

A Role for Tubular Networks and a COP I-independent Pathway in the Mitotic Fragmentation of Golgi Stacks in a Cell-free System

Tom Misteli and Graham Warren

Cell Biology Laboratory, Imperial Cancer Research Fund, London WC2A, 3PX, United Kingdom

Abstract. Golgi stacks were previously shown to be converted into tubular networks when incubated in mitotic cytosol depleted of the coatamer subunit of COP I coats (Misteli and Warren, 1994). Similar, though smaller, networks are now shown to be an early intermediate on the Golgi fragmentation pathway both in vitro and in vivo. Their appearance mirrors the disappearance of Golgi cisternae and at their peak they constitute 35% of total Golgi membrane. They are consumed by two pathways, the first involving the budding of COP I-coated vesicles described previously (Misteli

and Warren, 1994). The second involves a COP I-independent mechanism that leads eventually to a vesicle fraction that is larger in size and more heterogeneous than that produced by the COP I-mechanism. We suggest that both pathways operate concurrently at the onset of mitotic fragmentation. The COP I-independent pathway converts cisternae into tubular networks that then fragment. The COP I-dependent pathway partially consumes first the cisternae at the beginning of the incubation and then the tubular networks that form from them.

MEMBRANE traffic ceases when animal cells enter mitosis and this event is accompanied by fragmentation of the Golgi apparatus, the nuclear envelope and, to varying extents, the rest of the ER (Warren, 1993). The Golgi apparatus is converted into thousands of small vesicles that become randomly distributed throughout the mitotic cell cytoplasm (Zeligs and Wollman, 1979; Lucocq et al., 1989). Since the mother cell is then divided by cytokinesis into two, equally sized daughters (Rappaport, 1986), such extensive fragmentation of the Golgi apparatus will ensure nearly equal partitioning by a stochastic process (Birky, 1983).

We put forward a very simple model to explain the vesiculation of the Golgi apparatus based on the general inhibition of membrane traffic during mitosis in animal cells (Warren, 1985). COP I-coated vesicles bud from dilated cisternal rims (Weidman et al., 1993) and are involved in both anterograde (Rothman, 1994) and retrograde (Lettourneur et al., 1994; Pelham, 1994) transport through the Golgi apparatus. We proposed that, at the onset of mitosis, budding would continue but the vesicles formed would no longer be able to fuse with their target membrane. Since the membrane lost from a particular cisterna through budding is normally compensated by the fusion of an incoming vesicle, the necessary consequence of such an inhibition is

that the cisternal length will decrease and vesicles will accumulate.

Several lines of evidence support this hypothesis. The first is that intra-Golgi transport is inhibited during mitosis. In vivo, lactosyl-ceramide is synthesized in an early part of the Golgi apparatus and moves, by vesicle-mediated transport (Wattenberg, 1990), to a later part where it is converted into GA2 (GalNAc-lactosyl-ceramide). This conversion is inhibited in mitotic HeLa cells and only resumes once the cells have been allowed to enter telophase (Collins and Warren, 1992). In vitro, the transport of the G protein of vesicular stomatitis virus (VSV)¹, from the *cis* cisterna in one stack to the *medial* cisterna in another, is inhibited by mitotic cytosol. This inhibition is triggered, either directly or indirectly, by the mitotic kinase, p34^{cdc2} (Mackay et al., 1993; Stuart et al., 1993). The inhibited step is unknown but studies on other pathways show that either docking or fusion is inhibited. Endocytic vesicles and endosomes cannot fuse in a cell-free system under mitotic conditions (Tuomikoski et al., 1989; Woodman et al., 1993) and the secretory granules in a mast cell line cannot fuse with the plasma membrane during mitosis, even when secretion is triggered by added IgE (Hesketh et al., 1984).

The second line of evidence comes from a morphological analysis of the fragmentation process. Clusters of vesicles and free vesicles accumulate during the early phases of mitosis and have been shown to contain resident Golgi enzymes (Lucocq et al., 1987, 1989; Pypaert et al., 1993).

Dr. Misteli's current address is Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724.

Address correspondence to Graham Warren, Cell Biology Lab., Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A, 3PX, UK. Tel.: 0171 269 3561. Fax: 0171 269 3417.

1. Abbreviations used in this paper: GalT, β 1,4-galactosyltransferase; fPBS, fish skin gelatin in PBS; VSV, vesicular stomatitis virus.

Their diameter (47 ± 2.3 nm; Lucocq et al., 1987) is very similar to that of COP I-coated transport vesicles (~ 50 nm; Oprins et al., 1993) that have recently been shown to accumulate during the early phases of mitosis in FT210 cells when $[AIF_4]^-$ is added to prevent uncoating (Misteli and Warren, 1995). Furthermore, the accumulation of mitotic Golgi vesicles is accompanied by the predicted decrease in cisternal length (Misteli and Warren, 1995). These observations have been extended using a cell-free system that mimics many of the aspects of disassembly observed *in vivo*. Rat liver Golgi stacks are incubated with mitotic HeLa cytosol in the presence of an energy source, added in the form of ATP. Under these conditions, COP I-coated vesicles have been shown to bud and uncoat at the same rate as in interphase cytosol and also at the same rate as observed *in vivo* (Orci et al., 1986; Misteli and Warren, 1994). This strongly suggests that it is the docking or fusion of these vesicles with their target membrane that is altered under mitotic conditions as predicted by our hypothesis. The direct involvement of COP I-coats in the fragmentation process was confirmed using mitotic cytosol depleted of the coatomer subunit. No small vesicles were formed (Misteli and Warren, 1994).

The last, and indirect line of evidence comes from experiments in which mutated components of the vesicle-mediated transport machinery have been introduced in excess into interphase cells. The rab class of GTP-binding proteins are involved in the assembly of the SNARE complexes that determine the specificity of vesicle targeting (Lian et al., 1994; Sjøgaard et al., 1994). Rab1 is involved in the targeting of vesicles between the ER and Golgi and within the Golgi stack (Plutner et al., 1991). Microinjection of mutants that cannot bind GTP have been shown to vesiculate the Golgi apparatus most likely as a result of an inhibition of vesicle fusion (Nuoffer et al., 1994; Wilson et al., 1994). ARF (ADP-ribosylation factor) belongs to another class of GTP-binding proteins involved in vesicle-mediated transport and is a component of the COP I coat (Serafini et al., 1991). ARF1, bound to Golgi membranes in the GTP-bound form, recruits the other subunit, the coatomer, thereby assembling a COP I-coated bud (Orci et al., 1993). The Q71L mutant of ARF1 cannot hydrolyze bound GTP so the COP I-coated vesicles that form cannot uncoat (Tanigawa et al., 1993). Over-expression of this ARF1 mutant has been shown to fragment the Golgi apparatus most likely as an indirect consequence of an inhibition of fusion (Zhang et al., 1994).

There are, however, several observations that challenge this simple view of the Golgi fragmentation process. The first is that incubation of Golgi stacks with mitotic cytosol depleted of coatomer had the unexpected effect of converting the stacks into extensive tubular networks (Misteli and Warren, 1994). The significance of these networks during normal fragmentation (in the presence of coatomer) was not appreciated at that time because thin Epon sections mostly rendered these networks as tubular and vesicular profiles. We now show, using thicker sections and quantitative analysis, that these networks are important intermediates on the fragmentation pathway both *in vitro* and *in vivo*.

The second observation is that about one third of Golgi membrane could not be consumed by the COP I-mediated

budding mechanism (Misteli and Warren, 1994). We now show that this is not a technical limitation of the assay but represents a COP I-independent pathway for fragmentation. The intermediate tubular networks are broken down by both pathways yielding a population of transport vesicles and a larger, more heterogeneous population of vesicles. This explains the third observation, that cell-free fragmentation gives rise, not to one, but to two populations of vesicles (Misteli and Warren, 1994).

Materials and Methods

Materials

All reagents were of analytical grade or higher and purchased from Sigma or BDH UK unless otherwise stated. All electron microscopy reagents and accessories were purchased from Agar Scientific (Stansted, UK) unless otherwise stated.

Incubations

Rat liver Golgi membranes were prepared as described in Slusarewicz et al. (1994). Interphase and mitotic cytosols were prepared as described previously (Stuart et al., 1993; Misteli and Warren, 1994). Cell-free incubations were performed as described in Misteli and Warren (1994), except that they were scaled up fourfold to give enough material for electron microscopy. Briefly, cytosolic proteins were transferred to MEB buffer (50 mM Tris-HCl, pH 7.3, 50 mM KCl, 10 mM $MgCl_2$, 20 mM β -glycerophosphate, 15 mM EGTA, 2 mM ATP, 1 mM DTT) just before use by application to a 1.5 ml Bio-Rad P6-DG (Bio-Rad, Hemel Hempstead, UK) spin column. A typical assay comprised 20 μ l of a $10\times$ concentrated ATP regenerating system (100 mM creatine phosphate, 10 mM ATP, 0.2 mg/ml creatine phosphokinase), 20 μ l 2 M sucrose in H_2O , 10 μ l Golgi membranes (100 μ g/ml final concentration), and 150 μ l cytosol (7.5–10 mg/ml final concentration). In some experiments GTP γ S (Boehringer Mannheim, East Sussex, UK) was added from a freshly prepared 2 mM stock in MEB buffer to give a final concentration of 20 μ M. All incubations were carried out in borosilicate glass tubes and incubations performed in a waterbath set at 37°C.

