Intra-Golgi Protein Transport Depends on a Cholesterol Balance in the Lipid Membrane*

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Ernstpeter Stüven‡§, Amir Porat§%, Frida Shimron§, Ephraim Fass§, Dora Kaloyanovai, Britta Brügger‡, Felix T. Wieland‡, Zvulun Elazar§, and J. Bernd Helms**

From the ‡Department of Biochemistry and Cell Biology, University of Utrecht, P. O. Box 80176, 3508 TD Utrecht, The Netherlands
§ Biochemie-Zentrum Heidelberg, University of Heidelberg, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany, the ¶Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel, and the ¶¶Department of Biochemistry and Cell Biology, University of Utrecht, P. O. Box 80176, 3508 TD Utrecht, The Netherlands

Transport of proteins between intracellular membrane compartments is mediated by a protein machinery that regulates the budding and fusion processes of individual transport steps. Although the core proteins of both processes are defined at great detail, much less is known about the involvement of lipids. Here we report that changing the cellular balance of cholesterol resulted in changes of the morphology of the Golgi apparatus, accompanied by an inhibition of protein transport. By using a well characterized cell-free intra-Golgi transport assay, these observations were further investigated, and it was found that the transport reaction is sensitive to small changes in the cholesterol content of Golgi membranes. Addition as well as removal of cholesterol (10 ± 6%) to Golgi membranes by use of methyl-β-cyclodextrin specifically inhibited the intra-Golgi transport assay. Transport inhibition occurred at the fusion step. Modulation of the cholesterol content changed the lipid raft partitioning of phosphatidylcholine and heterotrimeric G proteins, but not of other (non) lipid raft proteins and lipids. We suggest that the cholesterol balance in Golgi membranes plays an essential role in intra-Golgi protein transport and needs to be carefully regulated to maintain the structural and functional organization of the Golgi apparatus.

Cholesterol is an essential lipid constituent in the membranes of mammalian cells and has a profound effect on the physical properties of these membranes. By interacting with the acyl-chain of other (phospho)lipids, cholesterol increases lipid packing in the membranes, thereby causing thickening of the membrane. Increased packing of the membrane affects the fluidity of the membrane and makes it less permeable for the transbilayer passage of small water-soluble molecules like glucose (1–3). Total cellular cholesterol levels are determined by de novo synthesis in the endoplasmic reticulum, by the uptake of extracellular cholesterol from, e.g. lipoproteins, and by esterification and subsequent release of cholesterol to the outside circulation or storage in lipid droplets (4). Regulation of these processes keeps total free cholesterol levels in cells precisely controlled. A crucial step in cholesterol homeostasis is action of a cholesterol-sensing machinery at the endoplasmic reticulum (5). Within the cell, the cholesterol distribution between membranes is also actively regulated, resulting in a concentration gradient along the secretory pathway, with low concentrations of cholesterol in the ER1 and high concentrations in the plasma membrane (6, 7). The Golgi apparatus contains intermediate levels of cholesterol (7), and there are indications of a cholesterol gradient even within the Golgi complex (8–11). Also along the endocytic route a heterogeneous cholesterol distribution has been observed (12).

The tight regulation of cholesterol homeostasis and subcellular distribution suggests a critical role of this lipid in biological processes. Due to the cholesterol gradient along the secretory pathway and its implication on lipid bilayer thickness, cholesterol has been postulated to be involved in the targeting of Golgi resident proteins to this organelle (3).

Cholesterol is also involved in the stabilization and function of lipid-enriched microdomains (lipid rafts) within a membrane. The scaffold of these microdomains is built by sphingolipids and cholesterol (13–16). Cholesterol tightly interacts with sphingolipids that contain predominantly long-chain saturated fatty acids. This results in the segregation of these lipids from other membrane lipids into microdomains. Due to the physical properties of these microdomains, distinct classes of membrane proteins are incorporated, causing their segregation as well (13).

