyme source. The transfer reaction can be measured using radioactively labeled UDP-Gal. Although the radioactive precursor cannot enter the cell, surface nucleotide phosphatases and sugar phosphatases can hydrolyse the nucleotide sugar. The labeled sugar can then be used intracellularly to label macromolecules. Thus, assays of surface GT must always control for potential intracellular utilization of labeled sugar. Despite extensive control experiments, solid evidence for a biologically functional involvement of GT is difficult to obtain.[5] Another potential difficulty in studies of the involvement of GT in extracellular interactions is the presence of enzymes released by the cells; HeLa cells for example release 2 to 3% of their intracellular GT per hour.[24] This enzyme may adhere to the cell surface and, as cell surface GT activity is never more than a few percent of total cell-associated GT, contribute significantly to enzyme activities measured at the outside of the cells or in the culture medium.

In spite of all the practical difficulties, many laboratories have documented the existence of surface GT. In particular, GT is implicated in mouse fertilization, embryonic cell adhesions as morula compaction and neural-retina specificity, in mesenchyme migration, limb bud morphogenesis, immune recognition, and growth control. Shur and Hall[115] demonstrated a possible involvement of sperm surface GT during in vitro capacitation. They observed a striking correlation between the activity of one particular surface enzyme (i.e., GT) and the T/t complex effects on fertilization and development. The T/t complex of the mouse is located near the centromeric end of chromosome 17 and contains over 100 known dominant and recessive mutations, some of them having a profound effect on spermatogenesis, fertilization, and embryonic morphogenesis. It was found that cell surface GT on uncapacitated sperm is occupied by a large molecular weight glycoconjugate substrate. In embryonal carcinoma cells, the sub-

strate occurs in great abundance, and there is evidence that surface GT is implicated in a surface receptor complex.[116] Also, the zona pellucida possesses substrates for sperm surface GT. The addition of UDP-Gal dissociates sperm-zona pellucida adhesions, while UDP-dialdehyde serves as a competitive inhibitor of the adhesion; also, alpha-lactalbumin inhibits sperm-zona binding.

Comparison of the enzymatic properties of Golgi and cell surface GT indicates that both enzymes are much alike. Cummings et al.[114] show that the kinetics of GT isolated from a plasma membrane fraction from 3T12 cells are similar to those from intact cells. Both enzymes have an absolute requirement for manganese and have the same K_m for UDP-Gal and for acetylglucosamine. In the presence of alpha-lactalbumin, both enzymes transfer galactose to glucose rather than to acetylglucosamine. The latter phenomenon is also observed for GT of intact 3T12 cells and of intact baby hamster kidney cells.[114,117] There may be a direct or evolutionary relationship between GT from Golgi and plasma membrane, but, at present, there is no enzyme purified from the plasma membrane to enable comparison of the different species.

Thus, although many lines of evidence suggest that GT activity is present on the cell surface of some cells, evidence for a role in intercellular recognition is largely correlative. By utilizing biochemical, immunological, and genetic probes of enzyme activity, more insight to a possible function of GT as an "ecto-enzyme" will be forthcoming.

V. METABOLISM OF GT

Study of the biosynthesis and metabolism of GT is the first step in understanding why GT is mainly present in the Golgi complex. This basic cell biological question of intracellular localization concerns many membrane proteins, whether they are present in the plasma membrane or in subcellular (endo)membranes. However, at present, there are no data available which provide a comprehensive explanation for the mechanisms that determine whether a membrane protein is destined for one of the subcompartments or for the plasma membrane. The only exceptions are the enzymes present in the mitochondrial membranes (reviewed by Doonan et al.).[118]

Most integral membrane proteins such as the glycoprotein of Vesicular Stomatitis virus, HLA, H_2 histocompatibility proteins, and erythrocyte band 3 are synthesized on membrane-bound polysomes in the RER and are transported via the Golgi complex to the plasma membrane mannose oligosaccharides. During their transport from the RER to the Golgi complex, the *N*-linked oligosaccharides undergo a number of processing steps: the three terminal glucose and six of the nine mannose residues are removed, and the residual oligosaccharide chains are elongated by sequential addition of *N*-acetylglucosamine, galactose, and sialic acid.[1] It is generally assumed that both secretory and membrane proteins share the pathway from the RER to the plasma membrane and make use of the same set of enzymes for oligosaccharide maturation present in the different compartments.[74,120-122] As stated above, it is not understood how secretory proteins like albumin, transferrin, IgG, and amylase, and integral membrane proteins like asialoglycoprotein receptor, erythrocyte band 3, and viral glycoproteins pass through the RER and the Golgi complex on their way to the plasma membrane while other membrane components such as ribophorin and GT take up their characteristic positions within the different intracellular membranes (RER and trans-cisternae of the Golgi complex, respectively), thus preserving the functional integrity of the respective organelles.[76,123,124]

Although most structural and kinetic studies of GT have been carried out on the secreted forms of the enzyme and on the GT isolated from whole organs, the metabolism of the enzyme has been studied mainly in tissue culture cells because they offer the opportunity to effectively label the enzyme biosynthetically. Strous and Berger[24]

have determined the amount of (^{35}S)-methionine-labeled GT to be 0.005% of total radioactivity incorporated into HeLa cell proteins. The amount of enzyme activity per cell may vary; in rat parotid glands, the level of Golgi GT can rise dramatically (six- to tenfold) following chronic administration of the beta-adrenergic receptor antagonist, isoproterenol.[31] The enzymes isolated from control and isoproterenol-treated animals have identical amino acid compositions and isoelectric points; there is no indication for induction of a specific isoenzyme. Also, the enzyme activity per cell was studied in gastric mucosa of rats following fasting and refeeding. At 3 hr after refeeding, the level of GT was enhanced 30% per cell as compared to the starved situation, again indicating that cells are capable of regulating the GT activity.[125]

