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Trimeric G proteins regulate the cytosol-induced redistribution of Golgi enzymes into the endoplasmic reticulum

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SUMMARY

Streptolysin O-permeabilized cells incubated with a high concentration (5-10 mg/ml) of cytosolic proteins and ATP-generating system exhibit redistribution into the endoplasmic reticulum (ER) of Golgi integral proteins (mannosidase II, galactosyltransferase, TGN 38), detected by immunofluorescence. In addition, mannosidase II is detected in the ER of cells exposed to a high concentration of cytosolic proteins and processed for immunolectron microscopy by immunoperoxidase. The redistribution process requires ATP and is not affected by previous microtubule depolymerization. Ultrastructural observations indicate that Golgi disassembly occurs by budding of coated vesicles. This stage of the process is inhibited by GTPγS, $AlF_{(3-5)}$, transducin βγ subunits, and mastoparan, indicating the involvement of trimeric G proteins. At a later stage, vesicles lose their coats and fuse with the ER. This part of the process does not occur in cells incubated at either 15°C or 20°C, or exposed to *N*-ethylmaleimide. In cells treated with either cholera or pertussis toxin Golgi redistribution into the ER shows a 50-fold lower requirement for cytosolic factors than in untreated cells. These data suggest a regulatory role for both α_s and α_i trimeric G proteins in the normal Golgi-ER retrograde transport taking place in intact cells.

Key words: Golgi complex, retrograde transport, G protein

INTRODUCTION

During interphase the cisternal organization of the Golgi complex is maintained by a controlled balance of membrane input and output. The input pathway consists of anterograde endoplasmic reticulum (ER)-Golgi membrane transport. The output depends on both the production of sorting and exocytic vesicles from the *trans*-Golgi network (TGN) and the existence of a Golgi-ER retrograde pathway (Klausner et al., 1992; Lippincott-Schwartz, 1993; Rothman and Orci, 1992). Currently there is great interest in the retrograde pathway because it is assumed to fulfill the role of returning escaped ER resident proteins, which eventually reach the Golgi complex (Lewis and Pelham, 1992; Pelham, 1988) as well as misfolded proteins that are not suitable to proceed along the exocytic pathway (Hammond and Helenius, 1994). On the basis of observations made on cells treated with the fungal metabolite brefeldin A (BFA), the retrograde pathway has been described to occur by the microtubule-dependent extension of tubule processes which emanate from the Golgi cisternae and fuse with the ER (Lippincott-Schwartz et al., 1990). By contrast, anterograde membrane transport is thought to be mediated by carrier vesicles which transport both proteins and lipids from the ER to the *cis*-Golgi and between the different Golgi compartments (Rothman and Orci, 1992; Takizawa and Malhotra, 1993). Conditions that alter the normal balance existing between anterograde and retrograde membrane pathways lead to disruption of the Golgi organization. Thus, treatment with BFA inhibits the ER-Golgi anterograde transport but not the retrograde pathway and consequently the Golgi complex is disassembled and redistributes into the ER (Klausner et al., 1992). These effects are also observed in two other situations: overexpression of a Golgi receptor for ER lumenal proteins (Hsu et al., 1992) and ADP-ribosylation of rho, a small GTP-binding protein (Sugai et al., 1992a,b). In addition, cell mutants have been described that exhibit a BFA-like phenotype (Kao and Draper, 1992; Zuber et al., 1991). Taken together these studies suggest that Golgi-ER retrograde transport is a highly regulated process that influences both Golgi structural maintenance and function.

The mechanism underlying anterograde transfer between membrane compartments of the exocytic pathway has been extensively investigated using both biochemical and genetic approaches (Rothman and Orci, 1992). By contrast, little attention has been given to the factors that regulate retrograde transport. Studies using BFA have indicated that redistribution of Golgi membranes to the ER is inhibited by both the non-hydrolyzable analog of GTP, GTPγS, and the activator of trimeric G proteins, $AlF_{(3-5)}$, suggesting that GTP-binding proteins might be involved (Donaldson et al., 1991b; Tan et al., 1992). However, both GTPγS and $AlF_{(3-5)}$ primarily interfere with the association/disassociation cycle of the coat proteins to Golgi membranes and therefore they also inhibit anterograde transport as well (Donaldson et al., 1991a; Melançon et al., 1987). Part of the difficulty in understanding the basic mechanism of retrograde transport is due to the lim-

itations imposed by the use of BFA, a drug that also induces tubule processes from the TGN, endosomes, and lysosomes (Lippincott-Schwartz et al., 1991; Tooze and Hollinshead, 1992; Wood et al., 1991). Thus there is no direct evidence that Golgi-ER retrograde transport in the absence of BFA is tubule-mediated (Pelham, 1991). Recently tubule formation from Golgi cisternae has been reported to occur in vitro in the absence of BFA and under conditions of low ATP or cytosol supply (Cluett et al., 1993; Weidman et al., 1993). Although these tubule processes have been assimilated to those formed in vivo during retrograde transport their physiological relevance is uncertain. In the present study we show disassembly of the Golgi complex and redistribution into the ER in Streptolysin O (SO)-permeabilized cells incubated with a high concentration (5-10 mg/ml) of cytosolic proteins. This process requires ATP, is independent of tubule formation, and is regulated by trimeric G proteins.

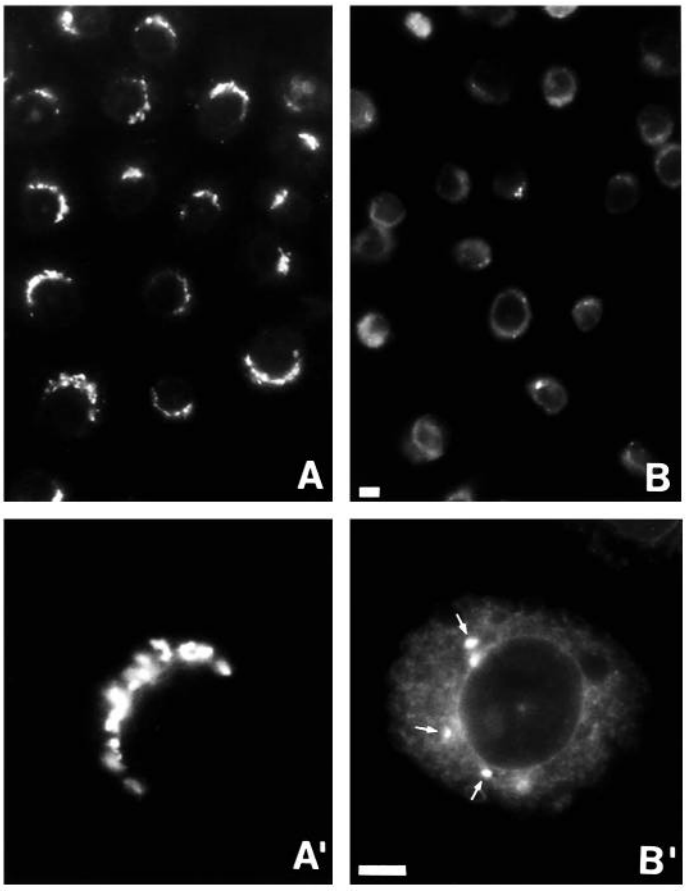

Fig. 1. Redistribution of Golgi α-mannosidase II in SO-permeabilized cells incubated with a high concentration of cytosolic proteins. NRK cells grown on coverslips were SO-permeabilized and incubated for 1 hour at 37°C in transport buffer containing ATP-generating system plus cytosol at either 0.1 mg/ml (A,A′) or 5 mg/ml (B,B′) protein concentration. Cells were fixed and processed for indirect immunofluorescence with anti-mannosidase II antibodies. Arrows in B′ indicate the localization of possible tubulovesicular networks seen in Fig. 5C. Bar, 20 μm.

