Localization of GTP-stimulated Core Glycosylation to Fused Microsomes

JACQUES PAIEMENT and J. J. M. BERGERON*
*Département d'anatomie, Université de Montréal, and *Department of Anatomy, McGill University, Montréal, Québec, Canada, H3C 3J7*

ABSTRACT Purified rough microsomes from liver maximally incorporated *N*-acetyl-[^{3}H]glucosamine into endogenous acceptors from UDP-*N*-acetyl-[^{3}H]glucosamine substrate, providing the associated ribosomes were removed and 0.5 mM GTP was added. These conditions also led to the coalescence of microsomes into large fused membranes. By measurement of membrane profiles on electron micrographs, a correlation was observed between GTP-stimulated glycosylation and microsomal membrane length ($r^2 = 0.92$). Membrane fusion was not observed in the absence of GTP, with sugar transfer inhibited by >90% for acid-resistant acceptors (protein), and ~50% for acid-labile acceptors (lipid-linked intermediates). When radiolabeled acceptors were localized by electron microscope radioautography, high concentrations of silver grains (83 grains/100 μm membrane length) were observed over fused membranes with lower grain densities observed over unfused membranes in the same preparation (20 grains/100 μm). These studies directly link microsomal membrane fusion to GTP-stimulated core glycosylation. The observations extend the suggestion of Godelaine et al. (1979, *Eur. J. Biochem.* 96:17–26) that physiological levels of GTP promote the translocation of substrate across endoplasmic reticulum membranes which, we propose, occurs via a membrane fusion phenomenon.

A central problem in asparagine-linked core glycosylation is the mechanism by which nucleotide sugars located in the cell sol gain access to glycoprotein acceptors located within the lumina of the endoplasmic reticulum. As the lipid-linked oligosaccharide precursors of the core sugar glycopeptides are also intraluminally oriented (1) then sugar translocation must occur before or during the synthesis of the lipid-linked oligosaccharides.

In 1977, Godelaine and colleagues (2) discovered that physiological concentrations of GTP enabled the translocation of sugar substrate across intact membrane vesicles derived from the rough endoplasmic reticulum (ER),[1] provided these membranes had been stripped of their ribosomes (2–4). Paiement, with these same investigators (5) showed that physiological concentrations of GTP coincidentally promoted fusion of the same stripped rough microsomes.

To ascertain whether the GTP-specific membrane fusion phenomenon was merely coincidental or more causally linked to core glycosylation, we have compared membrane fusion and glycosylation quantitatively and attempted to localize the sites of sugar translocation to acceptor glycolipids and glycopeptides. Electron microscope radioautography of ER vesicles after incubation with GTP and uridine diphosphate-*N*-acetyl-[^{3}H]-glucosamine (UDP-[^{3}H]GlcNAc) has revealed silver grains, indicative of glycosylated lipid-linked oligosaccharides and glycopeptides, predominantly over fused ER membranes, thereby providing a direct link between membrane fusion and core glycosylation. Portions of this work have been reported in abstract form (6).

MATERIALS AND METHODS

Preparation of Microsomal Subfractions: Microsomes from rat liver were prepared by differential centrifugation by using a slight modification of the procedure of Bergeron et al. (7). After an initial centrifugation at 8,700 g_{av} for 13 min, the supernatant was centrifuged at 25,000 rpm for 6 min 40 s in the Beckman type 50 rotor (8). Total microsomes were separated from the supernatant by centrifugation at 100,000 g_{av} for 60 min and then resuspended in sucrose to give a final concentration of 1.38 M (giving a density $\simeq$ 1.20 g·cm^{-3}, which is known to be the equilibrium density for rough microsomes, [9]). They were then placed under step-gradients of 1.0, 0.86, and 0.25 M sucrose, and centrifuged by using a Beckman SW 60 rotor at 300,000 g_{av} for 60 min. The rough microsomal pellet was recovered and resuspended in 0.25 M sucrose with 4 mM imidazole buffer, pH 7.4. These rough microsomes were stripped of their ribosomes with 5 mM sodium pyrophosphate as previously outlined (2) and the resulting membrane preparation was designated stripped rough microsomes. Protein concentra-

[1] *Abbreviations used in this paper:* Dol-P-P-GlcNAc, dolichol pyrophosphate GlcNAc; Dol-P-P-GlcNAc-GlcNAc, dolichol pyrophosphate *N,N'*-diacetylchitobiose; ER, endoplasmic reticulum; GlcNAc, *N*-acetylglucosamine; UDP-[^{3}H]GlcNAc, uridine diphosphate-*N*-acetyl-[^{3}H]glucosamine.

