Associations of Elements of the Golgi Apparatus with Microtubules

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ABSTRACT   The intracellular spatial relationships between elements of the Golgi apparatus (GA) and microtubules in interphase cells have been explored by double immunofluorescence microscopy. By using cultured cells infected with the temperature-sensitive Orsay-45 mutant of vesicular stomatitis virus and a temperature shift-down protocol, we visualized functional elements of the GA by immunolabeling of the G protein of the virus that was arrested in the GA during its intracellular passage to the plasma membrane 13 min after the temperature shift-down. Complete disassembly of the cytoplasmic microtubules by nocodazole at the nonpermissive temperature before the temperature shift led to the dispersal of the GA elements, from their normal compact perinuclear configuration close to the microtubule-organizing center (MTOC) into the cell periphery. Washout of the nocodazole that led to the reassembly of the microtubules from the MTOC also led to the recompaction of the GA elements to their normal configuration. During this recompaction process, GA elements were seen in close lateral apposition to microtubules. In cells treated with nocodazole followed by taxol, an MTOC developed, but most of the microtubules were free of the MTOC and were assembled into bundles in the cell periphery. Under these circumstances, the GA elements that had been dispersed into the cell periphery by the nocodazole treatment remained dispersed despite the presence of an MTOC. In cells treated directly with taxol, free microtubules were seen in the cytoplasm in widely different, bundled configurations from one cell to another, but, in each case, elements of the GA appeared to be associated with one of the two end regions of the microtubule bundles, and to be uncorrelated with the locations of the vimentin intermediate filaments in these cells. These results are interpreted to suggest two types of associations of elements of the GA with microtubules: one lateral, and the other (more stable) end-on. The end-on association is suggested to involve the minus-end regions of microtubules, and it is proposed that this accounts for the GA–MTOC association in normal cells.

It is well established that the Golgi apparatus (GA)[1] is centrally involved in the intracellular transport, processing, and sorting of secretory and lysosomal proteins, and of integral glycoproteins bound for the plasma membrane (for a recent review, see reference 15). However, the factors that control the intracellular traffic of these components into, through, and out of the GA are largely unknown. It is recognized, for example, that, in many types of interphase cells, the GA is a compact

organelle located close to the nucleus. How do components that are synthesized and sequestered in the rough endoplasmic reticulum, which is often spread throughout the cytoplasm, become channeled into the relatively small volume occupied by the GA? How important are the compact structure and perinuclear location of the GA to these transport processes? As a preliminary to exploring these questions, which are addressed in the following article (29), we may first ask: how are the structure and location of the GA achieved and maintained? These questions are considered in this paper.

A role for microtubules in determining the overall form and intracellular location of the GA can be inferred from a number of observations. It has often been noted, for example,

in electron micrographs of ultrathin sections of cells that the pair of interphase centrioles is found in general proximity to a stack of GA saccules (cf. reference 1). The centriole pair is recognized as an important component of the microtubule-organizing center (MTOC), the region of the interphase cell out of which the cytoplasmic microtubules grow (8, 23, 25). At the light microscopic level of resolution, corresponding observations have been made by double-immunofluorescence labeling showing that the GA and the MTOC of interphase cells are strikingly superimposed (16). Furthermore, the maintenance of the compact perinuclear arrangement of the GA has been shown to depend on the integrity of cytoplasmic microtubules. When microtubules undergo disassembly induced by colchicine (21, 27) or nocodazole (13), the Golgi complex appears to disperse throughout the cytoplasm, although the nature and mechanism of this dispersion are not known. All of these results suggest that there is some kind of cytoskeletal–GA relationship mediated by microtubules in interphase cells, but its precise nature and its significance for GA functions are not understood.

In this paper, we have investigated the relationship between the assembly status of cytoplasmic microtubules and the intracellular disposition of elements of the GA by immuno-fluorescence microscopy. As an immunolabeling marker for the GA, we have relied primarily on the G protein of vesicular stomatitis virus (VSV). In VSV-infected cells, the G protein is synthesized, processed, and transported to the plasma membrane by the same route as the integral membrane proteins of the host cell (18). We have earlier demonstrated (5) that, in cells infected with the temperature-sensitive mutant Orsay-45 (O-45) of VSV, a shift from the nonpermissive (39.9°C) to the permissive temperature led to a synchronous wave of transport of the G protein from the rough endoplasmic reticulum to the Golgi complex and later to the cell surface. By observing O-45-infected cells 13 min after the temperature shift, the G protein found in the GA served as a marker for that organelle in our present experiments (see Discussion). Along with the immunolabeling of the G protein in the GA, we labeled by double fluorescence the same cells for tubulin revealing the MTOC (8, 23), and, in some experiments, triply labeled for vimentin intermediate filaments as well.

These results have provided evidence for a microtubule-dependent dispersion, reassembly, and intracellular polarization of the GA. Furthermore, in cells treated with taxol, in which bundles of free cytoplasmic microtubules no longer associated with an MTOC are generated (12), our data suggest that elements of the GA associate preferentially with one end region of a bundle. This polar association of GA and microtubule bundles may reflect the molecular mechanisms involved in the normal association of the GA with the MTOC.

