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Early and late functions associated with the Golgi apparatus reside in distinct compartments

(oligosaccharide trimming/terminal glycosylation/fatty acylation/vesicular stomatitis virus glycoprotein/cell-free system)

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ABSTRACT **Enzymes that catalyze the two successive stages of Golgi-associated processing of asparagine-linked oligosaccharides distributed differently when membranes from Chinese hamster ovary cells were centrifuged in a sucrose density gradient. A mannosidase that removes only outer, α-1,2-linked mannose residues from the precursor oligosaccharides of the vesicular stomatitis viral G protein (to yield a "trimmed" oligosaccharide core) was separated from enzymes (galactosyl- and sialyltransferases) that act in the later, terminal stage of glycosylation. Freshly acylated G protein with newly trimmed oligosaccharides banded in the distribution of early-acting membranes, defined by the mannosidase, whereas G protein pulse-labeled with [^{3}H]galactose distributed in the profile of the late-acting membranes. G protein present in the early-acting membranes in crude fractions could be terminally glycosylated by incubation with exogenous Golgi membranes *in vitro*; G protein lost its ability to be processed *in vitro* as it appeared to enter the late-acting membranes *in vivo*. These experiments reveal the existence of two distinct compartments through which intracellularly transported proteins such as G pass in sequence as Golgi-associated processes are carried out. It is likely that these compartments consist of cisternae on the *cis* and *trans* sides of the Golgi stack.**

The stack of cisternae composing the Golgi apparatus is markedly asymmetric (1–7), its *cis* [or entry (8, 9)] and *trans* [or exit (9, 10)] faces differing in morphological and histochemical properties. This *cis–trans* polarity could signify a fundamental division of the stack into functionally distinct compartments between which proteins can be transported vectorially. Alternatively, this overall asymmetry could reflect a continuous gradient of composition, the result of a sorting process operating within a stack composed of multiple copies of the same kind of compartment.

We recently reported (11) a dramatic change in the behavior in a cell-free assay system (12, 13) of a plasma membrane-type glycoprotein [the G protein encoded by vesicular stomatitis virus (VSV)] that occurred as a result of passage through the Golgi. Specifically, the assay distinguished two intracellular pools through which G protein passed in rapid succession: an earlier "transferable" pool that can be donated by membranes from infected cells to exogenous Golgi for oligosaccharide processing *in vitro* and a later "nontransferable" pool that cannot. The transferable pool appeared to reside in Golgi membranes (11). If the other pool also resided in Golgi membranes, but in a different region, then the change in properties of G protein (transferable versus nontransferable) in going from one region of the Golgi to the other would signify a compartment boundary in between.

Asparagine-linked oligosaccharides (such as those of the VSV G protein) are processed in two major stages (14–16). First, the precursor oligosaccharide is "trimmed" by the removal of four α-1,2-linked mannose units. Later, terminal glycosylation is initiated by the addition of *N*-acetylglucosamine, and completed by galactose and sialic acid incorporation. An α-1,2-mannosidase (14) responsible for the early (trimming) stage and glycosyltransferases catalyzing the steps in the later ("terminal") stage (17) are all comparably concentrated in rat liver Golgi membrane preparations and thus can be considered as enzymatic markers of this organelle. Here we report the resolution on sucrose density gradients of two regions of intracellular membranes from Chinese hamster ovary (CHO) cells responsible for these two successive Golgi-associated stages of oligosaccharide processing. Moreover, each region appears to house one of the two pools of G protein distinguished in the cell-free processing assay.

MATERIALS AND METHODS

Cells and Viruses. CHO cell lines [two mutants, clone 15B (18) and clone 1021 (19) and the parent line] and the clone 6 mutant (20) of mouse L cells were kindly provided by S. Kornfeld, Washington University, and were grown in monolayer in α minimal essential medium (GIBCO) containing 7.5% fetal calf serum (12). VSV infections were carried out as before (12). All experiments were performed 4 hr after infection.