The absence of immunocytochemically detectable GT, at the level of the RER, and the cis-Golgi membranes raises questions about the site of synthesis and route of intracellular transport. In HeLa cells, Strous et al.[27] used metabolic labeling and antibodies raised against human milk GT to immunoprecipitate the Golgi enzyme. After a 10-min pulse-labeling in the presence of (^{35}S)-methionine, they found two precursor forms. These precursors occur only in subcellular fractions having a density identical to rough microsomes. After 20 min of chase, the precursor polypeptides migrate at a density equal to that of smooth microsomes. The precursor polypeptides have molecular weights of 44,000 and 47,000 daltons on SDS-PAGE. Following preincubation and labeling in the presence of tunicamycin, an inhibitor of *N*-glycosylation, precursor molecules are synthesized with apparent molecular weights of 42,000 and 45,000 daltons, indicating that both GT polypeptides are provided with one *N*-linked oligosaccharide chain.[24] In addition to using cell fractionation, the intracellular transport time between the RER and the Golgi complex can also be determined by monitoring the processing of the *N*-linked oligosaccharide: endo-beta-*N*-acetylglucosaminidase H has been used to determine the time at which GT arrives at the cis- or middle section of the Golgi stack. This enzyme cleaves the oligosaccharide side chain between the two core *N*-acetylglucosamine residues, leaving a single *N*-acetylglucosamine residue attached to the polypeptide only if the addition of terminal sugar residues has not yet begun.[126] Thus, the rate at which newly synthesized GT acquires resistance to the endo-glycosidase is a measure of the rate of its movement to the Golgi complex. In HeLa cells, GT is transported within 20 min from the RER to the Golgi complex as established in this way.[24] The transport of GT from the RER takes place in the precursor (endo-beta-*N*-acetylglucosidase H sensitive) form. This is consistent with the localization of alpha 1,2-mannosidase 1, primarily to the Golgi complex, as described by Tabas and Kornfeld[127] and by Tulsiani et al.[128] The precursors retain their "high mannose" configuration until arrival in the Golgi complex. Thus, GT shares this feature with proteins destined for the plasma membrane such as erythrocyte band 3, the receptor for asialoglycoproteins in hepatocytes, Vesicular Stomatitis virus glycoprotein, and lysosomal enzymes.[122,129-131] Therefore, the transport pathway for these different classes of proteins appears to be similar, although the transport rates may differ considerably.[122]

Thus, the GT precursor polypeptides migrate to the Golgi complex in 20 min, and, concomitantly, their apparent molecular weights increase to 54,000 daltons. On the basis of experiments using biosynthetic labeling in the presence of (^{3}H)galactose, Strous et al.[27,28] conclude that this molecular weight increase is due mainly to *O*-glycosylation. Although glycosylation of serine and threonine residues may begin at the level of the RER,[43,132] the bulk of sugar addition, including galactose, takes place in the Golgi complex. These investigators calculate on the basis of an apparent molecular weight increase of 7,000 to 9,000 daltons that GT may contain 5 to 10 *O*-linked oligosaccharides. However, Cummings et al.[43] showed recently that *O*-linked oligosaccharides, especially when galactose and sialic acid residues are present, may cause an anomalous behavior on SDS-PAGE, resulting in an overestimation of the degree of

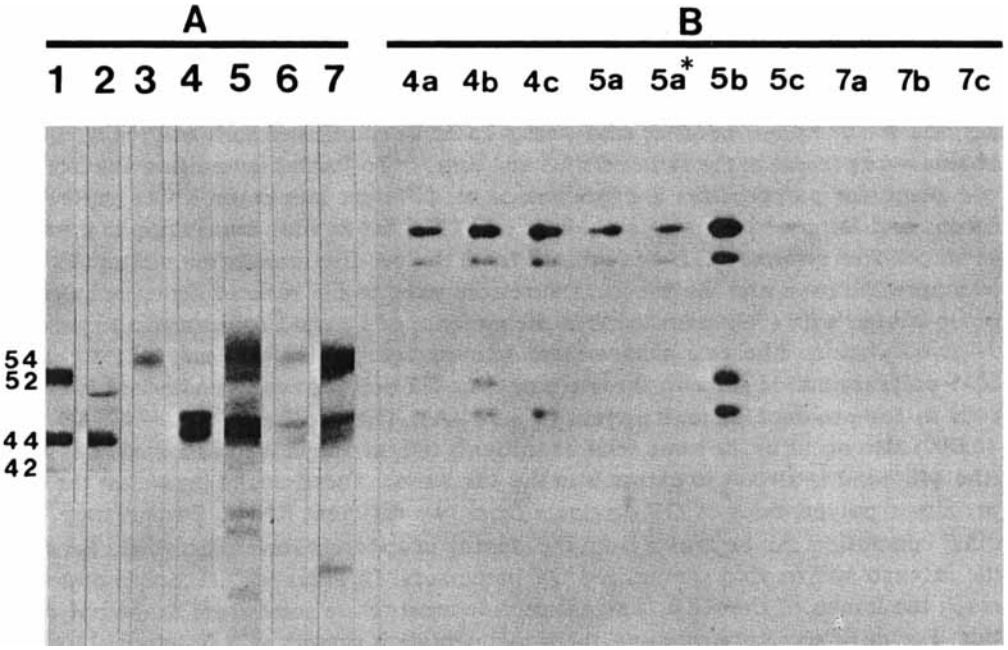

FIGURE 4. Biosynthesis of GT. Immunoprecipitated-GT polypeptides from a cell-free wheat germ translation system (lane 2) are compared with precursor-GT polypeptides isolated from HeLa cells biosynthetically labeled with (^{35}S)-methionine for 30 min (lane 1). The HeLa cells were preincubated and labeled in the presence of 5 μg/mℓ tunicamycin to prevent *N*-glycosylation. The cell-free system was programed with total RNA extracted from HeLa cells. In both lanes, the immunoprecipitated polypeptides (42,000 and 44,000 mol wt) have identical electrophoretic mobilities. Lane 3 shows GT immunoprecipitated from HeLa cells labeled with (^{3}H)palmitic acid for 30 min. Both the mature (54k) and the two precursor polypeptides are radioactively labeled. In lanes 4 to 7, GT polypeptides isolated from different human cell lines are compared. Cells were labeled in the presence of (^{35}S)-methionine for 40 min; lane 4: HeLa cells; lane 5: breast cancer cell line MCF-7; lane 6: human fibroblasts; and lane 7: hepatoma cells (HepG2). The electrophoretic mobilities of precursor and mature polypeptides are similar, except that mature GT from MCF-7 cells migrates slightly slower and as a more diffuse band. In panel B, primary structures of GT polypeptides from three cell lines are compared. Mature and precursor polypeptides isolated by immunoprecipitation and SDS-gel electrophoresis were partially proteolytically digested with Protease V-8, and the digests were separated on a 15% polyacrylamide gel according to the procedure of Cleveland et al.[141] Lanes a correspond to the mature (M_r = 54,000) polypeptides, lanes b to the M_r = 47,000 precursors, and the lanes c to the M_r = 44,000 precursors. The digestions result, in each case, in identical patterns for both mature and precursor polypeptides, except that digestions of the M_r = 47,000 bands (b) give one extra proteolytic fragment in all three cell lines. The numbers correspond to the GT from the cells depicted in A. Lanes 5a and 5a* are derived from the upper and lower part, respectively, of the mature MCF-7 band.

glycosylation, as is the case in the receptor for low density lipoprotein and in glycophorin.[133]