Recent advances suggest that cholesterol exerts many of its actions mainly by maintaining sphingolipid rafts in a functional state (16). Microdomains were postulated to exist at the trans-Golgi network, to explain the observed sorting of sphingolipids to the apical surface in polarized cells (17, 18). Functionally, sorting is explained by the transport of microdomain-associated proteins such as glycosylphosphatidylinositol-anchored proteins and influenza virus hemagglutinin to the apical surface (19–22). Likewise, microdomains have been implicated in sorting processes along the endocytic pathway (23, 24). At the plasma membrane, the most important role of microdomains may be their function in
signal transduction (14–16, 25, 26). Activation of signaling cascades can cause raft clustering into patches as well as change the partitioning of signaling molecules in lipid rafts. For example, in T-cell antigen receptor signaling, clustering of rafts is an essential feature in the formation of an immunological synapse between T-cells and antigen-presenting cells (27–29).

Here we report that small changes in cholesterol content of Golgi membranes specifically inhibit the fusion reaction of intra-Golgi protein transport. Thus, cholesterol levels at the Golgi complex must be precisely balanced to allow protein transport to occur. We considered the possibility that a change in cholesterol levels might affect the function of microdomains at the Golgi complex. Recently we obtained evidence for the existence of such microdomains at the early Golgi complex (30). We now show that changing the cholesterol content of membranes specifically affects the phase partitioning of heterotrimeric G proteins, suggesting a possible involvement of microdomains in the regulation of protein transport through the Golgi apparatus.

EXPERIMENTAL PROCEDURES

Materials

Methyl-β-cyclodextrin (average degree of substitution of 10.5–14.7), α-cyclodextrin, and brefeldin A were from Sigma. Stock solutions of cyclodextrins were freshly prepared at 50 mM in water. [3,4-3H]Cholesterol was kindly provided by Dr. Wolfgang D. Lehmann (German Cancer Research Center, Germany). 2,2,3,4,4,6-D6-cholesterol was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Cholesterol was from Merck (Darmstadt, Germany). Triton X-100 (purchased as a 10% solution) and GTP-S were from Roche Diagnostics GmbH (Mannheim, Germany).

Methods

Intra-Golgi Transport Assay—The standard intra-Golgi transport was performed as described previously (31). A standard intra-Golgi transport assay (25 μl) contained 2.5 mM Hepes buffer, pH 7.0, 15 mM KCl, 2.5 mM MgAcO4, 0.4 μCi of UDP-N-[3H]acetylglucosamine, 5 μl of 1:1 mixture of donor and acceptor Golgi membranes (2-3 μg of protein), 10 μM palmitoyl-coenzyme A, ATP- and UTP-regenerating systems, and 16.5–17.5 μg of rat or 16 μg of bovine brain cytosol. The transport reactions were incubated for 2 h at 30 °C. The incorporation of N-3H]acetylglucosamine into the VSV-G protein was determined as described previously (32).

Glycosylation Assay—This assay is based on a method described by Taylor et al. (33). Prior to isolation of Golgi membranes, CHO wild type cells were infected with the vesicular stomatitis virus. Isolated membranes were treated with 1 mM M6 of H2O and heated to 80 °C. To this solution, 150 μl of 10 mg/ml calf thymus phosphatidylcholine, sphingomyelin, and glucosylceramide analysis by ESI-MS/MS by scanning in the negative mode for the parent ions of [3,4-3H]acetylglucosamine transferase (39, 40). In control cells, the transGFP-tagged temperature-sensitive mutant of VSV-G (ts-G-GFP) (38).

RESULTS

Cholesterol Affects Transport within the Golgi Complex—Cholesterol (Chol) can be incorporated into cellular membranes by the addition methyl-β-cyclodextrin-cholesterol complexes (MβCD-Chol) to intact cells. To study the effect of cholesterol on the secretory pathway, CHO cells were incubated with MβCD-Chol. Such treatment resulted in a significant change in the subcellular distribution of the Golgi complex. In these experiments, the Golgi apparatus was visualized by using several Golgi markers, including p115, GM130, and GTP-tagged N-acetylglucosamine transferase (39, 40). In control cells, the Golgi complex exhibits a typical perinuclear localization (Fig. 1). However, upon loading of the cells with cholesterol the Golgi complex dissociated into small punctuated structures throughout the cells (Fig. 1A). When cells were depleted of cholesterol by incubation with MβCD (confirmed by determination of total cellular cholesterol levels, data not shown), the Golgi localization did not change as significantly, but a more condensed perinuclear localization was consistently observed.