MATERIALS AND METHODS

Materials

Tissue culture reagents were obtained from GIBCO BRL (Gaithersburg, MD). SO was purchased from Wellcome Diagnostics (Beckenham, UK). *N*-ethylmaleimide (NEM), GTPγS, nocodazole, 3,3′-diaminobenzidine tetrahydrochloride, saponin, and components of the ATP-generating system were from Sigma Chemical Co (St Louis, MO). Mastoparan was from Fluka Chemika (Buchs, Switzerland). Bacterial toxins were purchased from Calbiochem (San Diego, CA). Antibodies against α-mannosidase II, β-COP, KDEL, galactosyltransferase, and TGN38 were kindly provided by Drs M. G. Farquhar (University of California San Diego, CA), T. Kreis (Université de Genève, Switzerland), S. Fuller (European Molecular Biology Laboratory, Heidelberg, Germany), E. Berger (Universität Zürich, Switzerland), and G. Banting (University of Bristol, UK), respectively. FITC-, TRITC-, and peroxidase-conjugated goat secondary antibodies against rabbit and mouse IgG were purchased from TAGO (Burlingame, CA). Purified transducin βγ subunits were kindly provided by Dr Y. K. Ho (University of Illinois at Chicago, IL) (Jaffe et al., 1993).

Cytosol preparation

Cytosol was prepared as described (Taylor et al., 1992). Briefly, bovine brains were homogenized at 4°C in 25 mM Tris-HCl (pH 8.0), 500 mM KCl, 250 mM sucrose, 1 mM DTT, 1 mM PMSF and centrifuged at 125,000 ***g*** for 90 minutes. Supernatant was extensively dialysed against 25 mM Hepes-KOH (pH 7.2), 50 mM KAcO, 1 mM $Mg(AcO)_2$ and then centrifugated at 10,000 ***g*** for 20 minutes to remove precipitates. Aliquots (15-20 mg protein/ml) were frozen in liquid nitrogen and stored at −70°C.

Cell culture, permeabilization, and incubation conditions

Normal rat kidney (NRK) and HeLa cells were cultured in high glucose DME containing 2 mM glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 10% FCS. Cells grown on poly-L-lysine-coated glass coverslips were incubated on ice for 5 minutes with 1 unit/ml SO in 20 mM Hepes-KOH (pH 7.2), 110 mM KOAc, 2 mM $Mg(AcO)_2$, 1 mM DTT. Excess SO was removed by washing with this buffer at 0°C. Permeabilization was then achieved by incubating the cells at 37°C for 5 minutes in transport buffer (25 mM Hepes-KOH, pH 7.2, 75 mM KOAc, 2.5 mM $Mg(AcO)_2$, 5 mM EGTA, 1.8 mM $CaCl_2$). Cells were thoroughly rinsed in this buffer to release endogenous cytosolic proteins (i.e. more than 50% of the total lactate dehydrogenase activity was usually lost). They were then incubated at 37°C in transport buffer containing bovine brain cytosol (0.1-10 mg protein/ml) and ATP-generating system (1 mM ATP, 5 mM creatine phosphate, 0.2 i.u. rabbit muscle creatine phosphokinase).

Immunofluorescence

Cells were fixed for 30 minutes in 3% paraformaldehyde in phosphate buffer, pH 7.4, and then incubated for 10 minutes with 0.05% saponin and 0.5% BSA in PBS. Cells to be processed for β-COP staining were permeabilized with 0.2% Triton X-100 plus 0.5% SDS. Incubation with antibodies was performed at room temperature for 1-2 hours. Coverslips were mounted in 10% PBS/90% glycerol.

Immunoelectron microscopy

Conventional electron microscopy was performed on 2.5% glutaraldehyde fixed cells as described (Hidalgo et al., 1992). For immunoperoxidase, cells were fixed in periodate-lysine-paraformaldehyde fixative for 4 hours and permeabilized with 0.005% saponin in PBS/0.5% BSA. They were incubated overnight at 4°C with anti-mannosidase II antibody and 2 hours at room temperature with HRP-conjugated secondary antibody. Cells were then processed as described (Velasco et al., 1993).

RESULTS

Redistribution of the Golgi complex into the ER occurs in SO-permeabilized cells incubated with a high concentration of cytosolic proteins

SO-permeabilized NRK cells incubated with transport buffer containing 0.05-0.1 mg/ml cytosolic proteins and ATP-generating system maintained the organization of the Golgi complex as viewed by indirect immunofluorescence. Thus, under these conditions α-mannosidase II, a mid-Golgi resident in this cell type (Velasco et al., 1993), was detected in the perinuclear region (Fig. 1A,A′). In contrast, a reticular staining pattern, reminiscent of the ER, was observed in cells similarly incubated with a high concentation (5-10 mg/ml) of cytosolic proteins (Fig. 1B,B′). We studied the time-course of this process by performing double immunofluorescence staining of the cells with both anti-mannosidase II and an antibody recognizing the KDEL sequence, here used as an ER marker. Golgi disruption began after 10-15 minutes incubation and after 40 minutes or longer extensive immunofluorescence colocalization of both Golgi and ER antigens occurred (Fig. 2). This effect was not observed when normal cytosol was replaced by heat-inactivated cytosol or bovine serum albumin (not shown).

These results suggested that cytosolic factors induced the fusion of Golgi membranes with the ER. Indeed, we detected by electron microscopy mannosidase II immunoperoxidase staining in the ER and nuclear envelope of cells incubated with 5 mg/ml cytosolic proteins (Fig. 3). We concluded that cytosolic factors induce Golgi disassembly and fusion with the ER.

Golgi disruption induced by cytosolic factors involves vesicle budding

At the ultrastructural level the Golgi complex in intact NRK cells was found to be composed of 4-6 stacked cisternae and a number of associated vesicles (Fig. 4A). In general this organization was maintained in SO-permeabilized cells incubated with a low concentration (0.1 mg/ml) of cytosolic proteins (Fig. 4B). In contrast, Golgi structure was dramatically altered in permeabilized cells exposed to 5 mg/ml of cytosolic proteins. Disassembly seemed to occur by progressive vesiculation of the Golgi cisternae (Fig. 5A,B). Thus, significant increase in the number of small, 50-60 nm, vesicles and concomitant decrease in the number of cisternae was observed in the Golgi area during the first minutes of incubation with a high concentration of cytosolic proteins. Many of the vesicles produced were coated (Fig. 5B, insert). Convoluted tubules were also present in the Golgi area but no evidence was obtained that they were derived from Golgi cisternae; instead, connections between convoluted tubules and the nuclear envelope or ER cisternae were noted (Fig. 5C). Following 40-

