The Response of the Golgi Complex to Microtubule Alterations: The Roles of Metabolic Energy and Membrane Traffic in Golgi Complex Organization

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Abstract. A striking example of the interrelation between the Golgi complex (GC) and microtubules is the reversible fragmentation and dispersal of the GC which occurs upon microtubule depolymerization. We have characterized dispersal of the GC after nocodazole treatment as well as its recovery from the dispersed state by immunofluorescent localization of β1, 4-galactosyltransferase in Madin–Darby bovine kidney cells. Immunofluorescent anti–tubulin staining allowed simultaneous examination of the microtubule array.

Based on our results, dispersal can be divided into a three-step process: microtubule depolymerization, GC fragmentation, and fragment dispersal. In cells treated with metabolic inhibitors after microtubule depolymerization, neither fragmentation nor dispersal occur, despite the absence of assembled microtubules. Thus, fragmentation is energy dependent and not tightly linked to microtubule depolymerization. The slowing of fragmentation and dispersal by monensin or ammonium chloride, as well as progressive inhibition at <34°C, suggest that ongoing membrane traffic is re-

quired for these processes.

Similarly, recovery may be separated into four steps: microtubule repolymerization, GC fragment centralization, fragment coalescence, and polarization of the reticular GC network. Fragment centralization and coalescence were arrested by metabolic inhibitors, despite the presence of microtubules. Neither monensin nor ammonium chloride inhibited GC recovery. Partial inhibition of recovery at reduced temperatures paralleled the extent of microtubule assembly.

These data demonstrate that dispersal and recovery are multi-step operations, and that the individual steps differ in temperature dependence, energy dependence, and sensitivity to ionic perturbation. GC distribution and microtubule status have also been clearly dissociated, thereby proving that organization of the GC is an active process that is not simply determined by microtubule binding. Furthermore, the results indicate that ongoing intra-GC membrane traffic may participate in fragmentation and dispersal.

Tʜᴇ Golgi complex (GC)[1] of vertebrate cells is a single-copy organelle which colocalizes with the microtubule organizing center (MTOC) (43). Although the reason for this colocalization is unknown, the tight association of the GC and microtubules has been demonstrated in several systems. Examples include alterations in GC structure during reorganization of the microtubule array as myoblasts fuse to form myotubes (41), the continued association of the GC and MTOC as both reorient towards an experimental wound in a cell monolayer (14), and the unusual GC distributions which occur after pharmacological manipulation of the microtubule array (34).

A most dramatic example of this relationship is the reversible fragmentation and dispersal of the GC after treatment with agents such as nocodazole and colchicine which bind

tubulin heterodimers and inhibit their polymerization, thereby progressively depolymerizing microtubules (29, 31). Light microscopic histochemistry and immunocytochemistry show that the GC fragments into hundreds of islands distributed throughout the cytoplasm, and electron microscopic examination reveals that the islands are composed of stacked cisternae, identifiable as GC by ultrastructure and cytochemistry (26, 29, 43). Since the GC is normally composed of interconnected stacks of cisternae (27), these tubular interconnections may be severed after microtubule depolymerization, thus leading to dispersal of the individual stacks (41). Alternatively, lateral unfolding and extension of the tubular interconnections may result in GC redistribution throughout the cytoplasm without disrupting GC continuity (31). Despite its dispersed state, secretory and biosynthetic activities of the GC are remarkably unaffected (30, 31, 33, 40). These data suggest that each dispersed GC island is a competent functional unit.

One recent morphological study has suggested that GC is-

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1. *Abbreviations used in this paper:* GalT, β1, 4-galactosyltransferase; GC, Golgi complex; MDBK, Madin-Darby bovine kidney; MTOC, microtubule organizing center.

lands are translocated along microtubule-based tracks during recovery from the dispersed state (12). However, the mechanisms by which the GC is fragmented, dispersed, and, after nocodazole removal, reassembled, remain unknown.

We have used double-label immunofluorescence to investigate the mechanisms of GC fragmentation and dispersal after microtubule depolymerization, as well as recovery from the dispersed state in Madin–Darby bovine kidney (MDBK) cells. Polyclonal antiserum recognizing $\beta 1$, 4-galactosyl-transferase (GalT), an integral membrane GC-localized enzyme (32), and a monoclonal antibody to β-tubulin were used to label the GC and microtubules, respectively. The results indicate that both dispersal and recovery are energy dependent, and also implicate membrane traffic in dispersal. A model of GC dispersal and recovery is presented.