For some experiments, coatomer was depleted from cytosol by use of the anti-coatomer antibody CM1A10 (Orci et al., 1993) exactly as described previously (Misteli and Warren, 1994). In coatomer readdition experiments the final concentration of coatomer was 52 μ g/ml, which resulted in equally efficient budding of COP I-coated vesicles from membranes as in the presence of complete interphase cytosol (Orci et al., 1993).

Sedimentation Assay

The relative distribution of β 1,4-galactosyltransferase activity in the supernatant and pellet of a sample spun at 2,000 g_{av} for 10 min at 4°C was taken as a measure of the extent of fragmentation as described previously (Misteli and Warren, 1994). The activity of β 1,4-galactosyltransferase was determined as described by Bretz and Staubli (1977).

Synchronization of HeLa Cells

HeLa cells were grown at 37°C in DMEM medium (GIBCO BRL, Gaithersburg, MD) supplemented with 10% FCS, 200 mM glutamine, 50 IU/ml penicillin, 50 mg/ml streptomycin, and 1% nonessential amino acids, in an atmosphere of 5% $CO_2/95\%$ air. For G2/M arrest, cells were first grown for 24 h in 2.5 μ g/ml aphidicolin (Sigma) to prevent entry into S phase. The cells were then washed three times with fresh medium and grown for 8 h in normal medium. This was replaced with medium containing 200 μ g/ml and cells were grown for 10 h at 37°C. To permit entry into mitosis, the medium was removed and the cells washed five times in fresh medium, before incubation for 105 min (Misteli and Warren, 1995). Cells were then fixed on the dish and processed for electron microscopy as described below.

Electron Microscopy

Synchronized HeLa cells were fixed on the dish for 3 h with 2% paraformaldehyde, 0.2% glutaraldehyde (Fluka AG, Buchs, Switzerland), 0.2 M

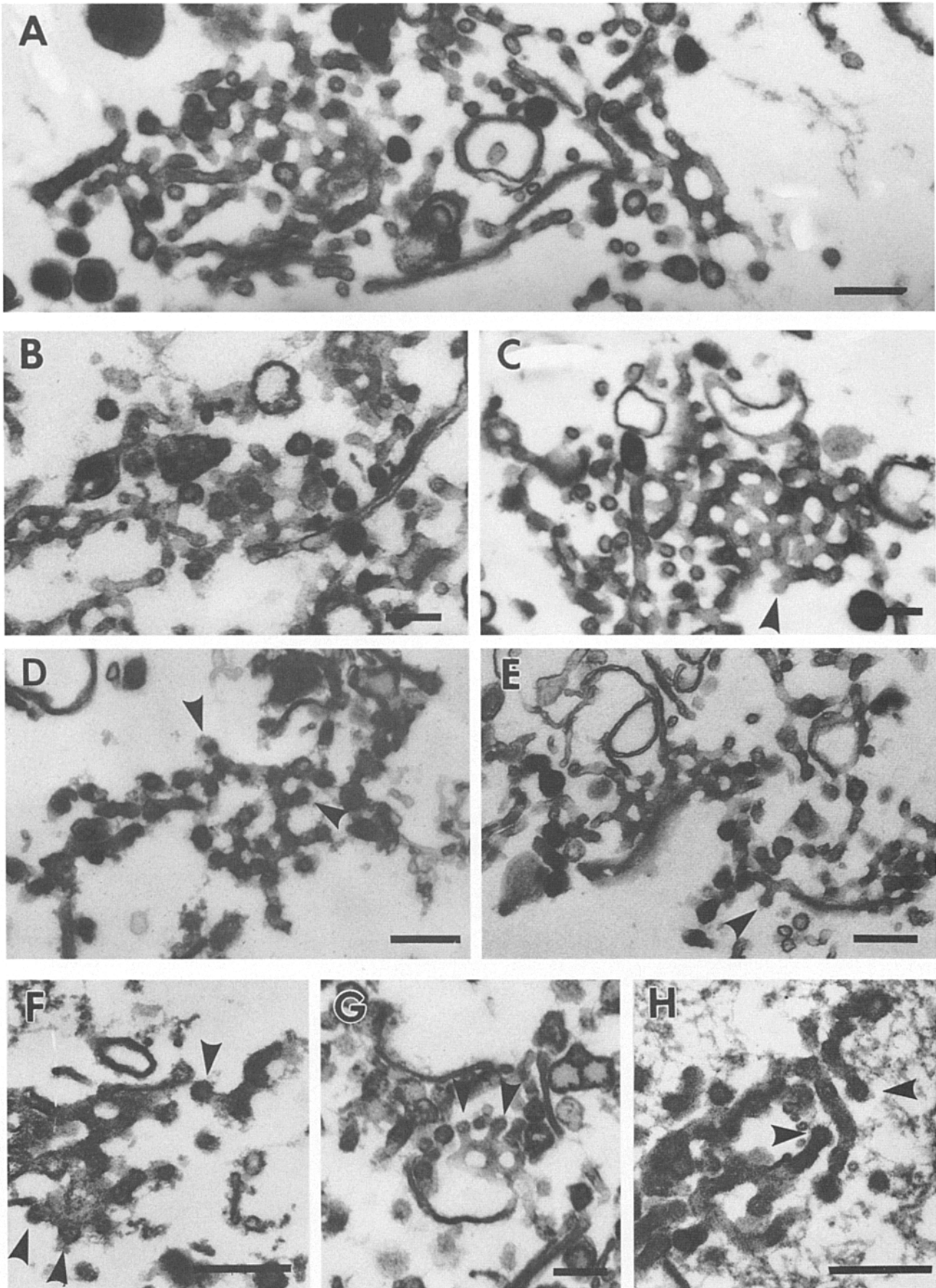


Figure 1. Morphology of tubular networks during normal cell-free fragmentation. Rat liver Golgi membranes were incubated with mitotic cytosol for 20–30 min then fixed and processed for Epon embedding using 80–100-nm-thick sections. A gallery of tubular networks is presented. Note the COP I-coated buds (C–H, arrowheads). Bar, 200 nm.

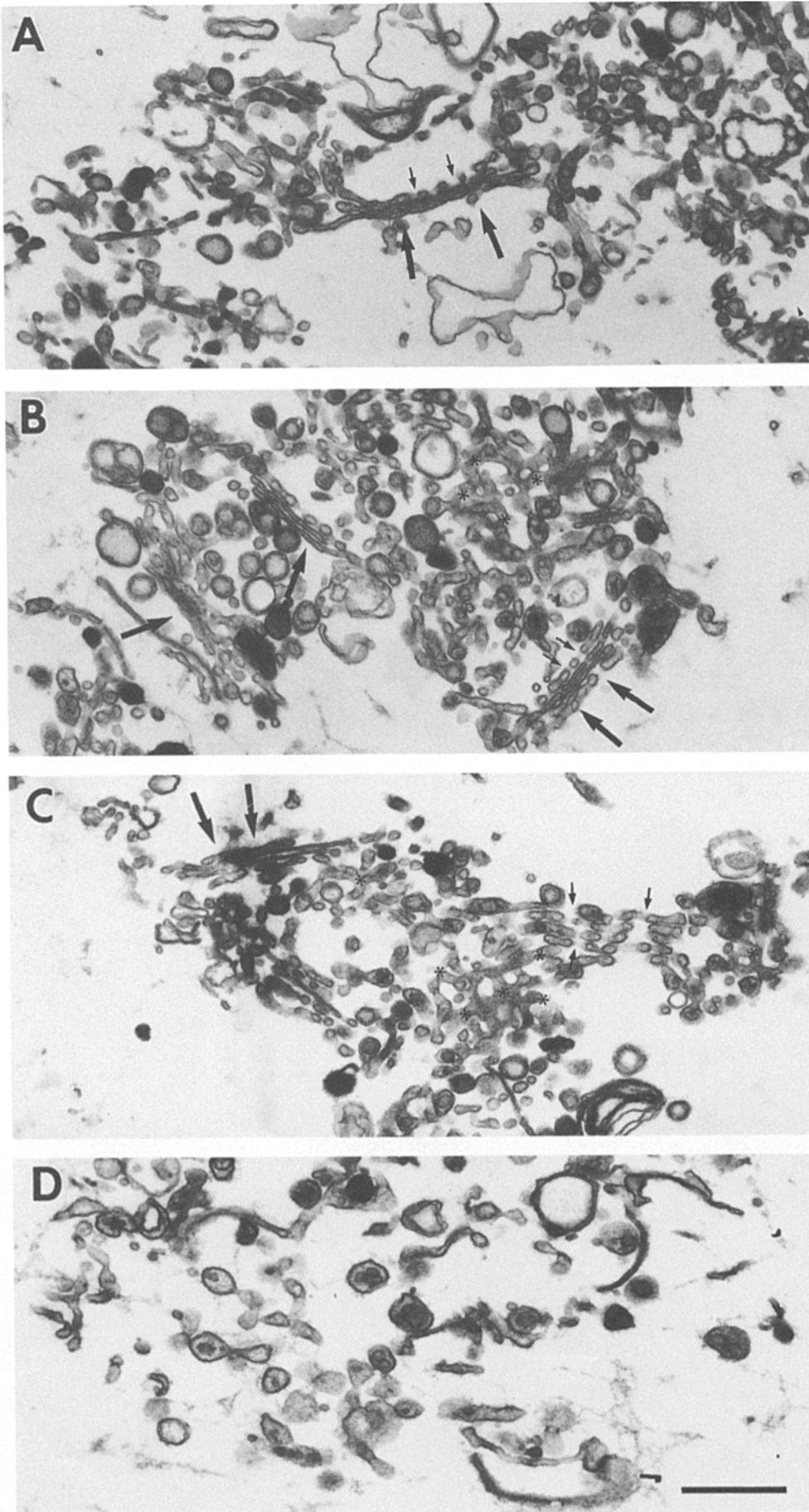


Figure 2. Tubular networks as intermediates during normal cell-free fragmentation. Golgi membranes were incubated with mitotic cytosol for (A) 10 min; (B) 20 min; (C) 30 min; or (D) 40 min, then fixed and processed for Epon embedding using 80–100-nm sections. The Golgi stacks (*large arrows*) became increasingly fenestrated between 10 and 30 min (*small arrows*) and had disappeared by 40 min. Tubular networks (*asterisks*) appeared by 20 min and had disappeared by 40 min. Bar, 1.0 μm .

