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Disruption of Endoplasmic Reticulum to Golgi Transport Leads to the Accumulation of Large Aggregates Containing β-COP in Pancreatic Acinar Cells

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When transport between the rough endoplasmic reticulum (ER) and Golgi complex is blocked by Brefeldin A (BFA) treatment or ATP depletion, the Golgi apparatus and associated transport vesicles undergo a dramatic reorganization. Because recent studies suggest that coat proteins such as β-COP play an important role in the maintenance of the Golgi complex, we have used immunocytochemistry to determine the distribution of β-COP in pancreatic acinar cells (PAC) in which ER to Golgi transport was blocked by BFA treatment or ATP depletion. In controls, β-COP was associated with Golgi cisternae and transport vesicles as expected. Upon BFA treatment, PAC Golgi cisternae are dismantled and replaced by clusters of remnant vesicles surrounded by typical ER transitional elements that are generally assumed to represent the exit site of vesicular carriers for ER to Golgi transport. In BFA-treated PAC, β-COP was concentrated in large (0.5–1.0 μm) aggregates closely associated with remnant Golgi membranes. In addition to typical ER transitional elements, we detected a new type of transitional element that consists of specialized regions of rough ER (RER) with ribosome-free ends that touched or extended into the β-COP containing aggregates. In ATP-depleted PAC, β-COP was not detected on Golgi membranes but was concentrated in similar large aggregates found on the cis side of the Golgi stacks. The data indicate that upon arrest of ER to Golgi transport by either BFA treatment or energy depletion, β-COP dissociates from PAC Golgi membranes and accumulates as large aggregates closely associated with specialized ER elements. The latter may correspond to either the site of entry or exit for vesicles recycling between the Golgi and the RER.

INTRODUCTION

The Golgi apparatus is a remarkably dynamic and labile organelle capable of undergoing rapid and reversible reorganization within minutes in response to a variety of experimental manipulations (for a review see Farquhar, 1991). We have used an in vitro system of freshly isolated pancreatic lobules to study the regulation of Golgi structure and function in acinar cells. We chose pancreatic acinar cells (PAC) because they are highly specialized—beyond any other cell type—for synthesis and transport of proteins along the exocytic pathway. Protein secretion in this cell is at least several orders of magnitude greater than in any cultured cell line, and, accordingly, the cell has highly amplified secretory compartments (rough endoplasmic reticulum [RER], transitional elements of the endoplasmic reticulum [ER], transport vesicles, Golgi complex) (Palade, 1975; Farquhar and Palade, 1981). This cell has been the model on which much of what we know about the exocytic pathway was worked out (Palade, 1975).

We reported earlier that when PAC are incubated under N_2, a treatment that leads to ATP depletion

and blocks ER to Golgi transport (Jamieson and Palade, 1967b, 1968), the Golgi complex undergoes a dramatic reorganization; the stacks of Golgi cisternae are essentially unchanged but the number of ER to Golgi transport vesicles on the cis side of the stacks is greatly reduced; their place is taken by fibrillar aggregates that contain small vesicle-free cages (Merisko *et al.*, 1986a,b).

Brefeldin A (BFA) is another treatment known to affect the Golgi complex. It is assumed to cause its complete disappearance in many cell types (Klausner *et al.*, 1992), but in PAC the Golgi stacks are replaced by Golgi remnants consisting of clusters of smooth vesicles and tubules surrounded by transitional elements of the ER (Hendricks *et al.*, 1992a). These vesicles and tubules have the density characteristic of Golgi membranes (1.14–1.16 g/ml) and contain Golgi membrane markers (α-mannosidase II, galactosyltransferase, and gp58). Thus, both treatments affect the Golgi complex dramatically but differently: in anoxic cells the dominant traits are the loss of vesicular carriers and the persistance of the stacks, whereas in BFA-treated cells the most striking effect is the replacement of stacks by Golgi remnants.

Recent evidence links the maintenance of the complex to the presence of nonclathrin coat proteins associated with certain Golgi membranes (Donaldson *et al.*, 1990; Duden *et al.*, 1991a). To find out if similar factors are involved in the maintenance of Golgi organization in PAC, we decided to investigate the distribution of β-COP, a 110-kDa major coat protein of nonclathrin-coated Golgi vesicles (Duden *et al.*, 1991b; Serafini *et al.*, 1991) in BFA-treated or ATP-depleted PAC.

MATERIALS AND METHODS

Materials

Animals, reagents, and supplies were obtained from the following sources: male rats (50–125 g) and female mice (15 wk or older) from Harlan Sprague-Dawley (Indianapolis, IN); BFA from Epicentre Technologies (Madison, WI); Ham's F-12 medium from the University of California, San Diego Core Facility; fetal calf serum from GIBCO (Grand Island, New York); HL-1 from Ventrex Labs (Portland, ME); Nutridoma from Boehringer Mannheim (Indianapolis, IN); Nu-serum IV from Collaborative Research (Bedford, MA); Nitex nylon monofilament (100-μm mesh) from Tetko, Inc. (Briarcliff Manor, NY), and the TiterMax adjuvant system from CytRx (Norcross, GA). Rhodamine-conjugated goat anti-rabbit F(ab)$'_2$ was purchased from TAGO (Burlingame, CA) and fluorescein isothiocyanate (FITC)conjugated goat anti-mouse F(ab)$'_2$ from Zymed (San Mateo, CA). [^{125}I]Staph protein A, immunological grade (>1.11 MBq/μg), was obtained from ICN Biomedicals (Irvine, CA). Goat anti-rabbit IgG conjugated to 5 nm colloidal gold particles was from Amersham (Arlington Heights, IL). All other reagents and chemicals were purchased from Sigma (St. Louis, MO).

Affinity-purified anti-β-COP (EAGE) IgG, a rabbit polyclonal antibody raised against a peptide (amino acids 496–513) of β-COP (Duden *et al.*, 1991b), and anti-β-COP monoclonal M3A5 (ascites fluid) (Allan and Kreis, 1986) were kindly provided by Dr. T. Kreis (European Molecular Biology Laboratory, Heidelberg, Germany). Mouse anti-gp58 antisera were prepared by immunizing mice with purified gp58.