Preparation of Postnuclear Supernatants for Fractionation on Sucrose Gradients. Cells (VSV-infected or uninfected) not labeled with radioisotopes were harvested in phosphate-buffered saline (3 ml per 10-cm plate; see ref. 12 for composition) from densely confluent monolayers with a rubber policeman, centrifuged at 600 × *g* for 5 min at 4°C, and then swollen (12). Postnuclear supernatants were prepared exactly as before (12), except that the cells were homogenized in a volume equal to 3.5 times that of the swollen cell pellet. Typically, 20 10-cm plates (2–3 × 10^7 cells per plate) yielded 3 ml of postnuclear supernatant (protein concentration, 5–7 mg/ml) that could be frozen in liquid N_2 and stored at −70°C for later use. Cells were labeled with radioisotopes in suspension as indicated in the figure legends, and then centrifuged, swollen, and homogenized as above.

Preparation of [^{3}H]Mannose-Labeled Oligosaccharides. Four confluent 10-cm plates of VSV-infected L cell clone 6 were pulse-labeled for 30 min with 2 ml per plate of Joklik's minimal essential medium (with 10% normal glucose level) containing nonessential amino acids (GIBCO) and [2-^{3}H]mannose (15 Ci/mmol, New England Nuclear; 1 Ci = 3.7 × 10^{10} becquerels) at 0.5 mCi/ml, and then chased for 1 hr in growth medium.

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Abbreviations: VSV, vesicular stomatitis virus; CHO, Chinese hamster ovary; ER, endoplasmic reticulum; Endo H, endo-β-*N*-acetylglucosaminidase H.

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VSV-infected clone 15B cells were labeled in the same manner, except the pulse was 15 min and the chase was 2.5 hr. After lipid extraction (21), [^{3}H]mannose-containing glycopeptides were prepared by Pronase digestion and gel filtration as described (21, 22). The size distribution of the oligosaccharides released by endo-β-*N*-acetylglucosaminidase (Endo H) (generously provided by P. W. Robbins, Massachusetts Institute of Technology) from these glycopeptide fractions was determined by gel filtration on Bio-Gel P-4 (400− mesh; Bio-Rad) (23). Ninety-three percent of the [^{3}H]mannose of the clone 6 glycopeptide preparation was present as a nearly equimolar mixture of $Man_9GlcNAc$ and $Man_8GlcNAc$. For the assays in Table 1, we further purified [^{3}H]$Man_5GlcNAc$ preparatively on the Bio-Gel column. Authentic ^{14}C-labeled $Man_9GlcNAc$, $Man_8GlcNAc$, and $Man_5GlcNAc$ standards used for calibration of the Bio-Gel column were the kind gift of Martin Snider (Massachusetts Institute of Technology).

Enzyme Assays. Galactosyltransferase was measured with ovalbumin as the acceptor according to the procedure of Brew *et al.* (24), modified as described (13). Sialyltransferase assays, based on a described procedure (19), contained (in 20 μl final volume): 50 mM Tris·HCl (pH 7.4), 10 mM $MnCl_2$, 0.5% Triton X-100, CMP-[^{3}H]sialic acid (18.9 Ci/mmol, New England Nuclear) at 2 μCi/ml, asialofetuin (25) at 2 mg/ml, and the membrane fraction to be assayed. Incubations (90 min at 37°C) were terminated by addition of 1% phosphotungstic acid (dissolved in 0.5 M HCl), and ^{3}H in the precipitate was measured. Intact fetuin was not active as an acceptor. Activity of α-1,2-mannosidase was measured as the amount of [^{3}H]mannose released from the [^{3}H]mannose-labeled clone 6 glycopeptide during a 90-min incubation at 37°C, essentially as described by Tabas and Kornfeld (14). Assays contained 50 mM sodium phosphate (pH 6.5), 0.1% Triton X-100, 5 mM $MgCl_2$, clone 6 glycopeptide (5000 cpm), and the membrane fraction to be assayed in a final volume of 22.5 μl.

RESULTS

Membranes from VSV-infected CHO clone 15B cells [the cell line employed as donor of G protein *in vitro* (11–13)] were centrifuged to equilibrium in a sucrose density gradient, and the distributions of enzymes believed to catalyze steps in the two stages of Golgi-associated oligosaccharide processing were determined (Fig. 1).

