MEMBRANE FUSION AND THE MECHANISM OF TERMINAL GLYCOSYLATION WITHIN THE GOLGI APPARATUS OF RAT LIVER HEPATOCYTES

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The anatomist, C. P. Leblond, first demonstrated that uptake of galactose into elaborating glycoproteins occurred within the cisterna of Golgi saccules (1). This discovery was extended by cell biologists and biochemists to the use of galactosyl transferase as a marker enzyme for purified Golgi apparatus (see references in 2 and 3). The enzyme transfers galactose to *N*-acetyl glucosamine residues on secretoary glycoproteins within the lumina of Golgi saccules. The substrate for the enzyme is uridine diphosphogalactose (UDP-gal), a cytosolically located nucleotide sugar. The question is, how is this membrane-impermeant molecule translocated across Golgi membranes to effect glycosylation? To address this question we have optimized an endogenous glycosylation assay which effectively transferred galactose from UDP-gal through intact Golgi membranes (purified Golgi fractions) to endogenous secretory glycoproteins in the Golgi lumina. Chemical studies have revealed that in addition to the luminally located secretory glycoproteins, transfer of galactose was also effected to a novel glycolipid, tentatively identified as dolichyl galactosyl phosphate (dol-P-gal), probably on the cytosolic surface (*vide infra*). Endogenous glycosylation required purified Golgi fractions, $MnCl_2$, sodium cacodylate buffer, and ATP. Removal of $MnCl_2$, sodium cacodylate, or ATP resulted in varying degrees of inhibition of the reaction. Addition of β-mercaptoethanol inhibited glycosylation by 40%.

The electron microscope (EM) revealed structural changes in Golgi membranes during endogenous glycosylation. Extensive membrane fusion was observed (Fig. 1). Stereology indicated a correlation between membrane fusion (mean membrane length) and glycosylation (picomoles of gal incorporated) for each of the conditions in which glycosylation had been inhibited ($r = 0.99$, $P < 0.001$). Furthermore, EM radioautography localized glycosylation exclusively to fused Golgi membranes (as visualized by silver grains from ^{3}[H]-galactose over fused membranes; $r = 0.92$, $P < 0.001$). Finally, preliminary

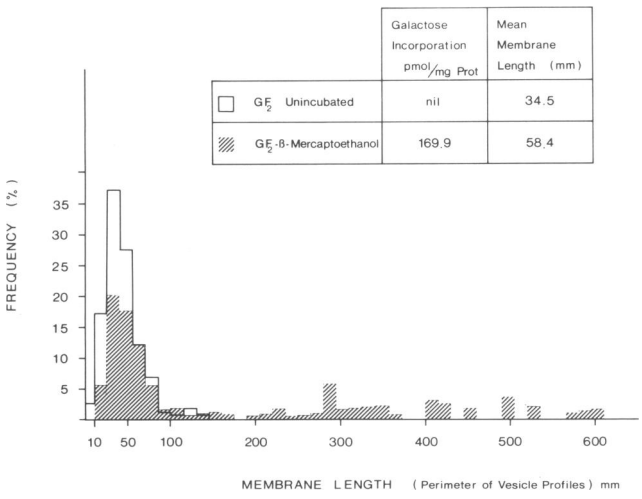

FIGURE 1 Distribution of Golgi membrane lengths (perimeters of vesicles) in freshly prepared Golgi intermediate fraction (GF_2, open histograms) and in the same fractions after incubation for 1 h in the endogenous glycosylation medium (hatched histograms). Stereology was carried out on electron micrographs (× 60,000) using Zeiss MOP-3 (Carl Zeiss Inc., Don Mills, Ontario). Scale, 60 mm = 1 μm.