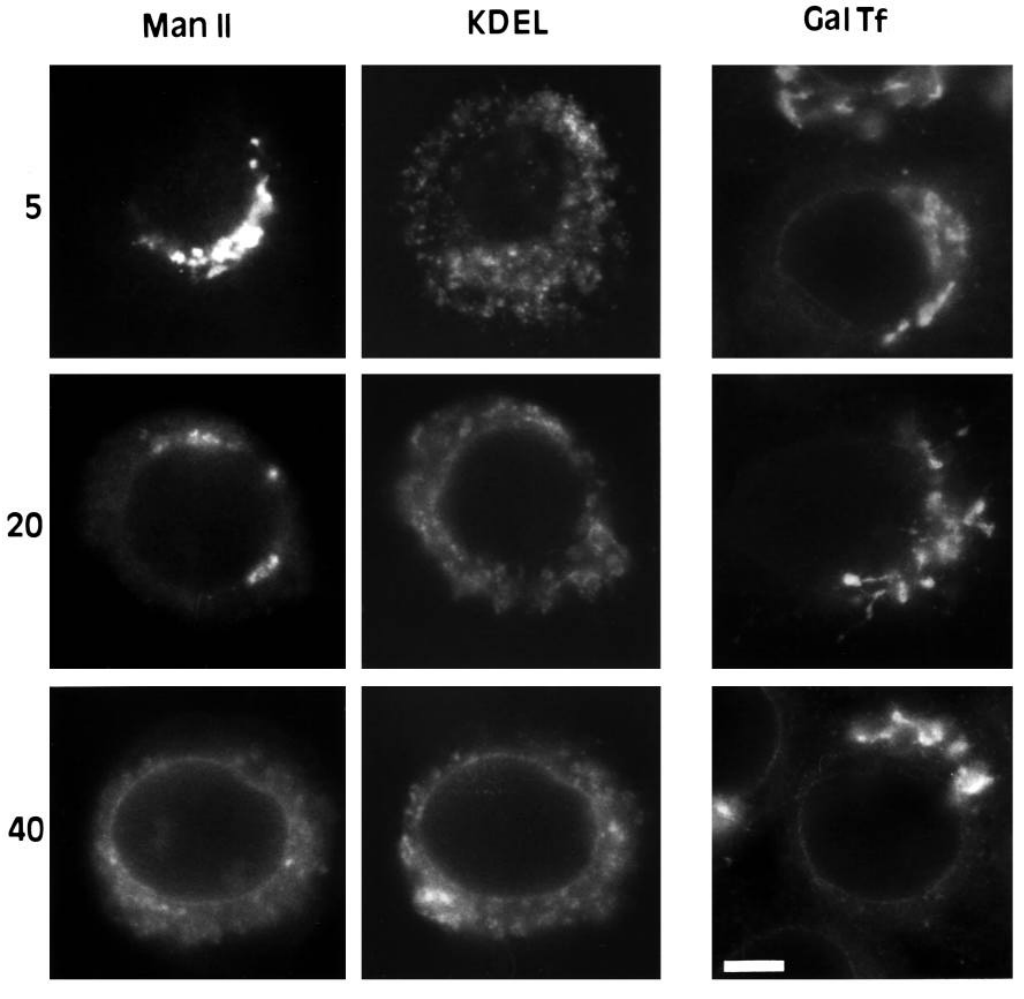

Fig. 2. Time-course of Golgi α-mannosidase II and galactosyltransferase redistributions into the ER. NRK and HeLa cells grown on coverslips were SO-permeabilized and incubated at 37°C in complete incubation medium containing 5 mg/ml cytosolic proteins and ATP-generating system. Coverslips were fixed at 5, 20 and 40 minutes of incubation and processed for immunofluorescence with antibodies against galactosyltransferase in the case of HeLa cells, or double-stained with antibodies against α-mannosidase II and the KDEL sequence (ER marker) in the case of NRK cells. Bar, 20 μm.

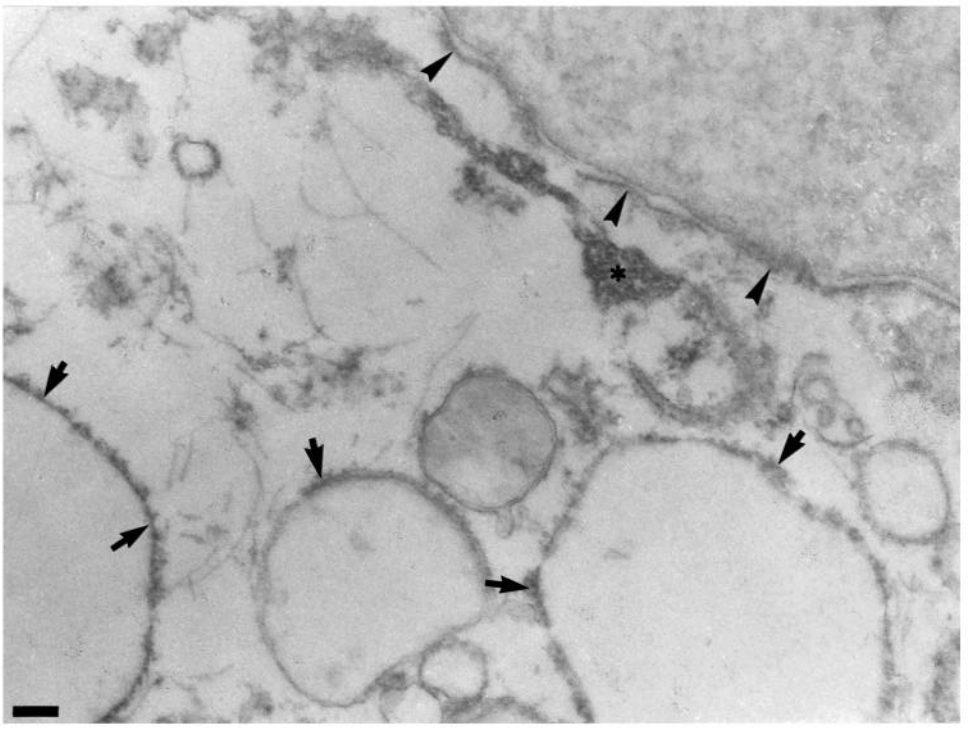

Fig. 3. Immunoperoxidase detection of α-mannosidase II in the cytoplasm of SO-permeabilized NRK cells incubated with 5 mg/ml cytosolic proteins. The outer membrane of the nuclear envelope (arrowheads) and the ER cistenae were stained. Immunoperoxidase reaction product was mainly localized at the ER membrane (arrows) although it also filled the lumen of some cisternae (asterisk). Bar, 0.2 μm.

60 minutes incubation the number of vesicles decreased as they supposedly fused with the ER (Fig. 5C). At this time extensive tubulovesicular networks were also seen. The latter were reminiscent of those observed in BFA-treated cells (Hidalgo et al., 1992). Similar structures were not detected in normal, intact cells. They have been proposed to represent Golgi remnants involved in Golgi reorganization during recovery from BFA treatment (De Lemos-Chiarandini et al., 1992; Hendricks et al., 1992; Hidalgo et al., 1992). The tubulovesicular networks stained weakly with anti-mannosidase II by immunoperoxidase (not shown). In addition, membrane structures that might correspond to tubulovesicular networks were often seen in the perinuclear region of cells stained with this antibody by immunofluorescence (Fig. 1B′).

Cytosolic factors induce the redistribution of different Golgi compartments into the ER

In addition to mannosidase II we also studied by immunofluorescence the redistribution of other Golgi integral proteins into the ER. Both the *trans*-Golgi enzyme galactosyltransferase (Roth and Berger, 1982) (Fig. 2) and the *trans*-Golgi network resident protein TGN38 (Luzio et al., 1990) (Fig. 6) showed substantial relocation after incubation with an excess of cytosolic proteins. Redistribution of these two proteins, however, occurred slower than that of mannosidase II and it never came to completion (Figs 2,6).

