Galactose Transfer to Endogenous Acceptors within Golgi Fractions of Rat Liver

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ABSTRACT The distribution of galactosyl transferase was studied using *trans* and *cis* Golgi fractions isolated by a modification of the Ehrenreich et al. procedure (1973. *J. Cell Biol.* 59:45–72) as well as an intact Golgi fraction isolated by a new one-step procedure. Two methods of assay were used. The first method analyzed the ability of Golgi fractions to transfer galactose (from uridine diphosphogalactose [UDP-gal] substrate) to the defined exogenous acceptor ovomucoid. The second method assessed the transfer of galactose from UDP-gal substrate to endogenous acceptors (endogenous glycosylation). The trans Golgi fraction (Golgi light) was highly active by the first method but revealed only low activity by the second method. Golgi fractions enriched in central and *cis* elements (the Golgi intermediate, heavy and especially the intact Golgi fraction) were highly active in both methods of assay. The endogenous glycosylation approach was validated by gel fluorography of the endogenous acceptors. For all Golgi fractions, transfer of galactose was revealed to secretory glycopeptides. It is concluded that galactosyl transferase activity in vivo occurs primarily in central and *cis* Golgi elements but not *trans* Golgi vesicles.

The discovery by Leblond and colleagues (26, 35) that i. v. injected [^{3}H]galactose was incorporated within the Golgi apparatus directly led to the use of galactosyl transferase as a marker enzyme for purified Golgi fractions (e.g. 4, 6, 11, 12, 14, 15, 22–25, 32). This enzyme activity is usually assayed in the presence of detergent and measures the transfer of galactose (from UDP-gal)[1] to a defined exogenous acceptor (either GlcNAc or free GlcNAc residues on specific glycopeptides). In such assays little account has been taken of the subcellular distribution of endogenous peptide acceptors.

In vivo, galactose transfer is expected to occur where there is both enzyme and endogenous acceptor. We have therefore attempted to localize the site of transfer of galactose to endogenous acceptors by assessing endogenous galactosyl transferase activity of a variety of Golgi fractions which differ in their morphological characteristics.

[1] Abbreviations used in this paper are: GlcNAc, *N*-acetylglucosamine; hGH, human growth hormone, SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; STKM, 0.25 M sucrose, 50 mM Tris HCl pH 7.4, 25 mM KCl, 5 mM $MgCl_2$; UDP-gal, uridine diphosphategalactose; VLDL, very low density lipoprotein.

MATERIALS AND METHODS

Isolation of Golgi Fractions

Golgi fractions from microsomal pellets were isolated exactly as described previously (4) from nonalcohol treated and overnight fasted rats, except for the addition of an additional 1.0 M sucrose layer in the final gradient between the 1.15 M sucrose load zone and 0.86 M sucrose layer. The Golgi fractions, as before, were pelleted from the 0.25/0.6/0.86/1.0 M/1.15 M sucrose interfaces and corresponded to the Golgi light, intermediate, heavy, and small vesicular fractions respectively. The residual load zone fraction (1.15 M sucrose) consisted of smooth microsomes and the residual pellet of rough microsomes.

An intact Golgi fraction was isolated from livers of 200–250 g Sherman rats starved overnight or fed ad libitum. After decapitation of rats, livers were rapidly removed and homogenized (15%) in 0.25 M STKM with a Potter-Elvehjem rotating homogenizer (Kontes Co., Vineland, N. J.). The homogenate was filtered through Nylon boulting cloth (Thompson B and S, Montreal, Quebec, Canada) and made to 1.02 M STKM and a linear gradient generated of 0.25 M STKM to 1.02 M STKM over the load zone (vol. of gradient: volume of load zone; 1:1). After a 1.5 h centrifugation at 190,000 g_{av} (SB 283 rotor, International Centrifuge, Fisher Scientific, Montreal, Quebec, Canada) a prominent band was observed to have floated to about ⅓ of the distance up the density gradient overlay with another band noted at the air/0.25 M STKM interface. Five fractions were removed from the tube with a bent, blunt needle (#16, Becton, Dickinson and Co., Rutherford, N. J.) and syringe and designated a, b, c, d, and e, as described in the legend to Table III. The intact Golgi fraction (b) was pelleted by dilution with TKM to a final concentration of 0.25 M STKM and pelleted (180,000 g_{max}) for 45 min. For all enzymic and chemical characterizations, the pelleted fraction

was suspended in 0.25 M STKM. Fractions a, c, d were removed from the gradient and assayed without pelleting. Fraction e (the pellet) was resuspended in 0.25 M STKM for biochemical assays.

Enzyme Assays

EXOGENOUS GLYCOSYLATION: Galactosyl transferase was assayed according to the method of Howell et al. (18) with ovomucoid as acceptor as described previously (4) except that the concentration of unlabeled UDP-gal was increased. The donor sugar consisted of UDP-[^{3}H]galactose (New England Nuclear, Montreal, Quebec, 12.3 Ci/mmol) that was dried and resuspended to a specific activity of 0.022 μCi/nmol. The incubation mixture contained UDP-[^{3}H] galactose, 330,000 dpm (6.69 nmol); ovomucoid (Schwarz/Mann, Orangeburg, N. Y.) 0.2 mg; $MnCl_2$ (Sigma Chemical Co., St. Louis, MO.) 3 μmol; sodium cacodylate buffer 3 μmol; β-mercaptoethanol 3 μmol; Triton X-100, 0.2% (vol/vol), adenosine triphosphate, disodium salt (Sigma Chemical Co.) 0.22 μmol, and cell fraction protein (5–100 μg) to a final volume of 0.1 ml. Incubations were carried out for 15 min at 37°C and stopped by the addition of 1 ml of ice-cold 1% phosphotungstic acid in 0.5 N HCl. After 30 min at 0°C, pellets were washed twice in phosphotungstic acid-HCl and once with absolute ethanol. Pellets were dissolved in 0.5 ml of Protosol (New England Nuclear, Boston, Mass.) and treated with H_2O_2, as recommended by the manufacturer. Radioactivity was determined in a Packard model 3003 Spectrometer (Packard Instrument Co., Downers Grove, Ill.) and cpm converted to dpm by the channels-ratio and external standard methods (34).

