Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1681–1685, March 1993
Cell Biology

Endoplasmic reticulum-through-Golgi transport assay based on O-glycosylation of native glycophorin in permeabilized erythroleukemia cells: Role for G_{i3}

BRIDGET S. WILSON, GEORGE E. PALADE, AND MARILYN G. FARQUHAR

Division of Cellular and Molecular Medicine and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093

Communicated by George E. Palade, November 4, 1992

ABSTRACT **An assay for endoplasmic reticulum (ER)-through-Golgi transport has been developed in streptolysin O-permeabilized murine erythroleukemia (MEL) cells. The reporter proteins are metabolically labeled native murine glycophorins, which display a distinctive shift in electrophoretic mobility after acquisition of O-linked oligosaccharides. The O-linked sugars are acquired at a site distal to a brefeldin A block, presumably in a cis Golgi compartment, and sialylation occurs in middle and/or trans Golgi compartments. In permeabilized cells supplemented with cytosolic proteins and an ATP-generating system, 20–50% of the radiolabeled precursor glycophorins can be converted to the mature, sialylated form. This maturation process is ATP- and cytosol-dependent and is blocked by guanosine 5′-[γ-thio]triphosphate (GTP[γS]). Electron microscopy of permeabilized MEL cells shows retention of ER elements, stacked Golgi cisternae, free polysomes, and other subcellular components. In the presence of GTP[γS], dilated vesicles accumulate around the Golgi stacks. Antisera to the carboxyl terminus of the Golgi resident α subunit of G_{i3} inhibit maturation of glycophorin. To our knowledge, a transport assay utilizing O-glycosylation of an endogenous protein as a monitor of ER-through-Golgi traffic in permeabilized cells has not been reported previously. Furthermore, the data provide evidence for heterotrimeric GTP-binding protein involvement in Golgi function.**

Recent progress in identifying the proteins of mammalian cells that comprise the machinery for vesicular trafficking from the endoplasmic reticulum (ER) through the Golgi apparatus has arisen from reconstitution assays using semi-intact cells or isolated Golgi fractions (reviewed in refs. 1 and 2). To demonstrate transport, these assays rely upon the Golgi-dependent modifications of the N-linked oligosaccharides of a reporter protein—namely, the vesicular stomatitis virus G protein. The signal obtained is highly amplified since the virus commandeers the translational and processing machinery of the infected cell. The results obtained indicate convincingly that vesicular traffic proceeds through a series of distinct steps that are dependent on Ca^{2+}, the hydrolysis of ATP or GTP, and the activity of a number of cytosolic proteins [*N*-ethylmaleimide-sensitive factor (NSF), soluble NSF-associated proteins (SNAPs), rab proteins, etc.] (1, 2).

We have developed an alternative reconstituted transport assay in murine erythroleukemia (MEL) cells, using as a reporter molecule, glycophorin 3 (gp3), a native membrane glycoprotein that is exclusively O-glycosylated (3, 4). With this assay we hope to define the subcellular location within the ER–Golgi system for the various steps in O-glycosylation, a process that is less well defined than N-glycosylation. We also believe it is important to establish alternative systems in cells of different lineages in order to identify cell type-specific variations in the basic transport machinery.

Previous studies have documented that glycophorins are integral plasma membrane glycoproteins expressed in all murine erythroid cells, including MEL cells. Glycophorins are assumed to influence the shape of erythrocytes through association with band 4.1 (5). Moreover, their high degree of sialylation confers considerable negative charge at the cell surface and may influence erythrocyte interactions with the endothelium, leukocytes, and macrophages. The major glycophorin of MEL cells, a tight doublet designated gp3, is synthesized and fatty acylated in the ER, O-glycosylated upon entry into the Golgi, and phosphorylated as a late modification (3, 4, 6–8). We have chosen gp3 as a monitor for ER-to-Golgi traffic because the mature O-glycosylated doublet can be easily resolved from two precursor forms by SDS gradient gel electrophoresis. We have successfully developed methods for permeabilizing MEL cells using the bacterial toxin streptolysin O (SLO) (9) and have determined specific conditions that support vesicular trafficking in the permeabilized cells. We have also explored the potential role in vesicular transport of the heterotrimeric GTP-binding protein (G protein), G_{i3}, recently localized to the Golgi apparatus (10).

MATERIALS AND METHODS

Materials. Reagents were from the following sources: RPMI medium, UCSD core facility; fetal calf serum, GIBCO; dimethyl sulfoxide (DMSO), ATP, GTP[γS], iodoacetamide, creatine phosphate, and phosphocreatine kinase, Sigma; [^{35}S]methionine, DuPont/NEN; protein A-Sepharose CL-4B, Pharmacia; polyacrylamide, National Diagnostics, Manville, NJ; other SDS/PAGE supplies, Bio-Rad; SLO, Burroughs Wellcome.