sucrose in 0.1 M Na-phosphate buffer, pH 7.2, at RT. Cells were then scraped using a rubber policeman and pelleted at RT for 15 min at 14,000 g in the Eppendorf centrifuge fitted with a horizontal rotor. The pellets were washed three times in PBS and postfixed in 1% osmium tetroxide, 1.5% cyanoferrate in 0.1M cacodylate buffer, pH 7.2, then dehydrated and embedded in Epon 812. Transverse sections through the entire pellet were cut on a Reichert ultramicrotome 2E set to 50–70 nm, picked up on a nickel grid and stained for 6 min with 2% uranyl acetate and for 2 min with lead citrate (Roth and Berger, 1982).

Golgi membranes after incubation were collected by centrifugation at top speed (14,000 g_{av}) for 20 min at 4°C in an Eppendorf table top centrifuge 5413 fitted with a horizontal rotor. For Epon embedding, the pellets were routinely fixed at room temperature for 30 min with 1% glutaraldehyde (Fluka, Gillingham, UK) in 0.1 M KH_2PO_4 -buffer, pH 6.7, 0.2 M sucrose, washed extensively with PBS, postfixed for 30 min with 1% osmium tetroxide, 1.5% cyanoferrate in 0.1 M cacodylate buffer, pH 7.2, and further treated with 1% tannic acid (Mallickrodt Inc., Paris, Kentucky) in 0.1 M cacodylate buffer for 30–60 min using the method of Simionescu and Simionescu (1976). Samples were then washed for 10 min in 1% Na_2SO_4 , dehydrated and embedded in the test-tube in Epon 812 (Taab Laboratories, Reading, UK). To ensure reproducible sampling for quantitation, the pellets were never dislodged from the bottom of the test tube during the embedding procedure and sections were always cut at the same position in the block. Transverse sections through the entire pellet were cut on a Reichert ultramicrotome 2E set to 50 nm or 80–100 nm as indicated in the text. Sections were picked up on a nickel or copper grid and stained with 2% uranyl acetate and lead citrate (Roth and Berger, 1982).

For cryoelectron microscopy, membranes were collected and either fixed in the same way as described or membranes were fixed overnight at 4°C in 1% paraformaldehyde in 0.1 M KH_2PO_4 -buffer, pH 6.7, 0.2 M sucrose. After fixation pellets were embedded in 10% gelatin in H_2O , cut into small blocks and infiltrated with 2.1 M sucrose in PBS overnight at 4°C. Blocks were cut on a Reichert ultramicrotome 2E with a nominal setting of 50–70 nm at –95°C and sections were collected on collodion/carbon coated copper grids. Sections were stained for 8 min at room temperature in 2% uranyl acetate and contrasted for 15 min with 2% methylcellulose, 0.4% uranyl acetate on ice.

For immunogold labeling, ultrathin-frozen sections of paraformaldehyde-fixed samples were prepared as described above and grids were incubated at room temperature in a moist chamber for 10 min on a drop of 50 mM NH_4Cl in PBS. Nonspecific binding was pre-blocked by incubation with 0.5% fish skin gelatin in PBS (fPBS) for 7 min. Sections were labeled with anti- ϵ -COP antibody (Hara-Kuge et al., 1994) at a 1:500 dilution in fPBS for 20 min, washed on six drops of fPBS over 15 min, and incubated with Protein A 10-nm gold (obtained from Drs. Slot and Geuze, Dept. of Cell Biology, University of Utrecht, The Netherlands). Sections were washed for 10 min in PBS and for 30 min in H_2O before staining with 2% uranyl acetate for 8 min at room temperature followed by incubation on a drop of 1.5% methylcellulose for 15 min on ice. Methylcellulose was removed using a filter paper and the grids air-dried.

All sections were observed in a Phillips CM10 electron microscope.

Stereological Definitions

Golgi cisternae were defined as membrane profiles with a cross-sectional length more than four times their width, the latter being not more than 60 nm. Golgi stacks were defined as membrane profiles comprising two or more cisternal profiles separated by a gap of no more than 15 nm and overlapping by more than half their cross-sectional length. Vesicles were defined as circular or oval membrane profiles with an average diameter of 30–200 nm where the ratio of the longest diameter of the profile to the longest diameter perpendicular to it was ≤ 1.5 . The average diameter of the profile was taken as the average of these two measurements. Tubules were defined as membrane profiles with a length of more than 1.5 times but less than four times their maximal width. On rare occasions, profiles with a width of more than 60 nm were observed that had a length more than four times this width. These were classified as tubules. Tubular networks were defined as tubular profiles with at least one branch point or, alternatively, as two or more intersecting tubular profiles. COP I-coated vesicles or buds were identified based on their morphological appearance. In cryosections, they were small vesicular profiles, typically 30–70 nm in diameter, or a bud with a characteristic non-clathrin, cytoplasmic coat ~ 10 nm in thickness (Orci et al., 1986; Oprins et al., 1993). In Epon sections, COP I-coated vesicles were visualized using the tannic acid method

(Simionescu and Simionescu, 1976; Orci et al., 1986). The coat was more fuzzy and slightly thicker than that seen in cryosections.

Stereological Methods

Sections of membranes were selected at low magnification in a systematic random fashion, photographed and printed to a final magnification of 52,500–95,000. For each experiment at least 20 Golgi regions were examined and results expressed as means from three to five experiments \pm SEM.

To determine the percentage distribution of a particular membrane class (cisternae, vesicles, COP I-coated vesicles, tubules, or tubular networks) photographs were overlaid with a lattice (10 mm spacing) or a line grid (30 mm spacing) on an overhead transparency. Quantitation was performed using a point-hit method (Weibel et al., 1969). The number of intersections (I_x) of a membrane profile with a test line was counted and the membrane structure assigned to one of the defined membrane classes. All membrane profiles on a photograph were counted except clearly identifiable contaminants such as nuclei, mitochondria or plasma membrane (which totaled less than 5% of all profiles). The total number of all intersections in all categories (I_t) represented 100% of total membrane. The percentage of membrane in each category (P_x) was determined as $P_x = \{ \Sigma I_x / I_t \} \times 100$.

To determine the diameter distribution of vesicular profiles (COP I-coated or uncoated), the longest diameter of the profile and the longest diameter perpendicular to it were measured with a ruler using sets of photographs from five experiments with a final magnification ranging between 52,500–92,000. The two diameter values were used to determine the average diameter of the profile and to calculate the ratio of the longer to the shorter diameter as an indicator of the circularity of the profile. The diameter distribution was expressed in 10-nm intervals, since in the lowest magnification photographs this represented ~ 0.5 mm, the smallest unit readable on the ruler.

Results

Tubular Networks as Intermediates during Mitotic Fragmentation

Mitotic fragmentation of the Golgi apparatus was mimicked in the test-tube by incubating highly purified rat liver Golgi membranes with mitotic cytosol derived from spinner HeLa cells arrested for one cell cycle by the addition of 100 ng/ml nocodazole (Misteli and Warren, 1994). In the presence of ATP and a regenerating system, Golgi

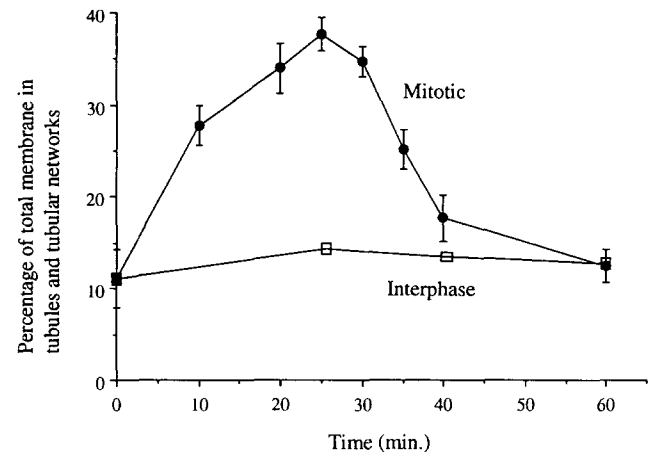


Figure 3. Quantitation of tubular profiles and tubular networks during normal fragmentation. Golgi membranes were incubated with interphase or mitotic cytosol for up to 60 min, fixed, and processed for Epon embedding. The amount of tubular profiles and tubular networks is expressed as a percentage of total membrane and results are presented as the mean \pm SEM of four experiments.