ENDOGENOUS GLYCOSYLATION: The assay was based on the galactosyl transferase assay (*vide supra*) except for a higher specific radioactivity of the donor sugar and a pH of 7 of the buffer (11). The constituents of the assay were: UDP-[^{3}H]galactose (sp act 11.5–12.3 Ci/mmol), 2.5 μCi; sodium cacodylate, 3 μmol, pH 7; $MnCl_2$, 3 μmol; β-mercaptoethanol, 3 μmol; the presence or absence of Triton X-100 (0.2% final concentration); adenosine triphosphate, disodium salt, 0.22 μmol and cell fraction protein (5–100 μg) in a final volume of 0.1 ml. Incubations were carried out for 30 min at 37°C (linear with time up to 60 min) and stopped with phosphotungstic acid-HCl as described for galactosyl transferase assays.

OTHER ASSAYS: Glucose-6-phosphatase, microsomal esterase, and 5′-nucleotidase activities were assayed exactly as described previously (4). Acid phosphatase was assayed as described by Smith (33) with β-glycerophosphate (disodium salt, pentahydrate, Sigma Chemical Co.) as substrate (5 μmol in 0.5 ml assay volume) in sodium acetate buffer (0.5 M, pH 5.0) at 37°C for 1 h. Reactions were terminated by the addition of ice-cold trichloroacetic acid (5%) and inorganic phosphate determined as described by Ames and Dubin (1). Protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Mississauga, Ontario) with bovine gamma globulin as standard.

Hormone Binding

Specific binding of ^{125}I-insulin and ^{125}I-hGH was carried out on fractions that were frozen and then thawed four times as described previously (9, 28). Incubations were carried out with freshly prepared ^{125}I-insulin (98–121 μCi/μg; 216,000–248,000 dpm per incubation) or ^{125}I-hGH (99 μCi/μg; 248,000 dpm per incubation), and 30–150 μg cell fraction protein (in triplicate) in a final volume of 0.5 ml. After steady-state incubation (48 h at 4°C), free hormone was separated from bound hormone by centrifugation (9, 28) and radioactivity assessed in a Packard Instrument Co. Autogamma spectrometer (efficiency, 40.5%). Nonspecific binding was determined by incubation of parallel tubes in the presence of 5 μg of unlabeled insulin or 1 μg of unlabeled hGH (again in a final volume of 0.5 ml). As before (9, 28, 29) the percentage of specific binding is defined as the difference between total binding and nonspecific binding and expressed as a percentage of the tracer added to the incubation mixture.

SDS-PAGE

Discontinuous SDS-PAGE (polyacrylamide gel electrophoresis) was carried out with a resolving gel of 7–20% acrylamide as described by Rachubinski et al. (30). Gels were stained in Coomassie Brilliant Blue. Fluorography of dried, impregnated gels was done as described by Laskey and Mills with Kodak RPX-O-Mat film (21).

Electron Microscopy

Subcellular fractions were fixed as described previously (4, 9) or following a protocol of 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 followed by reduced OsO_4 (19) and block staining in uranyl acetate (20). Either oriented pellets (13) or filtered samples (2) were embedded in Epon 812.

RESULTS

Morphologic and Biochemical Characterization

GOLGI SUBFRACTIONS: The Golgi subfractions were isolated from microsomal pellets without alcohol. Modification of the original (4, 13) method by the addition of a 1.0 M sucrose layer between the 1.15 M sucrose load zone and the 0.86 M sucrose region resulted in the easier recovery of the flattened saccule fraction (Golgi heavy, 0.86/1.0 M sucrose interface, not shown) leaving vesicular contaminants at the 1.0/1.15 M sucrose interface (not shown). The distribution of enzymic activities in the Golgi subfractions (Tables I and II) appeared similar to those described previously (4, 11). Addition of the 1.0 M sucrose layer resulted in a higher relative specific activity of galactosyl transferase in the flattened saccule fraction (Golgi heavy, Table II) and a lower concentration of galactosyl transferase activity in the vesicular fraction (sv).

INTACT GOLGI FRACTION: A comparison of the distribution of enzyme activities in the intact Golgi fraction (Table III) to that described for the Golgi subfractions (*vide supra*) revealed few noteworthy differences. The intact Golgi fraction, like the Golgi subfractions, revealed a high concentration of galactosyl transferase activity and markedly lower but detectable levels of glucose-6-phosphatase, microsomal esterase, 5′-nucleotidase, and acid phosphatase activities.