TABLE I

Biochemical Parameters of Subcellular Fractions

Fraction	Protein	RNA	Glucose-6-phosphatase	Acid phosphatase
	mg/g		*U/g*	
Homogenate	188.35 ± 16.31	8.80 ± 1.22	14.96 ± 3.64	6.43 ± 0.26
Microsomes	27.71 ± 3.59	3.50 ± 0.55	7.60 ± 1.85	0.97 ± 0.12
Rough microsomes	6.97 ± 1.28	1.48 ± 0.15	2.02 ± 0.47	0.15 ± 0.02
Stripped rough microsomes	3.54 ± 0.69	0.52 ± 0.06	1.66 ± 0.34	0.10 ± 0.01

Fractions were isolated as described in Materials and Methods and assayed for various constituents. Values are given as means ± SD. Protein and RNA determinations from seven experiments are given in milligrams. Glucose-6-phosphatase and acid phosphatase from four experiments are expressed as micromoles of phosphate liberated per minute per milligram of protein. Values are expressed per gram of liver wet weight.

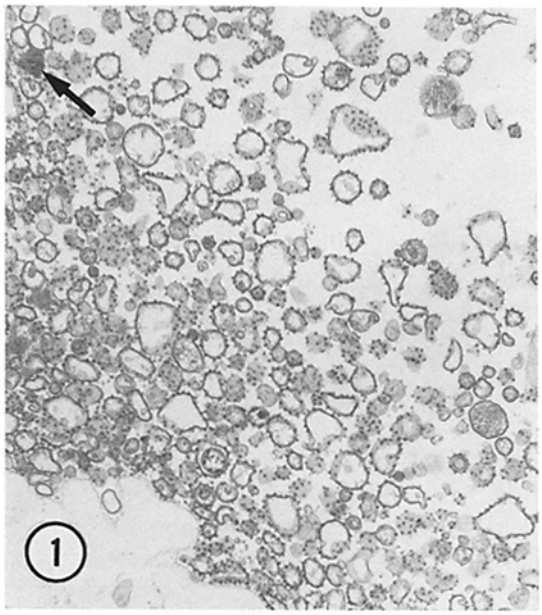


FIGURE 1 Electron micrograph of freshly purified rough microsomes resuspended in buffered 0.25 M sucrose. The vesicle profiles are homogeneous in appearance and size (measurement of 1,002 μm of membrane yielded a mean perimeter of 550 nm) and are surrounded by numerous electron-dense ribosomal particles. A peroxisomal core is indicated by the arrow. × 22,000.

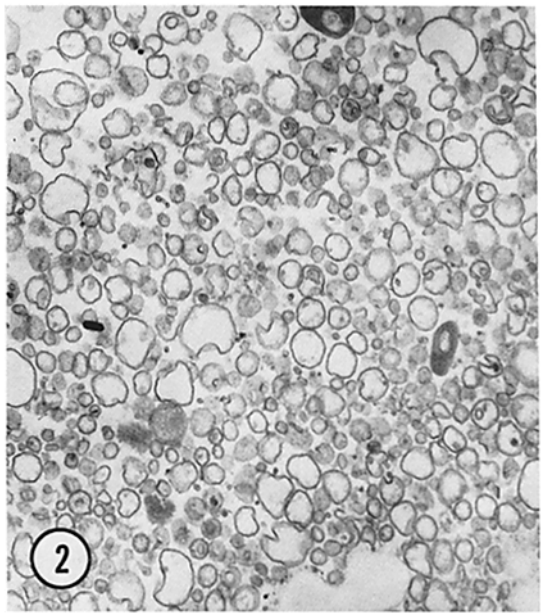


FIGURE 2 Rough microsomes after treatment with 0.25 M sucrose containing 5 mM sodium pyrophosphate. The microsomes appear similar in size to those in Fig. 1 and were determined to have a mean perimeter of 508 nm. × 20,000.

tions were determined by using the BIORAD protein assay kit (BIORAD Laboratories, Mississauga, Ontario) and RNA was monitored by the method of Fleck and Munro (10) as previously described (11).

Enzymic Assays: Glucose-6-phosphatase was assayed essentially as outlined by Nordlie and Arion (12). Reactions were carried out for 5 min at 37°C and stopped by the addition of ice-cold trichloroacetic acid (5%) with inorganic phosphate determined by the method of Ames and Dubin (13). Studies with acid phosphatase were based on the method of Gianetto and de Duve (14) except that the assay contained 0.1% Triton X-100. Reactions were carried out for 30 and 60 min and were stopped by the addition of ice-cold trichloroacetic acid. After centrifugation, phosphate content of supernates was assayed as for glucose-6-phosphatase.