Reconstituted Transport Assay. MEL cells, grown in RPMI plus 10% fetal calf serum, and induced with 2% DMSO 3–4 days before assay (3), were dispensed into fibronectin-coated 24-well plates. For pulse–chase studies, an 8-min pulse with [^{35}S]methionine (1 mCi/ml in methionine-free RPMI; 1 Ci = 37 GBq) at 37°C was followed by transfer to 4°C and addition of 0.5 ml of ice-cold RPMI with 1.5 mg of methionine per ml. Cell layers were washed with phosphate-buffered saline (PBS), followed by 20 min of incubation at 4°C with 2 units of SLO per ml. After washing with transport assay buffer [TAB; 20 mM Hepes, pH 7.2/140 mM K^+ (129 mM as potassium acetate/11 mM as KOH)/10 mM Na^+/0.5 mM Mg^{2+}/10.5 mM Cl^-], MEL cell cytosol was added to cover the cell layer. Ca^{2+} in TAB and in cytosol preparations was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: α_{i3}, α subunit of G_{i3}; BFA, brefeldin A; ER, endoplasmic reticulum; G protein, GTP-binding protein; G_{i3}, heterotrimeric G protein; MEL, murine erythroleukemia; NSF, *N*-ethylmaleimide-sensitive factor; SNAP, soluble NSF-associated protein; TAB, transport assay buffer; GTP[γS], guanosine 5′-[γ-thio]triphosphate; SLO, streptolysin O; DMSO, dimethyl sulfoxide.

50–80 nM, as determined by Quin-2 measurements (11). An ATP-generating system was supplied by the addition of 1 international unit of phosphocreatine kinase, 5 mM ATP, and 5 mM creatine phosphate, and supplements {guanosine 5'-[γ-thio]triphosphate (GTP[γS]), IgG} were added to a total volume of 200 μl. Cells were transferred to 37°C to initiate pore formation and follow intracellular transport after permeabilization as a function of different additives. Transport was stopped by lysis with 100 μl of 50 mM Tris·HCl (pH 7.5) containing Nonidet P-40 (at a final concentration of 1%) and lysates were processed through immunoprecipitation, gel electrophoresis, and fluorography.

Cytosol Preparation. MEL cells, induced with 2% DMSO for 3–4 days, were collected by centrifugation, washed with TAB, and resuspended in TAB (three or four times the volume of the cell pellet) with leupeptin and antipain at 50 μg/ml. Aliquots were passed 10 times through a ball-bearing homogenizer (12), and the homogenate was centrifuged at 4°C at 12,500 × *g* for 10 min. The postnuclear supernatant was centrifuged for 60 min at 100,000 × *g* in a Beckman TL-100 ultracentrifuge. The resulting supernatant was either used immediately as "cytosol" or stored in aliquots at −80°C. Protein concentrations were 4–5 mg/ml, as measured by the bicinchoninic acid protein assay (Pierce).

Immunoprecipitation. Glycophorins were immunoprecipitated by a modification of the method of Dolci and Palade (6). Immune complexes were released by boiling beads for 15 min in 2× Laemmli SDS/PAGE application buffer (13).

SDS/Page and Quantification of Radioactivity. Electrophoresis was carried out at 4°C for 20 hr at 250 V, using a 25-cm 12–15% linear gradient separating gel. Results were quantitated by 2–3 weeks of fluorography followed by densitometric scan or by 3- to 4-day exposure using a Molecular Dynamics Phosphor Imager.

Electron Microscopy. MEL cells were permeabilized with SLO at 4°C as described above, transferred for 30 min to 37°C in the presence of cytosol and an ATP-generating system plus or minus 500 μM GTP[γS], and then fixed for 1 hr at room temperature in 1.5% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) containing 5% sucrose. Cells, gently collected by scraping and pelleted at low speed (to minimize damage), were washed in the same buffer containing 7.5% sucrose, then postfixed 1 hr at 0°C in 1% OsO_4 in acetate/veronal buffer (pH 6.0), and stained in block with uranyl acetate. Samples were dehydrated, embedded in epon, and processed for electron microscopy.

Immunofluorescence. MEL cells, induced with 2% DMSO for 3 days, and collected by centrifugation, were fixed for 15 min with 2% formaldehyde/10 mM periodate/75 mM lysine, washed with PBS, infiltrated with polyvinylpyrrolidone, and frozen in liquid nitrogen. Semithin sections (0.5–1.0 μm) were cut on a Reichert F-4E ultramicrotome equipped with a cryoattachment and incubated with the anti-α_{i3} (where α_{i3} is the α subunit of G_{i3}) antibody, followed by a fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch). Micrographs were taken using an Axiophot (Zeiss) equipped for fluorescence microscopy.