We also examined the redistribution of the coat protein β-COP in SO-permeabilized NRK cells incubated with a high concentration of cytosolic proteins. As shown in Fig. 6 β-COP was primarily found associated with Golgi membranes and, in addition, in punctate accumulations distributed throughout the cytoplasm. During redistribution of mannosidase II into the ER β-COP remained associated with Golgi fragments. Once mannosidase II redistribution was completed β-COP localized in spots of different sizes. At no time did we observe diffuse, cytosolic staining for β-COP during the disorganization of the Golgi.

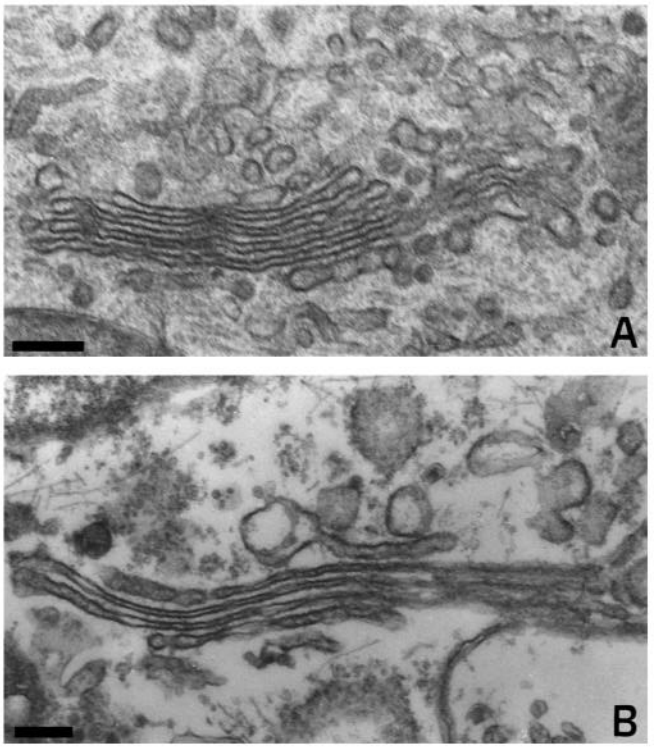

Fig. 4. Golgi complex organization in intact and SO-permeabilized NRK cells. (A) Golgi complex in a normal, intact NRK cell. (B) Structure of the Golgi complex after cell permeabilization and incubation for 1 hour at 37°C with 0. 1 mg/ml cytosolic proteins and ATP-generating system. Bar, 0.2 μm.

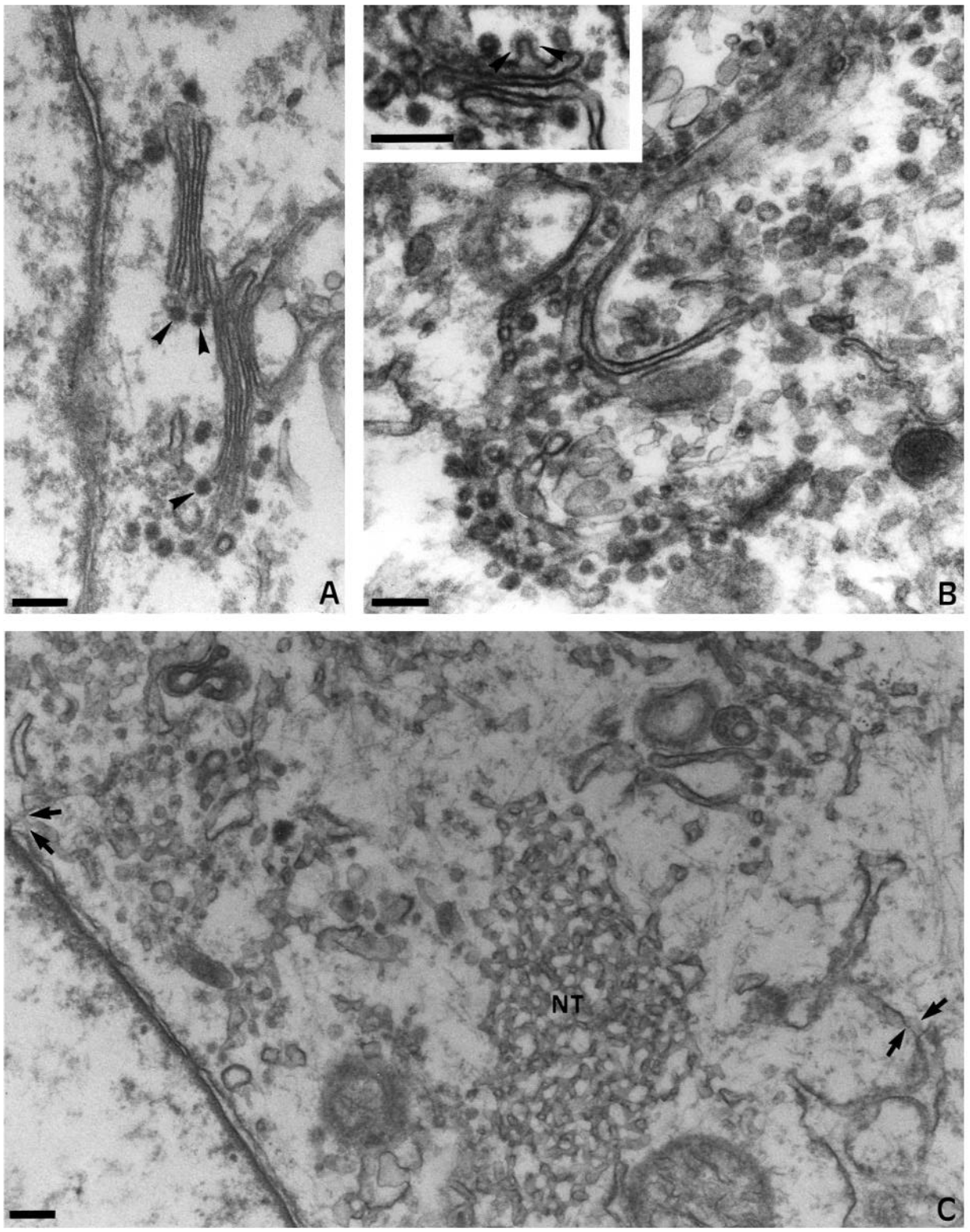

Fig. 5. Ultrastructural visualization of Golgi disassembly induced by cytosolic proteins. SO-permeabilized NRK cells were incubated at 37°C in complete incubation medium containing 5 mg/ml cytosolic proteins and ATP-generating system for 5 (A), 20 (B), and 60 minutes (C) before fixation and processing for electron microscopy. (A,B) Non-clathrin coated vesicles (arrowheads) are originated from Golgi cisternae during the initial disassembly of the Golgi complex. (C) After Golgi redistribution to the ER is completed extensive tubulovesicular networks (NT) persisted in the perinuclear region. Arrows indicate connections between convoluted tubules and ER elements. Bar, 0.2 μm.