Endogenous Glycosylation: This was carried out essentially as described by Godelaine et al. (2). Briefly, freshly purified, stripped rough microsomes (100–500 μg protein) were incubated with uridine diphosphate-*N*-acetyl-D-glucosamine (glucosamine-6-[^{3}H]*N*; UDP-[^{3}H]GlcNAc; 1–125 μCi, 24 Ci/mmol; New England Nuclear, Boston, MA) in 40 mM Tris-HCl, pH 7.4, 30 mM KCl, 7.5 mM $MgCl_2$, 2.5 mM $MnCl_2$, 2.5 mM dithiothreitol, 1 mM ATP, 10 mM phosphoenolpyruvate, 25 μg pyruvate kinase, with or without 0.5 mM GTP in a final volume of 0.1–0.5 ml. Reactions were carried out for varying periods of time and stopped with ice-cold 5% trichloroacetic acid and carrier albumin (0.1%). The total acid-precipitable products were washed twice with cold 5% trichloroacetic acid, then dissolved in 0.5 ml of Protosol (New England Nuclear) and radioactivity was determined in a Packard model 460 CD spectrometer. Hot acid-resistant, precipitable products were treated as described above except for heating at 90°C for 15 min as described by Godelaine et al. (2).

Morphological Procedures: Stripped rough microsomes were incubated in the endogenous glycosylation medium and then fixed and processed for electron microscopy as previously described (15). Stereology was carried out as previously described (15) using a Zeiss MOP-3 digitizer (Carl Zeiss, Inc., Don Mills, Ontario, Canada). For electron microscope radioautography, subcellular fractions were incubated with UDP-[^{3}H]GlcNAc for 120 min in the presence or absence of 0.5 mM GTP, then fixed and embedded in Epon. Losses of radioactivity in the various buffers and dehydrating solvents were monitored and found to be 1% of the total incorporated radioactivity for samples incubated with 0.5 mM GTP and 2.9% of the incorporated radioactivity for samples incubated in the absence of GTP (data not shown). Thin sectioning, application of nuclear emulsion, and radioautographic development for "fine" silver grains were as described previously (15). Morphometry was carried out on radioautographs having a final magnification of × 30,000 as calibrated with a grating replica of 54,800 lines/inch (J. B. EM Services Inc., Montreal, Canada). Using preparations developed for radioautography as indicated above, many of the silver grains consisted of a single, compact, round silver deposit, others consisted of two to four small silver deposits packed closely to one another. For grain distribution analysis, we have defined silver grains in two ways (16) and have carried out separate determinations based on these two definitions. In the first case, silver grains consisting of groups of two to four small silver deposits packed closely to one another were considered as constituting a single grain (16, 17). In the second case, all individual silver deposits were considered as separate silver grains irrespective of whether they were observed singly or in groups of two to four (16). With a defined ^{3}H-line source of 50-nm thickness, an HD (half-distance [18]) of 70 nm was determined in our laboratory conditions, using Ilford L-4 emulsion and solution-physical (fine-grain) development (Levine, G., B. Kopriwa, and N. J. Nadler. Unpublished observations). Therefore, a circle of 2.1-mm radius was