Blocking Antibodies. Specific antibodies to α_{i3} (EC) were kindly provided by Allen Spiegel (National Institutes of Health). IgG recognizing the carboxyl terminus of α_{i3} decapeptide sequence KNNLKECGLY was affinity purified on a peptide column as described (14). The affinity-purified rabbit IgG was concentrated in TAB by successive centrifugations using a Millipore filter unit with a 10,000 molecular weight exclusion limit. IgG concentration was determined by comparison to known concentrations of commercial IgG (Sigma) via SDS/PAGE in a Bio-Rad minigel apparatus. We found that the EC antibody immunoprecipitates a single protein species of ≈41 kDa that is distinct in electrophoretic mobility from immunoprecipitated G_{i2} (not shown).

RESULTS

gp3 as a Reporter Protein. The mature glycophorin species gp3 is exclusively O-glycosylated (3) and migrates as a tight doublet at 29 kDa upon SDS/PAGE in a 12–15% linear gradient gel (Fig. 1, lane 1). This mature form first appears as early as 10 min following an initial 8-min pulse with [^{35}S]methionine (Fig. 1, lane 1). When intact cells are collected immediately after the 8-min pulse, four additional glycophorin bands are seen. The predominant bands at 21 and 23 kDa (Fig. 1, lane 2) represent the early, ER forms of the gp3 doublet; these forms apparently lack sugars but are fatty acylated (6). The two weaker bands seen at 25 and 27 kDa were previously shown (7) to be glycophorin-related species unique to MEL cells. They are coprecipitated with gp3 and its precursors by anti-glycophorin polyclonal serum but do not contribute to the transport assay since they are incompletely translocated into the ER and, in intact cells, are almost completely degraded in the cytosol within 1 hr of synthesis (7).

SLO-Permeabilized Cells Retain Stacked Golgi Cisternae. Electron micrographs of permeabilized MEL cells show a considerable loss of cytoplasmic contents, but subcellular organelles remain largely intact (Fig. 2*A*). In general, the degree of preservation appears to be better than in cells permeabilized by hypotonic shock or mechanical disruption (7, 15). In the presence of cytosol and an ATP-generating system, the stacks of Golgi membranes were well maintained with a small population of vesicles in the near vicinity (Fig. 2*A*). When GTP[γS] was added together with cytosol and ATP, the Golgi cisternae appeared more dilated (Fig. 2*B*) and were surrounded by a larger number of dilated vesicles, a portion of which have thicker membranes interpreted as protein coats.

gp3 Is Formed as a Result of ER-to-Golgi Transport in Permeabilized Cells. gp3 transport was arrested immediately after the 8-min pulse by transfer to 4°C (16), followed by binding of SLO toxin at 4°C and subsequent return to 37°C (Fig. 3). Warming to 37°C promotes toxin-mediated pore formation in the plasma membrane and at the same time allows resumption of vesicular trafficking. In the presence of cytosol and an ATP-generating system, 20–50% of the 21- and 23-kDa precursor forms of glycophorin were converted to the mature form (Fig. 3, duplicate lanes 1 and 2); in a series of 17

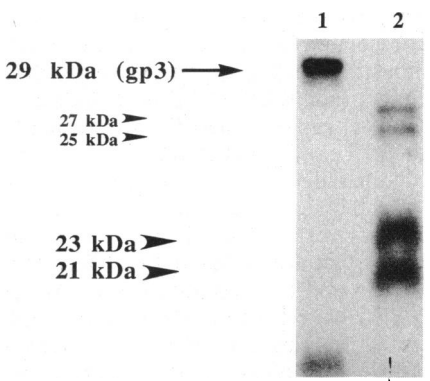

FIG. 1. gp3 as a reporter protein. MEL cells were metabolically labeled for 8 min with [^{35}S]methionine and either collected immediately (lane 2) or chased for 60 min (lane 1), followed by immunoprecipitation of glycophorin species and SDS/PAGE. The mature glycophorin species, gp3, is exclusively O-glycosylated (3, 6, 7) and migrates as a tight doublet at 29 kDa (arrow, lane 1). When collected after the 8-min pulse, two precursor forms of gp3 may be resolved. These newly synthesized proteins have yet to leave the ER, lack sugars, and migrate at 21 and 23 kDa (large arrowheads, lane 2). Note that two additional bands at 25 and 27 kDa (small arrowheads, lane 2) are also glycophorin species, but these are poorly translocated into the ER and are not further modified prior to degradation.