Preparation and Treatment of Pancreatic Lobules

Pancreatic lobules were prepared as previously described (Hendricks *et al.*, 1992a). Briefly, Ham's F-12 medium, supplemented with 1% each HL-1, Nutridoma, and Nu-Serum·IV; 0.1 TIU/ml aprotinin; and 10 mg/ml soybean trypsin inhibitor (SHF12), was injected into the interstitia of the gland to separate lobules, which were minced between scalpel blades into individual ~2-mm^3 units (Scheele, 1983). For microscopy, lobules were fixed directly or after the experimental treatments described below. For immunocytochemistry, groups of ~20 lobules were placed on Nitex rafts into vials containing 10 ml SHF12 and allowed to equilibrate in a 95% O_2: 5% CO_2 atmosphere at 37°C for 5–10 min before the addition of BFA. For cell fractionation, 0.48–0.51 g wet weight of lobules was added directly to the vials containing SHF12 and allowed to equilibrate. BFA treatment was for 60 min at 7.2 μM (2 μg/ml) unless otherwise indicated. We have previously established that in PAC, Golgi stacks are dismantled within 20–30 min at this BFA concentration (Hendricks *et al.*, 1992a). In ATP-depletion experiments, the medium was equilibrated (15–20 min) with N_2, and then lobules were added for 30-min treatment under N_2.

Electron Microscopy and Immunolabeling

For routine microscopy, lobules were fixed (2 h) with 2.5% glutaraldehyde in 100 mM cacodylate-HCl buffer (pH 7.2) containing 0.1 mM $MgCl_2$ and $CaCl_2$, postfixed with 1% OsO_4 in the same buffer, stained in block (2 h) with 2% uranyl acetate (pH 6.0), dehydrated in graded ethanols, and embedded in Epon.

For immunofluorescence or immunogold labeling, lobules were fixed for either 1 h with 3% paraformaldehyde, 0.05% glutaraldehyde in 0.1 M cacodylate-HCl buffer (pH 7.4) or for 3 h in 2% paraformaldehyde, 0.75 M lysine, 0.01 M sodium periodate in phosphate buffer (pH 7.4) (McClean and Nakane, 1974) and then processed as previously described (Hendricks *et al.*, 1992a). Semithin cryosections (1.0 μm) were cut on a Reichert ultramicrotome equipped with an FC-4 cryoattachment; they were collected on glass slides and incubated overnight (4°C) with rabbit anti-EAGE followed by FITC-conjugated goat anti-rabbit F(ab$'$)$_2$ (2 h) and examined in an axiophot (Zeiss, Thornwood, NJ). Some specimens were similarly incubated with mouse polyclonal gp58 antiserum followed by FITC-conjugated goat anti-mouse F(ab$'$)$_2$. Ultrathin cryosections were similarly prepared, collected on formvar/carbon-coated nickel grids, and incubated overnight at 4°C with rabbit anti-β-COP (EAGE) followed by goat-anti-rabbit IgG conjugated to 5-nm gold particles (1–2 h). Sections were absorption stained with 0.2% uranyl acetate and lead citrate (Tokuyasu, 1989), and examined at 80 kV on a Philips CM-10 electron microscope.

Cell Fractionation

Pancreas lobules were incubated at 37°C with or without BFA (1 h) and then homogenized at 4°C in 0.3 M sucrose (tissue concentration ~1:10) in a Brendler-type glass homogenizer (Saraste *et al.*, 1987). Lobule remnants, broken cells, and nuclei were sedimented at 600 $\times$ *g* (15 min) and discarded. The ensuing postnuclear supernatant was centrifuged at 100 000 $\times$ *g* for 60 min to yield a pellet containing primarily membranes and a final supernatant taken to represent the cytosol. To ensure that the membrane pellet was not contaminated with entrapped or absorbed cytosolic proteins, it was resuspended in sucrose by pipeting and sonicating in a water bath-type sonicator (Fisher Scientific, Tustin, CA) (4°C) and then centrifuged again at 100 000 $\times$ *g*. The final pellet and supernate fractions were solubilized and boiled (3 min) in sample buffer containing 10 mM dithiothreitol, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970), transferred to polyvinylidene difluoride membranes, and incubated with either M3A5 followed by

rabbit anti-mouse IgG or with anti-β-COP (EAGE) both followed by [^{125}I]Staph protein A. The immunoblots were exposed to film (X-OMAT; Kodak, Rochester, NY) for 7–24 h at −70°C with an intensifying screen. Gel loads of pellet and supernatant fractions were normalized to wet weight of starting tissue.

RESULTS

β-COP is Associated Primarily with Golgi Membranes in Exocrine Pancreas

In control specimens, β-COP was seen by immunofluorescence to be concentrated in the Golgi region of PAC (Figure 1, A–D) where it overlapped with the typical ring and loop staining pattern characteristic of Golgi membrane markers such as gp58 (Saraste *et al.*, 1987; Hendricks *et al.*, 1991). Punctate staining was also observed throughout the cytoplasm (Figure 1D). After immunogold labeling, β-COP was found in association with membranes of Golgi cisternae and transport vesicles (Figure 2, A and B), concentrated on their outer surface (Figure 2B), as expected for this peripherally oriented coat protein (Duden *et al.*, 1991b).

β-COP Dissociates from Golgi Membranes and Accumulates in Large Cytoplasmic Aggregates upon BFA Treatment

Upon BFA treatment, PAC Golgi cisternae disassemble and form collections of tubules and vesicles referred to as Golgi remnants (Hendricks *et al.*, 1992a). These remnants are surrounded by typical part-rough/part-smooth transitional elements of the ER (Jamieson and Palade, 1967a) (Figure 3A). Aggregates resembling those described previously in ATP-depleted pancreas (Merisko *et al.*, 1986b) were sometimes seen located in close proximity to the Golgi remnants (Figure 3, A and B). They were similar in texture but not located between transitional elements and Golgi membranes as is the case in anoxic cells (Merisko *et al.*, 1986b). In specimens treated for 60 min with 7.2 μM (2 μg/ml) BFA, immunofluorescence staining for β-COP was concentrated in the Golgi region as in controls, but it was distributed in globular masses rather than in ring-like patterns (compare Figure 1, D and F). By immunogold labeling, these masses were seen to correspond to the large (~0.5–1 μm) electron dense aggregates (Figure 4, A, B, and D). The Golgi remnants themselves did not contain detectable β-COP. Typically, the aggregates contained membranous profiles, and RER cisternae often surrounded them in spoke-like array. In plastic-embedded sections, it was evident that the surrounding ER cisternae typically had ribosome-free ends touching or embedded in the aggregates (Figure 3, A and B). These smooth ribosome-free regions of the ER represent another type of transitional element distinct in morphology and location from typical transitional elements that are characterized by the presence of broad ribosome-free regions with vesicular protrusions (Figure 3A).