Materials and Methods

Materials

The following were from Sigma Chemical Co. (St. Louis, MO), and were used at the specified dose(s): nocodazole (5 μM), NaN$_3$ (10 mM), 2-deoxy-D-glucose (1 mM), 2,4-dinitrophenol (1 mM), cycloheximide (100 μg/ml), monensin (1 and 10 μM), ouabain (1 mM), dibutyryl cyclic AMP (1 mM), dibutyryl cyclic GMP (1 mM), phorbol myristate acetate (10 nM and 1 μM), cytochalasin D (1 μg/ml), and cholera toxin (1 μg/ml). Normal goat serum and most other reagents were also from Sigma Chemical Co. Pertussis toxin (used at 500 ng/ml) was from List Biological Laboratories (Campbell, CA). Stock solutions were prepared as follows and stored in small aliquots at -20°C: nocodazole, 5 mg/ml in dimethyl sulfoxide; monensin, 50 mM in ethanol; phorbol myristate acetate, 1 mg/ml in dimethyl sulfoxide. Other reagents were freshly prepared.

Cell Culture

MDBK cells (kindly provided by J. Shaper, Johns Hopkins University) were maintained in Ham's F-12 media supplemented with 10% FBS (Gibco Laboratories, Grand Island, NY) at 37°C in 5% CO$_2$ and subcultured two to three times weekly. The dissection of GC recovery presented in this work was facilitated by the slow kinetics of this process in MDBK cells, relative to some other cells.

For experiments, cells were plated onto sterile 12-mm-diam glass coverslips (Bellco Glass, Inc., Vineland, NJ) and used in subconfluent condition after 2–3 d of growth. At the start of each experiment cells were shifted into F-12 media without serum. In dispersal experiments, cells were incubated at 4°C for 240 min (depending on the experiment) to depolymerize microtubules, and nocodazole was added 30 min before warming to prevent microtubule repolymerization upon warming. To disperse the GC before recovery experiments, cells were incubated with nocodazole for 180 min at 37°C. Nocodazole was removed by three to five changes of media over 15 min. During energy depletion experiments, culture was in F-12 salts (130 mM NaCl, 3 mM KCl, 1.9 mM Na$_2$HPO$_4$, 14 mM NaHCO$_3$, 0.3 mM CaCl$_2$, 0.6 mM MgCl$_2$, pH 7.5). Metabolic inhibitors were NaN$_3$, 2-deoxy-D-glucose, and, in some experiments, 2,4-dinitrophenol, although results were identical whether the inhibitor cocktail included 2,4-dinitrophenol or not. The metabolic inhibitors were added 30 min before warming (dispersal) or nocodazole removal (recovery). During culture after removal of metabolic inhibitors, F-12 medium was supplemented with D-glucose to 5 mg/ml. To regulate pH of the medium in temperature dependence experiments done in room air, culture was in F-12 salts, substituting 14 mM Hepes in place of NaHCO$_3$, supplemented with 1.8 mg/ml D-glucose. Monensin, ammonium chloride, ouabain, dibutyryl cyclic AMP, dibutyryl cyclic GMP, and phorbol myristate acetate, were added 15 min before warming (dispersal) or removal of nocodazole (recovery). Cells were incubated with cholera or pertussis toxin for 180 min at 37°C before dispersal for 180 min at 37°C in nocodazole and toxin. To assess recovery in the presence of toxins, the GC was dispersed with nocodazole for 180 min before the addition of toxin, toxin was added for 180 min at 37°C in the continued presence of nocodazole, and recovery examined after a 90-min incubation in nocodazole-free toxin-containing media.

Immunofluorescence

Cells were fixed for 10 min at -20°C in methanol, washed for 5 min at 25°C in TBS, and nonspecific sites blocked for 10 min in TBS containing 5% normal goat serum (TBS-5% NGS). Antibodies were diluted in TBS-10% NGS, and antibody incubations were 30 min at 37°C. Between incubations, coverslips were washed three to five times with TBS-2% NGS. GC was detected using 60 μg/ml polyclonal rabbit anti-bovine GalT IgG (37) purified by protein A affinity chromatography (generously provided by J. Shaper, Johns Hopkins University) followed by 1 μg/ml affinity-purified rhodamine-conjugated goat anti-rabbit IgG (Cappel Laboratories, Malvern, PA). Tubulin was subsequently stained with TUB2.1 monoclonal anti-β-tubulin (9) ascites diluted 1:200 (ICN Immunobiologicals, Lisle, IL) followed by 20 μg/ml affinity-purified FITC-conjugated goat anti-mouse IgG preabsorbed against rabbit and human IgG (Kirkegaard & Perry, Gaithersburg, MD).

Results

Kinetics and Temperature Dependence of GC Fragmentation and Dispersal

We have monitored the process of GC dispersal in cells preincubated at 4°C (to depolymerize the microtubules) and then warmed to 37°C in the presence of nocodazole. Dispersal did not begin until the cells were warmed (20). By 30 min after warming, the reticular GalT distribution had largely fragmented to generate small islands surrounding the nucleus. The GalT positive islands were no longer limited to perinuclear regions by 60 min, and were dispersed throughout the cell, including even the most peripheral cytoplasm, 120 min after warming to 37°C (Fig. 1 C).