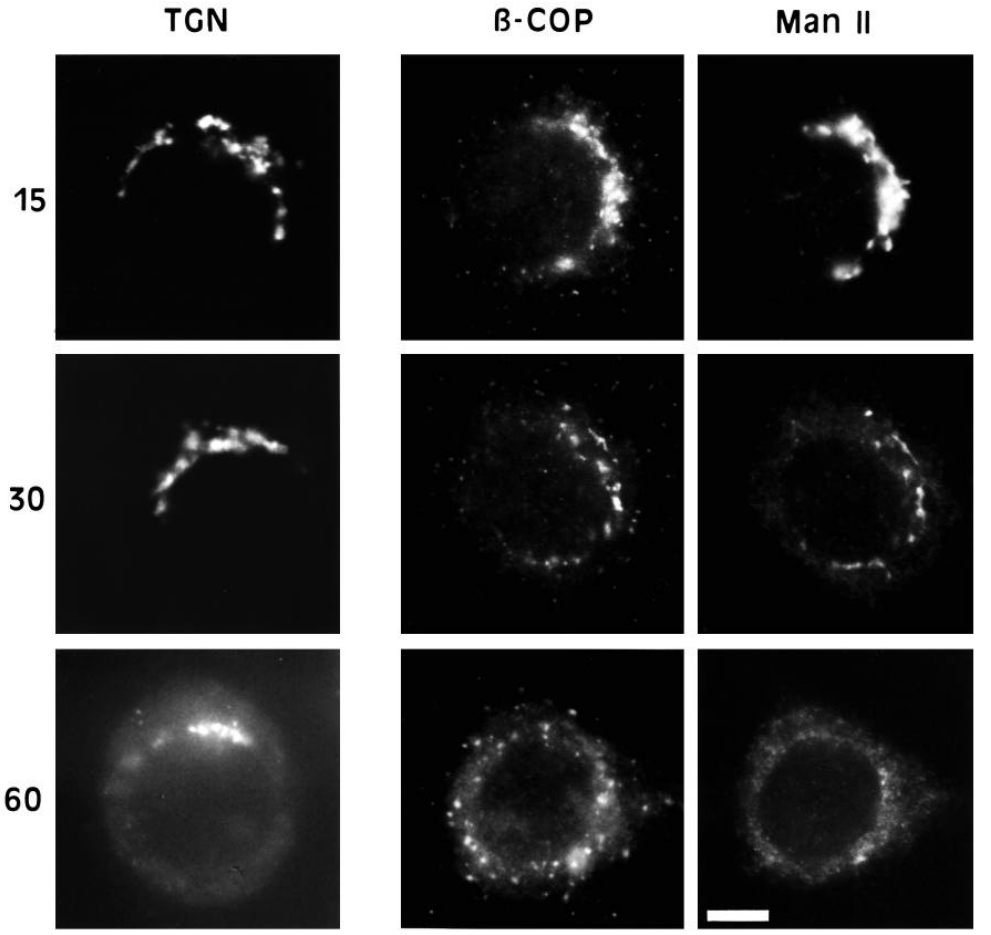

Fig. 6. Redistribution of β-COP and TGN38. SO-permeabilized NRK cells were incubated with 5 mg/ml cytosolic proteins and ATP-generating system. At 15, 30, and 60 minutes the cells were fixed and double-stained with antibodies against β-COP and α-mannosidase II or, alternatively, single-stained with an antibody against TGN38. Bar, 20 μm.

We therefore concluded that Golgi redistribution induced by cytosolic factors affects different Golgi compartments and it occurs without previous β-COP dissociation from Golgi membranes.

Requirements for Golgi redistribution induced by cytosol

Golgi redistribution into the ER required ATP as it did not occur when ATP was omitted from the incubation medium (Fig. 7A). It was also inhibited at low temperature. At either 15°C (Fig. 7B) or 20°C (not shown) the Golgi complex appeared partially fragmented as judged by the immunofluorescence detection of mannosidase II; this protein, however, failed to redistribute into the ER at these temperatures.

Golgi redistribution did not require intact microtubules. This was shown in cells which were treated with nocodazole prior to permeabilization and incubation with cytosol. Microtubule

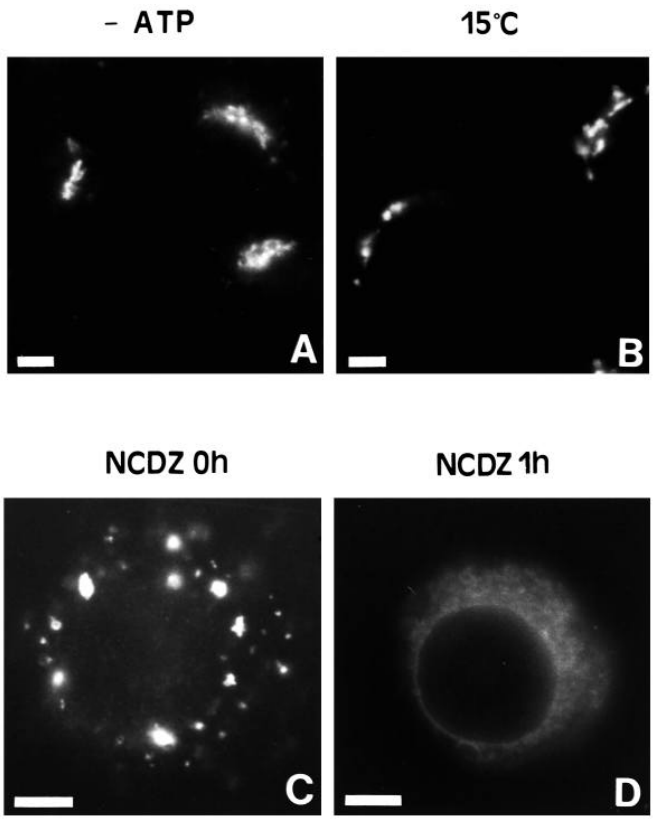

Fig. 7. Requirements for Golgi redistribution induced by cytosolic proteins. NRK cells were permeabilized and incubated with 5 mg/ml cytosolic proteins as indicated in Fig. 1 except that either ATP was omitted from the incubation medium or incubation was performed at 15°C. Cells were also treated at 37°C for 1 hour with 5 μM nocodazole before permeabilization with SO (NCDZ 0h). In addition, nocodazole-treated, SO-permeabilized cells were incubated at 37°C for 1 hour with ATP-generating system and 5 mg/ml cytosolic proteins in the presence of nocodazole (NCDZ 1h). The localization of α-mannosidase II is shown. Bar, 20 μm.

depolymerization by nocodazole is known to induce Golgi fragmentation into individual stacks (Turner and Tartakoff, 1989). Thus, when nocodazole treated cells were permeabilized and processed for indirect immunofluorescence with anti-mannosidase II antibody the Golgi complex appeared fragmented into spots distributed throughtout the cytoplasm (Fig. 7C). Staining of these cells with anti-tubulin antibody showed that microtubules were indeed depolymerized (not shown). Nocodazole-treated cells were also permeabilized and incubated with a high concentration of cytosolic proteins and in the continuous presence of nocodazole to prevent microtubule reassembly. In these cells mannosidase II staining showed an ER-like reticular pattern (Fig. 7D). This result indicated that Golgi redistribution into the ER induced by cytosolic factors did not depend upon an intact microtubule system for it to take place.

Inhibition by GTPγS and NEM

The above ultrastructural results provided evidence that redistribution of Golgi enzymes into the ER induced by cytosolic factors might be vesicle-mediated. Both GTPγS and NEM are potent inhibitors of the vesicular anterograde transport (Balch et al., 1984; Melançon et al., 1987). We determined by immunofluorescence the effects of these two agents on Golgi redistribution (Fig. 8). Permeabilized cells were incubated with a high concentration of cytosolic proteins. Either GTPγS or NEM was added at different time points and incubation continued for 1 hour in the presence of each agent. GTPγS completely blocked Golgi redistribution. However, inhibition was only effective if GTPγS was added at the begining (3-5 minutes) of incubation. Addition of GTPγS to the incubation medium after this time did not inhibit the process, suggesting that GTP-binding proteins are required at the time of Golgi breakdown. In contrast, NEM was inhibitory only if added during the first 15-20 minutes of incubation, after which the process became insensitive to this agent (Fig. 8). Therefore, we concluded that both GTPγS and NEM inhibit different steps of the Golgi redistribution process.