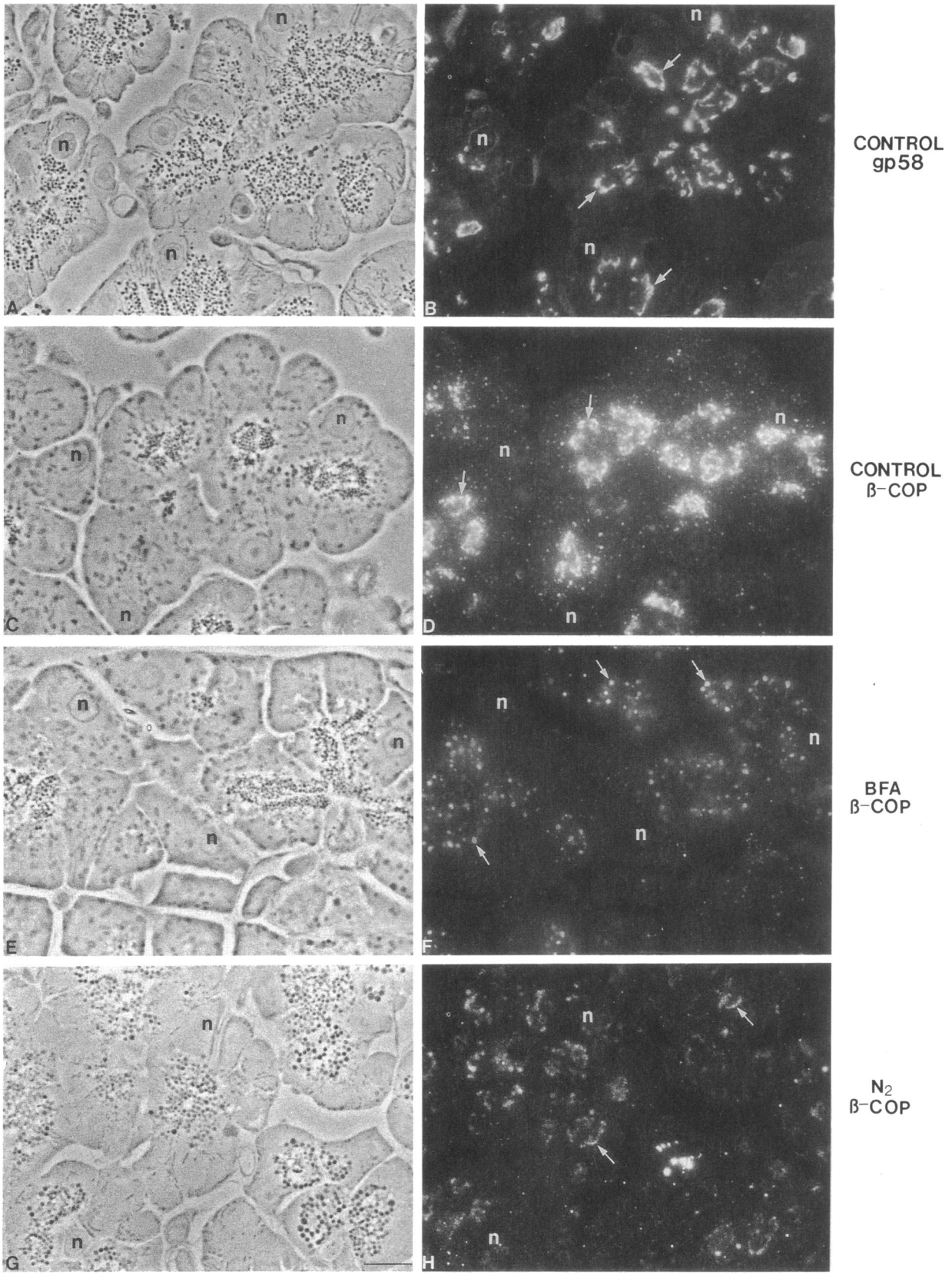
β-COP Aggregates are also Found in ATP-Depleted PAC

Under normal conditions, PAC rely primarily on fatty acid oxidation to generate the ATP needed for ER to Golgi transport, and when ATP synthesis is curtailed by incubation under N_2 or in the presence of uncouplers of oxidation phosphorylation, intracellular transport from the ER to the Golgi complex is blocked (Jamieson and Palade, 1967b; Jamieson and Palade, 1968). As mentioned earlier, stacked cisternae persist, but the number of vesicular carriers on the cis side of the Golgi stacks decreases dramatically, and fibrillar aggregates often containing small vesicle-free globular cages accumulate, preferentially on the cis side of the Golgi cisternae (Merisko *et al.*, 1986a,b). To determine whether β-COP accumulates in these aggregates, we determined the distribution of β-COP in PAC incubated under N_2 for 30 min. By immunofluorescence, we found that β-COP was concentrated in cytoplasmic aggregates (Figure 1, G and H) comparable in size and distribution with those found in tissue treated with BFA. By electron microscopy, the aggregates were seen to be preferentially located on the cis side (Figure 5, A–C) or at the lateral ends (Figure 5C) of the Golgi stacks. The stacked Golgi cisternae remained intact and were typically associated with transitional elements that lack vesicular protrusions in N_2-treated specimens (Figure 5B). The detailed arrangement of the aggregates was comparable to those found in BFA-treated PAC (see Figure 3, A and B). Some of them were surrounded by RER cisternae with smooth ribosome-free ends (Figure 5B). In addition, tubular extensions often appeared to penetrate or extend into the aggregates (arrows, Figure 5B).

These findings indicate that the large cytoplasmic aggregates that accumulate in the vicinity of Golgi stacks in ATP-depleted PAC contain β-COP and presumably other nonclathrin coat proteins. In size, texture, content of β-COP, and relationship to the specialized transitional regions of the RER, they are similar or identical to those seen in BFA-treated cells. This suggests that dissociation of β-COP from Golgi membranes and aggregate formation are early responses that accompany a block in the transport of secretory proteins from the ER to the Golgi complex, irrespective of the nature of the primary agent, i.e., BFA treatment or ATP depletion.