Morphologically, however, the intact Golgi fraction was quite different from the Golgi subfractions. The intact Golgi fraction revealed oriented stacks of fenestrated saccules with associated vesicles marked by VLDLs in the intracisternal content (Figs. 1–3). Of interest was the high degree of fenestration of the saccules and secretory vesicles in clear continuity with the fenestrated tubules (Figs. 2 and 3).

SDS-PAGE of the intact Golgi fraction compared to the Golgi subfractions (Fig. 4) revealed plasma peptides (the secretory content) as major constituents. However, a major band of 31,000 M_r was prominent in the intact Golgi fraction but only a minor constituent of the Golgi subfractions.

TABLE I

Distribution of Recovered Enzyme Activities from Homogenate

Fraction	Protein	Galactosyl transferase	Glucose-6-phosphatase	Microsomal esterase	5′-Nucleotidase	Acid phosphatase
	%	%	%	%	%	%
Nuclear + Mitochondrial	47.2	45.9	50.6	50.9	65.5	73.6
Microsomal	13.4	41.1	46.7	41.8	26.8	13.6
Supernatant	39.4	13.0	3.7	7.3	7.7	12.7

The absolute values for the microsomal fractions were: protein, 18.5 mg/g liver; galactosyl transferase, 0.224 nmol/min per mg protein; glucose-6-phosphatase 0.44 μmol Pi/min per mg prot; esterase, 9.7 U/min per mg prot; 5′-nucleotidase 0.12 μmol Pi/min per mg prot; acid phosphatase 0.02 μmol Pi/min per mg prot. Average of two fractionations.

TABLE II

Distribution of Recovered Enzyme Activities in Microsomal Subfractions

Fraction	Protein	Galactosyl transferase	Glucose-6-phosphatase	Microsomal esterase	5'-Nucleotidase	Acid phosphatase
	%	%	%	%	%	%
Golgi light	0.12 ± 0.02	4.11 ± 0.31	0.02 ± 0.00	0.04 ± 0.01	0.19 ± 0.02	0.49 ± 0.11
Golgi intermediate	0.42 ± 0.05	17.57 ± 1.01	0.14 ± 0.02	0.26 ± 0.02	1.35 ± 0.05	1.77 ± 0.27
Golgi heavy	0.75 ± 0.14	22.68 ± 3.02	0.48 ± 0.08	0.57 ± 0.12	3.96 ± 0.60	2.47 ± 1.41
Small vesicles	2.77 ± 0.68	23.12 ± 0.52	3.00 ± 0.85	2.68 ± 0.59	10.89 ± 1.39	3.42 ± 0.44
Smooth microsomes	53.28 ± 5.25	26.66 ± 3.32	53.18 ± 4.83	48.48 ± 2.51	60.91 ± 3.54	27.54 ± 1.80
Rough microsomes	42.67 ± 4.79	5.85 ± 0.55	43.17 ± 5.15	47.97 ± 2.60	22.72 ± 4.73	64.30 ± 2.08

The absolute values for the Golgi intermediate fraction were: protein, 0.072 ± 0.008 mg prot/g liver; galactosyl transferase, 8.4 ± 0.6 nmol/min per mg prot; glucose-6-phosphatase, 0.12 ± 0.01 μmol Pi/min per mg prot; microsomal esterase, 13.1 ± 8.6 U/min per mg prot; 5'-nucleotidase 0.26 ± 0.04 μmol Pi/min per mg prot; acid phosphatase, 0.15 ± 0.02 μmol Pi/min per mg prot. The results have been averaged from four fractionations ± one standard error of the mean.

TABLE III

Percentage Distribution of Recovered Enzyme Activities in Gradient for Isolation of Intact Golgi Fraction

Fraction	Protein (12)*	Galactosyl transferase (11)	Glucose-6 phosphatase (5)	Microsomal Esterase (3)	5'-nucleotidase (5)	Acid phosphatase (5)
a	0.1 ± 0.04	0.68 ± 0.17	0.07 ± 0.06	0.02 ± 0.01	0.06 ± 0.05	0.09 ± 0.07
b‡ (Intact Golgi)	0.2 ± 0.02	33.90 ± 2.90	0.84 ± 0.17	0.61 ± 0.11	1.21 ± 0.35	1.17 ± 0.03
c	2.3 ± 0.20	15.34 ± 1.70	2.10 ± 0.40	2.15 ± 0.40	2.32 ± 0.50	3.50 ± 1.40
d	54.1 ± 0.80	24.30 ± 1.90	25.90 ± 1.80	27.60 ± 2.40	16.80 ± 1.60	15.50 ± 1.50
e	40.8 ± 0.90	25.80 ± 1.90	71.10 ± 2.10	69.60 ± 2.70	79.60 ± 2.20	79.70 ± 1.70

Rat liver homogenate was made to 1.02 M STKM as described in Materials and Methods and a continuous sucrose gradient of 0.25 M STKM to 1.02 M STKM generated above the load. After centrifugation, a prominent band (*b*) floated ⅓ up the gradient. This band was removed, diluted with TKM and pelleted. This fraction corresponded to the intact Golgi fraction. Fraction *a* corresponded to a band found at the air/0.25 M STKM interface; fraction *c* to the remaining gradient after removal of band *b*; fraction *d* to the residual load zone and fraction *e* to the pellet.

* Number of fractionations

‡ The absolute values for the intact Golgi fraction were: protein, 0.24 mg prot/g liver; galactosyl transferase, 10.3 ± 1.5 nmol/min per mg prot; glucose-6-phosphatase, 0.57 ± 0.14 μmol Pi/min per mg prot; microsomal esterase, 14.7 ± 2.3 U/min per mg prot; 5'-nucleotidase, 0.44 ± 0.07 μmols Pi/min per mg prot; acid phosphatase, 0.228 ± 0.04 μmol Pi/min per mg prot.