We exploited these differences in order to characterize intermediates in the route by electron microscopy. In permeabilized cells exposed for 1 hour to 5 mg/ml cytosolic proteins and GTPγS we detected the presence of coated vesicles attached to intact Golgi cisternae (Fig. 9A). We tried to assess the fate of these coated vesicles. Following 10 minutes of incubation with cytosolic proteins and no inhibitor, NEM was added and incubation continued for 1 hour. This allowed for the budding process to occur during the preincubation period giving rise to disappearance of the Golgi complex (Fig. 9B). Noteworthy, instead of coated vesicles different kinds of uncoated vesicles were seen in these cells (Fig. 9B). We concluded that the redis-

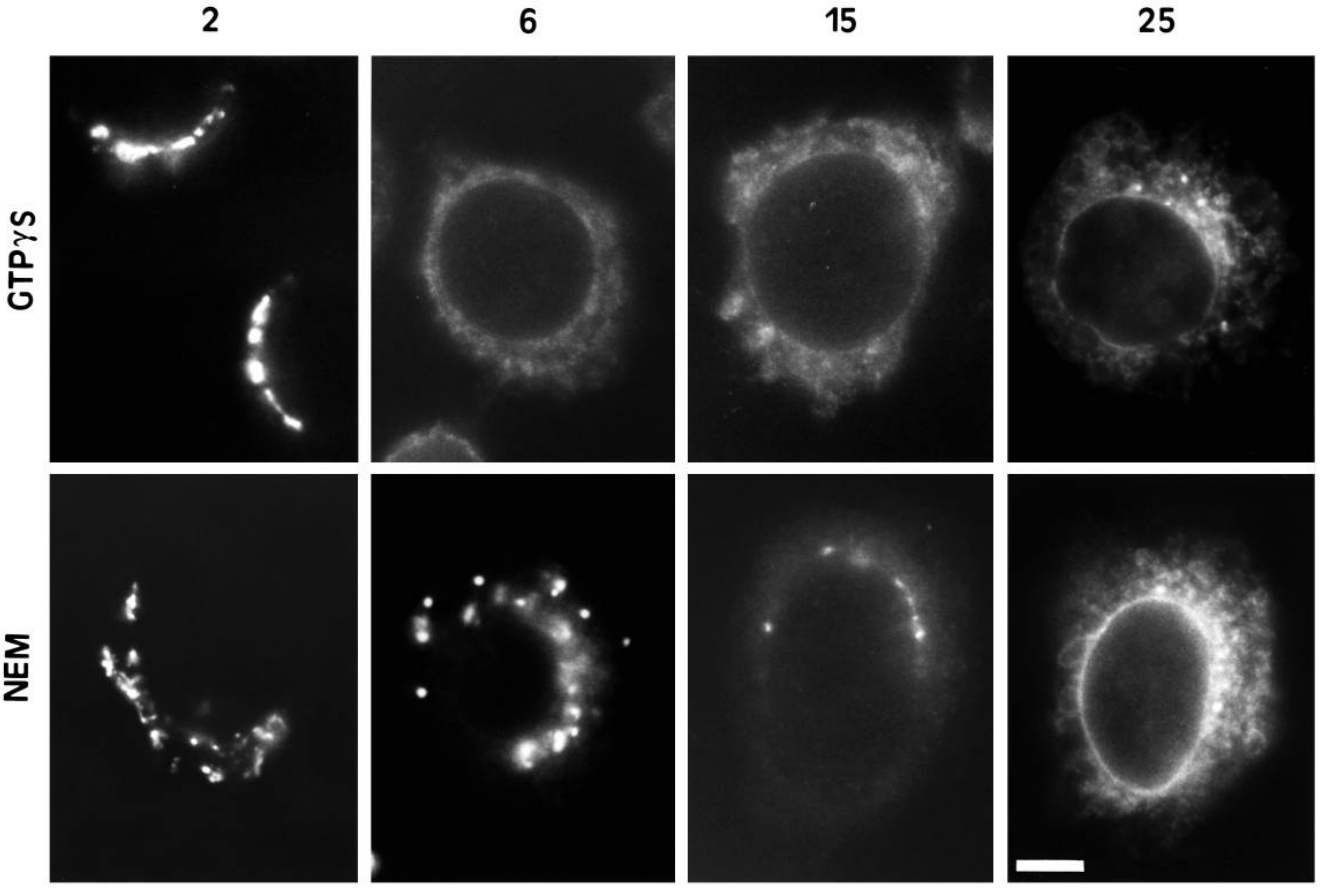

Fig. 8. Time-course of GTPγS and NEM inhibitions on Golgi redistribution induced by cytosolic proteins. SO-permeabilized NRK cells were incubated with 5 mg/ml cytosolic proteins and ATP-generating system at 37°C. Either 100 μM GTPγS or 1 mM NEM was added at 2, 6, 15, and 25 minutes and the incubation continued for 1 hour. Cells were then fixed and processed for immunofluorescence with anti-α-mannosidase II antibody. Bar, 20 μm.