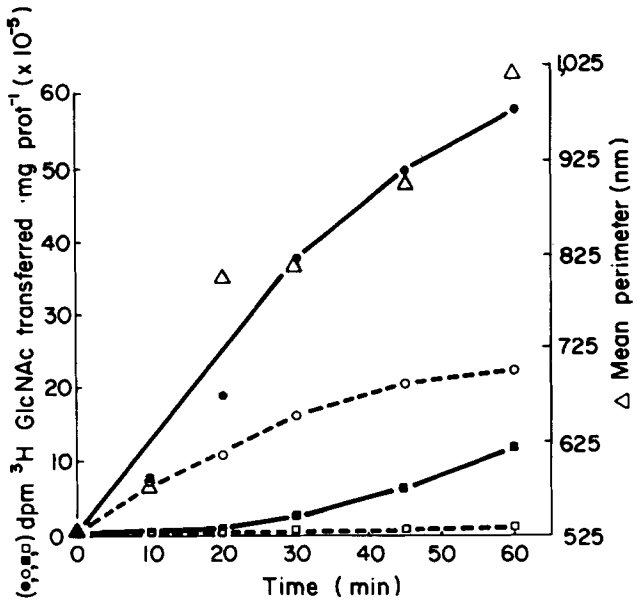


FIGURE 3 Transfer of [^{3}H]GlcNAc from UDP-[^{3}H]GlcNAc substrate to endogenous acceptors of stripped rough microsomes; comparison with membrane fusion occurring among same stripped rough microsomes. Two separate experiments were carried out yielding similar results. In this experiment, UDP-[^{3}H]GlcNAc (1 μCi) was added to 82 μg protein of stripped rough microsomes in a total volume of 0.1 ml in the endogenous glycosylation assay described in Materials and Methods. Incubations were carried out in the presence (●, ■) or absence (○, □) of 0.5 mM GTP. At various times, duplicate samples were removed and incorporation of label followed in ice-cold trichloroacetic acid precipitates (total incorporation; +GTP, ●; −GTP, ○) as well as trichloroacetic acid precipitates after prior heat treatment (acid-resistant, ■, □). For the membrane fusion analysis (right-hand ordinate, △) the same stripped rough microsomes were incubated (1,640 μg protein) in 0.5 ml of the endogenous glycosylation medium in the presence of 0.5 mM GTP. At various times, these samples were fixed and filtered for random analysis as described previously (15) and the perimeters of all sectioned profiles of the stripped rough microsomes determined (1,000 μm measured for each time interval) and the mean perimeter calculated (△). Corrections were carried out to account for possible errors in the morphometric analysis. Corrections for section thickness effect (23) were used and found not to significantly affect the correlation coefficient (0.958, corrected; compared with 0.956, uncorrected) when linear regression analysis was carried out to compare membrane lengths with sugar incorporation. Correction for section compression was deemed unnecessary since the pellicles had been cut with the knife edge parallel to the filtration direction (24).

centered on each silver grain of the micrographs at × 30,000 magnification.[2] When only one type of membrane (fused or unfused) was included, the grain was called "exclusive." If two types of membranes (fused and unfused) were within the circle, the grain was called "shared." Shared grains (31% of all grains defined as individual silver deposits) were allocated to fused and unfused membranes based on the proportion attributed exclusively to the individual fused and unfused membranes (21). All grains observed in micrographs were included in the analysis. Similar to a previous investigation on grain distribution (16), our analysis yielded similar results whether silver grains were defined as individual silver deposits or whether they included groups of two to four silver deposits. Only the results obtained with grains defined as individual silver deposits are presented.

[2] Using ^{3}H (19) or ^{125}I (20) radioactive sources, it was shown that varying the limits of the resolution boundary circle (by as much as twice the radius) had little effect on the interpretation of grain distributions over organelles.

RESULTS

Purified rough microsomes were analyzed morphologically (Fig. 1) revealing an homogenous population of ribosome-studded vesicles with a mean sectioned perimeter of 550 nm. Biochemical studies (Table I) indicated a recovery of 13.5% of homogenate glucose-6-phosphatase activity in this fraction, 3.7% of protein, and 16.8% of homogenate RNA, with an RNA/protein ratio of 0.21. Approximately 2% of the homogenate acid phosphatase activity was recovered in the fraction (Table I). Ribosomes were removed by washing with sodium pyrophosphate (2). This resulted in stripped rough microsomes (Fig. 2) with a mean perimeter of 508 nm of the sectioned vesicles. Biochemical studies (Table I) revealed a loss of 50.8% of protein and 64.8% of the RNA by the sodium pyrophosphate washing procedure, but with less of a loss of glucose-6-phosphatase activity (17.8%) and acid phosphatase (33.3%).

When stripped rough microsomes were incubated with UDP-[^{3}H]GlcNAc in the endogenous glycosylation medium, incorporation of label into total acid-precipitable products was greatly increased by the addition of 0.5 mM GTP (Fig. 3), in agreement with the work of Godelaine et al (2, 3). Furthermore, GTP enabled transfer of label to hot acid-resistant, precipitable material. GTP-stimulated incorporation of label into hot acid-resistant, precipitable material of stripped rough microsomes has previously been shown to be associated with endogenous