The presence of two different precursor polypeptides is notable. Strous et al.[27] used limited digestion of the biosynthetically labeled precursor polypeptides with protease V-8 in the presence of SDS to show total identity between the lower molecular weight precursor polypeptide and the mature protein. In addition, they noted one extra proteolytic fragment for the higher molecular weight precursor which differed from the two other polypeptides (Figure 4B). As already mentioned in Section III, the enzymatic galactosylation of biantennary complex *N*-linked oligosaccharides occurs in two steps: first, galactosylation of the acetylglucosamine beta 1,2-mannose alpha 1,3-branch, and in the next step, galactosylation of the acetylglucosamine beta 1,2-mannose alpha 1,6-

branch.[60] According to the "one linkage, one-enzyme" theory, there may be more than one, possibly two, different isoenzymes for terminal galactosylation of *N*-linked oligosaccharides.[56] Additional evidence for two different GT polypeptides comes from biosynthetic labeling of HeLa cells, using a very short-pulse (5 min) and short-chase periods. No precursor-product relationship could be established since both polypeptide chains were present in the same relative amounts.[134] To further investigate whether the two precursor polypeptides are synthesized on different messenger RNAs (mRNAs), Strous and Berger have used total HeLa cell RNA for in vitro translation in a wheat germ cell-free system.[63] GT was isolated from the cell-free translation mixture by immunoprecipitation and the products were compared to GT isolated from HeLa cells, pulse-labeled with (^{35}S)-methionine in the presence of 5 μg/mℓ tunicamycin to prevent *N*-glycosylation. The two predominant immunoprecipitated products migrate on a SDS-polyacrylamide gel with the two authentic GT polypeptides, synthesized in HeLa cells in the presence of tunicamycin (Figure 4A). The two bands (M_r = 42,000 and 44,000) also occur in the same relative amounts in both the in vivo and in vitro system (the 44k band is always in excess over the 42k band). These results show that the two precursor polypeptides of GT originate from two different RNAs. Furthermore, another conclusion can be drawn from the identity in apparent molecular weight between the in vitro and in vivo synthesized GT precursors. In order for GT polypeptides to reach the lumen of the RER, a signal peptide must reside somewhere in the polypeptide. For most secretory proteins, the signal peptide is present as a *N*-terminal hydrophobic peptide of about 20 amino acid residues, which is cotranslationally removed at the luminal side of the RER membrane. Since GT precursor polypeptides synthesized in tunicamycin-treated HeLa cells have molecular weights identical to the in vitro products, it is likely that GT polypeptides have a noncleavable, presumably, internal signal peptide. Two other laboratories have reported the identification of GT polypeptide(s) after translation in a cell-free system: Humphreys-Beher[31] has translated poly(A$^+$) RNA from rat parotid glands in a reticulocyte lysate and identified a 45,000-dalton band upon SDS-PAGE; it is not clear whether a specific band is also present at a slightly lower molecular weight. Giles and Browne[135] have shown different molecular weight polypeptides after translation of lactating rat mammary mRNA in the absence and presence of dog pancreas microsomes; they suggest the presence of a cleavable signal on GT. Thus, there is experimental evidence for two different precursors; however, it is not clear whether they have a cleavable signal peptide.

In addition to glycosylation of proteins, evidence is accumulating that many membrane proteins are post-translationally acylated. Although a definitive function for the fatty acid has not been identified, it has been suggested that this covalently bound lipid may help anchor proteins in the membrane, act as a signal in transport to the plasma membrane, or be involved in fusion events (reviewed by Magee and Schlessinger[136]). Schmidt and Schlessinger[137] first discovered that the proteins of many enveloped viruses are post-translationally fatty acylated. Since then, many nonviral plasma membrane proteins were found to be acylated: the transferrin receptor,[138] the major histocompatibility complex proteins,[139] and the major intrinsic protein of rat sciatic nerve myelin.[140] In all cases thus far described, palmitate is present close to or within the transmembrane region of the polypeptide, linked either to a serine, threonine, or cysteine residue. Incubation of HeLa cells in the presence of (^{3}H)palmitate for 30 min results in the labeling of all three GT polypeptides (Figure 4A).[134] The two precursors, in addition to the mature enzyme, are labeled in the presence of radioactive palmitate, suggesting that palmitation of GT begins at an early stage during the transport from the RER to the Golgi complex, prior to the acquisition of resistence to endo-beta-*N*-acetylglucosaminidase H. Similar findings have been reported for other membrane proteins.[138] The fact that a genuine Golgi membrane protein such as GT is palmitated

indicates that palmitation is not a prerequisite nor an exclusive feature of plasma membrane proteins.

As already discussed in Section II, GT is present in all tissues and its molecular weight differs between species and tissues. Since most purification procedures are based upon affinity chromatography on alpha-lactalbumin-immobilized supports, binding to this protein is the major selectivity criterion, while other features such as molecular weight or charge do not contribute to the purification. Modification of GT may occur during its lifetime or during the isolation procedure. Thus, it is not unexpected that a variety of species differing in molecular weight is reported (Table 1), even if the size and structure of GT, synthesized in most tissues and species, is about the same. Evidence for this hypothesis comes from experiments with different cell lines.[134] GT from human hepatoma cells was biosynthetically labeled and compared with the enzyme from human fibroblasts, from breast cancer MCF-7 cell, and with the enzyme from HeLa cervical carcinoma cells. All four cultured cell types synthesize GT in the same way, i.e., as two precursor polypeptides of 44,000 and 47,000 daltons, resulting in a mature protein of about M_r = 54,000 (Figure 4A). As all four GTs show a molecular weight difference of 7000 between the precursor forms and the mature enzyme, it is likely that they undergo *N*- as well as *O*-glycosylation. Of interest in this respect is the observation of Fujita-Yamaguchi and Yoshida[14] that GT from human serum does not contain *O*-linked oligosaccharides. If glycosylation of GT in hepatoma cells is comparable with that in normal hepatocytes, it follows that the majority of GT present in human serum is not a derivative of the liver enzyme. The mature enzyme produced by the MCF-7 cells migrates as a more diffuse band with a slightly higher molecular weight; whether this results from a slightly modified enzyme synthesized by mammary tissue or as a result of transformation of the cell line remains unclear. The primary structure of the different polypeptides has been compared by partial proteolytic digestion in SDS-PAGE according to the procedure of Cleveland et al.[141] There is a striking similarity among the three polypeptides derived from the different cell lines (Figure 4B). All three GT polypeptides have the same pattern, with the exception that the 47,000 species shows one extra proteolytic band in all three cell lines tested. These results are consistent with the concept that the polypeptide chains of GT are identical for all cells of the same species irrespective of transformation, while differences in glycosylation or other post-translational modifications may cause heterogeneity in molecular weight and/or charge. In particular, malignant transformation may cause changes in glycosylation patterns, as first indicated by Buck et al.[142] They emphasized that cell surface glycopeptides bear higher amounts of sialic acid residues following transformation.