As a marker of the first (trimming) stage, we assayed an α-1,2-mannosidase activity as described by Tabas and Kornfeld (14) by measuring the release of α-1,2-linked [^{3}H]mannose from precursor glycopeptides of G protein. This substrate was prepared from a variant line of L cells (clone 6) that accumulates $Man_8GlcNAc_2$ oligosaccharides (14). As markers for the subsequent stage of terminal glycosylation, we assayed both galactosyltransferase and sialyltransferase activities. Mannosidase and galactosyltransferase activities distributed differently in the gradient (Fig. 1), with the α-1,2-mannosidase peaking at a higher density ($\approx$1.13 g/ml) than galactosyltransferase ($\approx$1.11 g/ml). Uninfected clone 15B cells showed the same distributions (not shown). Sialyltransferase distributed in the same manner as galactosyltransferase (Fig. 1 *Inset*), as would be expected from the findings of Bretz *et al.* (17). All three enzyme activities distributed differently from the much denser rough endoplasmic reticulum (ER) membranes (identified as those containing newly translated G protein labeled with [^{35}S]methionine; see Fig. 4).

The activity we have measured [like the rat liver α-1,2-mannosidase (14)] can remove mannose units only from high-mannose precursor forms and not from the Man_5 intermediate that results from the first (trimming) stage of oligosaccharide processing *in vivo*. This was evident (Table 1) from the differential effects of inhibitors described by Tabas and Kornfeld (14). Hydrolysis of the high-mannose clone 6 glycopeptide by the α-1,2-

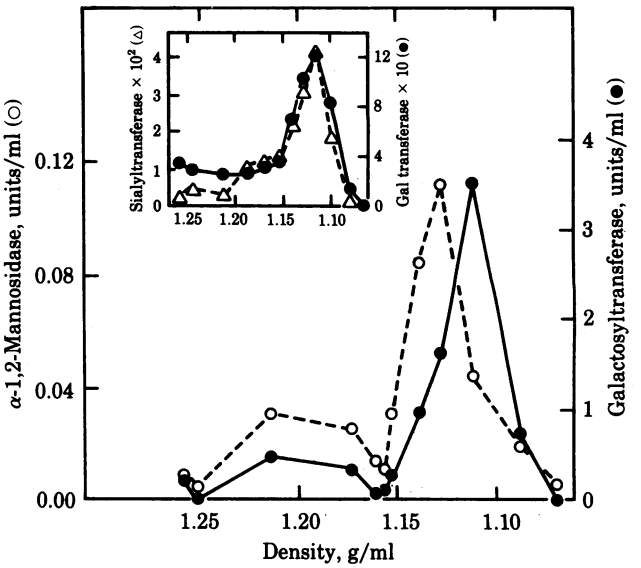

FIG. 1. Distributions of oligosaccharide-processing enzymes in membranes from VSV-infected clone 15B cells. The membrane pellet from 3 ml of postnuclear supernatant was washed and then fractionated on a six-step sucrose gradient exactly as described (11) except that the 20% (wt/wt) layer was replaced with an equal volume of 15% (wt/wt) sucrose. Membranes were harvested (11) from samples (0.8 ml of $\approx$9 ml total) of each fraction and suspended in 20 μl of water. Samples were assayed for α-1,2-mannosidase (○) and galactosyltransferase (●) activities, expressed as units per ml of gradient fraction. The α-1,2-mannosidase unit was defined by Tabas and Kornfeld (14). The galactosyltransferase unit is 1 pmol of [^{3}H]galactose incorporated per hr. Typically, 35% and 85% of the α-1,2-mannosidase and galactosyltransferase activities of the membrane pellet from postnuclear supernatant were recovered from the density gradients. (*Inset*) Distributions of galactosyltransferase (●) and sialyltransferase (△) activities (in units/ml). One milliliter of postnuclear supernatant of VSV-infected 15B cells was fractionated. One unit of sialyltransferase is 1 pmol of [^{3}H]sialic acid incorporated per hr. The refractive index of each fraction was measured for density determination.