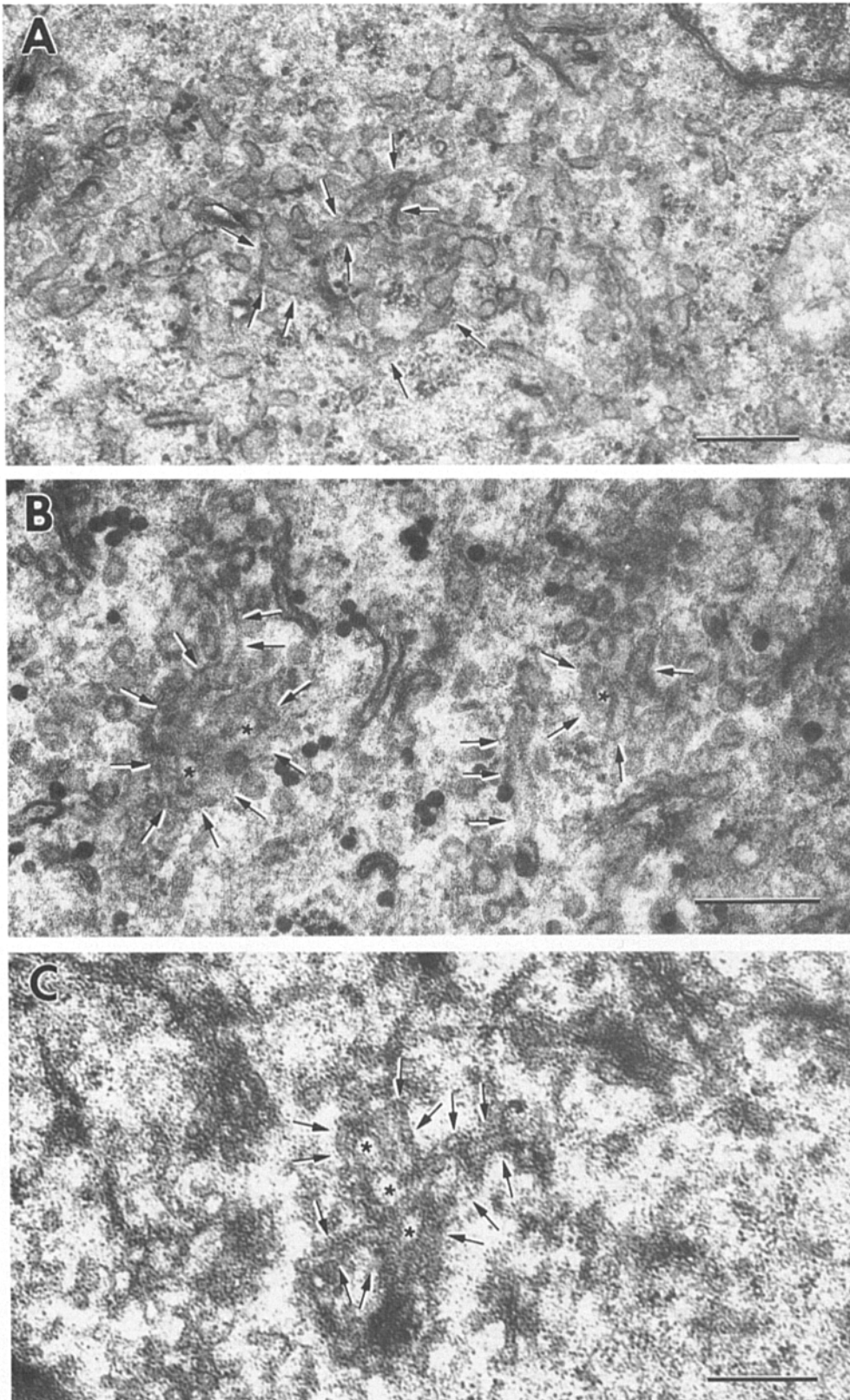


Figure 4. Tubular networks in mitotic HeLa cells. HeLa cells were synchronized at the G₂/M boundary using aphidicolin and olomoucine and then allowed to enter mitosis for 105 min. Sections through Golgi clusters in mid-prophase cells are shown. (A) Tubular profiles (arrows) not typically seen in interphase cells. (B and C) Two small tubular networks (arrows) containing fenestrations (asterisks) ~80 nm in diameter. Bar, 0.5 μ m.

stacks were converted within 60 min into membrane fragments indistinguishable from those found in Golgi clusters *in vivo* (Lucocq et al., 1987).

When the coatamer subunit of COP I coats was quantitatively removed from mitotic cytosol by immunodeple-

tion, Golgi stacks were converted into extensive tubular networks (Misteli and Warren, 1994). To determine whether these were part of the normal fragmentation process, Golgi stacks were incubated for 20–30 min in mitotic cytosol and samples fixed and prepared for conventional Epon

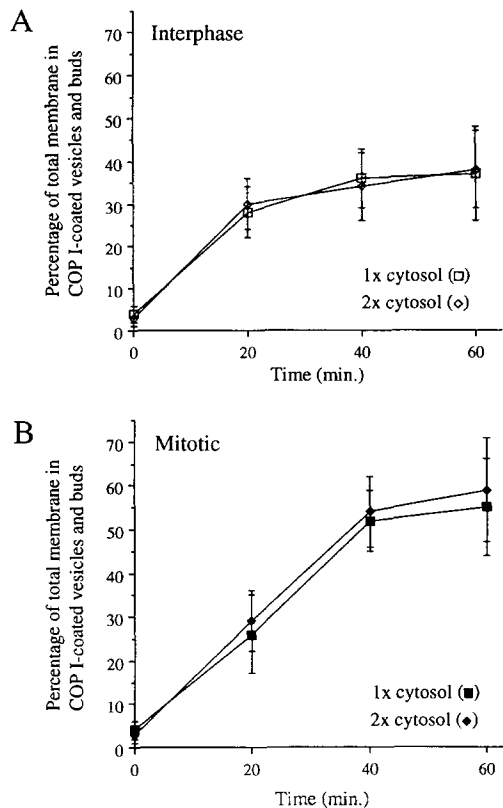


Figure 5. Consumption of Golgi membranes by COP I-coated vesicles. Golgi membranes were incubated with either (A) Interphase or (B) Mitotic cytosol for up to 60 min in the presence of 20 μM GTP γS . The ratio of cytosolic protein to Golgi membrane protein was either 100:1 (1 \times cytosol) or 200:1 (2 \times cytosol). Samples were then fixed, processed for cryoelectron microscopy and the percentage of membrane that was COP I-coated estimated using the point-hit method. Results are presented as the mean \pm SEM from three experiments.

microscopy. Thick sections (80–100 nm) were cut so as to permit better visualization of tubular networks and a representative selection of images is presented in Fig. 1. A typical network was made up of tubular profiles with a

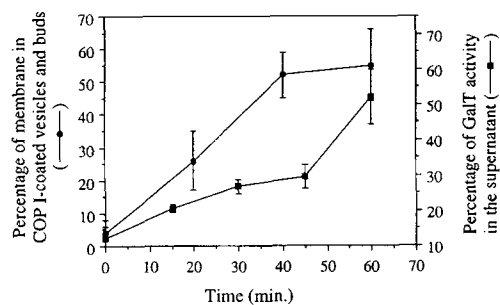


Figure 6. Time course of fragmentation. Golgi membranes were incubated with mitotic cytosol for up to 60 min in the absence (sedimentation assay) or presence (cryoelectron microscopy) of 20 μM GTP γS . Samples taken at different time points were either centrifuged at low speed and the percentage of GalT in the supernatant measured, or fixed, processed for cryoelectron microscopy, and then the percentage of COP I-coated membrane estimated. Results are presented as the mean \pm SEM from three (COP I-coated membranes) or five (GalT) experiments.

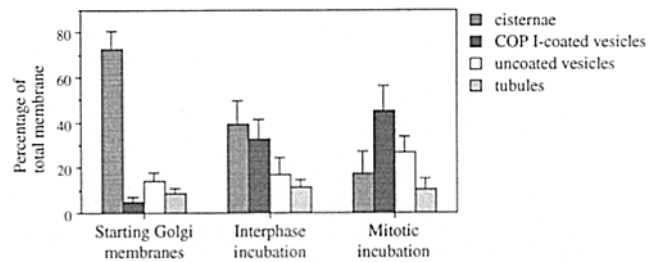


Figure 7. End products of cell-free fragmentation. Golgi membranes were incubated for 60 min with either interphase or mitotic cytosol in the presence of 20 μM GTP γS . Samples were then fixed and processed for cryoelectron microscopy. The percentage of total membrane in each category of membrane was estimated using the point-hit method. Results are presented as the mean \pm SEM from three experiments.

cross-sectional width of 50–80 nm, and branch points about 50–100 nm apart. Blind-ending tubules were frequently seen and often appeared to be coated (Fig. 1, C–H, arrowheads). The size of the fenestrations varied widely but on average appeared to be larger than the fenestrations that typically characterize the cisternal rims in interphase Golgi stacks (Weidman et al., 1993). The networks also differed from cisternae in that they were not planar structures but had what appeared to be a complex three-dimensional morphology. The morphology of these networks was identical when either paraformaldehyde alone or glutaraldehyde was used as the fixative.