When MDBK cells with 4°C depolymerized microtubules were warmed to 15°C for 120 min in the presence of nocodazole, fragmentation and dispersal did not occur (Fig. 1 A). There was some fragmentation in cells warmed to 20°C, but large juxtanuclear fragments persisted and dispersal was limited (Fig. 1 B). Fragmentation and dispersal were progressively more complete at higher temperatures, but dispersal was not comparable to 37°C (Fig. 1 C) until the cells were warmed to 34°C.

GC Dispersal Is Energy Dependent

To determine whether GC dispersal was energy dependent, microtubules were depolymerized at 4°C and cells were subsequently treated with metabolic inhibitors. It was important to completely depolymerize the microtubules by incubation at 4°C before treatment with metabolic inhibitors, since microtubule depolymerization in energy-depleted cells is resistant to nocodazole and colchicine (5, 20). When such cells were warmed to 37°C in the presence of nocodazole and the metabolic inhibitors, fragmentation and dispersal of the GalT distribution were completely inhibited (Fig. 1 E), although microtubules remained depolymerized (Fig. 1 D). Fragmentation and dispersal resumed upon removal of the metabolic inhibitors. Thus, dispersal does not automatically occur when microtubules are absent at 37°C.

Inhibition of Protein Synthesis Does Not Account for the Effects of Energy Depletion

To investigate the possibility that inhibition of protein synthesis was responsible for the energy dependence of dispersal, GC dispersal in cycloheximide-treated cells was examined
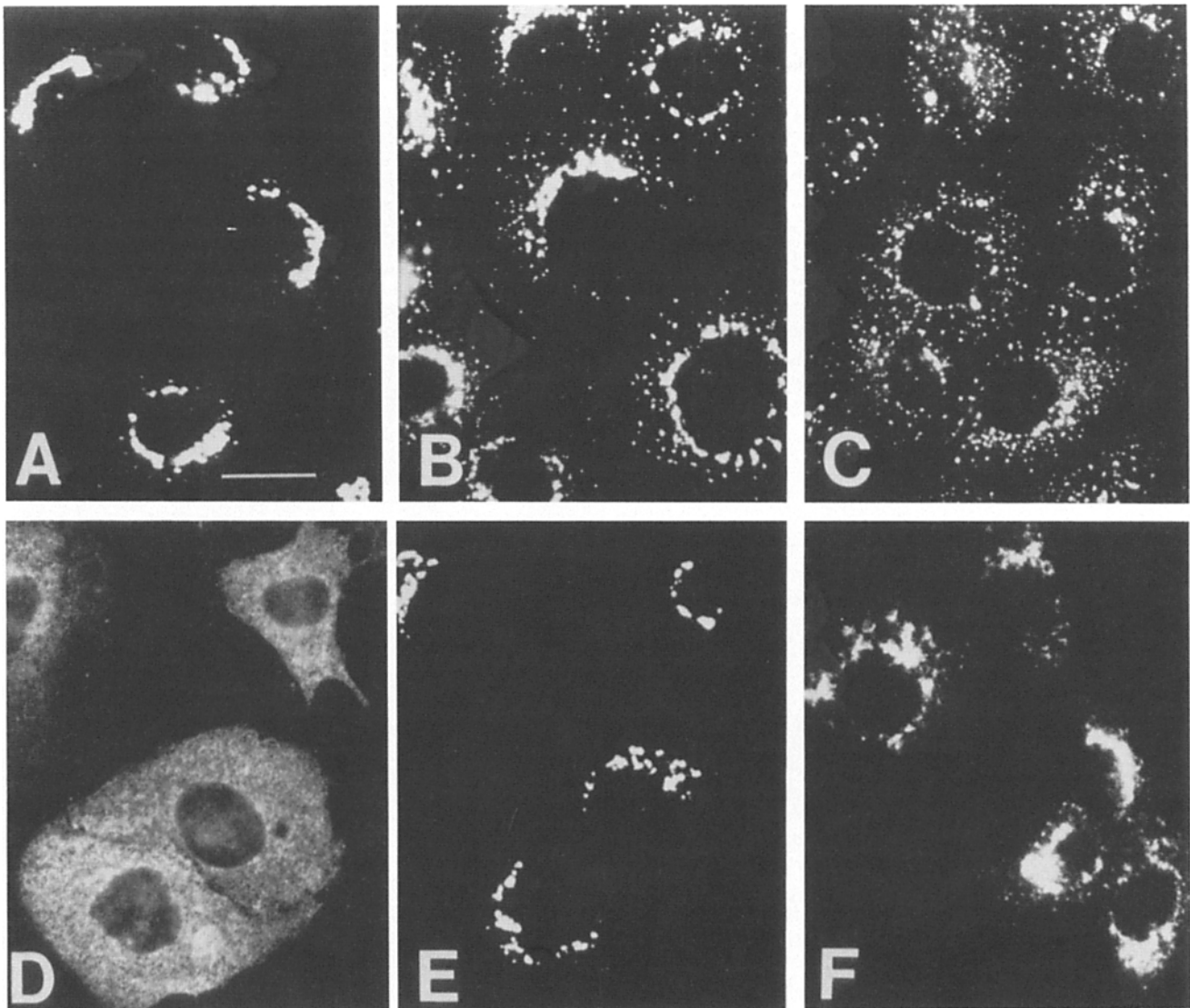
Figure 1. Golgi complex dispersal in MDBK cells. (*A–C*) Cells were incubated at 4°C for 240 min to depolymerize microtubules, and then warmed to 15°C (*A*), 20°C (*B*), or 37°C (*C*) in nocodazole-containing media for 120 min. GalT distribution is shown. (*D* and *E*) After microtubule depolymerization at 4°C, metabolic inhibitors and nocodazole were added for 30 min at 4°C before warming. The cells were then incubated at 37°C for 120 min in medium containing metabolic inhibitors and nocodazole. Tubulin (*D*) and GalT (*E*) distributions are shown. (*F*) After microtubule depolymerization at 4°C, 10 μM monensin and nocodazole were added for 15 min at 4°C before warming. The cells were then incubated at 37°C for 120 min in medium containing monensin and nocodazole. GalT distribution is shown. Bar, 20 μm.