β-COP is Concentrated in the Cytosol After Homogenization of BFA-Treated Pancreatic Lobules

To determine what proportion of β-COP was sedimentable in control vs. BFA-treated specimens, we first homogenized lobules in 0.3 M unbuffered sucrose and removed nuclei and tissue fragments (10 min at 600 × *g*). Because preliminary experiments demonstrated that the amount of β-COP recovered in the nuclear pel-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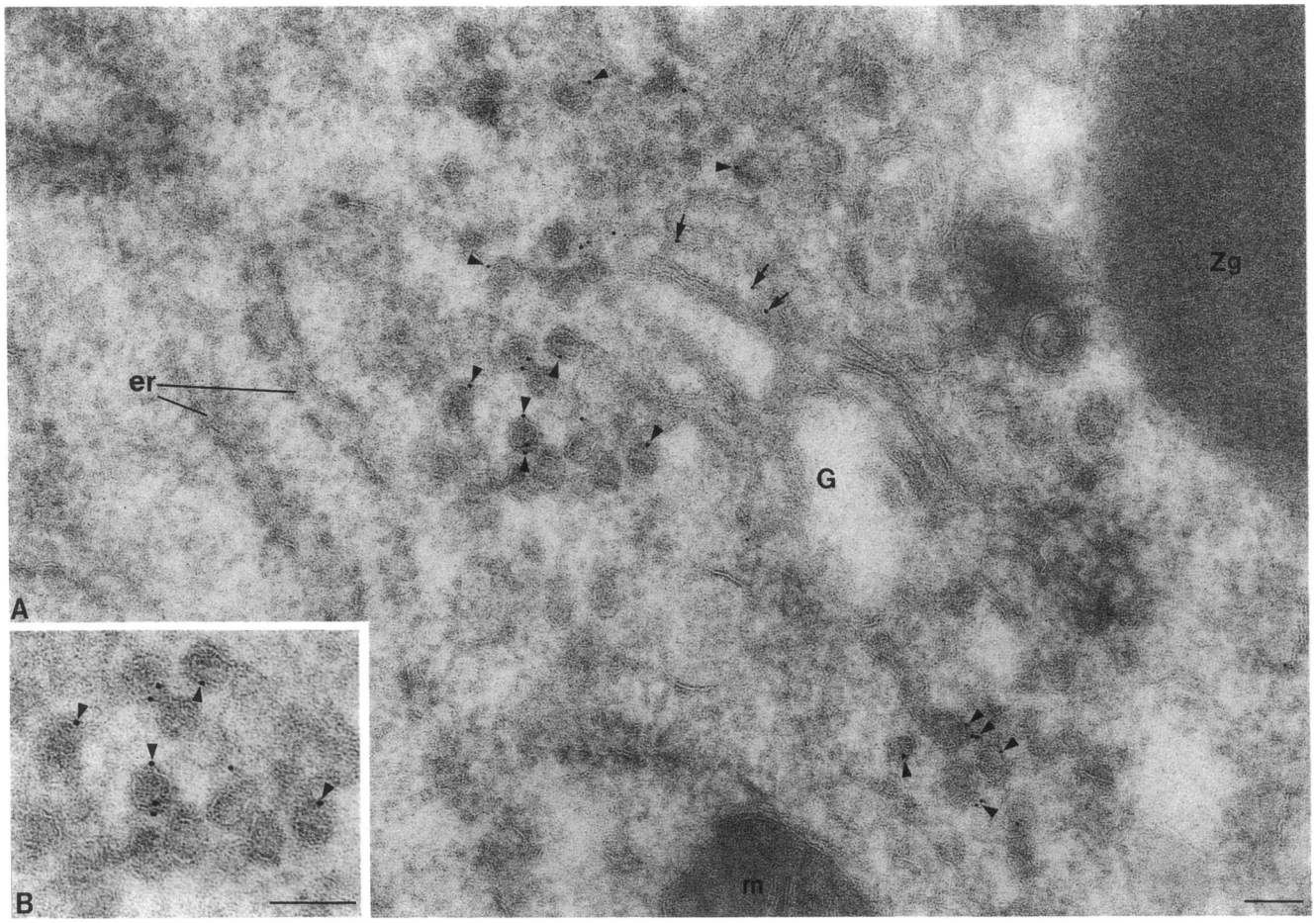


Figure 2. Immunogold labeling for β-COP in control PAC. (A) Gold particles are seen in association with both Golgi cisternae (arrows) and transport vesicles (arrowheads), but labeling of the vesicles is more extensive. (B) The gold is concentrated on the outer surface of the vesicle membranes (arrowheads). Ultrathin cryosection incubated with anti-β-COP (EAGE) and goat anti-rabbit IgG-gold (5 nm) conjugate. G, Golgi stack; m, mitochondrion; zg, zymogen granule. Bars, 0.1 μM.

let was insignificant, it was discarded, and postnuclear supernatants were centrifuged (1 h, 100 000 × *g*) to yield pellet and supernatant fractions. These fractions were solubilized and processed through SDS-PAGE, and transfer and immunoblotting was performed with the anti-β-COP monoclonal antibody M3A5. In both control and BFA-treated tissue (Figure 6), β-COP was detected at levels comparable with those found in total homogenate, primarily in the 100 000 × *g* supernate rather than the pellet fractions. When lobules were incubated under N_2 before homogenization and fractionation, β-COP was also detected primarily in the supernate (Figure 6). When autoradiograms were overexposed, small amounts of β-COP were detected in comparable amounts in the pellet fraction of all treatment groups. Similar results were obtained using polyclonal anti-EAGE (Figure 7).

These data suggest that the large aggregates disperse during lobule homogenization even though homogenization was done under conditions that retain membrane-associated β-COP in rat liver and Vero cells (Duden *et al.*, 1991b). The existence of a soluble pool of

Figure 1. Distribution of β-COP in control (A–D), BFA-treated (E and F), and ATP-depleted (G and H) PAC. Phase contrast (left) and immunofluorescence (right) microscopy. (A and B) Control lobules labeled with the cis Golgi marker, gp58, showing the apical location of the Golgi in PAC and its typical ring- or horseshoe-like staining pattern (arrows). (C and D) In control lobules β-COP is concentrated in the Golgi region (arrows), but the ring structures are broader and more diffuse than for p58. Punctate staining is also seen throughout the cytoplasm. (E–H) After incubation with 7.2 μM BFA for 60 min (E and F) or under N_2 for 30 min (G and H), β-COP remains concentrated in the Golgi region but has a globular staining pattern (arrows). Semithin sections were incubated with anti-gp58 or anti-β-COP (EAGE) followed by FITC-conjugated secondary antibodies as indicated in MATERIALS AND METHODS. n, nucleus. Bar, 10 μM.