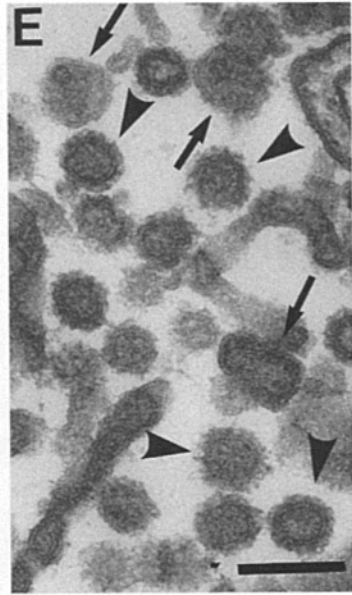
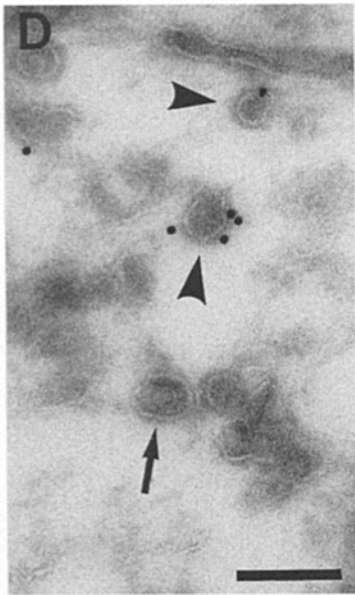
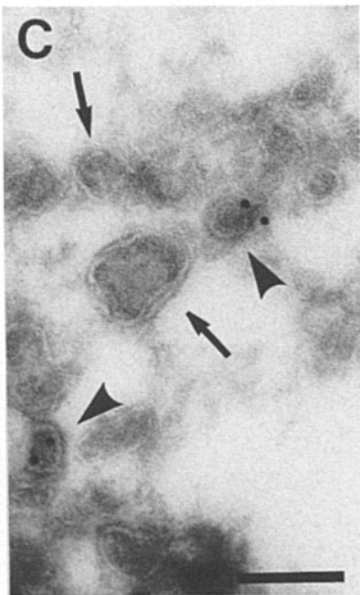
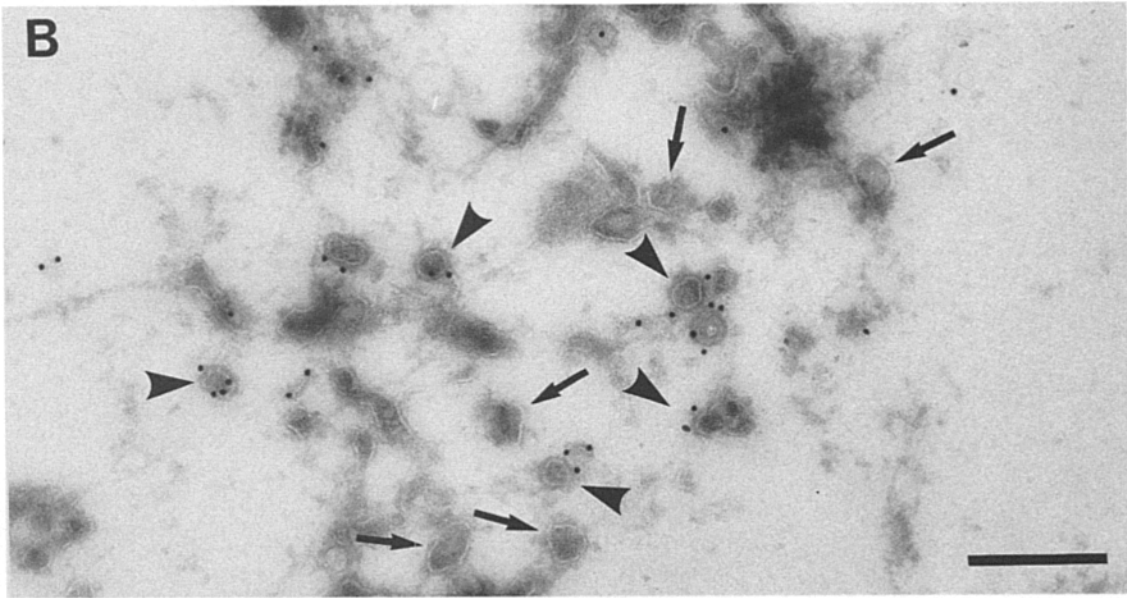
The transient nature of these networks during normal fragmentation is illustrated in Fig. 2 and quantitated in Fig. 3. Tubules were included in this quantitation since it was not possible to determine whether they were free or part of the tubular networks but at all times they constituted a minority of the population.

Networks began to appear within the first 10 min of incubation, most often at the periphery of cisternae. Cisternae on the *cis* and *trans* faces of the stack became increasingly fenestrated (Fig. 2, A and B, small arrows). Between 20 and 30 min of incubation, the networks were prominent (Fig. 2, B and C, asterisks) and constituted \sim 35% of total membrane (Fig. 3). From 30 to 40 min, the percentage of membrane in tubular networks fell to background levels (\sim 15% of total membrane) (Fig. 3) leaving remnants in the form of short tubules that were, occasionally, branched

Table 1. Change in the Percentage Distribution of Membranes after Incubation of Golgi Stacks with Mitotic Cytosol

	Cisternae	COP I-coated vesicles	Uncoated vesicles	Tubules
Change after incubation of Golgi membranes with:	%			
Interphase cytosol	-33.1	+28.0	+2.6	+3.0
Mitotic cytosol	-54.7	+40.6	+12.7	+2.0

Golgi membranes were incubated for 60 min with mitotic cytosol in the presence of 20 μM GTP γS . Samples were then fixed and processed for cryoelectron microscopy. The percentage of total membrane in each category of membrane was estimated using the point-hit method and the values presented are the differences between the means for the starting material and the 60-min time point.



(Fig. 2 D). The formation of tubules and small tubular networks was specific for the mitotic conditions, since there was no increase in the amount of membrane in tubular profiles and tubular networks over the same time period in the presence of interphase cytosol (Fig. 3).

Tubular networks were also identified *in vivo* in HeLa cells synchronized using a specific inhibitor of cyclin-dependent kinases. Olomoucine, a purine analogue, specifically inhibits p34^{cdc2} and p34^{cdk2} (Abraham et al., 1994; Glab et al., 1994; Vesely et al., 1994) and so arrests cells at the G1/S and G2/M boundaries. To block cells specifically at the G2/M boundary, HeLa cells were first incubated for one cell cycle with aphidicolin to prevent entry into S-phase. Removal of this drug for 8 h permitted passage through S phase and into G2. Addition of olomoucine for 10 h arrested ~85% of cells at the G2/M boundary. Removal of olomoucine then allowed reasonably synchronous passage of HeLa cells into mitosis (data not shown).

Fig. 4 shows sections of 80 nm nominal thickness through the Golgi region of cells in mid-prophase. Tubular profiles were clearly visible having a cross-sectional length of more than 200 nm and a width of 80–100 nm. At least some of these tubules were part of small tubular networks with fenestrations that were typically 80–100 nm in diameter (Fig. 4, B and C). These tubules and tubular networks were likely derived from the fragmenting Golgi apparatus since serial sectioning confirmed that they were mostly found in the centre of Golgi clusters. They were not seen in interphase cells nor at earlier or later stages of mitosis. They were not as prominent as those seen during fragmentation *in vitro*. This probably reflects the faster breakdown of the Golgi apparatus *in vivo* and the difficulty of tracing tubular connections in sections through fixed cells.

Together these data show that tubular networks are intermediates during Golgi fragmentation both *in vitro* and *in vivo*. Since their appearance mirrors the disappearance of cisternae, they are likely a major initial product of cisternal breakdown.

A COP I-independent Pathway for Mitotic Fragmentation

In the previous study, COP I-coated vesicles were shown to form and uncoat at the same rate under interphase and mitotic conditions (Misteli and Warren, 1994). This led us to suggest that the observed accumulation of vesicles in mitotic cytosol was likely the consequence of their inability to dock or fuse with their target membranes. Continued incubation in the presence of GTP γ S (to prevent uncoating) showed that 65 \pm 9% of the Golgi membrane could be incorporated into COP I-coated membranes under mitotic conditions. At that time, however, it was not clear whether the incomplete conversion of membranes into COP I-coated vesicles pointed to another mechanism, needed to complete the fragmentation process, or whether

it simply reflected a technical limitation of the cell-free system.

To address this problem, a more detailed analysis of the appearance of COP I-coated vesicles was carried out and the results are presented in Fig. 5. After 20 min incubation in the presence of GTP γ S, the percentage of COP I-coated membrane was almost the same in both interphase (28 \pm 6%) and mitotic (26 \pm 9%) cytosols, confirming earlier studies (Misteli and Warren, 1994). In interphase cytosol, the percentage began levelling off after 20 min reaching a plateau of 36 \pm 7% by 40 min of incubation (Fig. 5 A). Further consumption was most likely prevented because stacked cisternae are stable in interphase cytosol (Misteli and Warren, 1994). Complete unstacking occurs in mitotic cytosol and the percentage of COP I-coated vesicles and buds increased almost linearly up to 40 min of incubation and only then leveled off so that by 60 min the percentage of membrane consumed was 55 \pm 11% (Fig. 5 B). This value was similar to that observed previously (65 \pm 9%; Misteli and Warren, 1994). In neither case was the leveling off the consequence of a limitation in the amount of cytosolic coat or other components. When the ratio of cytosol to membranes was doubled, keeping the concentration of cytosol constant, the production of COP I-coated membranes followed almost exactly the same time course (Fig. 5).