Table 1. Properties of mannosidase activities present in CHO cell membranes

Conditions	Relative rate of hydrolysis: [^{3}H]Mannose labeled clone 6 glycopeptide	[^{3}H]Man_5-GlcNAc	4-Methylumbelliferyl α-D-mannoside
Control	[1]	[1]	[1]
+ $CuSO_4$ (1 mM)	1.17	0.09	0.12
Tris/maleate (50 mM, pH 6.8)	0.05	0.95	0.95
Sodium acetate (0.1 M, pH 4.4)	0.13	ND	0.61

Control incubations contained 50 mM sodium phosphate (pH 6.5), washed total membranes from the postnuclear supernatant of wild-type CHO cells (see Fig. 1), and clone 6 glycopeptide (5000 cpm), $Man_5GlcNAc$ (5000 cpm), or 1 mM 4-methylumbelliferyl α-D-mannoside (Sigma). All incubations contained 5 mM $MgCl_2$ and 0.1% Triton X-100. A direct comparison of the absolute rates of hydrolysis of the Man_5 oligosaccharide and clone 6 glycopeptide is not possible because the specific radioactivities of the substrates cannot be determined. Release of [^{3}H]mannose from the glycopeptide and the oligosaccharide was followed as described (14). Fluorescence of released 4-methylumbelliferone was measured as described (26). Values were normalized to the control. ND, not determined.

mannosidase was unaffected by 1 mM $CuSO_4$ but was completely inhibited at a pH of 4.4 or by 50 mM Tris/maleate (pH 6.8). In contrast, hydrolysis of $Man_5GlcNAc$ (which contains no α-1,2 linkages) and of the fluorogenic compound 4-methylumbelliferyl-α-D-mannoside was abolished by 1 mM $CuSO_4$ but unaffected by Tris/maleate buffer. The distribution and levels of α-1,2-mannosidase activity were the same as those shown in Fig. 1 when individual fractions were assayed in the presence of 1 mM $CuSO_4$ (data not shown).

We pulse-labeled VSV-infected cells to inquire whether G protein was present in the distributions defined by the marker enzymes. We considered a pulse of [^{3}H]palmitate likely to label G protein undergoing or about to undergo the first (trimming) stage of processing (27). [^{3}H]Galactose was chosen as a selective label of G protein undergoing the second (terminal) stage of processing. Because 15B cells terminate oligosaccharide processing before addition of galactose, CHO clone 1021 cells [which add galactose but not sialic acid to glycoproteins (19)] were used in this experiment. [Wild-type CHO cells were not used because a poorer (but nonetheless discernible) separation of early and late Golgi marker enzymes was routinely observed (not shown).]

We found that the [^{3}H]galactosyl-G protein distributed with a peak equilibrium density of about 1.11 g/ml (Fig. 2), just as did the galactosyltransferase activity (Fig. 2 *Inset*). Moreover, a large fraction of the [^{3}H]palmitoyl-G protein labeled in a 5-min pulse (Fig. 2) followed the pattern of the α-1,2-mannosidase (Fig. 2 *Inset*).