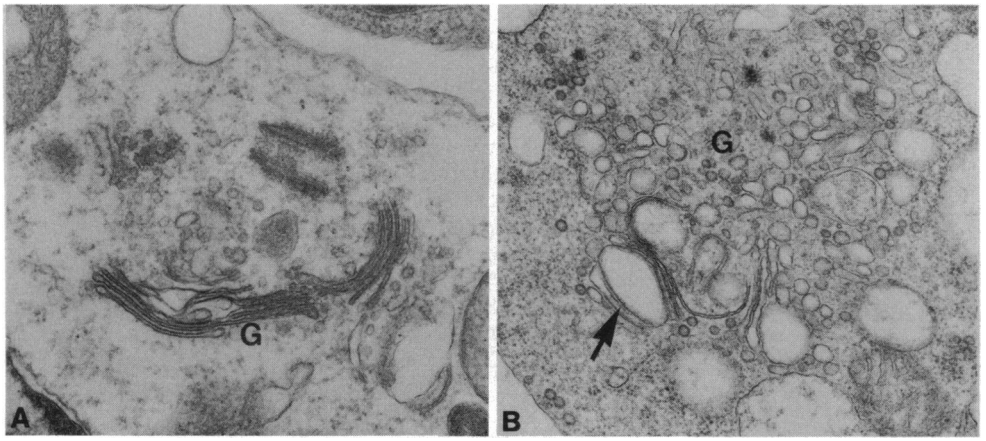

FIG. 2. Electron micrographs of SLO-permeabilized cells. SLO was bound at 4°C and pore formation was initiated upon warming to 37°C for 30 min in the presence of cytosol and ATP, minus (*A*) or plus (*B*) GTP[γS] (500 μM). Cells were then fixed and processed for routine electron microscopy. Permeabilized cells contained intact Golgi cisternae (G) (*A*). Dilated vesicles and/or cisternae (arrow) were more frequently seen in the Golgi region after exposure to GTP[γS] (*B*). (*A*, ×27,000; *B*, ×22,500.)

experiments the average percentage converted to the mature form was 25.1 ± 8. GTP[γS], the nonhydrolyzable analogue of GTP, effectively blocked transport (Fig. 3, duplicate lanes 3 and 4). As reported by others (17, 18), concentrations of GTP[γS] ranging from 10 μM to 1 mM were equally effective in blocking transport. Since GTP[γS] is impermeant, this indicates that practically all cells were permeabilized. This extent of permeabilization was also confirmed biochemically and by uptake of impermeant dyes (data not shown).

ER-to-Golgi Transport Is ATP- and Cytosol-Dependent. In agreement with transport requirements in other semi-intact cell systems (15), we found that cytosolic proteins are required for transport in SLO-permeabilized MEL cells (Fig. 4, compare bars 1 and 5); either MEL cell cytosol or rat liver cytosol (not shown) at 3–5 mg/ml optimally supports transport in our assay. Again, as in other reconstituted systems (15), transport was ATP-dependent (Fig. 4, compare bars 1 and 2). As in Fig. 3, GTP[γS] blocked the appearance of the mature form of gp3 (Fig. 4, bar 3). Ca^{2+} concentrations in MEL cell cytosol fractions were determined (11) and found to be close to normal cytosol levels (50–80 nM) (11), which made Ca^{2+} supplementation of the assay system unnecessary. Efficiency of transport was not significantly changed by the addition of an EGTA/Ca^{2+} (200 nM free Ca^{2+}) buffering system (Fig. 4, bar 4). However, at high EGTA concentrations transport was blocked (not shown).

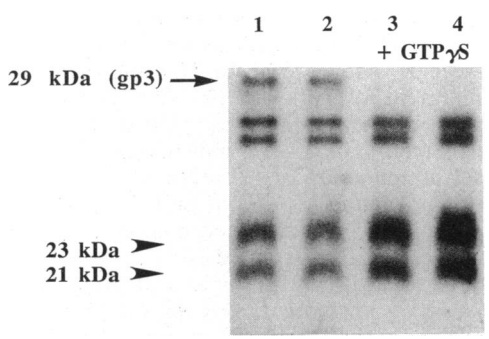

FIG. 3. gp3 is formed as a result of ER-to-Golgi transport in SLO-permeabilized cells. MEL cells were metabolically labeled for 8 min, followed by binding of SLO at 4°C and transfer to 37°C in the presence of cytosol and an ATP-generating system. The transport of newly synthesized glycophorin from the ER to Golgi was quantified by immunoprecipitation and SDS/PAGE (see Fig. 1) to detect a shift in electrophoretic mobility when O-linked sugars were acquired. Under these conditions ≈30% of gp3 is fully processed (arrow, lanes 1 and 2), indicating that glycophorin progressed from the ER through multiple compartments of the Golgi complex. The ER forms at 21 and 23 kDa are indicated by arrowheads. GTP[γS] (500 μM) prevented the Golgi-dependent maturation of gp3 (lanes 3 and 4). The transport assay is routinely performed in duplicate for each variable, as shown here.