These data argue that about 35–45% of Golgi membrane is consumed by a COP I-independent pathway. There is, however, still the possibility that the COP I-mediated mechanism is the sole device for consuming Golgi membranes and those membranes that remained at the end of the incubation had not undergone further fragmentation. Two lines of evidence argue against this. The first comes from a biochemical assay we devised to measure the fragmentation process (Misteli and Warren, 1994). This exploits the fact that fragmented Golgi membranes sediment less rapidly than the starting Golgi stacks. After incubation with cytosol, the mixture is centrifuged at low speed and the mitotic Golgi fragments that remain in the supernatant are measured by assaying for the resident Golgi enzyme, β 1,4-galactosyltransferase (GalT). The GalT activity in the supernatant was present within membrane vesicles because more than 85% could be sedimented by subsequent medium speed centrifugation and more than 75% was resistant to digestion with trypsin (data not shown). As shown in Fig. 6, the appearance of mitotic Golgi fragments in the supernatant was not a linear process. After 45 min incubation, 30 \pm 3% of the GalT was in the supernatant, but this increased sharply to 52 \pm 8% over the next 15 min. The pattern and extent of fragmentation was the same in the absence or presence of 20 μ M GTP γ S. Importantly, this 22% increase occurred over a time period when the percentage of COP I-coated vesicles and buds only increased by 3%, from 52 \pm 7% to 55 \pm 11% (Fig. 6). In other words, after the COP I-mediated budding mechanism had essentially finished consuming

Figure 8. Morphology of the mitotic fragmentation end products. After incubation with mitotic cytosol for 60 min in the presence of 20 μ M GTP γ S, Golgi membranes were fixed and processed either for cryoelectron microscopy (A–D) or for Epon embedding (E). Sections in B–D were labeled with antibodies against ϵ -COP followed by protein A coupled to 10 nm gold. Note COP I-coated (arrowheads) and uncoated (arrows) vesicular profiles. Bar, 250 nm (A and B) or 150 nm (C–E).

Golgi membranes, the membrane that was left still underwent a fragmentation process that resulted in a decrease in its sedimentability.

The second line of evidence comes from an analysis of the end-products of the fragmentation process. Golgi membranes were incubated with either interphase or mitotic cytosol for 60 min in the presence of 20 μM GTP γS , then fixed and processed for cryoelectron microscopy. Membranes were placed into four categories (cisternae, COP I-coated vesicles, uncoated vesicles and tubules) as defined in Materials and Methods and the amount of membrane in each category expressed as a percentage of the total. As shown in Fig. 7, $72 \pm 9\%$ of membrane in the starting material was present as cisternae and this fell to $39 \pm 10\%$ after a 60-min incubation in interphase cytosol. This decrease of 33% was almost exactly matched by the 28% rise in COP I-coated vesicles (Table I), from $5 \pm 2\%$ to $33 \pm 9\%$ (Fig. 7). In contrast, in the presence of mitotic cytosol, 55% of membrane was lost from cisternae after 60 min of incubation but only 41% appeared in COP I-coated vesicles (Table I). The difference of 14% was significant at the $P < 0.001$ level using the Student's *t*-test. The rest of the membrane lost from cisternae appeared as uncoated vesicles that increased by 13% over the same time period (Table I and Fig. 7). When added together, the increase in both COP I-coated and uncoated vesicles (53%) almost exactly matched the decrease in cisternal membrane (55%). The increase in uncoated vesicles was a unique feature of the mitotic incubation. Uncoated vesicles in interphase incubations increased by less than 3% over the same time period (Table I).

One remaining possibility is that the uncoated vesicles are simply COP I-coated vesicles that have somehow lost their coat, even in the presence of GTP γS . The two vesicle populations were, therefore, compared using mitotic samples processed for cryoelectron microscopy (Fig. 8, A–D) or Epon embedding (Fig. 8 E). COP I-coated vesicles were easily identified by their coat (Fig. 8, arrowheads) and their identity confirmed by labeling for one of the coatomer subunits, ϵ -COP (Fig. 8, B–D). Uncoated vesicles (Fig. 8, arrows) were generally more electron lucent than COP I-coated vesicles (arrowheads) and, on occasion, contained VLDL particles, a cargo never seen in COP I-coated vesicles. They were also more oval in shape. The average ratio of the longest diameter to that perpendicular to it was 1.18 compared to 1.04 for COP I-coated vesicles. Uncoated vesicles were also larger in size (Fig. 9). COP I-coated vesicles had an average diameter of 54 nm and more than 80% had a diameter between 40 and 60 nm. In contrast, uncoated vesicles were much more heterogeneous ranging in size from 70 to 200 nm (Fig. 9). The small and large vesicles identified in the previous study (Misteli and Warren, 1994) most likely correspond to the COP I-coated and uncoated vesicles, respectively. The differences in their size and morphology provide good evidence that the uncoated vesicles are not derived from COP I-coated vesicles but are the end-product of another fragmentation pathway.

Consumption of Tubular Networks

Since tubular networks are early intermediates it was im-

portant to determine whether they were consumed by the COP I-dependent or independent pathways, or by both.

The COP I pathway was clearly involved since coated buds were often observed emerging from tubular networks (Fig. 1, arrowheads) and though the thicker section made it difficult to identify the coat unambiguously, careful examination of thinner sections confirmed their identity as COP I coats (data not shown). Evidence for the involvement of the COP I-independent pathway was obtained by incubation of the tubular networks formed in the presence of mitotic cytosol depleted of coatomer (Misteli and Warren, 1994). When incubation was continued for a further 40 min these networks broke down yielding an apparently heterogeneous mixture of products including small tubular networks (Fig. 10 A, arrows) and short tubules, some of which were branched (Fig. 10 B, arrows). Small vesicular profiles were rarely, if ever, seen, unless purified coatomer was added during the incubation (Fig. 10, C and D). This indirect effect of the COP I-mediated budding mechanism on the production of uncoated vesicles will be discussed later.

The involvement of both pathways was also shown by stereological analysis of the consumption of tubular networks during normal fragmentation (Fig. 11). The percentage fall in tubular profiles ($16 \pm 5.3\%$), between 20 and 40 min of incubation, was larger than the rise in uncoated vesicular profiles ($8.7 \pm 4.7\%$) and this difference was significant at the $0.001 < P < 0.01$ level. The consumption of tubular networks could not, therefore, be solely the consequence of the COP I-independent pathway. On the other hand, more membrane appeared in COP I-coated vesicles ($19 \pm 4.1\%$) than was removed from cisternae ($13 \pm 5.4\%$) over the same time period (Fig. 11) and this was significant at the $0.01 < P < 0.02$ level. Since the fall in both cisternal and tubular membrane (29%) was closely matched by the rise in both COP I-coated and uncoated vesicles (28%) (Fig. 11), this

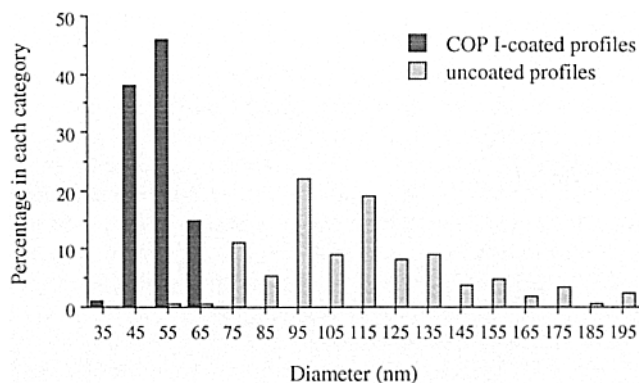


Figure 9. Frequency distribution of COP I-coated and uncoated vesicular profiles. Golgi membranes were incubated for 60 min with mitotic cytosol in the presence of 20 μM GTP γS . Samples were then fixed and processed for cryoelectron microscopy. The size distribution of COP I-coated vesicular profiles was compared to that of the uncoated ones. Each diameter (*d*) represents the percentage of profiles with an average diameter of $d - 5.0$ to $d + 4.99$ nm. 100 profiles were counted for the COP I-coated vesicles and 208 profiles for the uncoated ones.