using two distinct protocols. In the first, cycloheximide was included while microtubules were depolymerized at 4°C. GalT and tubulin distributions were examined at various times after warming to 37°C in medium containing cycloheximide and nocodazole. Alternatively, to drain content from the endoplasmic reticulum and GC before dispersal, a second protocol included a 180-min preincubation of cells with cycloheximide at 37°C before the addition of nocodazole at 37°C. In either case, GalT and tubulin distributions in cycloheximide-treated cells were identical to controls not treated with cycloheximide.

GC Dispersal Is Inhibited by Agents That Slow Golgi Traversal

To examine further the possible role of membrane traffic in

GC dispersal, we used the carboxylic ionophore, monensin, and the acidotropic amine, ammonium chloride, to slow transport through the GC without depleting cellular ATP (6, 17, 40). We also attempted to approximate the Na$^+$/K$^+$ alterations induced by monensin using ouabain (38), a specific inhibitor of the plasma membrane Na$^+$/K$^+$-ATPase.

To examine the effects of monensin, ammonium chloride, and ouabain on fragmentation and dispersal, microtubules were depolymerized at 4°C, and the cells briefly (15 min) treated with drug and nocodazole before shifting to 37°C. The GalT distribution of monensin- or ammonium chloride–treated cells examined 120 min after shifting to 37°C was incompletely fragmented relative to control cells, and polar reticular structures persisted (10 μM monensin, Fig. 1 *F*; control, Fig. 1 *C*). Very little fragmentation occurred, and
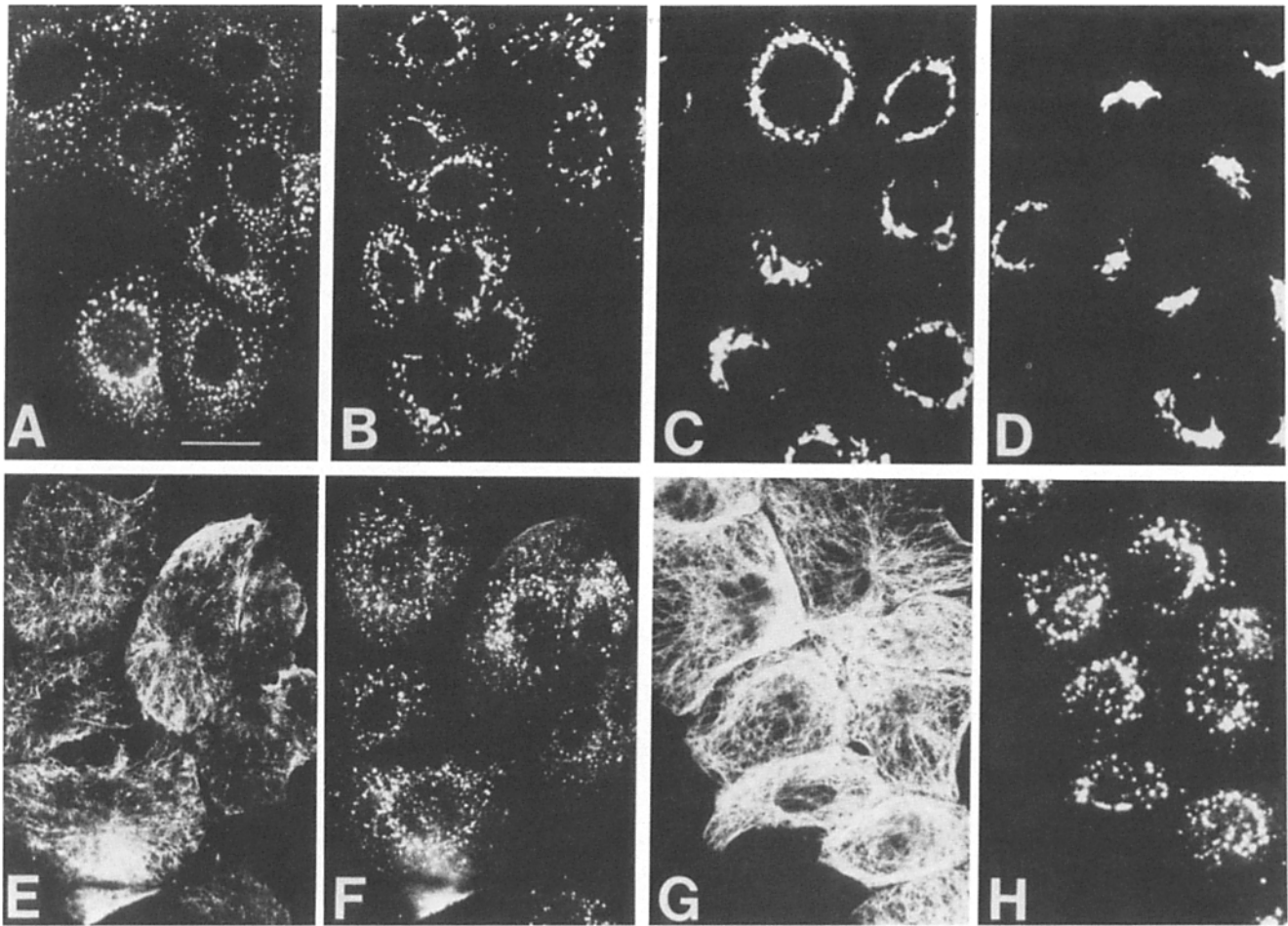
Figure 2. Golgi complex recovery in MDBK cells. (*A–D*) Cells were incubated at 37°C in nocodazole for 180 min to disperse the GC, and examined at that point (*A*) or incubated after nocodazole removal at 37°C for 15 (*B*), 90 (*C*), or 240 (*D*) min. GalT distribution is shown. (*E* and *F*) After GC dispersal, metabolic inhibitors were added for 30 min before