TABLE II

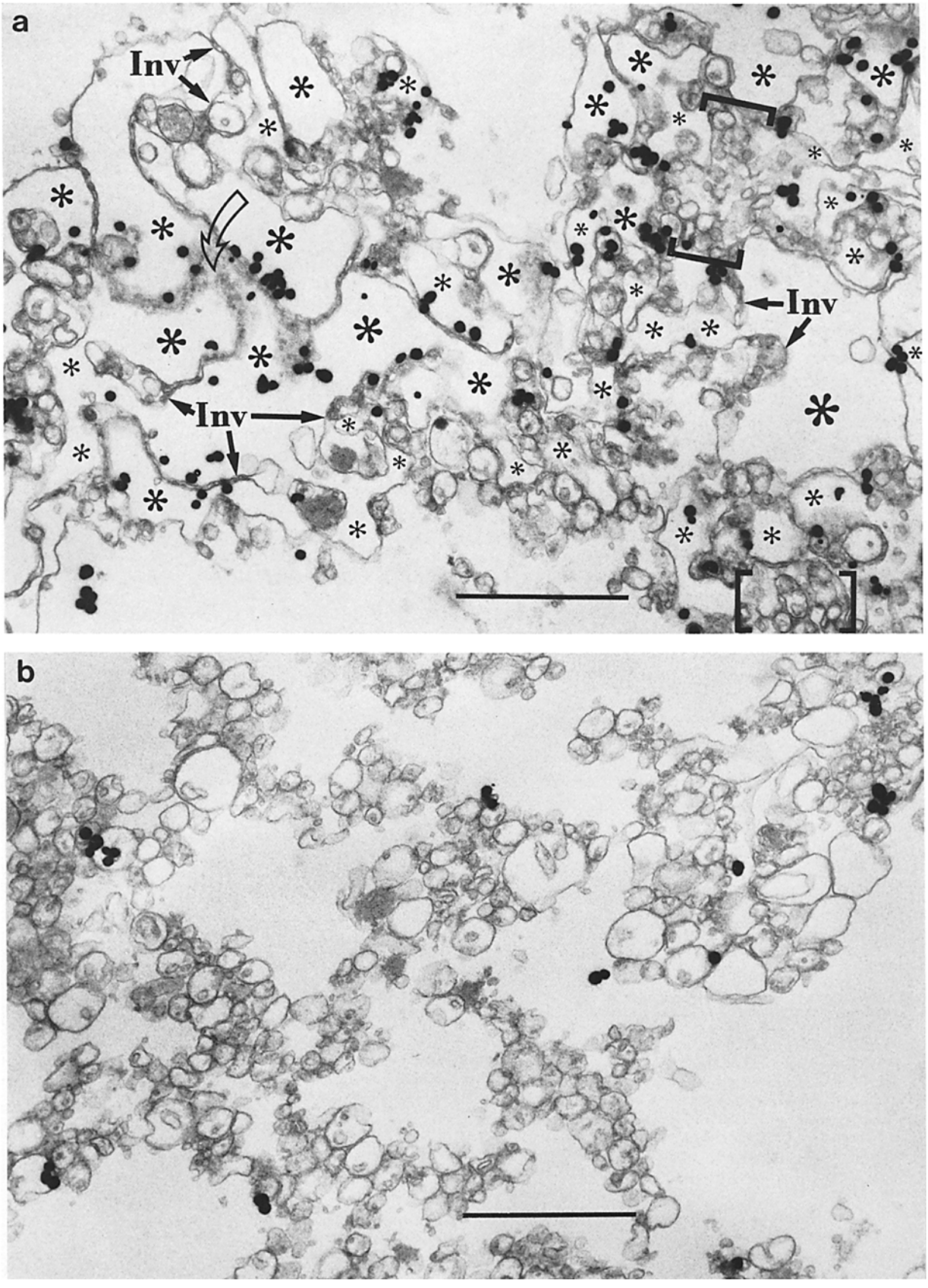
Relationship between GTP-stimulated Incorporation of [^{3}H]GlcNAc into Endogenous Acceptors of Stripped Rough Microsomes and Silver Grain Density over Sectioned Membranes

Parameter measured	+GTP	−GTP
Total incorporation of [^{3}H]GlcNAc	15.63 × 10^6*	3.62 × 10^6*
Acid-resistant incorporation	5.29 × 10^6*	0.36 × 10^6*
Fused membranes	43.9%	~0
Unfused membranes	56.1%	100%
Grain density over fused membranes	83.4‡	~0
Grain density over unfused membranes	20.4‡	14.3‡

125 μCi of UDP-[^{3}H]GlcNAc (24 Ci/mmol) was incubated with 600 μg protein of stripped rough microsomes in a total volume of 0.5 ml of the endogenous glycosylation medium in the presence or absence of GTP. At the end of a 2-h incubation, triplicate 20-μl samples were removed and assayed for total incorporation into ice-cold trichloroacetic acid precipitates, as well as incorporation into hot acid-resistant precipitates as described in Materials and Methods. The remaining material was fixed and filtered and thin sections processed for electron microscope radioautography and morphometry. Radioautographs having a final magnification of × 30,000 were used. Morphometry was carried out on 1,868 μm of membrane for the incubations carried out in the presence of GTP and 821 μm for the incubations in the absence of GTP. Unfused membranes were considered to be vesicles of perimeter <1.83 μm, i.e., the maximum perimeter of unincubated stripped rough microsomes (not shown). Silver grains were scored over the fused and unfused membranes by direct counting using micrographs of 34-d exposure (see Materials and Methods). A total of 685 grains were counted over fused membranes and 213 grains over unfused membranes in the samples incubated with GTP, while 117 grains were counted over unfused membranes in the samples incubated in the absence of GTP. Statistical analysis of mean grain densities indicated that the difference between the grain density over fused membranes (+GTP) and that over unfused vesicles (±GTP) was highly significant ($P < 0.001$) and remained significant ($P < 0.001$) after corrections for section thickness effect (23). The difference between the grain density over unfused vesicles +GTP and that over unfused vesicles −GTP was not statistically significant with or without corrections for section thickness (not shown).