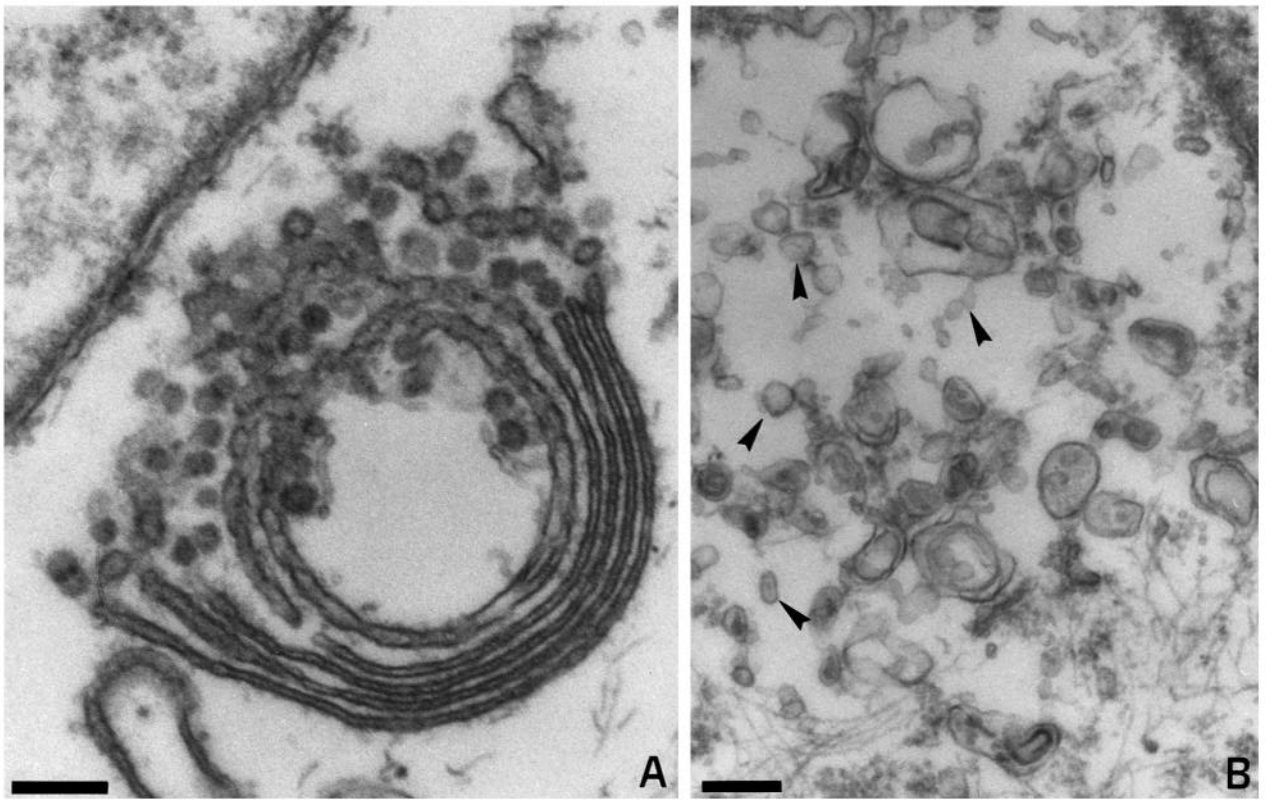

Fig. 9. Characterization of vesicular intermediates in the cytosol-induced Golgi-ER retrograde pathway. (A) Cells were SO-permeabilized and incubated for 1 hour at 37°C with 5 mg/ml cytosolic proteins, ATP-generating system, and 100 μM GTPγS. (B) Following a 10 minute incubation with 5 mg/ml cytosolic proteins, ATP-generating system, and no inhibitor, 1 mM NEM was added and incubation continued for 1 hour. Cells were fixed and processed for electron microscopy. Arrowheads indicate the presence of uncoated vesicles. Bar, 0.2 μm.

tribution of the Golgi complex into the ER induced by cytosolic factors is mediated by coated vesicles that bud from Golgi cisternae and then lose their coats before fusion with the ER.

Trimeric G proteins are involved in Golgi disassembly

Inhibition by GTPγS was indicative of the involvement of GTP-binding proteins in an early step of the cytosol-induced Golgi redistribution into the ER. GTPγS activates both monomeric and trimeric GTP-binding proteins whereas only trimeric G proteins are activated by $AlF_{(3-5)}$ (Kahn, 1991). We then tested the effect of adding $AlF_{(3-5)}$ to our assay. As shown in Fig. 10B $AlF_{(3-5)}$ inhibited mannosidase II redistribution into the ER. Further support for the involvement of trimeric G proteins in the process was obtained by adding transducin βγ subunits (Fig. 10C) and mastoparan (not shown), both of which separately inhibited Golgi redistribution.

Addition of βγ subunits should give rise to inactivation of G proteins as the βγ subunits complex with the free α subunits (Bomsel and Mostov, 1992). In contrast, mastoparan, which mimics an activated receptor, should render the opposite effect, i.e. activation of G proteins (Higashijima et al., 1990; Weingarten et al., 1990). Inhibition by both agents can be explained if Golgi redistribution into the ER would be controlled by several G proteins exerting opposite regulatory roles. To test this hypothesis we examined the effects resulting from incubating the cells with bacterial toxins known to carry out ADP-ribosylation of particular Gα subunits and hence affect their activity (Gilman, 1987). Pretreatment of cells with either cholera toxin or pertussis toxin stimulated Golgi redistribution. Thus, in both cases Golgi disassembly and redistribution into the ER occurred at a low concentration of cytosolic proteins (Fig. 10D-F). This was indicative of the involvement of both α_s and α_i G proteins, the former being irreversibly activated by cholera toxin treatment and α_i being inhibited by pertussis toxin. The emerging idea was that Golgi redistribution into the ER was stimulated by either α_s activation or α_i inhibition.

DISCUSSION

In this paper we have described the redistribution of Golgi components into the ER originated by the incubation of SO-permeabilized cells with an excess of cytosolic proteins. A similar phenomenon occurs in BFA-treated cells that also undergo Golgi disassembly and fusion with the ER (Klausner et al., 1992; Lippincott-Schwartz et al., 1989). However, important differences between both processes were noted. First, we did not observe by immunofluorescence tubule processes emerging from the Golgi cisternae during their redistribution into the ER. Instead, our ultrastructural observations indicate that the cytosol-induced Golgi redistribution occurs by budding of coated vesicles from the Golgi cisternae. In