nocodazole removal. Cells were then incubated for 90 min at 37°C in the continued presence of metabolic inhibitors (compare with *C*). Tubulin (*E*) and GalT (*F*) distributions are shown. (*G* and *H*) After GC dispersal, nocodazole was removed, and the cells were incubated for 15 min at 37°C (as in *B*). Metabolic inhibitors were added at this point (after 15 min of recovery), and incubation continued for 75 min (for a total of 90 min, compare with *C*). Tubulin (*G*) and GalT (*H*) distributions are shown. Bar, 20 μm.

dispersal was limited to relatively central, and often polar, areas of the cytoplasm, even after 240 min. This marked retardation of fragmentation and dispersal was evident in cells treated with 1 μM monensin, a low and relatively GC-specific dose, as well as with 10 μM monensin or 50 mM ammonium chloride. Ouabain had no effect on GalT fragmentation or dispersal.

Kinetics and Temperature Dependence of GC Recovery after Dispersal

Recovery of normal GC distribution and morphology involves microtubule reorganization, GC fragment centralization, coalescence, and polarization. MDBK cells incubated 180 min at 37°C with nocodazole and then reincubated for 0–240 min at 37°C without nocodazole are shown in Fig. 2. Just before nocodazole removal, the GalT positive fragments were found throughout the cytoplasm (Fig. 2 *A*) and the microtubules were completely depolymerized. Microtubules reassembled within 7.5 min, but alterations in the GalT

distribution were not apparent until 15 min after nocodazole washout. At this point, small GalT positive fragments remained, but had centralized to form a juxtanuclear cloud (Fig. 2 *B*). By 90 min after nocodazole removal the small fragments had coalesced into nearly continuous juxtanuclear rims composed of large reticular GalT containing structures (Fig. 2 *C*). These structures then began to polarize by moving towards the MTOC on one side of the nucleus. Polarization was complete 240 min after nocodazole withdrawal (Fig. 2 *D*).