Analysis of hormone binding sites (Figs. 5 and 6) in the intact Golgi fraction revealed binding of ^{125}I-insulin at the same level as that for the Golgi light fraction but less than that of the Golgi intermediate fraction and less than that of isolated plasmalemma. ^{125}I-hGH binding was, however, far greater in the intact Golgi fraction and all Golgi subfractions than that of plasmalemma.[2]

ENDOGENOUS GLYCOSYLATION: Having characterized the fractions morphologically and biochemically, studies were carried out to deduce the site of galactose incorporation in vivo. We attempted not only to assess the distribution of galactosyl transferase enzyme (*vide supra*) but also the distribution of endogenous acceptors. Thus the incorporation with UDP-[^{3}H]galactose into endogenous acceptors within the Golgi fractions was studied. Incorporation in all the Golgi fractions except the Golgi light fraction was high (Fig. 7). Smooth and rough microsomes showed negligible activity. Triton X-100 markedly increased uptake in the Golgi heavy, small vesicle, and intact Golgi fractions but lowered incorporation in the Golgi light and intermediate fractions.

Attempts were made to characterize the nature of the incorporated radioactivity by SDS-PAGE. Fluorography (Fig. 8) revealed some noteworthy differences between incubations carried out in the absence or presence of Triton X-100 at the level of a band equivalent to M_r 10,000. This band was labeled only when incubations were carried out without Triton X-100 and was common to both the Golgi intermediate and intact Golgi fractions. Reproducible differences between the bands labeled in the intact Golgi and Golgi intermediate fraction were also found as discussed in the legend to Fig. 8. Of note, however, was the lack of label in albumin, the major peptide present in the Golgi fractions and the transfer of label to a band of the mobility of transferrin (immediately above albumin).

DISCUSSION

A lack of correspondence between the distribution of galactosyl transferase enzyme (galactose transfer to ovomucoid) and galactosyl transferase activity (transfer to endogenous acceptors) was observed. As both enzyme and acceptor are required for galactose transfer in vivo, it was concluded that this phenomenon occurred primarily within central and *cis* elements of the Golgi apparatus. Morphologic studies indeed revealed some central and *cis* elements in those Golgi fractions active in endogenous galactosyl transferase activity i.e. the Golgi intermediate and heavy subfractions from microsomes and the new intact Golgi fraction. The *trans* nature of the Golgi light

[2] Considering the protein distributions for the microsomal Golgi subfractions (Table II) and the distribution of hormone receptors (Fig. 5) it can be calculated that the Golgi light, intermediate and heavy subfractions account for 0.12%, 0.94%, and 1.1% respectively of the total insulin receptors in microsomes. For hGH, these values increase to 1.5%, 4.5%, and 5% for the three Golgi subfractions. For the intact Golgi fraction (Table III, Fig. 6) it can be calculated that 0.89% of the total insulin receptors and 5.13% of the total hGH receptors are recovered in the fraction. These levels are similar to those described previously by us for Golgi fractions isolated from alcohol intoxicated rats (7–9, 28, 29). The present studies rule out crinophagy (due to the alcohol treatment) as an explanation of our previous results.