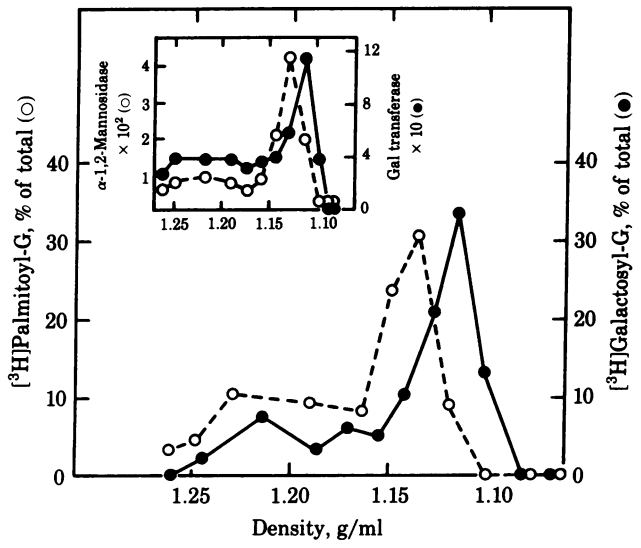

FIG. 2. Distribution in the sucrose gradient of the G protein of VSV-infected CHO 1021 cells pulse-labeled for 5 min with [^{3}H]palmitate (○) or [^{3}H]galactose (●). Membrane pellets from gradient fractions were electrophoresed in a sodium dodecyl sulfate/10% polyacrylamide gel (11). Gels were treated with EN^{3}HANCE (New England Nuclear). [^{3}H]G protein was quantitated from densitometer tracings of the autoradiographs and expressed as a percent of the total recovered from the gradient (40–50% of the total in the postnuclear supernatant). For [^{3}H]palmitate labeling, 8×10^7 infected clone 1021 cells were trypsinized into suspension as described (12) and incubated in 10 ml of serum-free α minimal essential medium containing 20 mM Hepes/NaOH (pH 7.4) and 2 mCi of [^{3}H]palmitate (11.8 Ci/mmol, New England Nuclear) at 37°C. Infected CHO 1021 cells (1.6×10^8) were labeled with [1-^{3}H]galactose (0.1 mCi/ml) as before (11) except the cell density was doubled. Postnuclear supernatants (0.5 ml for [^{3}H]palmitate; 1.0 ml for [^{3}H]galactose) were utilized for the gradients. (*Inset*) Distributions of α-1,2-mannosidase (○) and galactosyltransferase (●) activities in membranes of uninfected clone 1021 cells. Infected 1021 cells gave the same result. Postnuclear supernatant (2 ml) was fractionated. Enzyme activities are expressed as units/ml.

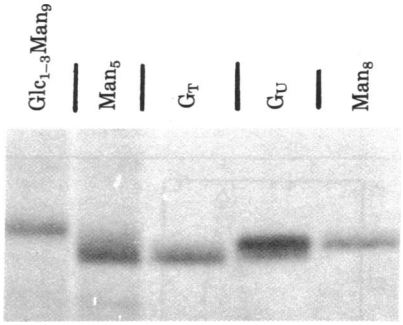

FIG. 3. Comparison of the apparent molecular weights of species of [^{3}H]palmitoyl-G protein with G protein markers containing known oligosaccharide structures. Electrophoresis of postnuclear supernatants prepared from: clone 15B cells pulse-labeled with [^{35}S]methionine for 3 min ($Glc_{1-3}Man_9$) or pulsed and then chased for 15 min (Man_5); clone 15B cells pulse-labeled for 5 min with [^{3}H]palmitate (G_U, denoting the major species present), or pulsed and then chased for 30 min (G_T, denoting the major species present); clone 6 cells pulse-labeled for 5 min with [^{3}H]palmitate and chased for 30 min (Man_8). The portion of the autoradiograph containing G protein is shown.

The presence of [^{3}H]palmitoyl-G protein in the distribution shown in Fig. 2 did not rule out a rapid accumulation of label there after acylation at an earlier site. In VSV-infected 15B cells labeled with [^{3}H]palmitate, we detected two major electrophoretic forms of tritiated G protein that distributed differently in the density gradient (Figs. 3 and 4). The lower molecular weight form (denoted G_T) predominated after a chase and electrophoresed in a polyacrylamide gel in the same position (Fig. 3) as G protein containing Man_5 trimmed sugar chains, the end product of processing in 15B cells. The form of [^{3}H]palmitoyl-G protein of higher molecular weight (denoted G_U) was most abundant after short pulses, yet migrated in electrophoresis faster than G protein pulse-labeled for 3 min with [^{35}S]methionine [labeling conditions under which G oligosaccharides would contain one to three glucose residues in addition to nine mannose units (28)]. Also, G_U electrophoresed slightly slower than G bearing $Man_8GlcNAc_2$ chains (the structures accumulated in clone 6 cells).† The oligosaccharides of G_U therefore most likely contain nine mannose units.