* Disintegrations per minute (milligrams of protein·2h)$^{-1}$

‡ Silver grains per 100 μm length.


a
Inv
Inv
Inv
b

glycopeptides (22). Morphometric analysis of the same stripped rough microsomes incubated in the endogenous glycosylation medium with additional GTP revealed a temporal increase in the mean perimeter of the sectioned vesicles (Fig. 3, right-hand ordinate). Linear regression analysis defined a high correlation between the GTP-stimulated incorporation of UDP-[^{3}H]-GlcNAc into total acid-precipitable products and increases in the mean membrane length of stripped rough microsomes. The coefficient of correlation $r = 0.956$ was found to be statistically significant at $P < 0.01$, as evaluated by the t test.

To test if the increase in vesicle size was directly related to the GTP-stimulated glycosylation, electron microscope radioautographic analysis was carried out. Stripped rough microsomes were incubated with UDP-[^{3}H]GlcNAc in the presence or absence of GTP and assayed for total incorporation into ice-cold trichloroacetic acid precipitates as well as hot acid-resistant precipitates (Table II). Samples from the same incubations were processed for electron microscope radioautography (Figs. 4 *a*, *b*). Morphometric analysis revealed a high grain density over fused membranes produced after incubation with GTP (83 grains/100 μm, Table II). Unfused membranes in the same preparation had a lower grain density (20 grains/100 μm, Table II). In samples incubated without additional GTP (Fig. 4 *b*), no membrane profiles were considered fused and the grain density was low (14 grains/100 μm, Table II).

DISCUSSION

The studies presented here have established a link between fusion among isolated liver microsomes and GTP-stimulated core glycosylation. The change in mean membrane length of the microsomal vesicles during glycosylation correlated with GTP-stimulated incorporation of [^{3}H]GlcNAc into total acid-precipitable products (Fig. 3). The time course of events as shown by quantitation, therefore, supports the previous suggestion (5) that the GTP-induced enhancement of glycosylation could be the result of fusion of the microsomal vesicles.[3] Since fusion correlated with the incorporation of labeled sugar into total acid-precipitable products and not with incorporation into acid-resistant products, these results also agree with the suggestion of Godelaine et al. (2) that the earliest effect of GTP and pyrophosphate treatment on core glycosylation, would be the stimulation of dolichol pyrophosphate N,N'-diacetylchitobiose (Dol-P-P-GlcNAc-GlcNAc) formation.

To test if the GTP enhancement of glycosylation could be localized to fused membranes, electron microscope radioautography was used to visualize the labeled acceptors. Incubations with 0.5 mM GTP revealed silver grains over unfused as well as fused microsomes but with a four-fold higher grain density over the latter (Table II, Fig. 4 *a*). No significant difference was noted for grain densities over the unfused vesicles in incubations carried out with or without 0.5 mM GTP. Within the limitations of these studies, therefore, GTP-stimulated glycosylation was localized to the fused membrane compartment.

Since fusion and glycosylation of stripped rough microsomal membranes depend on the same factors, exhibit the same kinetic properties, and occur within the same subcompartment we therefore suggest that GTP-stimulated fusion plays a causal role in core glycosylation. Godelaine et al. (3) originally proposed that GTP may stimulate translocation of sugar across the ER membranes. The GTP-stimulated membrane fusion may allow sugar translocation across the membrane, possibly through a change in membrane permeability to activated sugars (5), and/or membrane fusion may reorient the first dolichol derivative of core glycosylation, dolichol pyrophosphate GlcNAc (Dol-P-P-GlcNAc), from a cytosolic to a luminal orientation. This translocation could occur via inverted micelles (25) or inverted cylinders (lamellar-hexagonal phase transition, [26]) known to be formed at points of membrane fusion and suggested to be involved in the translocation of lipid across membranes (27). The location of Dol-P-P-GlcNAc-GlcNAc to the luminal surface of microsomes is well established (28, 29) but the topology of Dol-P-P-GlcNAc during glycosylation is as yet undefined.