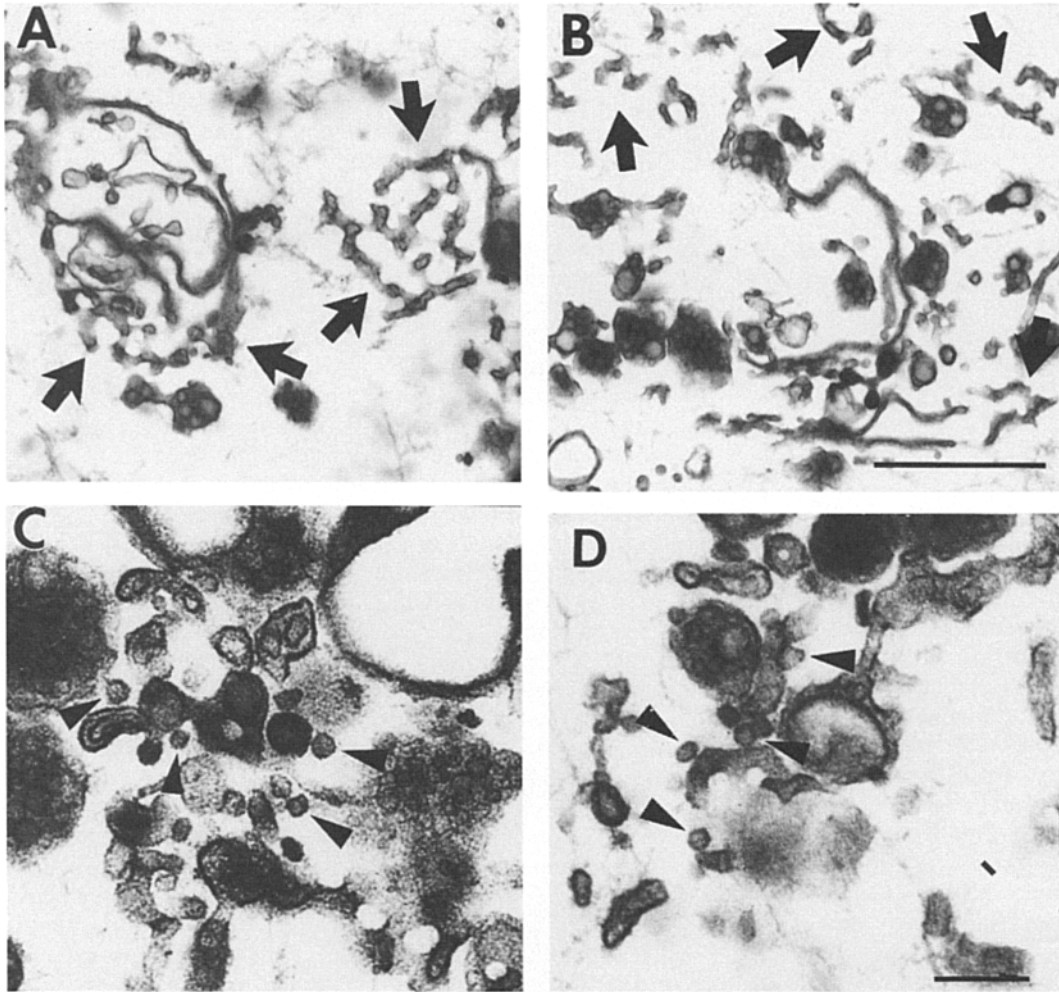


Figure 10. Fragmentation of tubular networks in the absence of coatomer. Golgi membranes were incubated with mitotic cytosol immuno-depleted of coatomer for 50 min and either incubated for a further 40 min in the absence (*A* and *B*) or presence (*C* and *D*) of purified coatomer (at a final concentration of 52 $\mu\text{g/ml}$). Note the partial fragmentation of the tubular networks in *A* and *B* (arrows). After readdition of coatomer, fragmentation was more complete and a larger number of vesicular profiles were apparent (arrowheads in *C* and *D*). Bars: (*A* and *B*) 1.0 μm ; (*C* and *D*) 250 nm.

strongly suggests that the tubular networks are consumed both by the COP I-dependent and independent pathways.

Discussion

We have shown that tubular networks are major intermediates on the Golgi fragmentation pathway. They are generated and partially consumed by a COP I-independent pathway. The rest are consumed by the COP I-dependent pathway described previously (Misteli and Warren, 1994).

Tubular networks were originally observed when Golgi stacks were incubated in the absence of coatomer (Misteli and Warren, 1994). Small networks were also seen very occasionally during normal fragmentation (see Fig. 5 *D* in Misteli and Warren, 1994). Their significance as genuine intermediates in the fragmentation process, however, was only appreciated once thicker Epon sections were examined. They peaked between 20 and 30 min, very similar to the half-time of 20 to 25 min measured in earlier experiments for the disappearance of cisternae. Since tubular

networks constituted 35% of total Golgi membrane at their peak, it is reasonable to suggest that most Golgi cisternae are converted into this intermediate before consumption by the COP I-dependent and -independent pathways. These networks were not artefacts of the cell-free system. They were observed *in vivo* during the early mitotic phases of synchronized HeLa cells (Misteli and Warren, 1995) and have also been observed in HeLa cells treated with okadaic acid to mimic the mitotic fragmentation of the Golgi apparatus (Lucocq et al., 1991).

Several lines of evidence point to a COP I-independent fragmentation pathway. The first is that the COP I-dependent mechanism consumed most, but not all, of the Golgi membrane. A time course of consumption at normal and twice normal levels of cytosol showed that this was not a technical limitation of the assay, caused by a deficiency of cytosolic coat or other components. At both levels of cytosol the amount of Golgi membrane consumed leveled off at between 55 and 65% of total membrane. In other words, between 35 and 45% of Golgi membrane could not

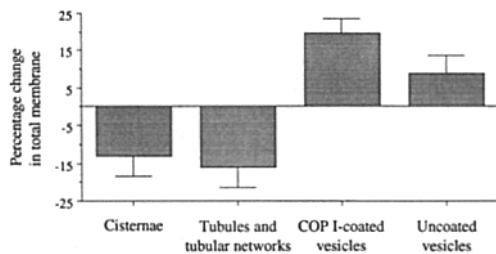


Figure 11. Consumption of tubular networks by the COP I-dependent and independent pathways. Golgi membranes were incubated with mitotic cytosol for 20 or 40 min in the presence of 20 μ M GTP γ S then fixed and processed for Epon embedding. The percentage change in different categories of membrane between these two time points is shown and results are presented as the mean \pm SEM of three experiments.

be consumed by the COP I-mediated budding mechanism. This residual membrane did, however, undergo further fragmentation during the later stages of the incubation, when COP I-mediated budding had nearly ceased, as measured by a decrease in its sedimentability. Furthermore, an analysis of the end products showed that the loss of cisternal membrane during mitotic fragmentation could not be accounted for solely by the increase in COP I-coated vesicles. The balance was made up of uncoated vesicles that differed in both size and morphology from the COP I-coated ones. The second line of evidence for a COP I-independent pathway came from incubation of the tubular networks formed in the presence of mitotic cytosol depleted of coatomer. Fragmentation continued generating branched tubules and smaller fragments. Stereological analysis during normal fragmentation (in the presence of coatomer) confirmed these qualitative observations. The consumption of tubular networks could only be explained if the COP I-independent pathway was involved in addition to the COP I-dependent pathway.

The mechanism employed by the COP I-independent pathway may be the same as that used by the COP I-dependent pathway. COP I-coated vesicles pinch off as the result of bringing the periplasmic (noncytoplasmic) surfaces of the membrane together. We have argued elsewhere (Rothman and Warren, 1994) that this periplasmic fusion process may be used by a number of devices that need to sever membranes. A consequence is that this process could also be triggered spontaneously. A flattened cisterna would become increasingly fenestrated, form tubular networks and eventually fragment into tubules. This process would normally be prevented by membrane scaffolds keeping the two surfaces apart, or the fenestrations repaired by homotypic fusion. At the onset of mitosis, cytoplasmic fusions (both homotypic and heterotypic) are inhibited so repairs would not be made to the cisternal rims, causing them to become increasingly fenestrated. Membrane scaffolds also break down making the cisternal cores more susceptible to periplasmic fusion. This again would result in increasing fenestration that would also not be repaired by homotypic fusion. The consequence of this COP I-independent, enhanced periplasmic fusion would be the conversion of cisternae first into tubular networks and then into tubular fragments as is observed.

The COP I-dependent mechanism would continue budding first from cisternae, then from tubular networks and would cease once two-thirds of the membrane had been consumed. Cessation may reflect a lack of further binding sites for both ARF and coatomer. The budding of COP I-coated vesicles from tubules might also help explain the formation of the uncoated vesicles. Tubules are the end-product of the COP I-independent pathway in the absence of coatomer, but uncoated vesicles are the end product in its presence. If COP I-coated vesicles, budding from tubules, remove more membrane than volume then the inevitable, geometric consequence would be to convert the remaining tubule into a more vesicular structure with an average diameter larger than that of the COP I-coated vesicle that budded from it. The heterogeneity of uncoated vesicles would be explained by the heterogeneity of the tubules produced by the COP I-independent mechanism.

It is not yet clear exactly what happens to the resident Golgi enzymes during the fragmentation process because immunocytochemical studies have so far proven too insensitive. The sedimentation data show that they are only released during the later stages of disassembly suggesting that they are associated with the central core regions. This is consistent with the observation that mannosidase II is normally excluded from the COP I-coated vesicles (Ostermann et al., 1993) that bud from the cisternal rims (Weidman et al., 1993). These rims appear to be consumed during interphase incubations in the presence of GTP γ S but even more membrane is consumed during mitotic incubations suggesting that some COP I vesicles might contain Golgi enzymes. Experiments are underway to test this possibility.

In summary, the simplest model of mitotic fragmentation involves an inhibition of cytoplasmic fusions and a relaxation of those Golgi membrane scaffolds that normally prevent periplasmic fusion. A combination of spontaneous and COP I-driven periplasmic fusion would ensure rapid and efficient fragmentation of the Golgi stack. This working model needs to be tested by determining the molecular basis of these events.

We would like to thank D. Palmer and J. Rothman (Sloan-Kettering, NY) for purified coatomer and the CM1A10 monoclonal anti-coatomer antibody; Dr. Hara-Kuge (National Institute of Health, Tokyo) for the anti-e-COP antibody; R. Watson for help with the electron microscopy and photography; Dr. L. Meijer (Station Biologique, Roscoff) for samples of olomoucine; and C. Rabouille and F. Barr for helpful comments on the manuscript.

Received for publication 16 February 1995 and in revised form 23 May 1995.