What is the fate of Golgi GT? Over a given period, this enzyme catalyzes galactosylation of glycoproteins either as soluble proteins or as integral membrane proteins passing through the lumen of the trans-Golgi cisternae; thereafter, the enzyme is released in the medium or fluid surrounding the cell. Because it is very difficult in a total organism to trace the secreted GT molecules back to their cells of origin, tissue culture cells offer an easy way to address this problem.

A. Secreted GT

LaMont et al.[117] observed that GT activity accumulated in tissue culture supernatants during cellular growth. This interesting finding strengthened the assumption that the enzyme is being released from the cells by a mechanism which converts the intracellular membrane-bound form to the extracellular soluble form.[17,18,23] Strous et al.,[24,28] using HeLa cells, examined the tissue culture medium after pulse-chase labeling. They could immunoprecipitate a protein of M_r = 52,000 on SDS-PAGE. After longer chase times, this protein was converted to a M_r = 50,000 species, probably as a

result of proteolytic degradation in the tissue culture medium. Both species were soluble proteins since they could not be sedimented by ultracentrifugation. There was a reciprocal relationship between the disappearance of GT from the cells and the appearance of the secreted forms in the medium. The exact location at which the conversion takes place is not certain. As the transport time for secretory proteins, such as albumin or transferrin, between the Golgi complex and the plasma membrane is less than 5 min in hepatoma cells,[122,123] GT, released in the Golgi, could reach the medium in the same short time. This makes assessment of the localization experimentally very difficult. In confluent HeLa cell cultures, pulse-labeled GT has an average half-life of 19 hr as judged from the rate of disappearance from the cells.[24] Since it takes GT 20 min to reach the Golgi complex after biosynthesis, it can be concluded that in a steady-state situation, less than 2% of the enzyme is present between the site of synthesis (RER) and the trans-Golgi cisternae. Alternatively, if the conversion of GT into a soluble form occurs in the Golgi region, less than 0.05% is associated with the cells in a soluble form.

Thus, GT, a genuine Golgi membrane component, is synthesized as two precursor polypeptides and processed in a manner analogous to plasma membrane and secretory proteins. However, its presence in the Golgi complex for approximately 19 hr, as opposed to the short cellular half-life of 20 to 60 min of secretory and plasma membrane proteins, implies a mechanism which retards GT at the level of the distal Golgi cisternae prior to its release into the medium.

The observation that tissue culture media in addition to body fluids contain GT polypeptides as well as enzymatic activity shows that the conversion from membrane protein to a soluble enzyme is a general phenomenon, probably not dependent upon the presence of certain proteolytic enzymes in the extracellular fluid. This implies that the conversion most probably takes place intracellularly. Support for this hypothesis comes from experiments with metabolically labeled HeLa cells incubated at 4°C with antibodies against GT. PAGE shows that only soluble GT was present at the exterior of the cells.[24]

The question of the origin of soluble GT is most important as Podolsky and Weiser[144] claim to have purified two forms of GTs, one of which is found only in malignant effusions. As their data are based upon electrophoretic separation of an enzyme activity with generally slow migration on polyacrylamide gels, it is not clear whether the difference in electrophoretic mobility relates to higher molecular size or lower charge density. In light of reports of pK-values ranging from 4.7 to 8, it is conceivable that the cancer-associated GT variant represents a subspecies. This may result from unusual oligosaccharides or another (post-translational) modification, resulting in a unique separation pattern after isoelectric focusing or electrophoresis under non-denaturing conditions. Recently, Podolsky and Isselbacher[39] have characterized the two GT species occurring in normal human serum and in sera from patients with a variety of cancers using several different monoclonal antibodies raised against soluble GT from normal serum. They conclude that the majority of their antibodies recognize both enzyme species equally well. It is therefore conceivable that the unusual GT found in sera from cancer patients is a derivative of the normal enzyme and that the only difference lies in abnormal glycosylation or another form of post-translational modification.

Since GT is released from (probably all) cells in a soluble form, it is important to stress the principal difference between GT as an integral membrane protein of the cell surface and the soluble enzyme loosely associated with the plasma membrane. The first configuration is required for the enzyme to play a role in cell-cell interaction phenomena; the second situation occurs because of the ability of the enzyme to bind surface or glycocalix glycoconjugates, having *N*-acetylglucosamine residues in ultimate posi-

tions. In this respect, care should be taken to interpret immunocytochemical localization studies on cells and tissues.

VI. IMPLICATIONS FOR GOLGI FUNCTIONS

Once GT has entered the trans-cisternae of the Golgi complex, it begins its contribution to one of the major functions of the Golgi complex: glycosylation of passing glycoproteins. The Golgi complex is in fact the best characterized compartment of the secretory route. It consists of a stack of smooth-surfaced cisternae.[121,145] In addition to its role in glycosylation, the Golgi complex harbors a host of macromolecules and enzymes delegated to fulfill many functions in the modification of molecules as well as in sorting processes. The stack of cisternae is comprised of several subcompartments on the basis of structural, histochemical, enzymological, and functional criteria. As discussed in Section IV, GT itself is mainly present in two or three cisternae at the trans-side of the complex, together with thiamin pyrophosphatase, and to a lesser extent in the trans-Golgi reticulum. Study of the intracellular transport, processing, and fate of the enzyme is important for an understanding of the Golgi complex for two reasons: (1) the enzyme undergoes several post-translational modifications on its way from the RER to the trans-side of the Golgi complex, including sialylation and galactosylation; these modifications are potentially important for the mechanism of the trans-Golgi localization of GT. (2) As a resident protein of a subcompartment of the Golgi complex, it serves as a probe for the study of that compartment.

In this review, it has become evident that the enzyme must have passed most of the functional sites between the RER and the trans-Golgi:

1. It becomes *N*-glycosylated and, thereafter, acquires resistance to endo-beta-*N*-acetylglucosaminidase H.
2. It is palmitated.
3. *O*-linked oligosaccharides are attached, including galactose and sialic acid residues.
4. It attains extensive charge heterogeneity, not only caused by sialylation, but presumably also due to sulfation.

Most of these modification reactions are functionally ascribed to different regions of the Golgi complex: processing of asparagine-linked oligosaccharides to the cis-side,[1,42] palmitation to an early Golgi compartment,[146] sulfation to the cis-side and the middle Golgi region,[147,148] and terminal sugar addition to the trans-side.[74,149-151] Studies of the biosynthesis and intracellular transport of GT show that the post-translational modifications occur according to the same scheme and at the same intracellular localizations, as is the case for other (membrane) proteins (summarized in Figure 5).