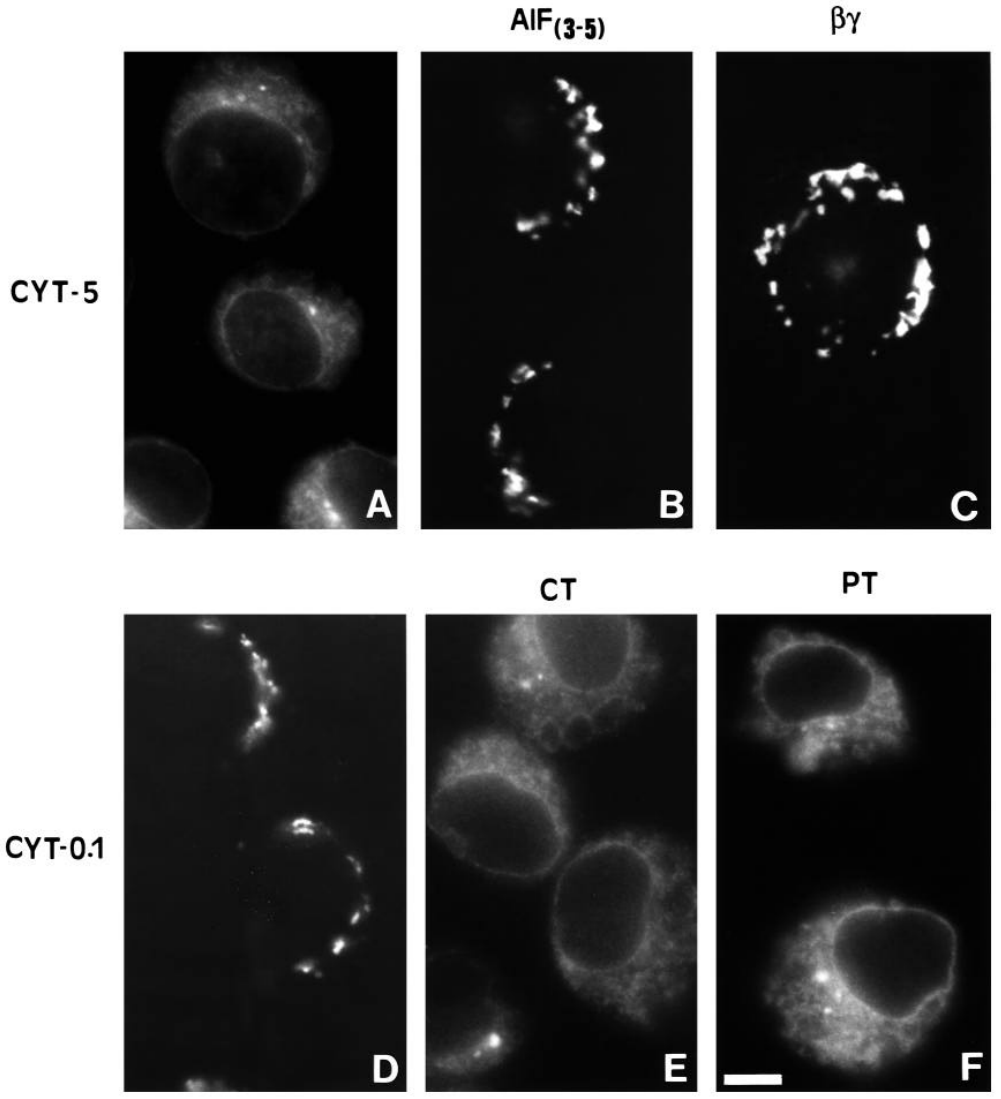

Fig. 10. Involvement of trimeric G proteins in Golgi disassembly. NRK cells were treated or not with 1 μg/ml cholera toxin (CT), 0.5 μg/ml pertussis toxin (PT) for 16 hours at 37°C. Following SO permeabilization the cells were incubated at 37°C for 1 hour in complete incubation medium containing low (0.1 mg/ml) or high (5 mg/ml) concentration of cytosolic proteins plus ATP-generating system. Effects resulting from adding either 50 μM $AlCl_3$ plus 30 mM NaF (B) or 5 μM of transducin βγ subunits (C) on Golgi redistribution are shown. Cells were fixed and stained for immunofluorescence with anti-α-mannosidase II antibody. Bar, 20 μm.

addition, the mechanism of Golgi disruption induced by cytosolic factors is different from that activated by BFA since it apparently occurs without previous β-COP dissociation from Golgi membranes. Furthermore, in the case of permeabilized cells incubated with a high concentration of cytosolic proteins intact microtubules are not required for Golgi redistribution into the ER. Finally, unlike the BFA effect, cytosol-induced Golgi redistribution does not seem to be a consequence of inhibition of the ER-Golgi anterograde transport. Instead, our results suggest the presence in the cells of cytosolic factors that may actively promote the Golgi-ER retrograde pathway by a still undetermined mechanism.

Role of cytosolic factors in promoting Golgi redistribution

Cytosol-induced Golgi redistribution into the ER may be the result derived from an exaggeration of the Golgi-ER retrograde pathway taking place in vivo during retrieval from the Golgi complex of either ER resident molecules (Pelham, 1988) or misfolded proteins (Hammond and Helenius, 1994). Alternatively, this redistribution process may be unrelated with the normal Golgi-ER retrograde pathway. However, this possibility seems unlikely since the fragmented Golgi membranes specifically fuse with the ER and morphological changes in other organelles were not observed during incubation of SO-permeabilized cells with cytosolic proteins.

The Golgi-ER retrograde pathway has been shown to be regulated by both the amount of ER proteins to be recycled from the Golgi and the number of specific receptors available for them. For instance, a BFA-like effect has been described in cells overexpressing ELP-1, a putative receptor for lumenal ER proteins bearing the KDEL sequence (Hsu et al., 1992). In addition, a striking redistribution of the KDEL receptor from the Golgi region to the ER occurs in cells overexpressing proteins with this retention sequence (Lewis and Pelham, 1992). The conclusion derived from these studies is that the retrograde pathway is somehow activated by the formation of ligand-receptor complexes in the Golgi. In this regard, involvement of cytosolic factors could introduce additional controls in the pathway. For instance, by influencing the activity of particular trimeric G proteins cytosolic factors might modulate the association of coat proteins with membranes. This, in turn, could be responsible for maintaining a controlled balance between anterograde and retrograde membrane routes. It has

been recently reported that both the association of coat proteins to Golgi membranes and the constitutive release of secretory vesicles are stimulated by the activation of cell surface receptors and by protein kinase C activators (De Matteis et al., 1993). In addition, Golgi-ER retrograde transport of shiga toxin has been shown to be stimulated by both butyric acid and cAMP (Sandvig et al., 1994). Therefore it is likely that second messengers and other cytosolic factors might regulate membrane traffic.

Steps in Golgi redistribution

The use of a permeabilized cell system allowed us to dissect the Golgi redistribution process into different stages. According to our data Golgi disassembly is achieved by budding of non-clathrin coated vesicles from Golgi cisternae. This process is regulated by trimeric G proteins. The vesicles thereafter lose their coat and fuse to the ER in a microtubule-independent, temperature- and NEM-sensitive manner.

Vesicle vs tubule-mediated retrograde transport

Golgi disassembly and redistribution into the ER induced by cytosolic proteins does not occur by the extension of tubule processes from Golgi cisternae. This is a significant difference with the redistribution caused by BFA treatment (Klausner et al., 1992). Thus we could not observe by immunofluorescence tubule processes emerging from the Golgi during incubation of SO-permeabilized cells with cytosol. Instead, ultrastructural observations indicated that Golgi disassembly was achieved by massive budding of 50-60 nm coated vesicles from Golgi cisternae. GTPγS was an efficient inhibitor of this budding process consistent with recent data indicating an inhibitory role for this agent in vesicle formation (Weidman et al., 1993). Treatment with NEM, on the other hand, inhibited targeting and/or fusion of these vesicles with the ER membranes. Therefore these results suggest the existence of a vesicle-mediated Golgi-ER retrograde pathway. In intact cells this route could coexist with others mediated by tubules and shown in studies with BFA or in cells depleted of ATP (Cluett et al., 1993; Lippincott-Schwartz et al., 1990). Alternatively, the vesicle pathway could be the normal route operating between the Golgi and the ER in cells whose coat proteins are not hindered from becoming associated with membranes; lack of such association is a characteristic common to both energy depletion and BFA treatment (Donaldson et al., 1991a,b). Indeed we observed association of β-COP with Golgi membranes during the disassembly process, indicating that retrograde transport can be initiated while (and perhaps because) coat proteins remain associated with the Golgi. A role for coat proteins in Golgi-ER retrograde transport has also been proposed from their interaction with the cytoplasmic retention signals of several ER membrane proteins (Cosson and Letourneur, 1994).

The functional role played by tubular extensions in membrane transport is controversial at present. It has been postulated that formation of uncoated tubules is a property inherent to Golgi membranes for which recruitment of coat proteins from cytosol is not required (Klausner et al., 1992). In contrast, it was recently shown that budding in vitro requires previous coat assembly on Golgi membranes. Furthermore, in the absence of coat proteins Golgi membranes do not form tubules as predicted by the above hypothesis (Orci et al., 1993). Other evidence indicates that tubule processes are normally formed in vivo. For instance, tubules have been described to occur in cells incubated at low (16°C) temperature (Lippincott-Schwartz et al., 1990; Tang et al., 1993). In this case, however, mid- and *trans*-Golgi proteins do not enter the tubules while p53, a protein localized at the intermediate compartment situated between the ER and the Golgi, does (Lippincott-Schwartz et al., 1990). Therefore, whereas it is evident that tubule processes can be originated from the Golgi region under certain conditions, it remains to be determined if they really account for the recycling pathway existing between the Golgi and the ER.

Regulation of Golgi redistribution by trimeric G proteins

Our data indicate that the Golgi-ER retrograde transport studied in SO-permeabilized cells is modulated by both α_s and α_i. These two Gα proteins, however, should have opposite regulatory roles, since either α_s activation (by cholera toxin treatment) or α_i inhibition (by pertussis toxin treatment) stimulates Golgi redistribution into the ER. Similar findings have been described for the production of secretory vesicles from the TGN (Leyte et al., 1992). This suggests the existence of a basic, G protein-regulated mechanism common to the two processes.

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