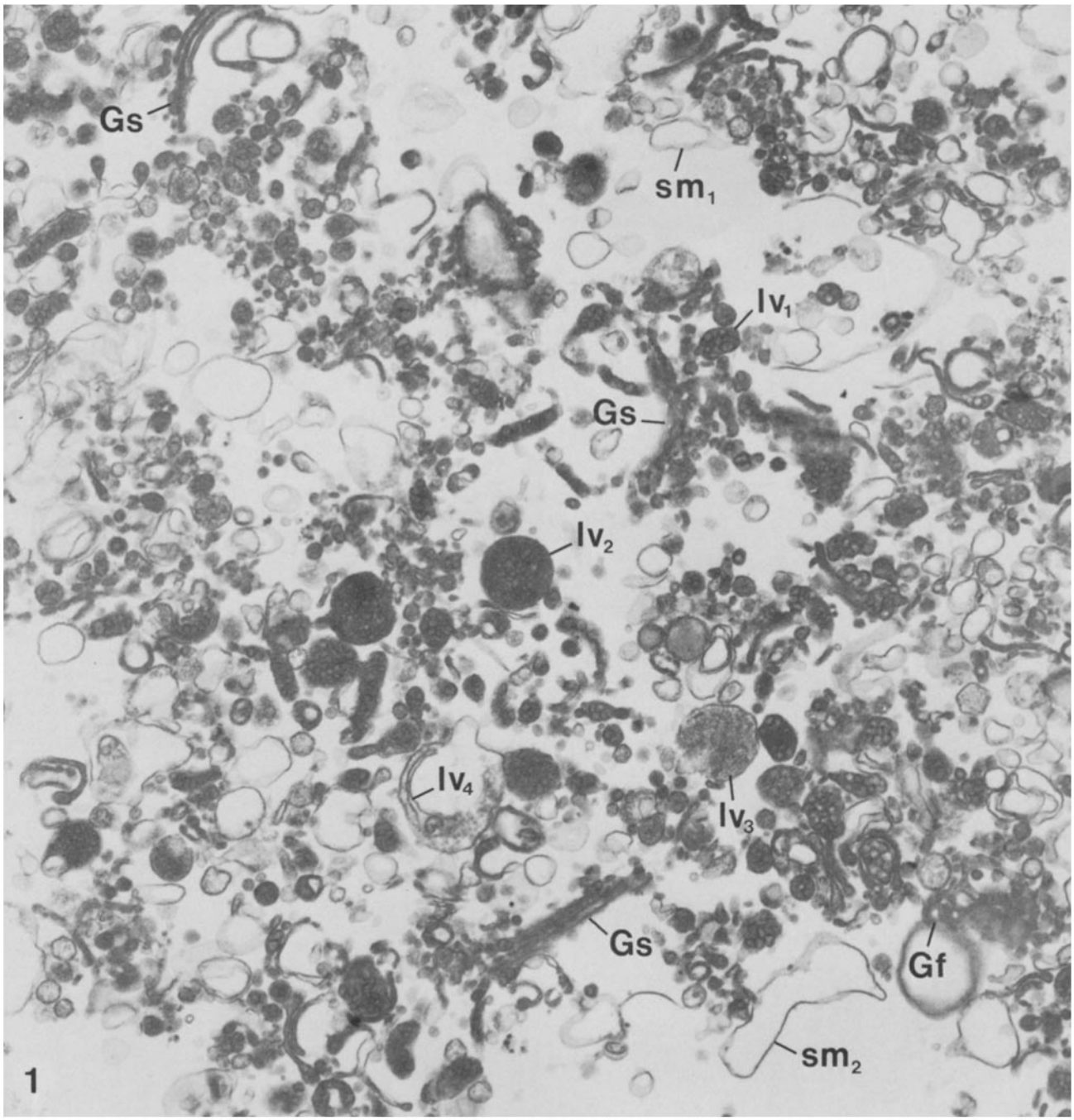

FIGURE 1 Representative view of the middle of a pellet of the intact Golgi fraction revealing stacks of Golgi apparatus (*Gs*) that appear fenestrated in oblique or tangential views (*Gf*). Various sized lipoprotein containing vesicles are found associated with the Golgi stacks. These vesicles vary in size from small vesicles (lv_1) with prominent lipoprotein-like particles in their content, larger vesicles (lv_2) with smaller more densely packed lipoprotein-like particles, large amorphous vesicles (lv_3) with the lipoprotein content less clearly demarcated but still visible, and heterogenous structures (lv_4) with an irregular content. Smooth membranous elements of small (sm_1) and large (sm_2) caliber were also noted. × 30,000.

fraction has been documented (3, 5, 11, 13) and it seems likely that despite the high galactosyl transferase enzyme level in this fraction that endogenous acceptors may be already fully glycosylated therein.[3]

To assess the validity of the endogenous glycosylation assay, we attempted to characterize the acceptors by fluorography. Studies were carried out on the Golgi intermediate and intact Golgi fractions. For both fractions, many of the acceptors corresponded in molecular weight on SDS-PAGE to plasma glycoproteins. This was also true for the acceptors in the Golgi light and heavy fractions (data not shown). The intact Golgi fraction was noteworthy in that additional acceptors at 57,000 M_r and especially at 16,500 M_r were observed. Such results may suggest the streaming of different acceptors to different

[3] The Golgi light fraction is probably analogous to the secretory vesicle fraction first pointed out by Merrit and Morré (22) to be highly enriched in galactosyl transferase enzyme.