We thank Dr. C. P. Leblond, Dr. N. J. Nadler, and Dr. C. E. Smith for helpful discussions and relevant comments on the manuscript. Appreciation is extended to Dr. B. Kopriwa for preparing radioautographs. We also thank Ms. Jeanne Cambron-Fournel, Ms. Lyne Roy, and Ms. Micheline Fortin for expert technical assistance and Ms. Jacqueline Drapeau for typing the manuscript.

This work was supported by grants from the Medical Research Council of Canada (MT-7325, MT-5605) as well as the National Cancer Institute of Canada.

Received for publication 11 January 1983, and in revised form 28 February 1983.

Note Added in Proof: The in vivo counterpart to the membrane fusion

[3] The absence of nucleotide sugar substrate or presence of tunicamycin had no effect on the GTP-stimulated fusion of stripped rough microsomes (5). Therefore, membrane fusion may stimulate glycosylation but not vice versa.

FIGURE 4 Location of endogenous acceptors by electron microscope radioautography of stripped rough microsomes after incubation with UDP-[^{3}H]GlcNAc in the presence or absence of GTP. Microsomes were incubated for 120 min at 37°C in 0.5 ml of medium (see Table II) then processed for electron microscope radioautography as described in Materials and Methods. Radioautographs were exposed for 42 d. Bars, 1 μm. × 30,000. (*a*) Stripped rough microsomes incubated with 0.5 mM GTP for 120 min. The vesicles are highly aggregated. Large irregular membrane-bound fusion products are marked by an asterisk over their lumina. Because of their irregular outlines, such large fusion products can be easily distinguished from the much smaller, more regular unfused vesicles. Unfused vesicles can be seen within some large invaginations (*Inv*) of the fusion products. Radioautographic "fine" silver grains representing sites of labeled endogenous acceptors are numerous along the length of the fused membranes (notice membrane regions surrounding the asterisks). A particularly high concentration of silver grains is seen along the closely apposed edges of three large fusion products (tangential zone indicated by the curved open arrow). In contrast, few silver grains are observed over regions containing many unfused vesicles (bracketed areas). (*b*) Stripped rough microsomes incubated without GTP for 120 min. Although the vesicles are aggregated, they resemble unincubated vesicles (see Fig. 2) and had a similar size based on a mean vesicle perimeter of 530 nm. In contrast with *a*, radioautographic silver grains are few and are randomly distributed over the unfused vesicles.

documented herein may be that core glycosylation is a posttranslational event occurring at points of fusion of ribosome-free regions of rough ER cisternae. A less obvious interpretation is that GTP in vivo may modulate at selected sites beneath ribosomes a local perturbation of membrane lipids allowing for nascent chain translocation and co-translational core glycosylation.