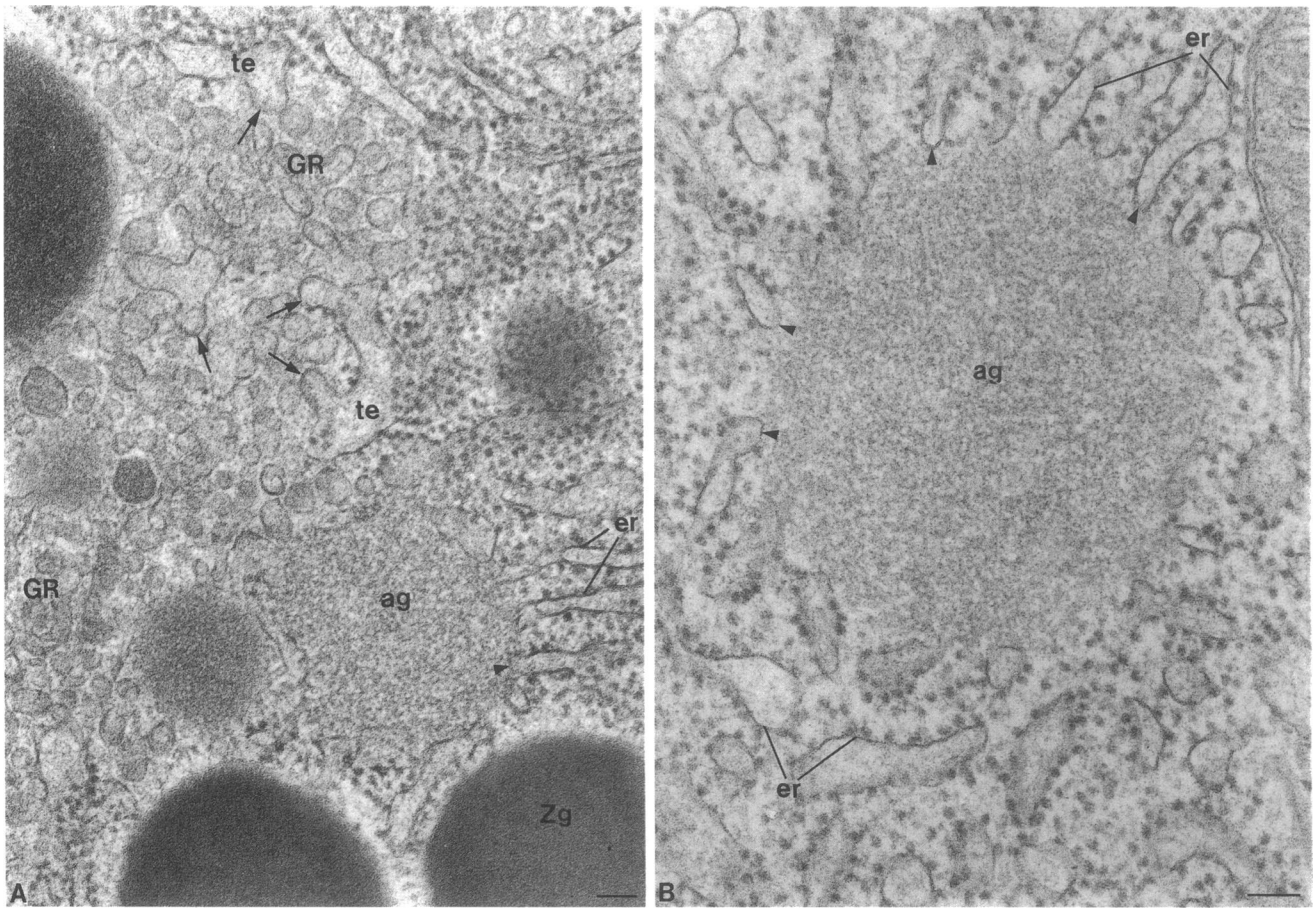


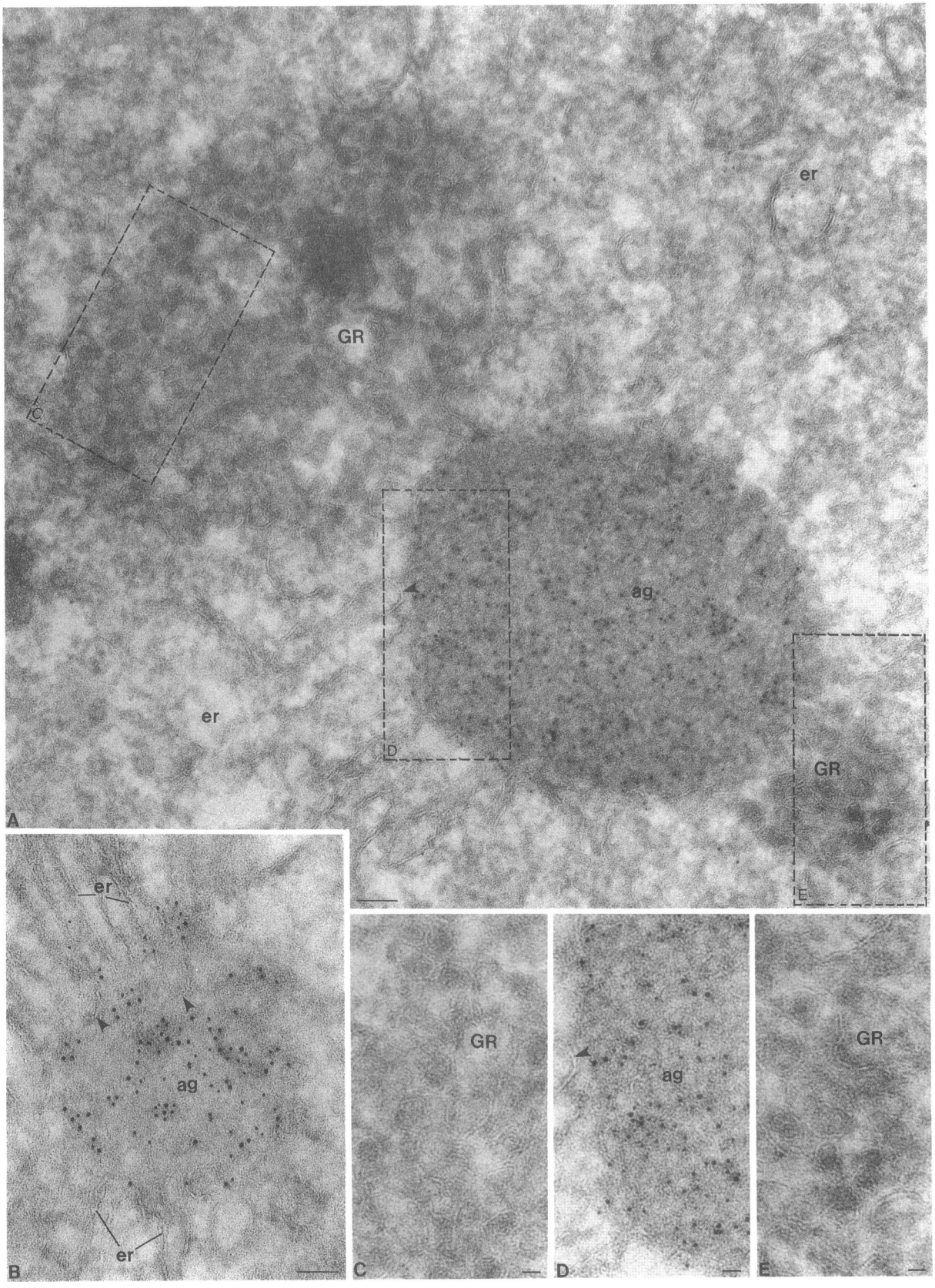
Figure 3. Plastic-embedded (Epon) sections from pancreatic lobules treated with BFA (7.2 μM, 60 min) and processed for routine electron microscopy. (A) Field showing a large fibrillar aggregate (ag) located in close proximity to both Golgi remnants (GR) and RER (er) cisternae. On one side, the aggregate faces a Golgi remnant composed of vesicles with transitional elements (te) of the ER at their periphery. The transitional elements are arranged with their smooth (ribosome-free) surface and vesicular protrusions (arrows) facing the Golgi remnants and their rough surface facing the RER. On the other side, the aggregate faces RER cisternae (er) arranged around it in spoke-like array. (B) Another aggregate showing that the surrounding RER cisternae (er) have ribosome-free ends that touch or extend into the aggregates (arrowheads). Bars, 0.1 μM.