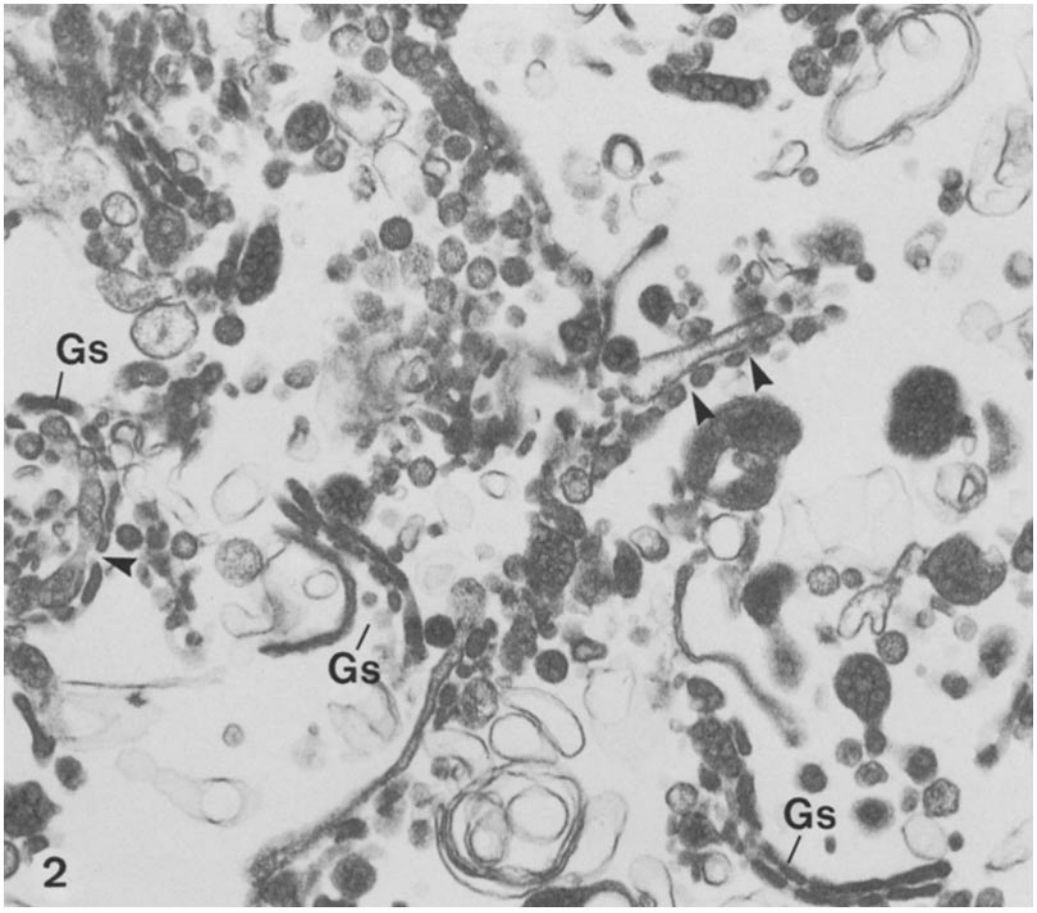

FIGURE 2 Higher power view of the intact Golgi fraction revealing Golgi stacks (*Gs*) and emphasizing the fenestrated nature (arrowheads) of the saccular components. × 41,000.

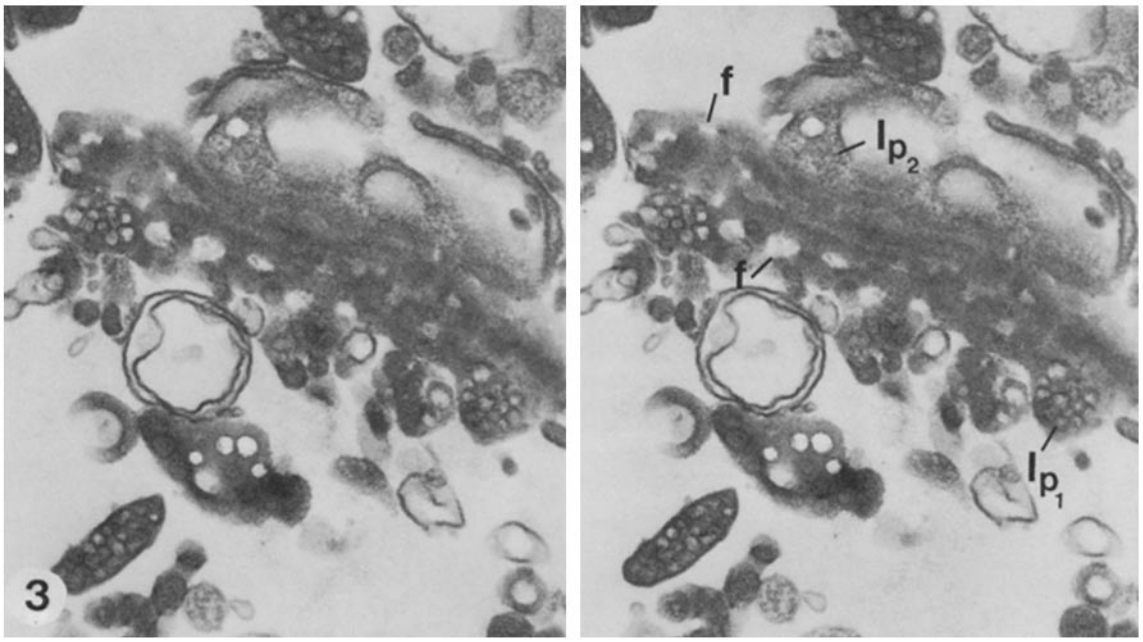

FIGURE 3 Stereopairs of a thin section of a Golgi stack from the intact Golgi fraction revealing apparent secretory vesicles appearing to bud off a highly fenestrated saccule. The secretory vesicles are marked by a content of lipoproteinlike particles. Less distinct lipoprotein content (lp_2) is shown in an apposing saccule with tubular distensions. Fenestrae are indicated (*f*). × 55,000.

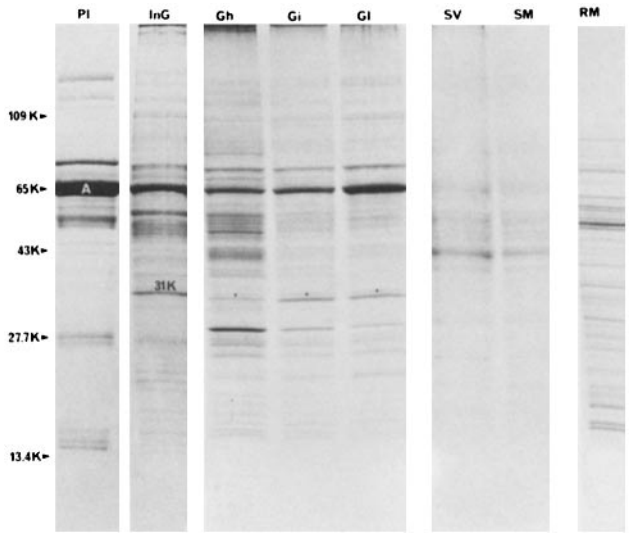

FIGURE 4 SDS-PAGE of Coomassie Blue-stained bands in the intact Golgi fraction compared to the subfractions derived from liver microsomes and plasma peptides. Molecular weight markers are indicated on the left and from left to right plasma peptides (*Pl*), the intact Golgi fraction (*InG*), the Golgi heavy (*Gh*), Golgi intermediate (*Gi*), Golgi light (*Gl*), small vesicular (*sv*), smooth microsomal (*SM*), and rough microsomal fractions (*RM*) were electrophoresed on 7-20% acrylamide gradient gels. The prominent band in plasma (*A*) corresponds to albumin and is the major constituent of the intact Golgi fraction as well as the Golgi subfractions derived from microsomal pellets. Interestingly, a major band of M_r = 31,000 is found in the intact Golgi fraction (*31K*) but is barely detectable in the Golgi subfractions (black dots are on the *31K* band itself in the Golgi subfractions).