References

- Abraham, R. T., M. Acquarone, A. Andersen, A. Asensi, R. Bellé, F. Berger, C. Bergounioux, G. Brunn, C. Buquet-Fagot, D. Fagot, et al. 1994. Cellular effects of olomoucine, an inhibitor of cyclin-dependent protein kinases. *Biol. Cell*. In press.
- Birky, C. W. 1983. The partitioning of cytoplasmic organelles at cell division. *Int. Rev. Cytol.* 15:49–89.
- Bretz, R., and W. Staubli. 1977. Detergent influence on rat-liver galactosyltransferase activity towards different acceptors. *Eur. J. Biochem.* 77:181–192.
- Collins, R. N., and G. Warren. 1992. Sphingolipid transport in mitotic HeLa cells. *J. Biol. Chem.* 267:24906–24911.
- Glab, N., B. Labidi, L.-X. Qin, C. Trehin, C. Bergounioux, and L. Meijer. 1994. Olomoucine, an inhibitor of the cdc2/cdk2 kinases activity, blocks plant cells at the G1 to S and G2 to M cell cycle transitions. *FEBS Lett.* 353:207–211.
- Hara-Kuge, S., O. Kuge, L. Orci, M. Amherdt, M. Ravazzola, F. T. Wieland, and J. E. Rothman. 1994. En-bloc incorporation of coatomer subunits during

- assembly of COP-coated vesicles. *J. Cell Biol.* 124:883–892.
- Hesketh, T. R., M. A. Beaven, R. J. B. Burke, and G. Warren. 1984. Stimulated release of histamine by a rat mast cell line is inhibited during mitosis. *J. Cell Biol.* 98:2250–2254.
- Letourneur, F., E. C. Gaynor, S. Hennecke, C. Demolliere, R. Duden, S. D. Emr, H. Riezman, and P. Cosson. 1994. Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic-reticulum. *Cell.* 79:1199–1207.
- Lian, J. P., S. Stone, Y. Jiang, P. Lyons and S. Ferro-Novick. 1994. Ypt1p implicated in v-SNARE activation. *Nature (Lond.)* 372:698–701.
- Lucocq, J. M., J. G. Pryde, E. G. Berger, and G. Warren. 1987. A mitotic form of the Golgi apparatus in HeLa cells. *J. Cell Biol.* 104:865–874.
- Lucocq, J. M., E. G. Berger, and G. Warren. 1989. Mitotic Golgi fragments in HeLa cells and their role in the reassembly pathway. *J. Cell Biol.* 109:463–474.
- Lucocq, J., G. Warren, and J. Pryde. 1991. Okadaic acid induces Golgi-apparatus fragmentation and arrest of intracellular-transport. *J. Cell Sci.* 100:753–759.
- MacKay, D., R. Kieckbusch, J. Adamczewski, and G. Warren. 1993. Cyclin A-mediated inhibition of intra-Golgi transport requires p34^{cdc2}. *FEBS Lett.* 336:549–554.
- Misteli, T., and G. Warren. 1994. COP-coated vesicles are involved in the mitotic fragmentation of Golgi stacks in a cell-free system. *J. Cell Biol.* 125:269–282.
- Misteli, T., and G. Warren. 1995. Mitotic disassembly of the Golgi apparatus *in vivo*. *J. Cell Sci.* 1.8:2715–2727.
- Nuoffer, C., H. W. Davidson, J. Matteson, J. Meinkoth, and W. E. Balch. 1994. A GDP-bound form of rab1 inhibits protein export from the endoplasmic-reticulum and transport between Golgi compartments. *J. Cell Biol.* 125:225–237.
- Oprins, A., R. Duden, T. E. Kreis, H. J. Geuze, and J. W. Slot. 1993. β -COP localizes mainly to the cis-Golgi side in exocrine pancreas. *J. Cell Biol.* 121:49–59.
- Orci, L., B. S. Glick, and J. E. Rothman. 1986. A new type of coated vesicular carrier that appears not to contain clathrin: its possible role in protein transport within the Golgi stack. *Cell.* 46:171–184.
- Orci, L., D. J. Palmer, M. Amherdt, and J. E. Rothman. 1993. Coated vesicle assembly in the Golgi requires only coatomer and ARF proteins from the cytosol. *Nature (Lond.)* 364:732–734.
- Ostermann, J., L. Orci, K. Tani, M. Amherdt, M. Ravazzola, Z. Elazar, and J. E. Rothman. 1993. Stepwise assembly of functionally active-transport vesicles. *Cell.* 75:1015–1025.
- Pelham, H. R. B. 1994. About turn for the COPs. *Cell.* 79:1125–1127.
- Plutner, H., A. D. Cox, S. Pind, R. Khosravi-Far, J. R. Bourne, R. Schwaninger, C. J. Der, and W. E. Balch. 1991. Rab1b regulates vesicular transport between the endoplasmic reticulum and successive Golgi compartments. *J. Cell Biol.* 115:31–43.
- Pypaert, M., T. Nilsson, E. G. Berger, and G. Warren. 1993. Mitotic Golgi clusters are not tubular endosomes. *J. Cell Sci.* 104:811–818.
- Rappaport, R. 1986. Establishment of the mechanisms of cytokinesis in animal cells. *Int. Rev. Cytol.* 105:245–281.
- Roth, J., and E. G. Berger. 1982. Immunocytochemical localization of galactosyltransferase in Hela cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. *J. Cell Biol.* 92:223–229.
- Rothman, J. E. 1994. Mechanism of intracellular protein-transport. *Nature (Lond.)* 372:55–63.
- Rothman, J. E., and G. Warren. 1994. Implications of the SNARE hypothesis for intracellular membrane topology and dynamics. *Curr. Biol.* 4:220–233.
- Serafini, T., L. Orci, M. Amherdt, M. Brunner, R. A. Kahn, and J. E. Rothman. 1991. ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: A novel role for a GTP-binding protein. *Cell.* 67:239–253.
- Simionescu, N., and M. Simionescu. 1976. Galloylglucoses of low molecular weight as mordant in electron microscopy. *J. Cell Biol.* 70:608–621.
- Slusarewicz, P., N. Hui, and G. Warren. 1994. Purification of rat liver Golgi stacks. In *Cell Biology: A Laboratory Handbook*. J. E. Celis, editor. Academic Press Inc., Orlando, Florida. 509–516.
- Sogaard, M., K. Tani, R. R. Ye, S. Geromanos, P. Tempst, T. Kirchhausen, J. E. Rothman, and T. Sollner. 1994. A rab protein is required for the assembly of snare complexes in the docking of transport vesicles. *Cell.* 78:937–948.
- Stuart, R. A., D. Mackay, J. Adamczewski, and G. Warren. 1993. Inhibition of intra-Golgi transport *in vitro* by mitotic kinase. *J. Biol. Chem.* 268:4050–4054.
- Tanigawa, G., L. Orci, M. Amherdt, M. Ravazzola, J. B. Helms, and J. E. Rothman. 1993. Hydrolysis of bound GTP by ARF protein triggers uncoating of Golgi-derived COP-coated vesicles. *J. Cell Biol.* 123:1365–1371.
- Tuomikoski, T., M. A. Felix, M. Doree, and J. Gruenberg. 1989. Inhibition of endocytic vesicle fusion *in vitro* by the cell-cycle control protein kinase cdc2. *Nature (Lond.)* 342:942–945.
- Vesely, J., L. Havlicek, M. Strand, J. J. Blow, A. Donella-Deana, L. Pinna, D. S. Letham, J. Kato, L. Detivaud, S. Leclerc, and L. Meijer. 1994. Inhibition of Cyclin-dependent kinases by purine analogues. *Eur. J. Biochem.* 224:771–786.
- Warren, G. 1985. Membrane traffic and organelle division. *Trends Biochem. Sci.* 10:439–443.
- Warren, G. 1993. Membrane partitioning during cell division. *Annu. Rev. Biochem. Sci.* 323–348.
- Wattenberg, B. W. 1990. Glycolipid and glycoprotein transport through the Golgi complex are similar biochemically and kinetically: reconstitution of glycolipid transport in a cell free system. *J. Cell Biol.* 111:421–428.
- Weibel, E. R., W. Stäubli, H. R. Gnägi, and F. A. Hess. 1969. Correlated morphometric and biochemical studies on the liver cell. I: morphometric model, stereological methods and normal morphometric data for rat liver. *J. Cell Biol.* 42:69–91.
- Weidman, P., R. Roth, and J. Heuser. 1993. Golgi membrane dynamics imaged by freeze-etch electron microscopy: Views of different membrane coatings involved in tubulation versus vesiculation. *Cell.* 75:123–133.
- Wilson, B. S., C. Nuoffer, J. L. Meinkoth, M. McCaffery, J. R. Feramisco, W. E. Balch, and M. G. Farquhar. 1994. A rab1 mutant affecting guanine-nucleotide exchange promotes disassembly of the Golgi-apparatus. *J. Cell Biol.* 125:557–571.
- Woodman, P. G., J. P. Adamczewski, T. Hunt, and G. Warren. 1993. *In vitro* fusion of endocytic vesicles is inhibited by cyclin-a-cdc2 kinase. *Mol. Biol. Cell.* 4:541–553.
- Zeligs, J. D., and S. H. Wollman. 1979. Mitosis in rat thyroid epithelial cells *in vivo*. I. Ultrastructural changes in cytoplasmic organelles during the mitotic cycle. *J. Ultrastruct. Res.* 66:53–77.
- Zhang, C. J., A. G. Rosenwald, M. C. Willingham, S. Skuntz, J. Clark, and R. A. Kahn. 1994. Expression of a dominant allele of human ARF1 inhibits membrane traffic *in vivo*. *J. Cell Biol.* 124:289–300.