The morphology of the Golgi apparatus is dynamically regulated by membrane flow processes at the cis- (incoming) and trans-site (outgoing) of the complex. This is dramatically visualized by the addition of Brefeldin A to intact cells. Brefeldin A inhibits anterograde transport to the Golgi complex and causes a complete disruption of the perinuclear Golgi structure (41).
Concomitant with changes in cholesterol content and Golgi morphology, protein transport through was the Golgi apparatus was also inhibited. As shown in Fig. 1B, loading of cells with cholesterol resulted in a strong inhibition of transport of GTP-tagged VSV-G protein (a secretary protein), as illustrated by its accumulation at the Golgi complex (Fig. 1B, middle panel) and sensitivity to Endo H (Fig. 1C). Under normal conditions, this protein accumulates at the plasma membrane (Fig. 1B, left panel) and is processed normally in the Golgi apparatus as shown by its resistance to Endo H (Fig. 1C). We could not observe inhibition intra-Golgi protein transport by extraction of cholesterol using MβCD alone. At high concentrations of MβCD, it becomes toxic to the cells resulting in cell death (data not shown). To determine the effect of cholesterol extraction on intra-Golgi protein transport, cells were pre-treated with 1 h with lovastatin, an inhibitor of cholesterol synthesis (42). After treatment of the cells with lovastatin and MβCD, protein transport through the Golgi complex was inhibited (Fig. 1, B [right panel] and C). Upon prolonged incubation with Lovastatin (24 h), addition of MβCD was not required to inhibit protein transport at the Golgi complex, further indicating the specificity of cholesterol extraction with MβCD (data not shown).

To study an effect of cholesterol on transport processes related to the Golgi complex in more detail, we made use of a well characterized cell-free system that reconstitutes intra-Golgi protein transport (31). As shown in Fig. 2A, addition of increasing amounts of MβCD-Chol complexes to the transport reaction causes a strong inhibition of intra-Golgi protein transport, with half-maximal inhibition at 0.17 mM MβCD-Chol.

To exclude the possibility that the inhibition of the assay signal by MβCD-Chol resulted from decreased glycosylation activity of GlcNAc transferase rather than from inhibition of transport, we used a glycosylation assay that determines GlcNAc activity under conditions that block fusion (32). MβCD-Chol, however, did not inhibit the glycosylation of VSV-G protein under these assay conditions (Fig. 2B, indicating that MβCD-Chol specifically inhibits the transport process. Another criterion for specificity of inhibition is the existence of transport intermediates, resistant to the inhibitor. In the experiment described in Fig. 2C, the transport reaction was either terminated by placing the reaction on ice, or at these time points the inhibitor MβCD-Chol was added and the reaction was allowed to proceed for 2 h (see, e.g. Refs. 32, 43 for similar kinetic determinations). Control samples treated with buffer only were incubated likewise at 30 °C until the end of the 2-h incubation period and served to represent the maximal transport signal. When MβCD-Chol was added 20–30 min after initiation of the reaction, a significant portion of the transport reaction became resistant to MβCD-Chol. Furthermore, 80 min (60–80 min in different experiments) after the beginning of the reaction, the full assay signal was obtained. Taken together, these data indicate that MβCD-Chol acts within the time frame of the transport process and that MβCD-Chol inhibits the transport reaction in a specific manner.