The time course of glycophorin maturation was greatly slowed in permeabilized cells. Significant amounts of the mature form of gp3 did not appear until 60 min after returning the cells to 37°C (not shown). The transport assay was routinely carried out for 2 hr at 37°C, at which point the system apparently reaches its limits. Electron micrographs of permeabilized cells incubated beyond 2 hr indicated a marked deterioration of intracellular organelles (not shown).

Evidence for Regulation of Golgi Functions by G_i. One potential target of GTP[γS] is the heterotrimeric G protein G_{i3}, recently shown to be a resident of the Golgi area in LLC-PK_1 cells (10, 19). We examined the localization of the α_{i3} subunit in MEL cells using an antibody (EC) developed by Spiegel and colleagues (14) and found that α_{i3} is concentrated in the Golgi region (Fig. 5). Other investigators have shown (20–24) that antibodies to the carboxyl terminus of α subunits specifically inhibit receptor/G protein interactions in reconstitution assays. Based upon these data, we concentrated IgG affinity-purified on the decapeptide sequence KNNLKECGLY from the carboxyl terminus of α_{i3} to 1–2 mg/ml and added it at 1:20–1:50 in our transport assay. In four separate experiments, each performed in duplicate, inhibition was observed (Fig. 6); it averaged 61% but ranged from

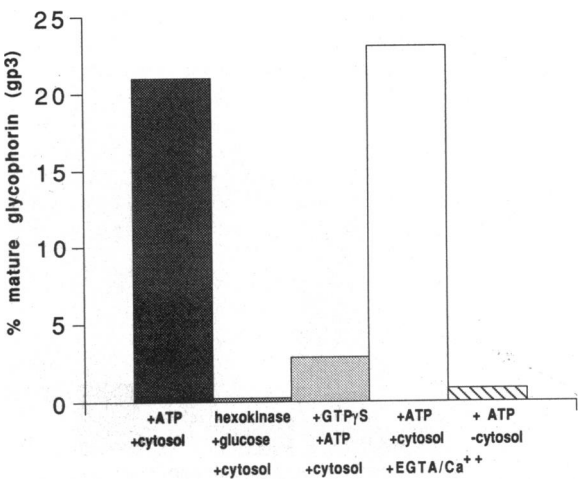

FIG. 4. Requirements for transport in permeabilized MEL cells. Conditions for the support of ER-to-Golgi transport were defined by varying the cytosol, nucleotide, and Ca^{2+} concentrations in the transport assay. Transport was supported only in the presence of an ATP-generating system and cytosol (bar 1). Addition of an ATP-depleting system (bar 2) or 500 μM GTP[γS] (bar 3) or the absence of cytosol (bar 5) completely blocked transport. Ca^{2+} concentration in unsupplemented cytosol is 60–80 nM. Addition of an EGTA/Ca^{2+} buffering system (200 nM Ca^{2+}) did not alter the efficiency of transport (bar 4). Values are averages of duplicates in the same experiment in which all parameters were compared concurrently.

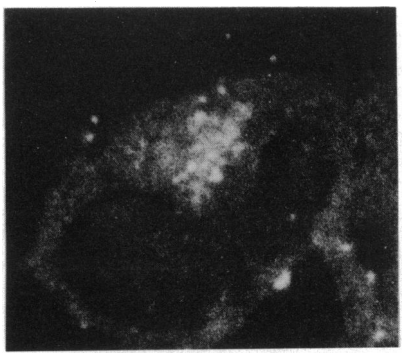

FIG. 5. Immunofluorescence localization of G_{i3} to the Golgi region. MEL cells were fixed, processed as semithin cryosections, and incubated with EC antibody followed by fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. Labeling of α_{i3} is restricted to the Golgi region in these cells. (×1450.)

25% to 81% for three different IgG preparations. This degree of inhibition compares favorably with published data on inhibitory antibodies in other experimental systems (23–25).

DISCUSSION

We have described an assay for intracellular transport that follows glycophorin from the site of synthesis and translocation into the ER through multiple compartments of the Golgi apparatus. To our knowledge, a transport assay in permeabilized cells that is based upon O-glycosylation reactions of a native glycoprotein (as opposed to an exogenous viral glycoprotein) has not been reported previously. In principle, the assay may help in analyzing and understanding the O-glycosylation process. We have also demonstrated that reconstitution of transport can be manipulated through introduction of blocking antiserum and, in doing so, have strengthened the mounting evidence that heterotrimeric G proteins are regulators of Golgi traffic.