REFERENCES

1. Snider, M. D., and P. W. Robbins. 1982. Transmembrane organization of protein glycosylation. Mature oligosaccharide-lipid is located on the luminal side of microsomes from chinese hamster ovary cells. *J. Biol. Chem.* 257:6796–6801.
2. Godelaine, D., H. Beaufay, and M. Wibo. 1977. Incorporation of *N*-acetylglucosamine into endogenous acceptors of rough microsomes from rat liver: stimulation by GTP after treatment with pyrophosphate. *Proc. Natl. Acad. Sci. USA.* 74:1095–1099.
3. Godelaine, D., H. Beaufay, M. Wibo, and A. Amar-Costesec. 1979. The dolichol pathway of protein glycosylation in rat liver. Stimulation by GTP of the incorporation of *N*-acetylglucosamine in endogenous lipids and proteins of rough microsomes treated with pyrophosphate. *Eur. J. Biochem.* 96:17–26.
4. Godelaine, D., H. Beaufay, and M. Wibo. 1979. The dolichol pathway of protein glycosylation in rat liver. Incorporation of mannose into endogenous lipids and proteins of rough microsomes. *Eur. J. Biochem.* 96:27–34.
5. Paiement, J., H. Beaufay, and D. Godelaine. 1980. Coalescence of microsomal vesicles from rat liver: a phenomenon occurring in parallel with enhancement of glycosylation activity during incubation of stripped rough microsomes with GTP. *J. Cell Biol.* 86:29–37.
6. Paiement, J., and J. J. M. Bergeron. 1982. In vitro transfer of UDP-GlcNAc to endogenous acceptors in stripped rough microsomes occurs within fused membranes. *J. Cell Biol.* 95(2, Pt. 2):247*a*. (Abstr.).
7. Bergeron, J. J. M., R. A. Rachubinski, R. A. Sikstrom, B. I. Posner, and J. Paiement. 1982. Galactose transfer to endogenous acceptors within Golgi fractions of rat liver. *J. Cell Biol.* 92:139–146.
8. De Duve, C., B. C. Pressman, R. Gianetto, R. Wattiaux, and F. Appelmans. 1955. Tissue fractionation studies. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.* 60:604–617.
9. Beaufay, H., A. Amar-Costesec, D. Thinès-Sempoux, M. Wibo, M. Robbi, and J. Berthet. 1974. Analytical study of microsomes and isolated subcellular membranes from rat liver. Subfractionation of the microsomal fraction by isopycnic and differential centrifugation in density gradients. *J. Cell Biol.* 61:213–231.
10. Fleck, A., and H. N. Munro. 1962. The precision of ultraviolet absorption measurements in the Schmidt-Thannhauser procedure for nucleic acid estimation. *Biochim. Biophys. Acta.* 55:571–583.
11. Rachubinski, R. A., D. P. S. Verma, and J. J. M. Bergeron. 1980. Synthesis of rat liver microsomal cytochrome b_5 by free ribosomes. *J. Cell Biol.* 84:705–716.
12. Nordlie, R. C., and W. J. Arion. 1966. Glucose-6-phosphatase. *In* Methods in Enzymology. W. A. Wood, editor. Academic Press, Inc., New York. 9:619–625.
13. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. *J. Biol. Chem.* 235:769–775.
14. Gianetto, R., and C. De Duve. 1955. Tissue fractionation studies. Comparative study of the binding of acid phosphatase, β-glucuronidase and cathepsin by rat-liver particles. *Biochem. J.* 59:433–438.
15. Paiement, J., R. A. Rachubinski, N. M. K. Ng Ying Kin, R. A. Sikstrom, and J. J. M. Bergeron. 1982. Membrane fusion and glycosylation in the rat hepatic Golgi apparatus. *J. Cell Biol.* 92:147–154.
16. Bergeron, J. J. M., R. A. Sikstrom, A. R. Hand, and B. I. Posner. 1979. Binding and uptake of ^{125}I-insulin into rat liver hepatocytes and endothelium. An in vivo radioautographic study. *J. Cell Biol.* 80:427–443.
17. Kopriwa, B. M. 1975. A comparison of various procedures for fine grain development in electron microscopic radioautography. *Histochemistry.* 44:201–224.
18. Salpeter, M. M., L. Bachmann, and E. E. Salpeter. 1969. Resolution in electron microscope radioautography. *J. Cell Biol.* 41:1–20.
19. Nadler, N. J. 1979. Quantitation and resolution in electron microscope radioautography. *J. Histochem. Cytochem.* 27:1531–1533.
20. Paiement, J., and C. P. Leblond. 1977. Localization of thyroglobulin antigenicity in rat thyroid sections using antibodies labeled with peroxidase or ^{125}I-radioiodine. *J. Cell Biol.* 74:992–1015.
21. Nadler, N. J. 1971. The interpretation of grain counts in electron microscope radioautography. *J. Cell Biol.* 49:877–882.
22. Paiement, J., D. Godelaine, and H. Beaufay. 1979. The transfer of *N*-acetylglucosamine and mannose to endogenous proteins of rough microsomes from rat liver. *In* Abstracts of the Eleventh International Congress of Biochemistry. Toronto. 05-9-R19:403.
23. Weibel, E. R., and D. Paumgartner. 1978. Integrated stereological and biochemical studies on hepatocytic membranes. II. Correction of section thickness effect on volume and surface density estimates. *J. Cell Biol.* 77:584–597.
24. Boldender, R. P., D. Paumgartner, G. Losa, D. Muellener, and E. R. Weibel. 1978. Integrated stereological and biochemical studies on hepatocytic membranes. I. Membrane recoveries in subcellular fractions. *J. Cell Biol.* 77:565–583.
25. Verkleij, A. J., C. Mombers, J. Leunissen-Bijvelt, and P. H. J. Th. Ververgaert. 1979. Lipidic intramembranous particles. *Nature (Lond.).* 279:162–163.
26. Cullis, P. R., and M. J. Hope. 1978. Effects of fusogenic agent on membrane structure of erythrocyte ghosts and the mechanism of membrane fusion. *Nature (Lond.).* 271:672–674.
27. Cullis, P. R., and B. De Kruijff. 1979. Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta.* 559:399–420.
28. Hanover, J. A., and W. J. Lennarz. 1978. The topological orientation of *N,N'*-diacetylchitobiosylpyrophosphoryldolichol in artificial and natural membranes. *J. Biol. Chem.* 254:9237–9246.
29. Hanover, J. A., and W. J. Lennarz. 1982. Transmembrane assembly of *N*-linked glycoproteins. Studies on the topology of saccharide-lipid synthesis. *J. Biol. Chem.* 257:2787–2794.