**Cholesterol Distribution in Golgi Membranes**—The loading of Golgi membranes with cholesterol by the MβCD–CD complex was quantitated by nano-electrospray ionization tandem mass spectrometry (ESI-MS/MS) as described by Sandhoff *et al.* (35). As shown in Fig. 3, isolated CHO Golgi membranes (0.6 μg of
protein) contain 320 pmol of cholesterol, in agreement with previously reported values (35). Pre-loaded MβCD dramatically changes the cholesterol content of Golgi membranes. As shown in Fig. 3, with increasing concentrations of pre-loaded MβCD, we found an increased association of cholesterol with Golgi membranes, up to a 4.5-fold increase (at 2 mM MβCD) over the endogenous pool of cholesterol.

Cholesterol is a major lipid in CHO Golgi membranes (0.57 mol of cholesterol/mol of phospholipid) (36) and has been implicated in the formation of microdomains (13–16). Golgi-derived microdomains can be isolated as detergent-resistant membranes, enriched in sphingolipids and cholesterol (30). To determine the amount of cholesterol that partitions in microdomains, Triton X-100 was added to solubilize Golgi membranes. After centrifugation, a detergent-soluble (soluble) and detergent-insoluble (DRM) fraction was obtained and their cholesterol contents quantified. Under these conditions, 62 ± 7% of the cholesterol partitions in DRMs (Fig. 3). This partitioning changes significantly in the presence of exogenously incorporated cholesterol. At 2 mM MβCD-Chol, 89% of the cholesterol is present in the detergent-insoluble phase (resulting in a 5.4-fold increase of this pool). 11% of the cholesterol is present in the detergent-soluble phase (resulting in a 2.5-fold increase of this pool). These results show that most of the exogenous cholesterol is incorporated in the detergent-insoluble phase.

The capacity of Golgi membranes to absorb severalfold its endogenous cholesterol content suggests that under these conditions the general membrane fluidity might also be affected.
A Cholesterol Balance in Golgi Membranes

We therefore determined the partitioning of other known microdomain-enriched lipids such as sphingomyelin (SM) and glucosylceramide (GlcCer) and of a bulk lipid (phosphatidylcholine (PC)) upon addition of exogenous cholesterol. In Golgi membranes, 85% of SM and GlcCer were present in DRMs. In contrast, most of the PC was detergent-soluble and only 28% partitioned to the detergent-insoluble phase (Table I). When Golgi membranes were loaded with cholesterol prior to detergent solubilization, the detergent insolubility of SM and GlcCer did not change significantly. Surprisingly, the pool of PC in the detergent-insoluble fraction was increased 1.5-fold to 42%. Because PC is a major membrane lipid (representing 37% of all phospholipids in Golgi membranes (36)), these data indicate a significant increase of total Golgi surface area of microdomains.

MβCD has also been used to selectively and rapidly extract cholesterol from membranes (46–48). We determined the efficiency of cholesterol extraction by MβCD from Golgi membranes by ESI-MS/MS (35). As shown in Fig. 4A, increasing amounts of cholesterol were extracted from isolated Golgi membranes with increasing concentrations of MβCD. The extraction was specific for MβCD, because αCD did not extract cholesterol from the membranes, in accordance with the much higher affinity of MβCD for cholesterol as compared with αCD (46). It was determined whether under these conditions cholesterol is extracted from the fluid- or liquid-ordered phase in the membrane. To this end, increasing amounts of cholesterol were extracted from Golgi membranes with MβCD, and subsequently the partitioning of the remaining membrane-bound cholesterol between the detergent-soluble and detergent-insoluble membranes was measured. As shown in Fig. 4B, both pools of cholesterol are similarly affected by MβCD. Because the detergent-insoluble pool represents the larger pool of cholesterol (62%, see above), most of the extracted cholesterol is derived from this pool. Comparison of Fig. 4A and Fig. 4B shows that at low concentrations of MβCD (e.g. 1 mM MβCD), the efficiency of cholesterol extraction from Golgi membranes varied to a significant extent (between 45% (Fig. 4A) and 10% (Fig. 4B) at 1 mM MβCD). For unknown reasons, these variations were observed between different batches of isolated Golgi membranes (data not shown).