The site of initiation of O-glycosylation is still an open issue: it has been placed in an early Golgi compartment (26–29), in a smooth-membrane subcompartment of the ER, or in a pre-Golgi compartment (30–32), possibly transitional elements and transporting vesicles located between the ER and Golgi. These apparently conflicting results may reflect

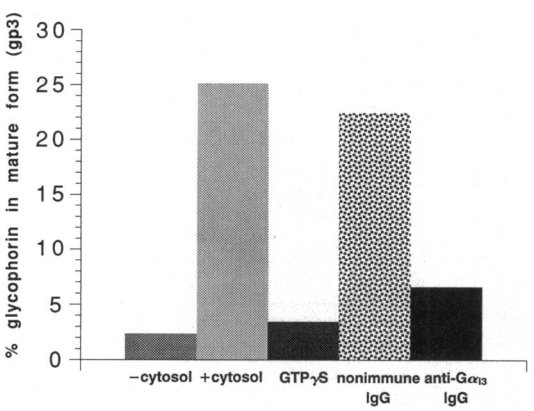

FIG. 6. Antibodies to G_{i3} block glycophorin processing. MEL cells were labeled and permeabilized, and transport of newly synthesized glycophorin was analyzed after immunoprecipitation of glycophorins and SDS/PAGE, as in Fig. 3. When added to permeabilized cells capable of reconstituting ER to Golgi transport, anti-α_{i3} antibodies inhibited Golgi-dependent maturation of glycophorin by up to 70%. Nonimmune IgG did not inhibit processing. Values are average of duplicates in a single experiment; inhibition with anti-α_{i3} antiserum was seen in three additional experiments. Duplicates omitting cytosol or containing GTP[γS] are shown for comparison.

variations in Gal*N*Ac-transferase localization among different cell types (33).

Our results show that in MEL cells O-glycosylation of ectodomain serine/threonine residues is initiated primarily or exclusively after exit from the ER. There are strong arguments for this assumption. First (see Fig. 1), a shift in the molecular weight for gp3 occurs only in transport-competent permeabilized cells. Second, disruption of the Golgi complex with brefeldin A (BFA) for up to 1 hr effectively blocks the glycosylation of glycophorin (8); Ulmer and Palade (8) demonstrated that glycophorin trapped in the ER acquires incomplete O-linked oligosaccharide chains only after 2–6 hr of BFA treatment, presumably due to migration of sufficient Golgi glycosyltransferases to the ER. Third, the binding of *Helix pomatia* lectin-gold to terminal Gal*N*Ac residues in MEL cells was found to be restricted to a few cisternae on the cis side of the Golgi complex (32).

Elongation of O-linked glycans is generally assumed to occur in middle and trans Golgi cisternae. Elhammer and Kornfeld (34) proposed that galactose addition occurred in a separate and subsequent compartment since *N*-acetylgalactosaminyl- and galactosyltransferases could be separated by subcellular fractionation on sucrose gradients. In addition, terminal Galβ1,3Gal*N*Ac residues have been detected by peanut agglutinin lectin in middle and trans cisternae (32, 35). Sialylation of *N*-linked glycans has been placed in the trans Golgi or the trans Golgi network (36) but sialylation of O-glycans may occur in trans cisternae or in earlier cisternae, depending upon the cell type (37). In our assay, all inhibitors tested block gp3 processing in the earliest precursor forms that are typical of newly synthesized ER forms. In the presence of cytosol and ATP, glycophorin shifts to the distinct gp3 band that corresponds to mature, sialylated glycoprotein. It follows that in our reconstituted system gp3 is apparently fully processed to its mature form, which is expected to be completed in the middle/trans Golgi cisternae.

Similar to the human glycophorin A, which has 15 O-glycosidically linked chains (38), murine gp3 is heavily glycosylated by a large number of tri- and tetrameric glycans (4) and detection of intermediary forms would be expected, especially when transport and maturation are slowed down as in our assay. Several minor forms are occasionally detected; they could represent partially glycosylated, intermediate forms of gp3 (not shown).

We assume that this assay system may also help in analyzing the role of factors such as low molecular weight G proteins recently shown to operate at different relays in the intracellular transport pathways (39, 40). The hypothesis that additional G proteins are involved in Golgi traffic is based on well-documented data (17, 18) indicating that GTP[γS] and AlF_4^- block protein transport at the ER–Golgi junction as well as between Golgi stacks. AlF_4^- is an activator of heterotrimeric G proteins (41) but not (according to a recent study) of small G proteins (42). We added AlF_4^- to intact MEL cells immediately after a brief pulse of metabolic labeling and found that newly synthesized glycophorin failed to become O-glycosylated in the Golgi, indicating a complete block in ER-to-Golgi transport (data not shown). AlF_4^- was shown to promote the association of the vesicular coat protein β-COP with Golgi membranes (43), and preincubation with AlF_4^- prevented BFA-induced redistribution of β-COP into the cytosol (44). In addition, excess G protein $\beta\gamma$ subunits inhibited GTP[γS]-induced association of adenosine diphosphate ribosylation factor (ARF) and β-COP with membranes (44). It was also reported that mastoparan (a peptide that activates heterotrimeric G proteins) plus GTP could block the BFA-induced disassociation of β-COP from Golgi membranes (45). Mastoparan or $\beta\gamma$ subunits have also been shown to inhibit endosome fusion (46) and exit of newly synthesized protein from the ER (47). More directly, α_{i3} has been local-

ized to Golgi membranes by immunocytochemistry, and overexpression of α_{i3} was shown to slow the rate of heparan sulfate proteoglycan secretion in LLC-PK1 cells concomitant with the accumulation of partially processed core protein in the middle/trans cisternae of the Golgi (19).