β-COP cannot be ruled out, but its actual size remains to be determined.

DISCUSSION

We have examined the distribution of β-COP, one of the coat proteins of nonclathrin-coated vesicles, in PAC under conditions known to block early events in secretory protein transport. Using immunofluorescence and immunogold procedures, we have found that in controls β-COP is associated with Golgi cisternae and transport vesicles in agreement with previous localizations in cultured cell lines (Duden *et al.*, 1991b; Robinson and Kreis, 1992). After BFA treatment, which rapidly inhibits early events in protein transport in PAC (Hendricks *et al.*, 1992b) and other cell types (Takatsuki and Tamura, 1985; Misumi *et al.*, 1986; Magner and Papagiannes, 1988; Oda and Nishimura, 1989; Oda *et al.*, 1990), β-COP dissociates from Golgi membranes in PAC and is found at high concentration in large aggregates located in close proximity to Golgi remnants. These aggregates are associated with part-rough/part-smooth regions of

Figure 4. β-COP redistributes and forms large cytoplasmic aggregates after BFA treatment of PAC. Pancreatic lobules were treated with BFA as in Figure 3 and processed for immunogold labeling. (A) A large electron-dense aggregate (ag) that is heavily labeled for β-COP is seen in close proximity to Golgi remnants (GR). The latter consists of clusters of vesicles and tubules. The aggregates are typically surrounded by RER (er) cisternae (arrowheads) and contain membrane profiles. (B) Another cytoplasmic aggregate heavily labeled for β-COP is surrounded by ER cisternae (er) whose ribosome free ends (arrowheads) are in contact with the β-COP aggregates. (C–E) Enlargements of fields shown in A. Bars: A and B, 0.1 μM; C–E, 0.025 μM.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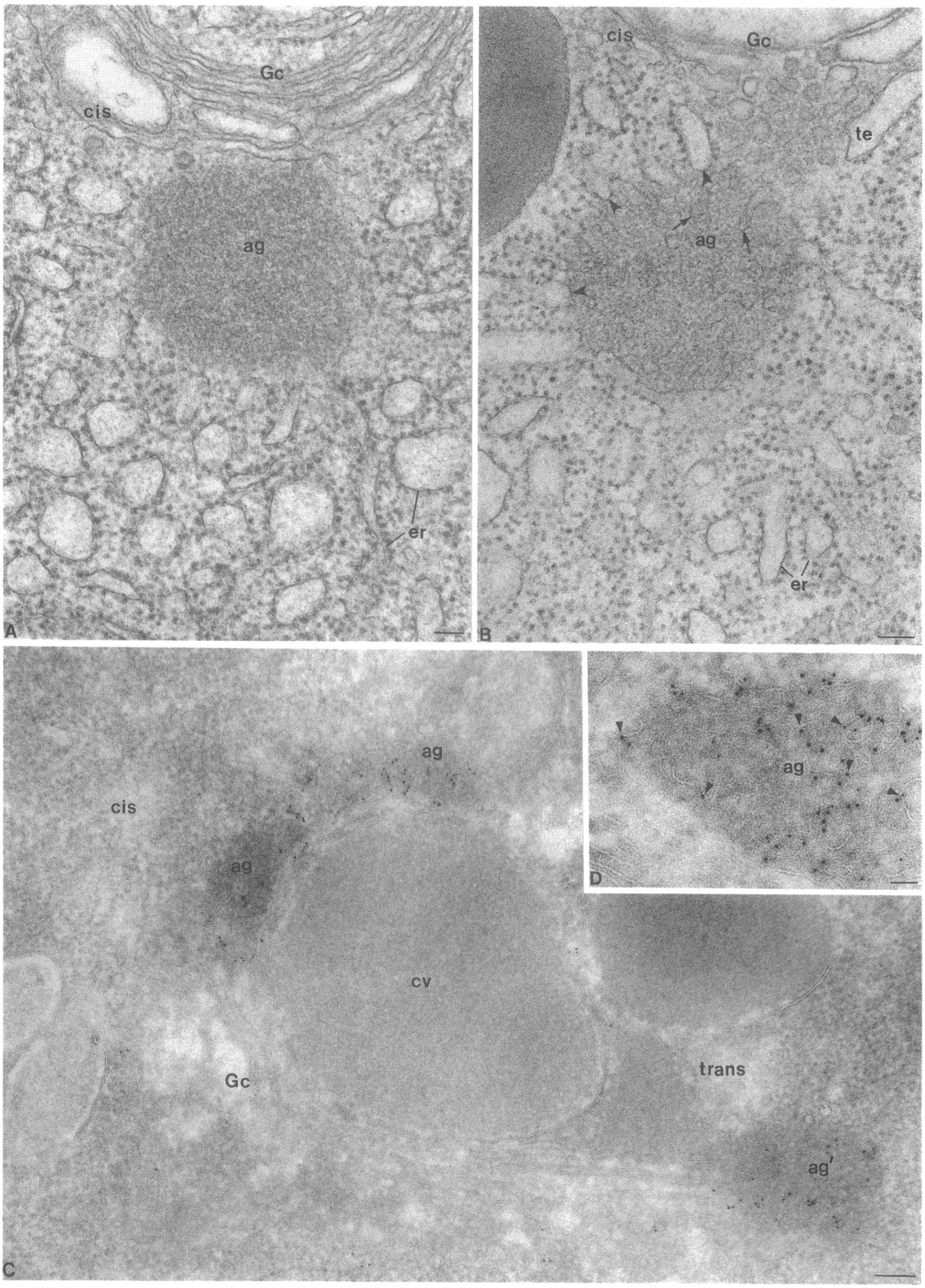
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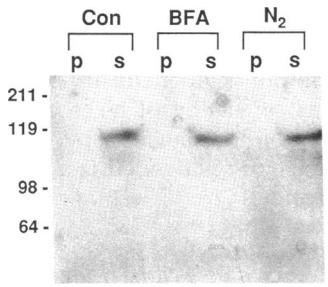