Removal of Cholesterol from Golgi Membranes Blocks Late Stages of Intra-Golgi Transport—Because MβCD and pre-

**Table I**

| Lipid    | PC nmol/μg | SM nmol/μg | GlcCer nmol/μg |
|----------|------------|------------|---------------|
| Golgi    | 313 ± 27   | 170 ± 16   | 4.7 ± 0.5     |
| Golgi + Chol | 380 ± 45 | 173 ± 25   | 5.7 ± 0.8     |
| Insol. complex | 88 ± 16 | 144 ± 21   | 4.0 ± 0.2     |
| Insol. complex + Chol | 158 ± 36 | 145 ± 24   | 4.3 ± 0.1     |

**Fig. 3.** Incorporation of cholesterol in Golgi membranes affects the solubility of membranous cholesterol in Triton X-100.

Golgi membranes were incubated with increasing concentrations of MβCD-Chol for 2 h at 30 °C (the concentration mentioned for MβCD-cholesterol complexes is based on the MβCD concentration). After centrifugation, the membrane pellet was resuspended in PEN buffer, and detergent-soluble and -insoluble phases were prepared as described under “Experimental Procedures.” Total cholesterol (Total, black bars), cholesterol in the detergent-insoluble phase (DRM, gray bars), and detergent-soluble cholesterol (Soluble, white bars) were determined by ESI-MS/MS after sulfatation, as described under “Experimental Procedures.” Values have been corrected for precipitation of cholesterol in the absence of membranes (under all conditions less than 5% of the data shown).

**Fig. 4.** Extraction of cholesterol from Golgi membranes by cyclodextrins. A, Golgi membranes were incubated with increasing amounts of methyl-β-cyclodextrin (closed circles) or α-cyclodextrin (open circles) for 2 h at 30 °C. After 100,000 × g centrifugation, the membranes were resuspended in 1,4-dioxane. The pellets were resuspended in 1,4-dioxane, and, after sulfatation, the cholesterol content was determined by ESI-MS/MS. B, Golgi membranes were incubated with increasing concentrations of methyl-β-cyclodextrin for 2 h at 30 °C. After centrifugation, the membrane pellet was resuspended in PEN buffer, and detergent-soluble and -insoluble phases were prepared as described under “Experimental Procedures.” For cholesterol determination, the membrane pellet (Total, squares), the detergent-insoluble material (DRM, circles), and the lyophilized material of the supernatant (Soluble, triangles) were resuspended in 1,4-dioxane, and after sulfatation the cholesterol content was determined by ESI-MS/MS.
Methyl-β-cyclodextrin have opposite effects on the cholesterol content of membranes and have differential effects on the phase partitioning of cholesterol, we tested the effect of MβCD on the cell-free intra-Golgi transport assay. As shown in Fig. 5, MβCD also efficiently inhibited the transport assay with half-maximal inhibition at 0.3 mM and 80–90% inhibition at 1–2 mM MβCD, respectively. The inhibition was specific for methyl-β-cyclodextrin, because α-cyclodextrin inhibited the transport assay only at much higher concentrations (Fig. 5A, IC50 = 2.2 mM). With this batch of Golgi membranes, 10 ± 6% cholesterol was extracted from the membranes at 1 mM MβCD (data not shown). MβCD did not artificially inhibit the glycosylation of VSV-G protein under the assay conditions (Fig. 5B). Other cholesterol-binding agents such as filipin also affected the transport assay. At a concentration of 40 μg/ml, filipin III inhibited transport by 90% (data not shown).

The transport reaction becomes fully resistant to the inhibition by MβCD when added 60 min after onset of the incubation (Fig. 6A). Resistance to MβCD inhibition occurred after the GTPγS inhibition, which acts on the budding step, indicating that MβCD inhibits a later stage in the transport process, possibly the fusion process. This is supported by experiments with the cell-free intra-Golgi transport assay in the presence of brefeldin A. Under these conditions, an assay signal is obtained in the absence of COPI vesicles, and therefore monitors predominantly the docking and fusion processes. The fusion machinery operating under these conditions is the same as the machinery involved in the fusion of COPI-coated vesicles (49, 50). As shown in Fig. 6B, MβCD (and MβCD-Chol, data not shown) inhibits the cell-free assay with the same efficiency in the absence and presence of brefeldin A, suggesting that MβCD and MβCD-Chol both act at the same transport step, i.e. the fusion machinery of intra-Golgi transport. In accordance with this, we find that MβCD has no significant effect on vesicle formation in the cell-free intra-Golgi transport system as determined by the accumulation of COPI-coated vesicles in the presence of GTPγS (data not shown).