The fact that GT itself is galactosylated and contains sialic acid residues is especially worthwhile. Geren et al.[152] reported the incorporation of galactose into the enzyme in the presence of UDP-(^{14}C)Gal. In order to function as an effective acceptor molecule, these investigators first had to remove terminal sialic acid and galactose residues. Since GT can only galactosylate terminal *N*-acetylglucosamine residues, this provided evidence that the *N*-linked oligosaccharide, present on GT, not only contained galactose, but also terminal sialic acid residues. The presence of both galactose and sialic acid residues in oligosaccharide side chains of GT implies that within the context of Golgi subcompartmentalization the sialylation of *N*- and probably also *O*-linked chains occurs at the same place as galactosylation.

The localization of GT to the trans-side of the Golgi complex also helps elucidate the transport route of lysosomal enzymes. These enzymes not only contain phospho-

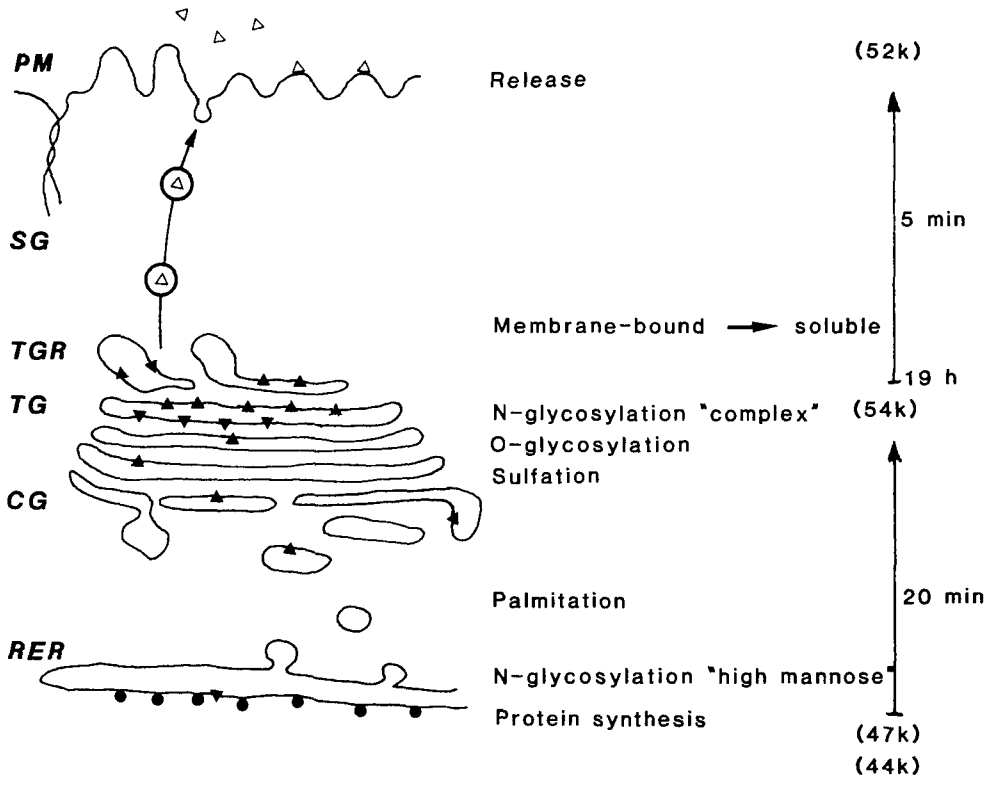

FIGURE 5. A schematic view of the pathway of GT. The steps illustrated include: biosynthesis in the rough endoplasmic reticulum, cotranslational *N*-glycosylation, palmitation before or at the entrance to the Golgi complex, *O*-glycosylation and complex *N*-glycosylation in the *trans*-Golgi, loss of achoring peptide, and release into the medium via secretory vesicle. Approximate resident periods and apparent molecular weights are indicated on the right margin. Solid triangles indicate membrane-bound GT, open triangles are the soluble species, and solid circles are membrane bound ribosomes. RER, rough endoplasmic reticulum; CG, cis-Golgi cisternae; TG, trans-Golgi cisternae; TGR, trans-Golgi reticulum; SG, secretory granules or vesicles; and PM, plasma membrane.

rylated high mannose oligosaccharides, but also carry complex-type chains, thus implying that lysosomal enzymes linked to the mannose 6-phosphate receptor pass the trans-cisternae of the Golgi complex enroute to the lysosomes.[131,153,154] As the presence of mannose 6-phosphate on lysosomal enzymes is a prerequisite for transport of these enzymes to the lysosomes via the mannose 6-phosphate receptor, absence of one of the factors involved in this sorting mechanism may cause severe lysosomal storage disorders. Enzymes involved in the synthesis of the recognition system are mainly present in the Golgi complex. In I-cell disease (mucolipidosis II), the formation of the recognition marker is hampered by the absence of the enzyme activity *N*-acetylglucosamine-1-phosphotransferase.[155] Immunocytochemistry with antibodies against GT offers the opportunity to monitor Golgi behavior in human fibroblasts from a patient with diminished lysosomal function caused by I-cell disease (Figure 6). It is clear that absence of the phosphotransferase causes not only malfunction of the lysosomes, but also severely disturbs Golgi morphology.

The complexity of Golgi operation is compounded by the fact that the Golgi complex is a center where multiple routes of vesicular traffic converge. The best established route is the secretory pathway, wherein the Golgi functions as a chemical processor producing molecules ready to be sent to different regions of the cell or to different