GC and microtubule recovery after nocodazole treatment occurred at lower temperatures than dispersal. After complete fragmentation and dispersal, nocodazole was removed and the cells incubated at various temperatures. At 15°C, microtubule reassembly was limited to aster formation and the GalT staining remained disperse. At 20°C, microtubules appeared to be of normal length, but the network was more sparse than in control cells. Despite this partial microtubule network, GC recovery was comparable to 37°C controls.

Both Centralization and Coalescence of GC Fragments during Recovery Are Energy Dependent

To determine the energy dependence of GC recovery in nocodazole-treated cells, two protocols were used. In the first, cells received metabolic inhibitors before nocodazole removal. When nocodazole was subsequently washed out in the continued presence of the metabolic inhibitors, an assembled microtubule array was formed (Fig. 2 E), although microtubules without relation to the MTOC were common (5). Under these conditions, despite the presence of assembled microtubules, GalT positive fragments remained extensively dispersed after 90 min at 37°C (Fig. 2 F), and were indistinguishable from those in nocodazole-treated cells which were not allowed to recover (Fig. 2 A). No centralization occurred. Recovery continued normally upon removal of the metabolic inhibitors.

A second protocol was used to study the energy dependence of recovery in the presence of a more normal microtubule network. In these experiments nocodazole-treated cells were allowed to recover briefly (15 min) from nocodazole before the addition of metabolic inhibitors. Centralization of the GalT positive fragments and coalescence to form larger fragments were arrested shortly after addition of the metabolic inhibitors (Fig. 2 H, compare with Fig. 2 B), despite the presence of a normal appearing microtubule network (Fig. 2 G). Thus, this second protocol suggests that both coalescence and centralization are energy dependent.

When cells were pretreated with cycloheximide and nocodazole for 180 min followed by recovery in nocodazole-free cycloheximide-containing medium, recovery of the juxtanuclear GalT distribution was identical to control cells not treated with cycloheximide.

We also examined the effects of monensin, ammonium chloride, and ouabain on recovery. Monensin, at either dose, and ammonium chloride, caused vacuolization of GalT-containing regions of the GC within 15 min. Vacuolization was less obvious as treatment exceeded 90 min, but the normally compact GC took on a somewhat loosened distribution. This loosened GC organization was indistinguishable from the GalT distribution in cells allowed to recover from nocodazole treatment in the presence of monensin or ammonium chloride. Ouabain had no effect on normal GalT distribution or GC recovery. Thus, monensin, ammonium chloride, and ouabain had no effect on recovery.

Are G Proteins, Kinases, Actin Involved in GC Dispersal or Recovery?

Several studies have suggested associations between the GC and protein kinases (2, 3, 22), as well as GTP-binding proteins (1, 19). Therefore, we examined the effects of dibutyryl cyclic AMP, dibutyryl cyclic GMP, phorbol myristate acetate, cholera toxin, and pertussis toxin on dispersal and recovery. None of these agents had any effect on dispersal or recovery. However, GTP-binding proteins cannot be ruled out, since sodium fluoride inhibited GC dispersal (see note added in proof).

There is currently no evidence supporting a role for actin in GC organization in animal cells. Consistent with a lack of actin involvement, it has been shown that normal GC morphology and the early stages of recovery after nocodazole treatment are unaffected by cytochalasin (12, 42). Transport

of newly synthesized proteins through the GC is also unaffected by cytochalasin (33). We examined the role of actin in each stage of GC dispersal and recovery using cytochalasin D. Both processes continued normally, confirming that actin is not involved in GC reorganization after microtubule alterations.

Discussion

We have used a polyclonal antiserum to GalT, a well-characterized integral membrane protein of trans-Golgi cisternae (32), as a marker for the GC. GC stacks are known to remain functionally and cytologically intact after nocodazole- or colchicine-induced microtubule depolymerization (26, 30, 40, 43), and we have confirmed that, for each of the conditions explored, the GC of MDBK cells is composed of stacks of cisternae (Turner, J. R., and A. M. Tartakoff, unpublished observations).[2] Additionally, the distribution of GalT before and after nocodazole or colchicine treatment is similar to that of thiamine pyrophosphatase (29) as well as cis-, medial-, and trans-Golgi localized antigens (4, 47; Turner, J. R., and A. M. Tartakoff, unpublished observations). Finally, viral glycoprotein and fluorescent ceramide concentrated within the GC have distributions similar to GalT both in control cells and in cells with depolymerized microtubules (15, 30). We therefore believe that GalT indicates the distribution of the entire stack under the conditions studied.

GC Dispersal

GC dispersal can be divided into three stages: (a) microtubule depolymerization, (b) GC fragmentation (30–60 min), and (c) fragment dispersal (120 min). Through the use of reduced temperature, metabolic inhibition, and ionic perturbation, we have been able to arrest GC dispersal at each of these stages, thereby demonstrating that they are distinct processes.