If MβCD inhibits the transport assay by extraction of cholesterol from membranes, then preincubation of the membranes with MβCD and subsequent incubation of these membranes in the transport reaction in the absence of MβCD should also inhibit the transport assay. As shown in Fig. 7A, preincubation of the membranes with MβCD inhibited the transport assay with the same efficiency. In contrast, preincubation of cytosol did not affect the transport reaction (Fig. 7B). To test directly whether MβCD acts by removing cholesterol from the Golgi membranes, we performed similar two-stage assays in which the Golgi membranes were preincubated under various conditions. When Golgi membranes were preincubated with either MβCD-Chol or MβCD, the transport reaction was effi-
the detergent-insoluble and detergent-soluble phases. The partitioning of a non-raft transmembrane protein of the Golgi complex, p23, was also not affected by these treatments and remained in the detergent-soluble fraction. Typical Golgi raft proteins such as Flotillin-1 and GAPR-1 (30, 56) localized predominantly to the detergent-insoluble fraction and were also not affected by the relatively small cholesterol variations in the membrane. However, affected at higher cyclodextrin concentrations, see e.g. Ref. 30. However, the phase partitioning of heterotrimeric G proteins did change upon cholesterol addition or depletion. Upon cholesterol depletion both the Gaα and the Gβ subunits favored the detergent-soluble phase (Fig. 8). In contrast, both G protein subunits relocalized predominantly to the detergent-insoluble phase upon cholesterol addition. Because localization of proteins to microdomains can affect their activity, these data indicate that alterations in the cholesterol content of membranes might affect the function of heterotrimeric G proteins. These data support previous reports on the involvement of heterotrimeric G proteins in the fusion reaction of COPI vesicles with Golgi membranes (45).

**DISCUSSION**

**Maintenance of a Cholesterol Balance**—Here we have shown that cholesterol levels in Golgi membranes play a crucial role in intra-Golgi protein transport. Because both cholesterol extraction from Golgi membranes and cholesterol insertion into Golgi membranes inhibits the transport assay, we suggest that a critical cholesterol concentration needs to be maintained to allow protein transport to occur. It is not known how cholesterol is transported to and from the Golgi complex, nor is it known how maintenance of a balanced concentration is regulated. There is convincing evidence that differences between cholesterol contents of organelles are maintained dynamically, because cholesterol can be rapidly transferred between membranes (4). Vesicular transport processes do not seem to play a major role in intracellular cholesterol transport (57–59). This is in agreement with the recent observation that cholesterol segregates from COP1-coated vesicles during budding from Golgi membranes (36). Current candidates for mediating intracellular cholesterol transport include caveolin (26), possibly in a complex with other proteins (60).

Several other intracellular transport pathways are affected by increased or decreased cholesterol levels. These pathways include clathrin-dependent endocytosis, caveolae endocytosis, biogenesis of synaptic-like microvesicles, plasma membrane to Golgi apparatus, trans-Golgi network to apical surface, endosome to Golgi transport, recycling endosome-mediated transport, and yeast vacuole fusion (reviewed in Refs. 61–63). Most of these transport systems are inhibited by modulation of the cholesterol content of the membranes. A remarkable exception is the stimulation of vacuolar fusion by addition of ergosterol, but these membranes have a low ergosterol to phospholipid ratio. Finally, ER to Golgi and the trans-Golgi network to basolateral plasma membrane transport pathways are not affected by changes in the cholesterol content of membranes (20, 64, 65). It is not clear why cholesterol might have such a differential effect on various transport events, but there seems to be a tendency that membranes with a high cholesterol content are more sensitive to changes in cholesterol concentrations. This again suggests that the cholesterol content of various membranes in the cells must be carefully balanced to allow intracellular transport to occur.