The proportion of [^{3}H]G in the G_T form in [^{3}H]palmitate labeling experiments was highly variable, ranging between 22% and 75% after a 5-min pulse. This would be expected from the temporal proximity of acylation to trimming. G_T always distributed in the gradient in the same manner as α-1,2-mannosidase activity (Fig. 4). G_U distributed differently in the gradient from G_T, but similarly to rough ER membranes (Fig. 4). This suggests that most of the [^{3}H]palmitate is added to G protein just prior to trimming, and in different membranes, perhaps in a still earlier region of the Golgi, or in the ER. Acylation at random in the ER would be unlikely because of the lag after protein biosynthesis (27), and it might take place instead in a specialized region such as the transitional elements.

What is the relationship between the two sequentially acting membranes responsible for the early and late stages of oligosaccharide processing (Figs. 1, 2, and 4) and the two pools (transferable and nontransferable) of G protein revealed by assays using the cell-free system (11)? Depending on the preparation, between 35% and 60% of the total [^{3}H]palmitoyl-G protein labeled in a 5-min pulse in 15B cells was converted from

† The electrophoretic mobility differences in Fig. 3 are due to carbohydrate heterogeneity, and not to palmitate attachment, because all of these forms of G comigrate after digestion with Endo H (data not shown).

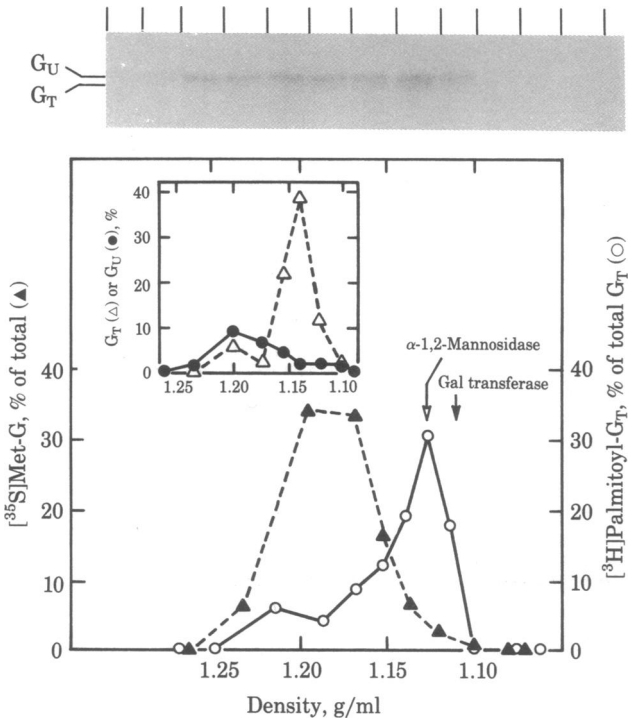

FIG. 4. VSV-infected clone 15B cells were pulse-labeled with [^{3}H]palmitate for 3 min (see Fig. 2), and membranes from the postnuclear supernatant (0.5 ml) were fractionated on the sucrose gradient. (*Autoradiograph*) Gel of the electrophoresed fractions (only the portion containing G protein is shown). Plotted below (○), from this gel, is the distribution of the faster-migrating, trimmed form of G protein (G_T, 18% of the total [^{3}H]G recovered from the gradient in this experiment). For reference, the locations of the peaks of α-1,2-mannosidase (open arrow) and galactosyltransferase (closed arrow) activities from Fig. 1 are indicated. ▲, Rough ER marker, the distribution of the ^{35}S-labeled G protein (determined as for [^{3}H]G protein) in 15B cell membranes (from 0.1 ml of postnuclear supernatant) after a 3-min pulse-label with [^{35}S]methionine as described (11). (*Inset*) a different [^{3}H]palmitate labeling experiment (5 min pulse) in which G_T predominated (now 75% of the total G) as illustration of the variability in this ratio. Here, the distribution of both [^{3}H]G_T (△) and [^{3}H]G_U (●) forms are shown, expressed as a percent of the total [^{3}H]G protein (G_T plus G_U) recovered from the gradient.