Fragmentation is energy dependent, since it is blocked by metabolic inhibitors. This effect may be due to inhibition of intra–GC transport, a process known to require energy (24, 39). Arrest of protein synthesis does not account for the effects of metabolic inhibition.

Fragmentation is also inhibited at 15–20°C, even though ATP levels should be similar to those at 37°C (39). These

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2. We have examined the ultrastructure of MDBK cells and confirmed that morphologically small GC stacks are distributed throughout the cytoplasm after nocodazole treatment, rather than confined to the juxtanuclear region. In agreement with other reports, we noted (a) that the cisternae seemed shorter than in control cells, and (b) that some of the cisternae were not as flat as controls (26). The GC was composed of stacks arranged in the expected distribution after 240 min at 4°C or after treatment at 37°C with metabolic inhibitors to prevent dispersal or recovery. Also consistent with the fluorescent GalT distribution, monensin or ammonium chloride induced pronounced dilation of cisternae within 15 min. After 90 min of monensin treatment, many swollen vesicles, some of which did not appear to be Golgi-derived, had accumulated in the juxtanuclear cytoplasm. This suggests that the loss of compact GalT distribution in such cells may have resulted from overcrowding of this region and intercalation of the swollen vesicles among dilated GalT-containing cisternae. Vacuolization of the GalT staining pattern in nocodazole-treated cells after 15 min of monensin treatment was not obvious by fluorescence microscopy, but electron microscopic examination confirmed that marked cisternal dilation had occurred. Since the GC stacks of nocodazole-treated cells appeared smaller than controls, it may be that they contained less membrane and, therefore, were incapable of the extensive dilation necessary for vacuolization to be visible by light microscopy.
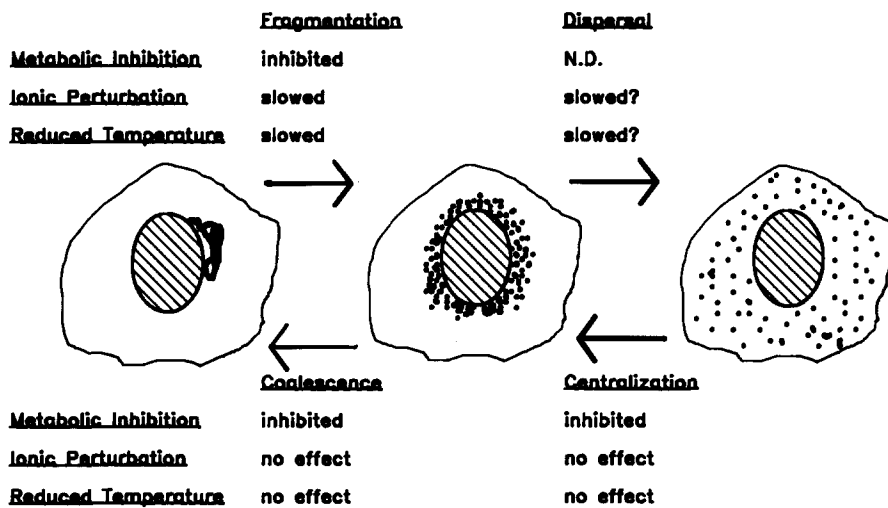
Figure 3. The effects of metabolic inhibition, ionic perturbation, and reduced temperature on Golgi complex dispersal and recovery.

results may implicate membrane traffic in GC fragmentation, since budding and fusion of membranes are inhibited at 10–15°C, and a block in GC traversal and exit persists up to 20–22°C (35, 39).

The inhibition of GC fragmentation and dispersal by the ionic perturbants monensin and ammonium chloride, both of which slow intra–GC membrane traffic, also suggests that membrane traffic is required for GC fragmentation. These two agents may not have the same effects in all cells (23), but they both caused rapid vacuolization of the GC in MDBK cells, and are known to neutralize acidic compartments, alter cytoplasmic pH, and, most importantly, slow GC traversal and exit (11, 17, 40). The effects of monensin and ammonium chloride are not related to recent work showing that these agents promote redistribution of lysosomes and secretory granules (11, 13), since the latter phenomena are dependent on intact microtubules.

The arrest of fragmentation and dispersal by reduced temperature, metabolic inhibition, and ionic perturbation (Fig. 3) clearly demonstrates that, although microtubule depolymerization is necessary, it is not sufficient for GC fragmentation and dispersal. Thus, fragmentation is an active process which is not tightly linked to microtubule depolymerization, although under "normal" conditions (37°C, no metabolic inhibitors or ionic perturbants) GC fragmentation follows microtubule depolymerization. These observations separate the processes of microtubule depolymerization and GC fragmentation for the first time, thereby proving that GC organization does not follow the microtubule distribution directly, but through energy and temperature dependent processes.