**Inhibition of the Fusion Machinery**—Extraction from membranes of cholesterol with MβCD results in a specific inhibition of intra-Golgi transport. Kinetic analysis of the inhibition and the experiments with brefeldin A suggest that MβCD inhibits...
the fusion machinery. Various mechanisms for the observed inhibition can be postulated: (i) Cholesterol is required for the catalytic activity of proteins active in the fusion machinery. So far, however, this has not been reported for any of the fusion proteins involved, including NSF, SNAPs, SNAREs and Rab proteins (for reviews see Refs. 66 and 67). (ii) Cholesterol affects the fusion machinery by affecting membrane fluidity. A general effect of cholesterol on membrane fluidity seems un-

**Fig. 7.** MβCD inhibits intra-Golgi protein transport by extraction of cholesterol. A, Golgi membranes (2.4 μg) were preincubated (lanes 4 and 5) in the presence of buffer (lane 4) or 3 mM MβCD for 10 min at RT. The membranes were then isolated by centrifugation, washed, and tested for their transport activity in the presence of fresh cytosol. As a control, Golgi membranes were not preincubated but added directly to the transport reaction and incubated at 0 °C (lane 1), under standard conditions (lane 2), or as lane 2, but in the presence of 2 mM MβCD. B, 6.3 mg/ml bovine brain cytosol in PEN buffer was preincubated with buffer (lane 1) or 1 mM MβCD (lane 3) for 60 min at 30 °C. After removal of MβCD by gel filtration, the pre-treated cytosol was added to the transport reaction under standard conditions. As a control for the efficiency of inhibition, MβCD was added to a standard transport reaction without preincubation (lane 2). C, Golgi membranes were preincubated for 20 min at 30 °C with MβCD-Chol complex (ratio 12:1, mol:mol) (lane 1), MβCD-Chol complex (ratio 48:1, mol:mol) (lane 2) or 3 mM MβCD. Preincubation with buffer served as a control (assay signal of 100%). After incubation the membranes were isolated by centrifugation, washed, and tested for their transport activity in the presence of fresh cytosol. The concentration mentioned for MβCD-cholesterol complexes is based on the MβCD concentration.
likely, because depletion of cholesterol increases the membrane fluidity, and this should, if anything, facilitate membrane deformations that occur during the fusion reaction (13). In addition, reconstitution of SNARE-mediated fusion of liposomes functions in the absence of cholesterol, and thus, removal of cholesterol should not affect the SNARE-mediated fusion step (68). Finally, such a general mechanism should affect other membrane deformations as well, such as budding of transport vesicles. The formation of COPI-coated vesicles in the cell-free system (data not shown) and a reconstituted assay system (69) does, however, not dependent on cholesterol. This is in contrast to the formation of clathrin-coated vesicles along the endocytic pathway and the formation of synaptic vesicles, which are affected by MβCD treatment (70, 71). In the latter case, a specific cholesterol-binding protein has implicated in the budding process. (iii) In the presence of other lipids such as sphingomyelin, cholesterol is involved in stabilization of microdomains. The involvement of microdomains in the regulation of intra-Golgi protein transport will be discussed below.

**Microdomains and Intra-Golgi Protein Transport**—There are several indications for the existence of microdomains at the Golgi complex. Originally, microdomains were postulated to exist at the trans-Golgi network, to explain the observed sorting of sphingolipids in polarized cells to the apical surface (17, 18). Subsequently, it was found that glycosylphosphatidylinositol-anchor proteins become associated with DRMs during Golgi passage (72). Microdomains have also been suggested to occur as a result of the cholesterol and sphingomyelin gradient along the secretory pathway to explain protein sorting at the Golgi complex (3). Recently, it was found that cholesterol and sphingomyelin are segregated from Golgi-derived COPI-coated vesicles, implicating a segregation process that might involve microdomains within the Golgi complex (36). By detergent extraction, Golgi-derived detergent-soluble complexes (GIC mi-
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