We have localized α_{i3} by immunofluorescence to Golgi regions in MEL cells and a few other (but not all) cell lines tested and found that in SLO-permeabilized MEL cells, affinity-purified antibodies to the KNNLKECGLY sequence from α_{i3} inhibit maturation of gp3. The use of anti-carboxyl-terminal antibodies for the specific inhibition of G proteins has been well established (20–24) and is attributed to their ability to block interaction between activated receptors and the respective G protein (48) or, alternatively, to block G-protein interaction with GTP or its analogs (21). If G_{i3} is involved in a mechanochemical sense, to promote assembly/disassembly of vesicle-associated protein complexes (43–45) or to facilitate vesicle fusion, competition for binding to a resident Golgi protein is a probable explanation for the inhibitory effects of the blocking antibodies. The mechanism of action is still speculative, however, and we must at least consider the possibility that G_{i3} could regulate other crucial functions, such as the maintenance of ionic conditions in Golgi cisternae that are supportive of either glycosylation reactions or protein sorting, either of which would conceivably prevent glycoprotein maturation. Moreover, since we have observed a few cell types in which G_{i3} could not be detected by immunofluorescence on the Golgi apparatus (unpublished observation), its function there is likely to be cell-type specific.

The results obtained so far are in keeping with current findings that implicate multiple G proteins, including heterotrimeric G proteins, in intracellular traffic. Although interesting and challenging, the data require further work to (*i*) consolidate the findings, (*ii*) rationalize the apparent differences in effects obtained in different cell systems, (*iii*) identify the full complement of GTP-dependent events, (*iv*) identify the receptor-like proteins in Golgi membranes, and (*v*) explore the possibility that proteins like G_{i3} control intra-Golgi environmental conditions rather than vesicular transport through the Golgi complex.

We thank Drs. Allen Spiegel and Teresa Jones (National Institutes of Health) for providing anti-α_{i3} (EC) antisera and valuable discussion. Access to the Phosphor Imager was kindly provided by Charles Zuker (University of California, San Diego). Technical assistance of Michael McCaffery, Pauline Liang, and Yves Terrazas was greatly appreciated. Ca^{2+}/EGTA bufr program was courtesy of Bruce Edwards (LoveLace Medical Foundation) and fluorimeter was courtesy of Roger Tsien (University of California, San Diego). This work was supported by National Institutes of Health Grants CA46128 (to M.G.F. and G.E.P.) and DK17780 (to M.G.F.). B.S.W. was supported by postdoctoral fellowships from Cancer Research Coordinating Committee, University of California, and American Cancer Society (California Division).