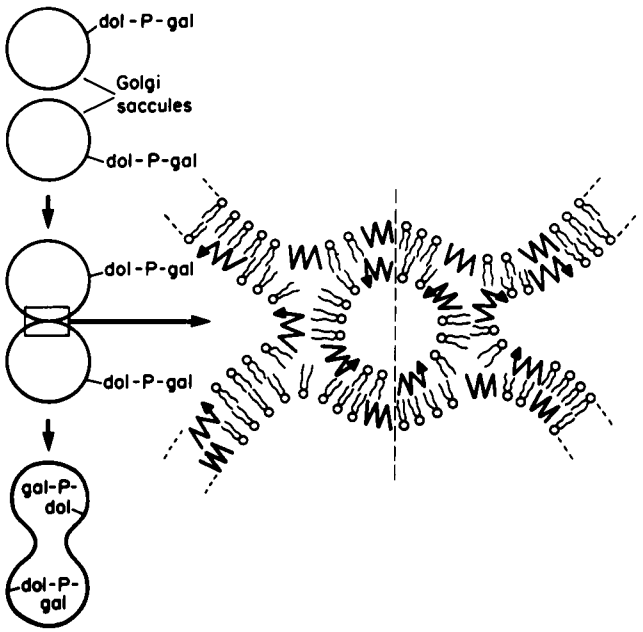

FIGURE 2 Model for terminal glycosylation within Golgi saccules. Galactose is transferred from UDP-gal to dol-P on the cytosolic surface of Golgi saccules to form dol-P-gal. Membrane fusion then allows translocation of dol-P-gal or an analogous intermediate across the lipid bilayer by means of the inverted micelle intermediate of Cullis (4). The dashed line indicates the polarity of rupture of the micelle. The postulated relationship of dol-P-gal within the inverted micelle is indicated. Symbols: ⩙, dol-P-gal; ⩛, dol-P.

biochemical studies have showed 50–80% inhibition of glycosylation by prior trypsin treatment of Golgi membranes. Preliminary EM studies have revealed that trypsin-treated Golgi membranes were morphologically indistinguishable from control membranes.

The results implicate transfer of UDP-gal to dol-P-gal on the cytosolic surface of Golgi membranes. Membrane fusion then allows translocation of dol-P-gal across the lipid bilayer by inverted micelles (4) at points of fusion (Fig. 2). The model, although speculative, satisfactorily resolves the topological questions of glycosylation in vivo.

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VOLTAGE-DEPENDENT CHANGES OF A MEMBRANE PROTEIN IN LIPID MODEL MEMBRANES

STUDIES WITH THE HEPATIC ASIALOGLYCOPROTEIN RECEPTOR

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A major question about the interaction of membrane proteins with lipids is whether the biosynthetic process determines once and for all the orientation of the protein with respect to the bilayer, or whether later processes can affect the protein's disposition. We show here that a membrane receptor protein for asialoglycoproteins (the hepatic binding protein, or HBP [1]) can undergo voltage-dependent changes in its disposition with respect to the lipid bilayer.

RESULTS AND DISCUSSION

This protein mediates endocytosis of desialylated glycoproteins by hepatocytes. It has the great advantages of being obtainable in relatively pure form and of being soluble in aqueous solution free of detectable lipid and detergent (2). In its water-soluble, detergent-free form it does not bind ligands (galactose-terminal glycoproteins such as asialo-orosomucoid, ASOR). When we mixed HBP with phosphatidyl choline vesicles, the protein associated nonelectrostatically with the lipid, and specific ligand binding activity indistinguishable from that of the native receptor was restored (3).

Because of this spontaneous reconstitution, we thought that HBP might insert into preformed black lipid membranes (BLMs); it did (4). An increase in conductance was observed when a *trans*-positive voltage was imposed across a BLM (Fig. 1 *a*). The nature of this conductance increase pointed strongly to lipid perturbation in that: (*a*) no discrete step size of conductance was observed; (*b*) the conductance tracing fluctuated irregularly, in a way not suggesting true channels; (*c*) the conductance was not linearly proportional to the first, or any higher, power of the HBP concentration; and (*d*) the conductance displayed little ion selectivity. Also consistent with a lipid perturbation was the observation that HBP lowered and broadened the phase transition of dipalmitoyl phosphatidyl choline vesicles (3).

For a signal to be transduced by a receptor after ligand binding, a conformational change in the receptor is usually assumed to take place. Given the possibility that such a change could alter lipid-protein interactions, we examined the effect of ligand binding on the conductance in an HBP-doped BLM. When specific ligand was used, we noted a dramatic voltage-dependent increase in conductance (Fig. 1 *b*). In contrast to the observation with HBP alone, both a *trans*-positive and a *trans*-negative potential induced a conductance increase. The monovalent uncharged ligand *N*-acetyl galactosamine also produced this effect. Thus, ligand binding results in an alteration of the receptor which can be monitored by the conductance measurements.

It is well known that calcium is specifically required for the binding activity of this receptor. Calcium (and not other divalent cations) had an interesting effect on HBP induced conductance. Although the magnitude of conductance was not increased, the voltage-dependent conductance was again made symmetrical as in the presence of ligand (Fig. 1 *c*).

Our model for the stages of interaction between HBP and lipid bilayers is shown in Fig. 2. In Fig. 2 *a*, the protein (added to the *cis* side) associates with the lipid bilayer in