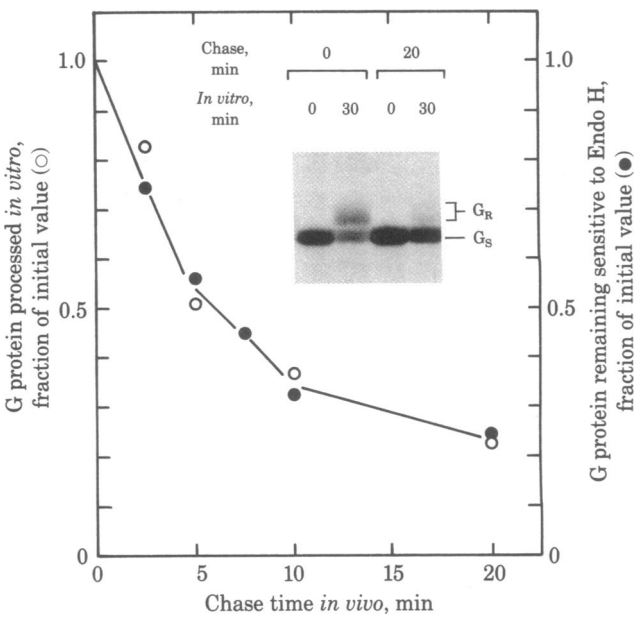

FIG. 5. Processing of [^{3}H]palmitoyl-G protein by exogenous Golgi *in vitro* as a function of time after acylation *in vivo*. (*Autoradiograph*) VSV-infected 15B cells were pulse-labeled for 5 min with [^{3}H]palmitate as in Fig. 4 (0 min chase) or pulsed and then chased (in the presence of 30 μM unlabeled palmitate) for 20 min, and postnuclear supernatants were prepared. Incubation mixtures for cell-free processing contained 20 μl of [^{3}H]palmitate-labeled postnuclear supernatant, 20 μl of postnuclear supernatant from wild-type cells, and 60 μl of reaction cocktail (11). After incubation at 37°C for either 0 or 30 min, aliquots were taken, digested with Endo H, and electrophoresed as before (12). G_R, the forms of G protein bearing Endo H-resistant oligosaccharides; G_S, the Endo H-sensitive form. A total of 59% of the [^{3}H]G was processed to Endo H resistance after 30 min *in vitro* with no chase *in vivo*, and 8.4% when 20-min chase had been allowed. The graph shows an independent experiment of the same design in which postnuclear supernatants prepared from [^{3}H]palmitate-labeled VSV-infected 15B cells after various times of chase (in the presence of unlabeled palmitate and cycloheximide at 5 μg/ml) were tested as donors *in vitro* (○) as described above. The percent of [^{3}H]G protein processed to G_R *in vitro* did not change between 40- and 60-min incubation, indicating that an endpoint had been reached. G_R values (% of total) were 35%, 29%, 18%, 13%, and 8% at 0, 2.5, 5, 10, and 20 min of chase, and are plotted (○) as a fraction of the value at 0 min of chase. In a parallel experiment (●), the parent of the 15B mutant (VSV-infected) was pulse-labeled for 5 min with [^{3}H]palmitate and chased as above, but now the percent of [^{3}H]G remaining sensitive to Endo H at the time of homogenization was determined. G_S values (% of total) were 50%, 37%, 28%, 23%, 16%, and 12% of total at 0, 2.5, 5, 7.5, 10, and 20 min of chase and are expressed as a fraction of the value at 0 min of chase.