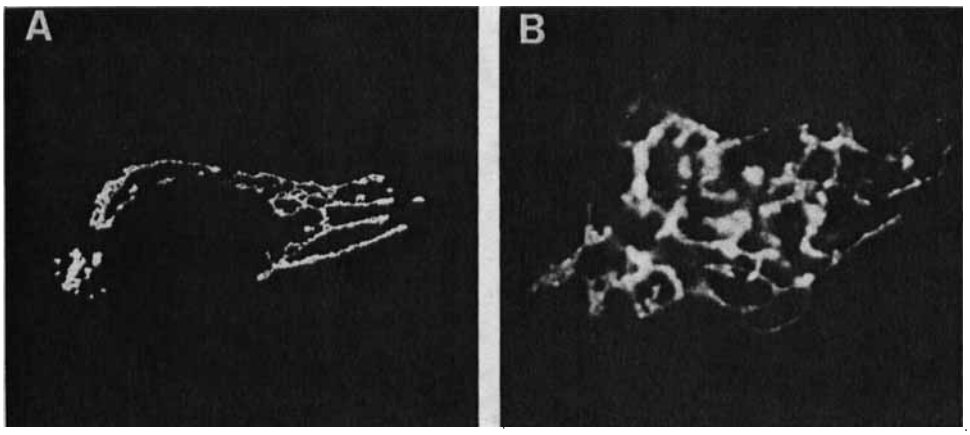

FIGURE 6. Immunofluorescence of human fibroblasts. Nonconfluent cells were fixed in acetone, incubated with anti-GT antibodies, and stained with goat anti-rabbit IgG conjugated with fluorescein. (A) normal fibroblast and (B) fibroblast from patient with I-cell disease. In normal fibroblasts, the Golgi complex exhibits a very extended Golgi complex next to the nucleus. In I-cell fibroblasts, the Golgi complex loses its regular shape and is now spread over the whole cell.

plasma membrane domains. A second and less documented role as a way station is its involvement in the endocytotic route.

A. GT and the Secretory Pathway

The overall metabolism and turnover of the Golgi system is not easy to establish. Study of the metabolism of GT as a resident membrane protein does not allow a direct approach because the enzyme is released from the Golgi complex as a soluble protein after 19 hr. Two different theories on the mechanism of transport through the Golgi complex to advance soluble and membrane proteins from the cis- to the trans-side have been proposed: (1) cisternal progression, proposing that each cisterna matures across the Golgi stack and (2) transport via a vesicle shuttle between the different cisternae (reviewed in Reference 121). In the cisternal progression model, the membrane and luminal-content proteins move with the cisterna in a synchronous manner from cis- to trans-Golgi. Saraste and Kuismanen[156] have studied immunocytochemically the transport of Semliki Forest virus membrane proteins at different temperatures; they can block both the entrance and exit of the membrane protein reversibly. They propose that membrane proteins enter the Golgi stack via tubular extensions of the pre-Golgi vacuolar elements which generate the Golgi complex. The proteins then pass across the Golgi complex following cisternal progression and enter the post-Golgi vacuolar elements to be routed to their different destinations. As the transport of these proteins across the stack is very rapid (less than 5 min),[157] there must exist recycling processes between neighboring cisternae in the trans-cis direction in order to reconstitute the continuous presence of resident Golgi proteins in the different subcompartments. However, there is no such evidence at present.[74,149] The alternative movement would be vesicular traffic operating between the edges of individual cisternae.[158] In this concept, individual cisternae are stable entities maintaining their resident enzymes. This vectorial transport is supported by reconstitution experiments with Vesicular Stomatitis virus membrane protein using isolated Golgi elements.[159] In both concepts, sorting mechanisms are needed to prevent GT from leaving the Golgi complex. In the cisternal progression theory, GT must carry signals to select and shuttle the enzyme back to one of the immature cisternae. If vesicular transport of secretory and plasma membrane

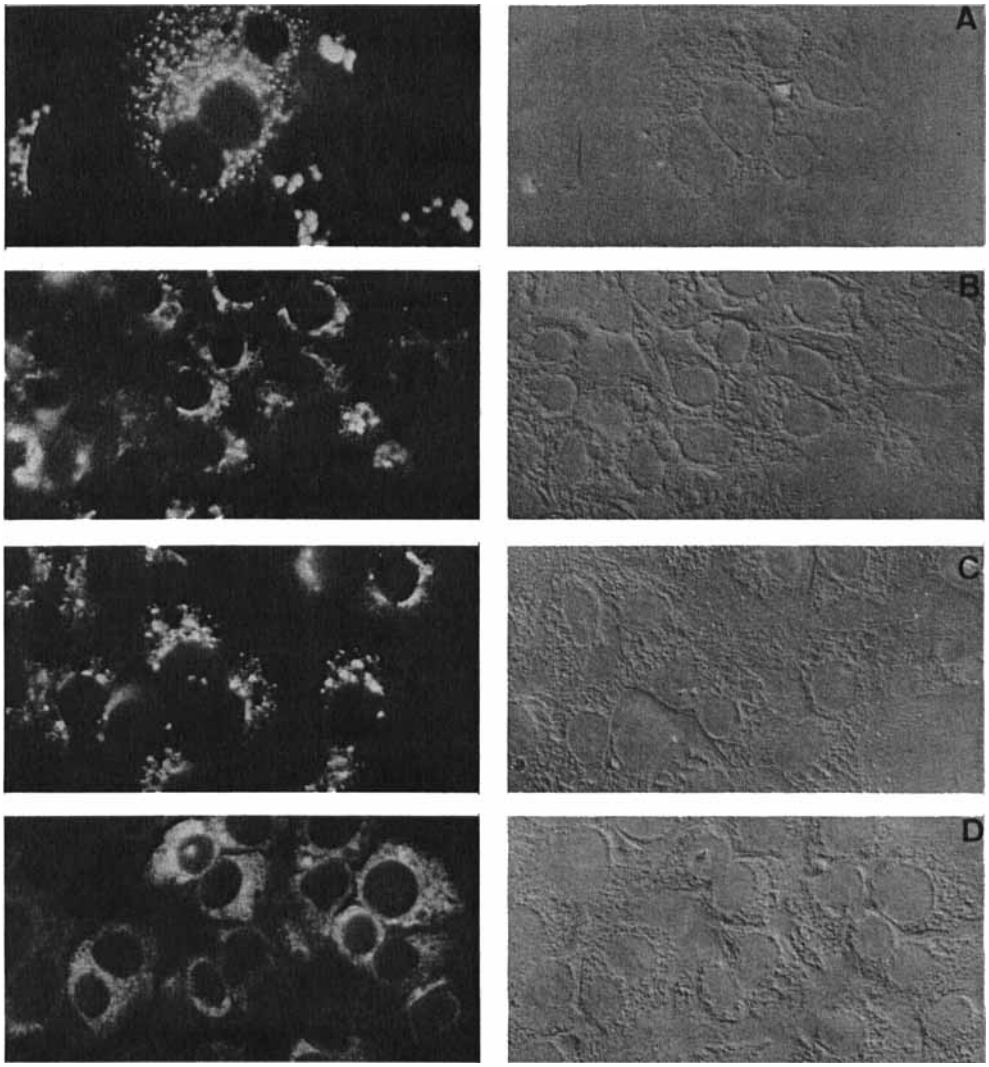

FIGURE 7. Effect of different conditions on the distribution of GT and albumin in HepG2 cells. Near confluent cells were fixed in acetone and incubated with antibodies against either GT (A, B, and C) or against albumin (D, E, and F) and stained with goat anti-rabbit IgG conjugated with fluorescein. A and D, control cells; B and E, cells treated with 1.0 μM of monensin for 2 hr; and C and F, cells treated with 10 mM of NH_4Cl for 2 hr. The right panels show the phase-contrast micrographs. (A) Golgi portions are scattered over the whole cell during mitosis. Monensin causes dilation of Golgi cisternae, but not translocation (B); under these conditions intracellular transport of albumin is inhibited in the same dilated Golgi cisternae (E). In the presence of ammonium chloride, again the Golgi complex is disorganized (C), but albumin is now present all over the cell, indicative for accumulation in vesicles beyond the Golgi complex (F).

proteins is assumed, the enzyme must have a high affinity for the trans-Golgi cisternae, while (membrane) proteins destined for the plasma membrane can move freely within the Golgi membranes and are selected in areas which bud off and fuse again with the next cisterna.