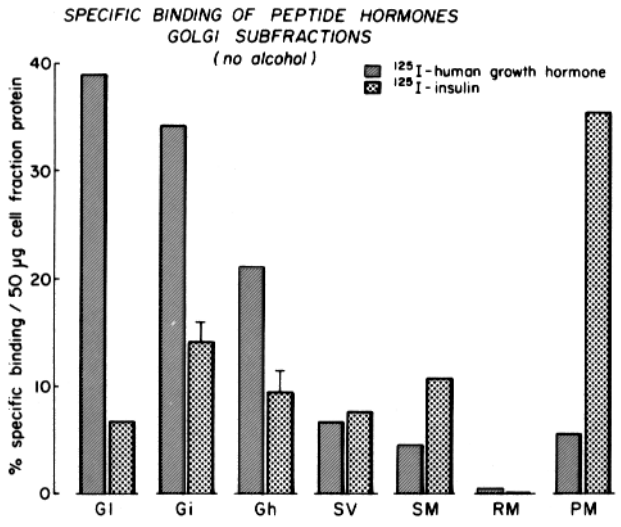

FIGURE 5 Specific binding of near physiologic levels of ^{125}I-*insulin* and ^{125}I-*human growth hormone* to Golgi fractions isolated from microsomal pellets as compared to the plasmalemma fraction—all fractions isolated from female animals. The Golgi fractions reveal high binding for ^{125}I-*hGH* but lower binding for ^{125}I-*insulin*. The converse is true for the plasmalemma fraction. It has been speculated (8) that this may be related to the higher turnover of the lactogen (*hGH*) receptor than the insulin receptor. Data based on two or more fractionations. Where more than two fractionations have been carried out, standard errors of the mean are included (T).

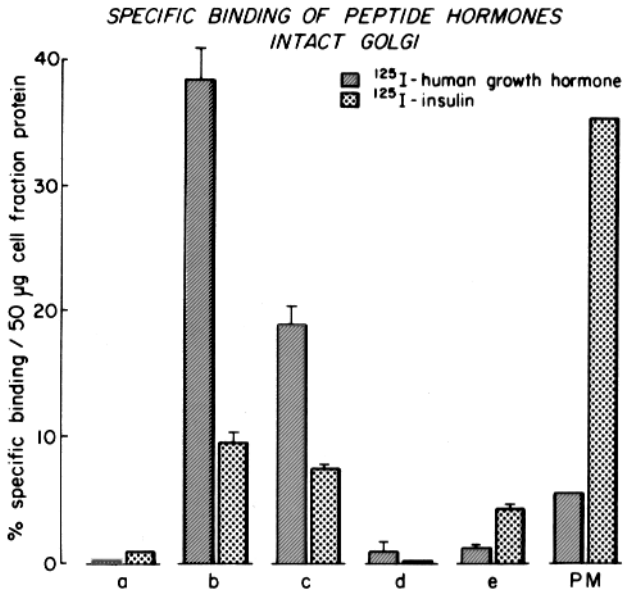

FIGURE 6 Specific binding of ^{125}I-*hGH* and ^{125}I-*insulin* to the intact Golgi fraction (*b*) as compared to other fractions (*a, c, d, e*) removed from the gradient described in Table III. The data is compared to specific binding of the same hormones to purified plasmalemma (*PM*)—all fractions from livers of female rats. As for the Golgi subfractions (Fig. 5) binding is high for hGH and lower for insulin in the intact Golgi fraction (*b*) whereas the converse is true for the plasmalemma fraction.