1. Balch, W. E. (1989) *J. Biol. Chem.* **264,** 16965–16968.
2. Rothman, J. E. & Orci, L. (1992) *Nature (London)* **355,** 409–415.
3. Ulmer, J. B., Dolci, E. D. & Palade, G. E. (1989) *J. Cell Sci.* **92,** 163–171.
4. Krotkiewski, H. & Lisowska, E. (1988) *Carbohydr. Res.* **184,** 27–38.
5. Anderson, R. A. & Lovrien, R. E. (1984) *Nature (London)* **307,** 655–658.
6. Dolci, E. D. & Palade, G. E. (1985) *J. Biol. Chem.* **260,** 10728–10735.
7. Ulmer, J. B. & Palade, G. E. (1989) *J. Biol. Chem.* **264,** 1084–1091.
8. Ulmer, J. B. & Palade, G. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6992–6996.
9. Gravotta, D., Adesnik, M. & Sabatini, D. (1990) *J. Cell Biol.* **111,** 2893–2908.
10. Ercolani, L., Stow, J. L., Boyle, J. F., Holtzman, E. J., Lin, H., Grove, J. R. & Ausiello, D. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 4635–4639.
11. Tsien, R. Y., Pozzan, T. & Rink, R. J. (1982) *J. Cell Biol.* **94,** 325–334.
12. Balch, W. E. & Rothman, J. E. (1985) *Arch. Biochem. Biophys.* **240,** 413–425.
13. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
14. Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C. G. & Spiegel, A. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7809–7813.
15. Beckers, C. J. M., Keller, D. S. & Balch, W. E. (1987) *Cell* **50,** 523–534.
16. Saraste, J., Palade, G. E. & Farquhar, M. G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6425–6429.
17. Melançon, P., Glick, B. S., Malhotra, V., Weidman, P. J., Serafini, T., Gleason, M. L., Orci, L. & Rothman, J. E. (1987) *Cell* **51,** 1053–1062.
18. Beckers, C. J. M. & Balch, W. E. (1989) *J. Cell Biol.* **108,** 1245–1256.
19. Stow, J. L., de Almeida, J. B., Narula, E. J., Holtzman, E. J., Ercolani, L. & Ausiello, D. A. (1991) *J. Cell Biol.* **114,** 1113–1124.
20. McKenzie, F. R., Kelly, E. C. H., Unson, C. G., Spiegel, A. M. & Milligan, G. (1988) *Biochem. J.* **249,** 653–659.
21. Nair, B. G., Parikh, B., Milligan, G. & Patel, T. B. (1990) *J. Biol. Chem.* **265,** 21317–21322.
22. LaMorte, V. J., Goldsmith, P. K., Spiegel, A. M., Meinkoth, J. L. & Feramisco, J. R. (1992) *J. Biol. Chem.* **267,** 691–694.
23. Murray-Whelan, R. & Schlegal, W. (1992) *J. Biol. Chem.* **266,** 2960–2965.
24. Gutowski, S., Smrcka, A., Nowak, L., Wu, D., Simon, M. & Sternweis, P. C. (1991) *J. Biol. Chem.* **266,** 20519–20524.
25. Plutner, H., Cox, A. D., Pind, S., Khosravi-Far, R., Bourne, J. R., Schwaninger, R., Channing, J. D. & Balch, W. E. (1991) *J. Cell Biol.* **115,** 31–43.
26. Johnson, D. C. & Spear, P. G. (1983) *Cell* **32,** 987–997.
27. Roth, J. (1984) *J. Cell Biol.* **98,** 399–406.
28. Cummings, R. D., Kornfeld, S., Schneider, W. J., Hobgood, K. K., Tolleshaug, H., Brown, M. S. & Goldstein, J. L. (1983) *J. Biol. Chem.* **258,** 15261–15273.
29. Abeijon, C. & Hirschberg, C. B. (1987) *J. Biol. Chem.* **262,** 4153–4159.
30. Tooze, S. A., Tooze, J. & Warren, G. (1988) *J. Cell Biol.* **106,** 1475–1487.
31. Pathak, R. K., Merkle, R. K., Cummings, R. D., Goldstein, J. L., Brown, M. S. & Anderson, R. G. W. (1988) *J. Cell Biol.* **106,** 1831–1841.
32. Perez-Vilar, J., Hidalgo, J. & Velsaco, V. (1991) *J. Biol. Chem.* **266,** 23967–23976.
33. Carraway, K. L. & Hull, S. (1989) *BioEssays* **10,** 117–120.
34. Elhammer, A. & Kornfeld, S. (1984) *J. Cell Biol.* **98,** 327–331.
35. Lotan, R., Skutelsky, E., Danon, D. & Sharon, N. (1975) *J. Biol. Chem.* **250,** 8518–8523.
36. Roth, J., Taatjes, D. J., Weinstein, J., Paulson, J. C., Greenwell, P. & Watkins, W. M. (1986) *J. Biol. Chem.* **261,** 14307–14312.
37. Locker, J., Griffiths, G., Horzinek, M. C. & Rottier, P. J. M. (1992) *J. Biol. Chem.* **267,** 14094–14101.
38. Yoshima, H., Furthmayr, H. & Kobata, A. (1980) *J. Biol. Chem.* **255,** 9713–9718.
39. Bourne, H. R. (1988) *Cell* **53,** 669–671.
40. Balch, W. E. (1990) *Trends Biochem. Sci.* **15,** 473–477.
41. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56,** 615–649.
42. Kahn, R. A., Kern, F. G., Clark, J., Gelmann, E. P. & Rulka, C. (1991) *J. Biol. Chem.* **266,** 2606–2614.
43. Donaldson, J. G., Lippincott-Schwartz, J. & Klausner, R. D. (1991) *J. Cell Biol.* **112,** 579–588.
44. Donaldson, J. G., Kahn, R. A., Lippincott-Schwartz, J. & Klausner, R. D. (1991) *Science* **254,** 1197–1199.
45. Ktistakis, N. T., Linder, M. E. & Roth, M. G. (1992) *Nature (London)* **356,** 344–346.
46. Colombo, M. I., Mayorga, L. S., Casey, P. J. & Stahl, P. D. (1992) *Science* **255,** 1695–1697.
47. Schwaninger, R., Plutner, H., Bokoch, G. M. & Balch, W. E. (1992) *J. Cell Biol.* **119,** 1077–1096.
48. Hamm, H. E., Deretic, D., Hofmann, K. P., Schleicher, A. & Kohl, B. (1987) *J. Biol. Chem.* **1987,** 10831–10838.