Immunocytochemistry allows for the study of Golgi complex behavior using antibodies against GT as probes. For an overview, whole cells fixed in acetone can be used (Figure 7). In nonconfluent HeLa cells, fluorescent staining is concentrated near the nucleus as a thin band or as a few bright spots (Figure 7A). In mitotic cells, the GT-

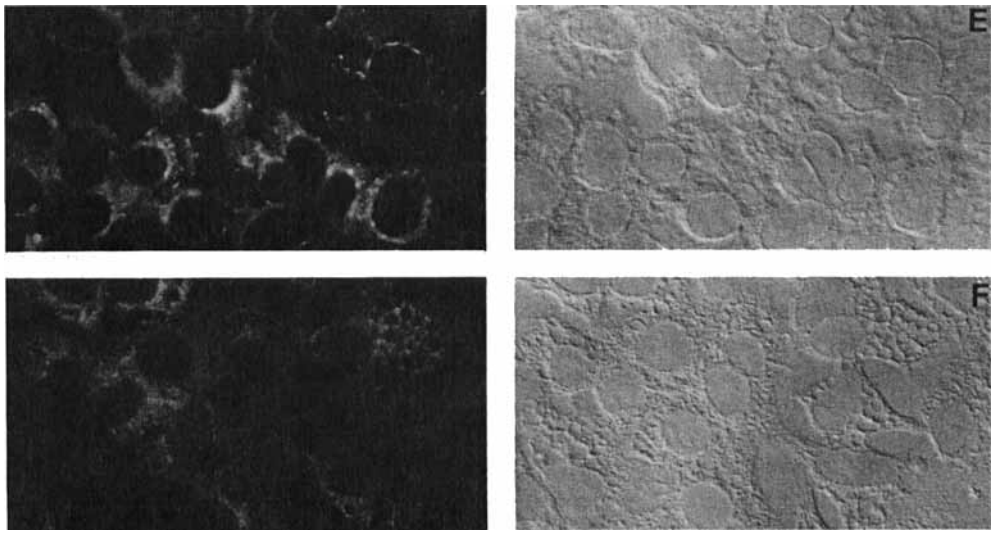

FIGURE 7

containing Golgi cisternae are completely vesiculated (Figure 7A). In addition, it is likely that the cis-cisternae fragment into (the same) smaller units. After mitosis, the Golgi portions will then rearrange to form two different Golgi complexes in the daughter cells. Warren et al.[160] have demonstrated that the intracellular transport of the membrane protein of Vesicular Stomatitis virus is inhibited during mitosis. They observed that the viral protein is distributed differently in mitotic normal rat kidney (NRK) cells and does not reach the plasma membrane. Intracellular transport is apparently impossible under these circumstances. Berlin et al.[161] studied the fate of surface-bound concanavalin A during mitosis in several mammalian cell lines. They showed that endocytotic processes are profoundly depressed during mitosis. Internalization by phagocytosis of IgG-opsonized sheep erythrocytes is completely suppressed throughout all phases of mitosis, extending partly into early G1. Also, fluid-phase pinocytosis, as observed by the uptake of soluble horseradish peroxidase, as well as the uptake of concanavalin A which follows lectin binding to surface receptors, is markedly depressed during pinocytosis. As both the exocytotic and the endocytotic pathway are inhibited during mitosis, a more general mechanism is probably responsible for this phenomenon. It is tempting to speculate that the scattered Golgi complex is responsible for both seemingly unrelated phenomena and that a common mechanism may be responsible for this inhibition.

As the study of the metabolism of resident proteins provides no direct answer to the issues of Golgi function, perturbation of the Golgi complex has been used to study the Golgi behavior. Monensin has been used in many studies to study intracellular transport.[121,122,162,163] The primary effect of the ionophore monensin is partial equilibration of sodium ions across the Golgi membranes, followed by a rapid dilatation of the individual cisternae. This ion exchange counteracts acidification by an electrogenic proton pump present in the Golgi system.[164,165] Strous et al.[28] showed that monensin primarily affects GT-containing Golgi cisternae. Although monensin effectively blocks entry of Semliki Forest virus glycoprotein into the trans-Golgi compartment,[149,150] GT can still reach its position in the trans-Golgi, and, in addition, it is both *O*- and *N*-glycosylated normally. Continuous growth in the presence of monensin results in a longer half-life of GT, suggesting that in the presence of the perturbant the enzyme is less sensitive to proteolytic cleavage. Figure 7B shows the effect of monensin on the

distribution of GT in hepatoma cells. It is clear that the trans-Golgi cisternae are now vesiculated and distributed in an area around the nucleus, clearly different from the localization during mitosis. When the cells are stained for albumin (Figure 7E), the same distribution is obtained, confirming earlier observations that transport of secretory proteins is inhibited at the trans-side of the Golgi complex.[74]

Another group of agents, the so-called lysosomotropic amines (e.g., ammonium chloride, chloroquine, and primaquine), are weak bases which freely diffuse and accumulate within lysosomes as well as other acidic compartments as a consequence of being protonated.[166] In endocytosis of receptor-ligand complexes, acidification probably plays an important role in dissociation and recycling of plasma membrane receptors.[167] As the Golgi complex also contains proton-pump activity, and as a consequence its pH is slightly acidic,[164,165] ammonium chloride causes the Golgi cisternae to dilate, as is demonstrated by immunocytochemical localization of GT (Figure 7C). In contrast to the effect of monensin on the distribution of GT-containing cisternae, ammonium chloride also causes these cisternae to spread farther away from the nucleus. When hepatoma cells are immunocytochemically stained for albumin, it is clear that accumulation of this secretory protein also occurs in vesicles beyond the Golgi complex, indicating that other acid compartments are involved in transportation of albumin in addition to the Golgi (Figure 7F).[143]