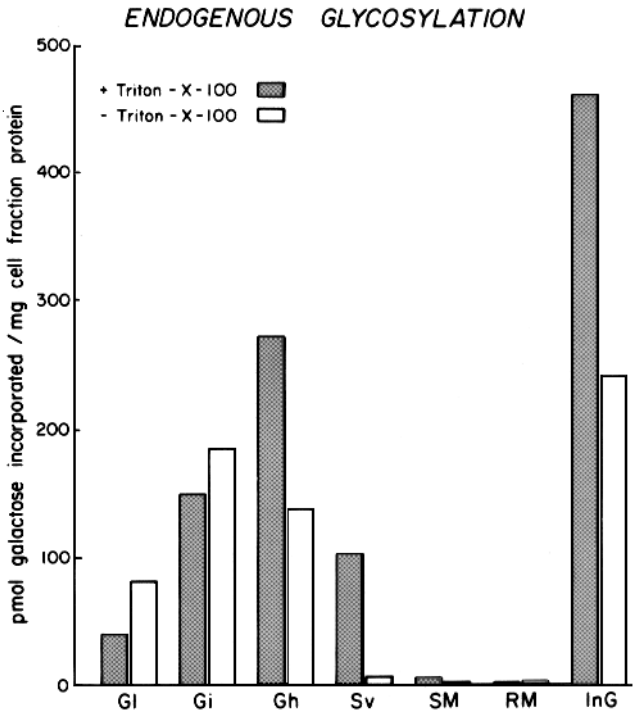

FIGURE 7 Endogenous incorporation of UDP-[^{3}H]galactose into the subfractions (*Gl, Gi, Gh, Sv, Sm, RM*) derived from the microsomal pellet of homogenates or into the intact Golgi fraction (InG) obtained directly from liver homogenates. The results are expressed as picomoles galactose incorporated during the 30 min incubation. The effect of *Triton X-100* in the incubation medium is noted with marked activation of transfer for the intact Golgi fraction, the Golgi heavy and small vesicular fractions. *Triton X-100* has an inhibitory effect on the Golgi light and intermediate fractions.

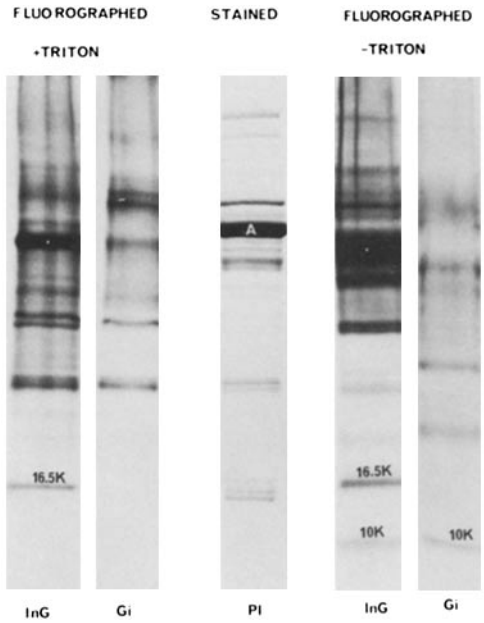

FIGURE 8 Incorporation of UDP-[^{3}H]galactose into endogenous macromolecules of the Intact Golgi (*InG*) fraction and the Golgi intermediate (*Gi*) fraction. From left to right is indicated a fluorograph of the intact Golgi fraction (4×10^5 cpm) and the Golgi intermediate fraction (2.9×10^5 cpm) after incubations carried out in the presence of Triton X-100 as described in Materials and Methods. The center gel corresponds to Coomassie Blue-stained plasma peptides of which albumin (*A*) is the most prominent (M_r = 65,000). The band immediately above is transferrin. The two gels on the right are fluorographs of the Intact Golgi fraction (2.5×10^5 cpm) and Golgi intermediate fraction (1.24×10^5 cpm) after incubations carried out in the absence of Triton X-100 also as described in Materials and Methods. Each fraction and the presence or absence of Triton X-100 in the incubation medium resulted in differences in macromolecules labeled. Albumin the major peptide in the Golgi light and intermediate was unlabeled although other plasma peptides, including transferrin, were labeled. Endogenous transfer by intact Golgi fractions was prominent to a macromolecule of M_r = 57,000 (indicated by a white dot). A difference between *InG* and *Gi* was noted by the radiolabeled band at M_r = 16,500 (*16.5K*) for the intact Golgi fraction and finally both the intact Golgi fraction and the Golgi intermediate fraction incorporated galactose into a band of M_r = 10,000 (*10K*) only when incubations were carried out in the absence of Triton X-100. Although the *Gi* fluorograph on the far right is from a separate experiment, each gel was calibrated with electrophoresis molecular weight standards. The Golgi light and Golgi heavy subfractions revealed an identical distribution of acceptor peptides to that shown for the *Gi* fraction (data not shown).

regions of the Golgi apparatus, a fact represented in the intact Golgi fraction but not in the disrupted Golgi subfractions derived from microsomal pellets.

A curious observation concerned the detection of radioactivity at M_r = 10,000 only when endogenous glycosylation was carried out in the absence of Triton X-100. This has previously been considered as a probable region of migration for dolichol (core-sugar) intermediates by Pless and Lennarz (27) or as a region of migration of retinyl (core-sugar) intermediates in glycosylation on SDS-PAGE by Rosso et al. (31). The disappearance of only this band when incubations were carried out in the presence of Triton X-100 (Fig. 8) has also been noted by Pless and Lennarz (27) in their system.

The results obtained with the new intact Golgi fraction helped support the conclusions as to the intra-Golgi location of glycosylation. This fraction retained the most prominent morphologic feature of the Golgi apparatus, namely, the stacked saccules.[4] The fraction was highly active in exogenous and endogenous glycosylation as well as revealing several other properties (e.g. hormone receptors) characteristic of the Golgi fractions isolated from microsomal pellets. The major difference was revealed on SDS-PAGE by a Coomassie Blue-stained band at 31,000 M_r present only in the intact Golgi fraction and may be a peripheral membrane protein involved in the stacking of cisternae.

In conclusion, endogenous glycosylation appears restricted to Golgi fractions enriched in central and *cis* regions of the Golgi apparatus. It is therefore in these regions and not the *trans* Golgi region that galactose transfer in vivo is expected to be prevalent.

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[4] We believe this method may be an improvement over most other methods for isolating intact Golgi apparatus (e.g. refs. 12, 14–17, 22–25) based on its simplicity, rapidity and reproducibility as well as on the high levels of galactosyl transferase activity.

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