Endo H-sensitive (G_S) to Endo H-resistant (G_R) forms (Fig. 5 *Autoradiograph*) upon incubation with wild-type membranes (11). No processing was observed when wild-type membranes were replaced by 15B cell membranes in the assay (data not shown). In light of our earlier work showing that the G protein processed *in vitro* originated in Golgi-like membranes (11), these findings imply that acylated G protein present in the early marker distribution (Figs. 2 and 4) is transferable *in vitro*. Furthermore, as was the case for [^{35}S]methionine-labeled G after its trimming (11), [^{3}H]palmitoyl-G lost its ability to be processed *in vitro* (entering a nontransferable pool) rapidly after acylation. In a parallel experiment (also Fig. 5), we found that in wild-type cells the timing with which the oligosaccharides of [^{3}H]palmitate-labeled G underwent the first step of terminal glycosylation [assessed by the conversion to Endo H resistance (15, 21)] was similar. The data for processing *in vitro* and Endo H sensitivity *in vivo* in Fig. 5 have been expressed as a fraction of the respective initial values at the start of the chase period. If it can be assumed that the lack of *N*-acetylglucosamine glycosyltransferase I in 15B cells (15) does not seriously affect the kinetics of transport of G within the Golgi, then the coincidence of the data would suggest that G protein enters the nontransferable pool as it encounters the terminal glycosyltransferases present in the late-acting marker distribution in the sucrose gradient.

DISCUSSION

That the two major stages of asparagine-linked oligosaccharide processing (trimming of peripheral mannose and terminal glycosylation) occur within distinct intracellular compartments follows because: (*i*) Membranes carrying out these two stages can be physically separated (Figs. 1, 2, and 4). (*ii*) They differ in enzymatic composition (Figs. 1 and 2). (*iii*) They act upon transported glycoproteins in a definite sequence (16). (*iv*) The capacity of G protein to be terminally glycosylated *in vitro* by exogenous Golgi is permanently lost as G appears to pass between them (Fig. 5). A compartment boundary can be likened to the interface between two immiscible liquids in that molecules of identical structure can exist in different states on its two sides. The data suggesting that a molecule of G protein under-

goes a transition in its state [from transferable to nontransferable *in vitro* (item *iv*)] as it changes locations (items *i–iii*) therefore provide telling evidence of an intervening compartmental boundary. The permanent nature of the switch to the nontransferable state further suggests that transport of G protein across this boundary is a vectorial process.

The boundary between these compartments almost certainly resides within the Golgi apparatus because both stages of oligosaccharide processing are associated with Golgi membranes in other instances (1–7, 17, 20). In the case of CHO cells, electron microscopic immunocytochemistry has demonstrated that G protein enters Golgi stacks after its exit from rough ER (8); and, as described here, the two compartments responsible for oligosaccharide processing in these cells fractionate distinctly from rough ER (Figs. 1, 2, and 4). Crude fractions of CHO cells were analyzed in our experiments for the distribution of the relevant enzymes and their glycoprotein products. Lack of purity does not diminish the biochemical demonstration of two compartments, but it does prevent an unequivocal link from being made between them and morphologically defined elements of the Golgi, a deficiency that will be important to remedy in future work. Therefore, we will refer to the compartments as "Golgi-associated."

Because exported proteins are believed to traverse the Golgi stack in the *cis* to *trans* direction (1–10), an appealing interpretation is that the early and late compartments consist of distinct sets of cisternae on the *cis* and *trans* sides. A large body of indirect evidence [considered elsewhere (6)] supports this view by implying that the terminal glycosyltransferases that act in the late compartment are concentrated in the *trans*-most cisterna or two of the stack. The compartment boundary would then divide the Golgi stack into two distinct portions, explaining the *cis–trans* asymmetry so evident in earlier morphological and histochemical studies (1–10). It is entirely possible, however, that there are additional Golgi-associated compartments.

Subfractions of rat liver Golgi enriched in *cis* and *trans* elements have been described (29, 30), but were characterized before the processes of oligosaccharide trimming (14, 16) and fatty acylation (27) were known.

Why are Golgi-associated functions carried out in at least two stages in separate compartments? One explanation—that the compartments are receptacles for exported proteins already sorted according to final cellular destination—seems inconsistent with our findings. Examples of each major type of exported protein (secretory, lysosomal, and plasma membrane) possess oligosaccharide chains that appear to have undergone both trimming and terminal glycosylation (16, 31). These proteins must all have passed through both early and late Golgi-associated compartments. If, as we suggest, the late compartment consists of the *trans*-most cisternae of the stack, the implication would be that sorting of exported proteins is postponed until these proteins are on the verge of leaving the Golgi.

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