Since monensin and ammonium chloride inhibit membrane fission (38), their effects, as well as the 20°C block, support two models of GC fragmentation. In the first, a critical step involves severing of the tubular intercisternal connections between Golgi stacks (41). Upon elimination of these interconnections, the GC would be transformed from a single organelle into a loose collection of individual stacks which would be free to disperse. The second presupposes the existence of many "stacking templates" or "Golgi complex organizing centers." If these were normally clustered in the MTOC by microtubules, they might become separated in cells with depolymerized microtubules. GC membranes would follow the templates via continual budding with peripheral reformation of multiple stacked islands.

Fragment dispersal is the final step in generating the dispersed GC. Although dispersal begins soon after fragmentation, the kinetics are slower than either microtubule depolymerization or GC fragmentation. Thus, dispersal may reflect diffusion of the individual GC islands, either along defined tracks or randomly. Since energy depletion completely blocks fragmentation, characterization of the energy dependence of fragment dispersal is difficult. Fragment dispersal is progressively inhibited below 34°C or by monensin or ammonium chloride, but, since fragmentation is also slowed, there observations do not prove that fragment dispersal was affected directly.

GC Recovery

Recovery can be divided into four steps: (a) microtubule repolymerization (<7.5 min), (b) GC fragment centralization (15–30 min), (c) fragment coalescence (30–90 min), and (d) GC polarization (90–240 min). Microtubules assemble into a normal appearing network before GC alterations are obvious. This observation is consistent with the suggestion (12) that GC fragments travel along microtubules during recovery, since microtubules initiate reassembly from the centriole (25), and, therefore, must grow outward to reach dispersed GC fragments.

Centralization is an active process, since it is rapid and energy dependent. These results support the hypothesis that a retrograde, dynein-like, ATP-dependent motor (8, 18, 36) is responsible for GC fragment movement (12, 31). The possibility that active microtubule cycling is essential must also be considered, since microtubules in energy-depleted cells are nocodazole-resistant and, therefore, probably do not cycle (5).

Membrane functions which are blocked at 0–22°C (e.g., the 20–22°C block in GC traversal; references 35, 39) are not involved in centralization, since recovery occurs at 20°C, despite incomplete microtubule reassembly.

The coalescence of centralized GC fragments to form a larger reticular GC is also energy dependent, since GC fragments in cells allowed to recover briefly from nocodazole before the addition of metabolic inhibitors appeared "ready" to

coalesce, but did not. It is possible that this energy requirement reflects reestablishment of interconnecting cisternal bridges severed during fragmentation, and thus involves energy dependent membrane fusion.

Why Is the Golgi Complex Associated with Microtubules?

These studies emphasize many unanswered questions. For example: (a) is association of the GC with microtubules important during mitosis? One might hypothesize that the GC islands which are generated by nocodazole treatment represent the smallest functional GC unit, and that their formation is the essential first step in formation of the mitotic GC (16). This is plausible if the GC associates preferentially with cytoplasmic microtubules, rather than mitotic spindle microtubules, as has been proposed for secretory granules (45). During mitosis, the small Golgi stacks generated after the depolymerization of cytoplasmic microtubules could be disassembled into vesicles (16), and, once the vesicles reassemble into small stacks, the microtubule- and energy-dependent recovery process we describe may mediate reformation of an intact GC. (b) Although the dispersed islands remain stacked, do all structures normally associated with the GC remain with the islands, or are postcisternal elements altered or absent, as has been suggested (44)? Since postcisternal elements sort proteins destined for distinct plasma membrane domains in polarized epithelia (10), such a deficiency might explain the inaccurate sorting in nocodazole-treated epithelial cells (7, 28). (c) Are the GC islands generated by nocodazole treatment equivalent to plant dictyosomes, which are distributed throughout the cytoplasm (21)? Since microtubules in many plant cells do not converge on a single juxtanuclear MTOC, but are arranged circumferentially beneath the plasma membrane (46), the dispersed dictyosome distribution, with frequent clustering near the surface, suggests that the plant GC is associated with microtubules.

Note added in proof: Sodium fluoride is known to inhibit intra-GC membrane traffic (19). Treatment of cells with sucrose-supplemented hypertonic medium inhibits both membrane traffic along the secretory path and endocytosis (Docherty, P. A., and M. D. Snider, personal communication). We have recently found that GC fragmentation is markedly inhibited by 10 mM sodium fluoride or hypertonic medium containing 0.2 M sucrose. The effects of these agents are not due to energy depletion or binding of AlF$_4^-$ to tubulin, since the results are similar whether microtubules are depolymerized by nocodazole at 37°C or by preincubation at 4°C. These observations support our contention that ongoing intra-GC membrane traffic is essential for fragmentation to occur, and suggest that a nontubulin GTP-binding protein is involved.

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