B. GT and the Endocytotic Pathway

The Golgi complex is probably also involved in endocytotic processes. Geuze et al.[168] report the presence of a considerable amount of receptor for asialoglycoproteins in rat liver Golgi. Snider and Rogers[169] show that the receptor for transferrin can recycle via a compartment, which contains sialyltransferase, presumably the Golgi complex. They demonstrated that in neuraminidase-treated cells (containing galactose-terminal transferrin receptors) the transferrin receptor is resialylated intracellularly and is then returned to the plasma membrane. It is well known that membrane proteins are exchanged between the cell surface and intracellular organelles during the uptake of membrane by pinocytosis and phagocytosis and during addition of membrane by fusion of intracellular vesicles with the plasma membrane (for reviews, see References 170 to 172). The experiments with the transferrin receptor strongly suggest that certain membrane proteins can recycle between the plasma membrane and the Golgi complex. Endocytosis of plasma membrane receptors can be demonstrated by incubating human hepatoma cells containing receptors for transferrin and asialoglycoproteins at their cell surface (HepG2) at 37°C in the presence of the different antireceptor antibodies for several hours.[134] Immunofluorescent staining is then present intracellularly, presumably in the endosomal or the compartment of uncoupling ligand and receptors (CURL), and at the plasma membrane. If these cells are incubated under the same conditions in the presence of affinity-purified antibody raised against GT, no fluorescence is detectable, either at the plasma membrane or intracellularly, indicating that the Golgi enzyme does not recycle between the plasma membrane and the Golgi complex. This finding is consistent with the presence of a stable Golgi system, wherein the communication between the individual cisternae is mediated by shuttling vesicles. It strongly suggests that resident proteins of the Golgi complex do not return to their places in the Golgi complex once they reach the plasma membrane.

Thus, although membrane proteins can pass through the cisternae of the Golgi complex either from the RER or from other intracellular compartments and can arrive at the plasma membrane, GT is constrained; in fact, its presence is restricted to the trans-Golgi and trans-Golgi reticulum.

VII. PERSPECTIVES

More than 80 years after the discovery of the Golgi complex, many of its essential roles are well established, but many functions remain to be elucidated. Among these is the crucial question of its involvement in the complex process of directing proteins to different destinations within or outside of the cell. It is now clear that the Golgi complex comprises most of the system involved in post-translational modifications of proteins passing through the complex. As it emerges from a huge number of publications, most of the post-translational modifications affect in one way or another the ultimate destination or at least the transport rate of (glyco)proteins on their way from the RER through the Golgi complex to the different sites of the cell. Some proteins (e.g., alpha$_1$-antitrypsin and alpha$_1$-antichymotrypsin synthesized by hepatoma cells) need "high mannose" *N*-linked oligosaccharides without the three terminal glucose residues for transportation between the RER and the Golgi complex.[173] Also, different proteins travel at different rates between the RER and the Golgi complex, indicating that selection can take place at this stage of the secretory pathway.[122,174,175]

Although the Golgi complex contains the tools for imposing signals on passing proteins, it is not clear whether sorting itself takes place in the cisternae of the complex. A well-known example is the addition of the mannose 6-phosphate marker onto lysosomal enzymes: a Golgi enzyme can discriminate between proteins destined for lysosomal function and others and provide them with the signal. However, careful examination shows that those proteins once enroute to the lysosomes also contain *N*-linked oligosaccharides of the "complex" configuration, indicating that they also passed through the trans-side of the Golgi complex. There are additional suggestions that targeting of proteins occurs after the proteins have left the trans-Golgi cisternae: hormones and enzymes, destined for storage in secretory granules until they are secreted upon a stimulus by a secretagogue, often need a proteolytic cleavage to yield active hormones (e.g., insulin in pancreatic islet cells or opioid peptides in different regions of the pituitary). These proteolytic cleavages probably occur in the Golgi complex, after which the unprocessed (pro-)hormones leave the cells in an inactive form via the "constitutive" pathway, while the correctly tailored peptides are sorted to storage granules.[176]

Acidification of intracellular compartments may well be a factor in directing proteins to certain destinations as is seen in studies on sorting mechanisms in receptor-mediated endocytosis. In addition to lysosomes, it is now apparent that endosomes, CURL, and the trans-Golgi reticulum also have a pH between 6.5 and 5.5.[165,177,178] If an acidic environment is important for sorting, certainly the trans-Golgi reticulum is an obvious candidate for the major sorting center since this compartment apparently contains lysosomal enzymes and receptors for mannose 6-phosphate and for asialoglycoproteins in addition to secretory proteins.[74,96] In this respect, the trans-Golgi reticulum can be regarded as an extension of the Golgi stack because it also contains small amounts of GT and thiamin pyrophosphatase.

While some insight exists into the movement of proteins and membrane systems in both the exocytotic and the endocytotic direction, there is currently a lack of understanding as to how cells maintain their different endomembranes with specific sets of enzymes in order to function properly (the only exception being mitochondrial membrane proteins). It is tempting to believe that membrane proteins contain cytoplasmic extensions that play a role in the destination and residence of organelle membrane proteins. However, in spite of many different approaches, including gene modification by molecular engineering, there is no information available on these putative determinants required for proteins to reside within a certain compartment. This must be the case for GT. If it contains a cytoplasmic fragment, it must be so small that even non-

specific proteolytic enzymes cannot recognize it. It is provided with palmitate residues, as are many proteins in various membrane systems. It is *N*- and *O*-glycosylated and probably also contains sulfate residues, but again these features are probably not distinctive for Golgi localization. Thus, this major question remains unanswered. The Golgi complex is polarized in many respects. Thus, lipid composition should also be implicated in the range of factors involved in membrane protein arrangement within the Golgi complex. It is known that from cis to trans the relative concentration of cholesterol increases. Clearly, one promising way to shed light on the problem of GT localization is the establishment of the primary structure of a few Golgi membrane proteins, particularly that of GT. This information will clarify how the enzyme is anchored in the Golgi membrane and how detachment from the membrane occurs. It will also resolve the issue of the two (or more) different precursor molecules. And last, but not least, it will shed light on the question of the ecto-GT, involved in cell-cell interactions. In order to function at a location distinct from the Golgi complex, this GT species must have different specifications for membrane anchoring since it must pass the Golgi complex and reach the plasma membrane as an integral membrane protein.

During the past 15 years, a substantial body of knowledge has accumulated regarding the biochemistry of GT. The general mechanisms which govern galactosylation of glycoproteins and the synthesis of lactose establish this enzyme as a valuable model system. At the same time, our understanding of membrane protein metabolism and of Golgi complex functions has increased correspondingly. Certainly, future studies with GT will continue to address these and other important issues.

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