Figure 6. β-COP appears in the supernatant fraction in control, BFA, and ATP-depleted pancreatic lobules. Control, BFA- (7.2 μM, 60 min), and N_2-treated (30 min) lobules were homogenized in 0.3 M sucrose, and postnuclear supernatants were prepared and centrifuged (100 000 × *g*, 60 min) to yield pellet (p) and supernatant (s) fractions that were solubilized, separated by SDS-PAGE, and immunoblotted with monoclonal antibody M3A5. In all cases, β-COP is detected largely in the 100 000 × *g* supernatant.

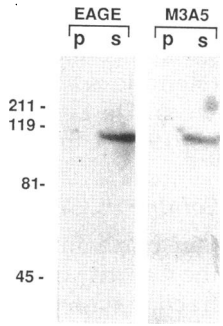

Figure 7. Immunoblot similar to that in Figure 6 prepared from BFA-treated (60 min) lobules. β-COP is detected primarily in the supernatant with both anti-EAGE polyclonal or M3A5 monoclonal antibodies.

RER distinct from typical transitional elements that are concentrated at the periphery of the Golgi remnants in PAC (Hendricks *et al.*, 1992a). The Golgi remnants themselves do not contain detectable β-COP. Under N_2, which curtails ATP synthesis and blocks protein transport presumably at the level of ER transitional elements (Jamieson and Palade, 1968), β-COP also accumulates in large cytoplasmic aggregates similar to those found after BFA treatment. Typically, the aggregates are closely associated or in direct contact with cis cisternae or the lateral ends of the Golgi stacks. These findings suggest that concomitant with the arrest of secretory protein transport, β-COP dissociates from Golgi membranes and self-associates into large cytoplasmic aggregates in both BFA-treated and ATP-depleted PAC.

The dense aggregates we observed in BFA-treated or ATP-depleted PAC are similar to the fibrillar aggregates previously reported to accumulate preferentially on the cis side of the Golgi complex in N_2- or dinitrophenol-treated PAC (Merisko *et al.*, 1986b). The fact that the aggregates frequently contained vesicle-free nonclathrin cages led to the assumption that they contain proteins that play a role in ER to Golgi transport of newly synthesized proteins (Merisko *et al.*, 1986b). We conclude that the fibrillar aggregates previously described correspond to the β-COP-containing aggregates identified in this study. In addition to β-COP, these aggregates may contain other proteins involved in vesicular transport such as ADP-ribosylation factor (ARF) and the Sec23p homologue. However, in other systems, it has been assumed that both β-COP and ARF, a GTP-binding protein associated with nonclathrin-coated vesicles (Orci *et al.*, 1991b), dissociate from Golgi membranes and are dispersed throughout the cytoplasm in BFA-treated and ATP-depleted cells (Donaldson *et al.*, 1991a,b). The mammalian homologue of Sec23p, a protein required for ER to Golgi transport in yeast (Kaiser and Schekman, 1990), was localized to electron-dense "transitional zones" in PAC (Orci *et al.*, 1991a) that were thought to represent the fibrillar masses described by Merisko *et al.* (1986b). Because both the BFA- and N_2-induced blocks in secretory protein traffic are rapidly (within minutes) reversible, the accumulation of aggregates containing β-COP (and presumably other coat proteins) in close association with Golgi remnants and Golgi stacks, respectively, may facilitate the efficient recruitment of coat proteins to budding sites (Merisko *et al.*, 1986b; Orci *et al.*, 1991b) or they may provide a scaffolding for Golgi membranes on resumption of vesicular transport (Duden *et al.*, 1991a).

It should be noted that in BFA-treated PAC, Golgi remnants persist and there is no detectable redistribution of Golgi proteins to the ER (Hendricks *et al.*, 1992a), even though β-COP dissociates from Golgi membranes. It follows that β-COP dissociation does not automatically lead to the return of Golgi membranes to the ER. In other systems, redistribution of Golgi markers such as α-mannosidase II has been detected by immunocytochemistry. This finding led to the assumption that the Golgi complex disappears and Golgi membrane proteins redistribute to the ER because retrograde transport continues whereas anterograde transport stops (Klausner

Figure 5. β-COP aggregates are also seen in ATP-depleted PAC. (A and B) Plastic-embedded sections from lobules treated with N_2 for 30 min, showing dense aggregates very similar to those observed after BFA treatment. These aggregates are typically associated with the cis side (cis) of the stacked Golgi cisternae (Gc), which remain intact after this treatment. ER elements comparable with those observed in proximity to BFA-induced aggregates have ribosome-free ends that face the aggregates (arrowheads, B). Smooth tubular membranes extend into one of the aggregates (arrows). A classical part-rough/part-smooth transitional ER element (te) is also present in proximity to residual transport vesicles which are uncommon in N_2-treated pancreas. (C) Immunogold labeling of ultrathin cryosection from a similar N_2-treated lobule. Two β-COP containing aggregates are seen, one on the cis side of the intact Golgi cisternae (ag) and the other on the lateral end of the stack (ag'). (D) Enlargement of another aggregate that is heavily labeled for β-COP. Membranous profiles are seen with the aggregate (arrowheads). cv, condensing vacuole; trans, trans side of Golgi stack; Gc, Golgi cisternae. Bars, 0.1 μM.

et al., 1992). However, by electron microscopy, residual Golgi membranes have been detected not only in PAC (Hendricks *et al.*, 1992a) but also in several other cell types (De Lemos-Chiarandini *et al.*, 1992; Hidalgo *et al.*, 1992; Ulmer and Palade, 1991).

Soluble vs. Sedimentable Pools of β-COP

Our biochemical results indicate that in control PAC, the majority of the β-COP is present in the supernatant fraction with only a very small amount associated with membranes. This is in accord with many but not all findings in other systems. In rat liver and Vero cells, a significant fraction of the β-COP sedimented with Golgi fractions in low ionic strength buffers (Allan and Kreis, 1986; Duden *et al.*, 1991b). However, 80% of the β-COP was recovered in a 125 000 × *g* supernatant on homogenization in the high ionic strength buffers used to isolate stacked Golgi membranes (Duden *et al.*, 1991b). In the cytosolic fraction, β-COP was found to be present in 13–14S complexes in both rat liver and Vero cells and as "coatomers," i.e., heteropolymers with other coat proteins (which precipitated at low pH) in brain cytosol (Waters *et al.*, 1991). When bovine brain cytosol (used as a provider of β-COP) was incubated with Golgi fractions derived from CHO cells in the presence of an ATP-generating system, the Golgi membranes bound a small amount of β-COP that was released in nonsedimentable form on BFA treatment (Donaldson *et al.*, 1991a). In the presence of membranes and GTPγS, only 10% of the total β-COP became sedimentable in 10 min at 14 000 × *g*. Thus, the majority of the findings to date suggests that there is a large soluble pool of β-COP and a variable but a much smaller pool of membrane-associated β-COP.

Dissociation of β-COP from Golgi Membranes on BFA Treatment

There is general consensus that in control (untreated) cells, staining for β-COP is concentrated in the Golgi region where it is associated with Golgi vesicles and cisternae (Allan and Kreis, 1986; Donaldson *et al.*, 1990; Duden *et al.*, 1991b; Robinson and Kreis, 1992). There is little agreement, however, as to the state of β-COP after BFA-induced dissociation from Golgi membranes. By immunofluorescence, β-COP was found distributed throughout the cytoplasm in a diffuse (Donaldson *et al.*, 1990, 1991a) or grainy (Robinson and Kreis, 1992) pattern in BFA-treated NRK and Vero cells. In our case, β-COP was concentrated in large aggregates located in the Golgi region after both BFA treatment and ATP depletion. This is despite the fact that very little β-COP was sedimentable in either controls or in PAC in which ER to Golgi transport was blocked. We can speculate that the aggregates represent cytoplasmic pools of coatomer complexes that self-associate after disruption of ER to Golgi transport. This is in keeping with the presence of vesicle-free globular cages seen previously in the aggregates (Merisko *et al.*, 1986b). Our findings indicate that by immunocytochemistry β-COP is localized to discrete areas consisting of large deposits, whereas by cell fractionation it appears dispersed in the cytosol fraction. Further work is needed to clarify the reasons for this discrepancy.

Two Different Types of Transitional Domains of the ER can be Distinguished After Transport Arrest

In ATP-depleted and BFA-treated PAC, we were able to identify two different types of transitional elements of the ER. One type, originally described in PAC (Palade, 1975; Jamieson and Palade, 1967a), consists of part-rough/part-smooth elements; typically their smooth membrane domains possess vesicular protrusions, presumably profiles of budding or fusing vesicles. These elements have been assumed to represent the site of exit of transport vesicles destined for the Golgi and/or intermediate compartment. In BFA-treated PAC, such elements are located at the periphery of the Golgi remnants and are oriented with their ribosome-studded surfaces facing out to the surrounding RER and their smooth surfaces with vesicular protrusions facing inward toward the vesicles and tubules of the Golgi remnants (Hendricks *et al.*, 1992a). The other part-rough/part-smooth ER elements described here can be distinguished from "classical" transitional elements by their morphology, location, and relationship to β-COP aggregates: they lack vesicular protrusions, typically surround the β-COP containing aggregates in a spoke-like pattern, and have ribosome-free ends that touch or extend into the aggregates. Occasionally, tubular profiles extend deeper into the aggregates. These two different transitional domains of the ER also can be recognized in ATP-depleted PAC where they have very similar or identical properties to those in BFA-treated PAC except that, as noted earlier (Merisko *et al.*, 1986b), in ATP-depleted PAC the classical transitional elements lack vesicular protrusions.

What is the nature of these two different transitional ER domains? It is tempting to speculate that one domain may correspond to the exit site for vesicles departing from the ER for the Golgi and/or intermediate compartment and the other might represent the site of entry of recycling vesicles returning from the Golgi. If so, which represents the exit and which represents the entry site? In the past, the classical transitional elements have been assumed to represent both the site of budding and the site of fusion of recycling vesicles returning to the ER. The second type of transitional ER domain is novel and has not been described previously. The fact that the latter are associated with tubular extensions might suggest that they correspond to the site of entry of recycling vesicles involved in retrograde transport from the Golgi to the ER, because long tubular structures

associated with retrograde transport have been seen in BFA-treated NRK cells (Lippincott-Schwartz *et al.*, 1990). Yet a reverse role cannot be excluded because these elements resemble those seen in stable transfectants of CHOE1 cells expressing rubella virus E1 glycoprotein (Hobman *et al.*, 1992). In these cells the presumptive ER exit site has been amplified by overexpression of E1. The latter accumulates at the exit site because it cannot leave the ER in the absence of the rubella E2 glycoprotein. At present, there are no available markers for these post-ER pre-Golgi membranes as they do not contain ER, Golgi, or intermediate compartment membrane proteins (Hobman *et al.*, 1992). Further investigation of the nature of these transitional domains awaits the development of appropriate markers. For the present it is intriguing to note that after BFA treatment, there is a sorting out of these transitional elements and that β-COP associates with one but not the other. In keeping with the original discovery of β-COP in association with taxol-polymerized microtubules, it may be that the aggregates maintain their close association with transitional elements and Golgi remnants through interactions with cytoskeletal elements.

In conclusion, under conditions where ER to Golgi transport is blocked, β-COP and other coat proteins dissociate from Golgi membranes, but their distribution appears to vary from cell type to cell type. The large aggregates of these proteins seen in PAC may reflect the large volume and high rate of secretory protein transport that characterizes these cells. The coat proteins are restricted in their distribution and remain in close proximity to both Golgi and ER elements, thereby being readily available for reassembly to these membranes on resumption of transport. Perhaps our most salient finding is that although β-COP dissociates from Golgi membranes and transport vesicles as a result of these treatments, Golgi membranes and transitional regions of the ER remain segregated from other membrane compartments, i.e., they do not disappear and Golgi membrane proteins do not mix with ER membranes in BFA-treated PAC.

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