In the following article (29), the same system of O-45-infected cells is used to investigate the effect of microtubule assembly status on the intracellular transport, processing, and surface expression of the G protein. A preliminary account of this work has appeared (28).

MATERIALS AND METHODS

Cell Culture and Virus Production: Normal rat kidney cells (NRK) were obtained from Dr. Peter K. Vogt (University of Southern California). They were routinely cultured in Coon's modified F-12 media supplemented with 10% fetal calf serum in a 10% CO_2 atmosphere at 37°C. Cells were trypsinized and plated at a density of 1–2 × 10⁴ cells/ml on 18-mm² glass coverslips and were generally grown for 48 h before infection experiments. The

O-45 mutant of VSV was obtained from Dr. Harvey Lodish (Massachusetts Institute of Technology). It was routinely grown in Vero cells cultured in Coon's F-12 media with 2% fetal calf serum to titers of 1 × 10⁸ plaque forming units/ml.

Preparation of Affinity-purified Primary and Secondary Antibodies: Guinea pig and rabbit antibodies were prepared against purified chick brain tubulin (31) and subsequently affinity purified on a Ultrogel acrylamide-agarose-22-linked tubulin column. Guinea pig antibodies were raised against electrophoretically purified baby hamster kidney cell vimentin (32). They were affinity purified on a column prepared with purified vimentin that was conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) in the presence of 0.5% SDS. Rabbit and guinea pig antibodies to the G protein of VSV were as described (4, 5). They were subsequently affinity purified on a *Lens culinaris* lectin-cross-linked G protein column. Affinity-purified goat antibodies to rabbit IgG or to guinea pig IgG were cross-absorbed on the heterologous antigen column before conjugation with dichlorotriazinylaminofluorescein (Research Organics, Inc., Cleveland, OH) or rhodamine lissamine sulfonylchloride according to the methods of Brandtzaeg (7).

Use of the G Protein of the VSV as a Marker for the Golgi Complex: Coverslip cultures were infected with the O-45 mutant at low multiplicity (10 plaque forming units/cell) for 40 min at 32°C. They were rinsed three times with media and immediately transferred to tubes with stirrers and further maintained at the nonpermissive temperature of 39.9°C in a circulating water bath. During all infection experiments, cells were maintained in Coon's F-12 media with 5% CO_2 buffered with 15 mM HEPES at pH 7.4. This minimized rapid changes in pH levels when microtubule-altering agents were eventually added. As previously established by light and electron microscopic immunolabeling studies (4, 5), a temperature shift from 39.9°C to 32°C results in a synchronized movement of the G protein from the rough endoplasmic reticulum to fill the Golgi complex by 13 min and then on to the cell surface by 30 min. In experiments involving the drug treatments described below, all manipulations affecting microtubules were carried out with the O-45-infected cells at 39.9°C, after which the cells were shifted to 32°C for 13 min before fixation with paraformaldehyde. For the G protein to serve as a Golgi marker in all of these experiments requires the assumption that the intracellular transport of the G protein from the rough endoplasmic reticulum to the GA after the temperature shift is unaffected by the prior drug treatments. This assumption is considered further in the Discussion and is justified in the following article (29).

Manipulation of Cytoplasmic Microtubule Assembly Status in O-45-Infected NRK Cells: The microtubule-depolymerizing agent nocodazole (Aldrich Chemical Co., Milwaukee, WI) was introduced at a concentration of 10 μg/ml (30 μM) in medium during the last 90 min of a 3-h incubation at the nonpermissive temperature of 39.9°C. Under these conditions complete disassembly of cytoplasmic microtubules was achieved as monitored by immunofluorescent microscopy with antibodies directed to tubulin. If nocodazole-containing medium was removed and replaced with drug-free medium, reassembly of cytoplasmic microtubules immediately commenced and continued for ~30 min. During this time, the positioning of the Golgi complex was determined. Experiments involving taxol treatment were of two kinds. In the first type, taxol (obtained through the courtesy of Dr. M. Suffness, Natural Products Branch, Division of Cancer Treatment, National Institutes of Health) was introduced at a concentration of 10 μM in medium immediately after the removal of nocodazole, after which microtubule repolymerization was allowed to proceed for another 20 min. In other experiments, taxol was introduced directly instead of nocodazole during the last 90 min of incubation of 39.9°C. As indicated above, each of these treatments was then followed by a 13-min incubation of the cells at 32°C to move the G protein into the GA.

Double and Triple Immunofluorescent Microscopy: In all experiments cells were fixed for 30 min at room temperature with 3% paraformaldehyde in PBS, pH 7.4. After three 10-min rinses in PBS supplemented with 0.2 M glycine, cells were permeabilized with 0.1% Triton X-100 in PBS for 5 min. After three rapid rinses in PBS, they were either singly labeled with rabbit antibodies to the G protein, doubly labeled with guinea pig antibodies to the G protein and rabbit antibodies to tubulin, or triply labeled with rabbit antibodies to tubulin and to the G protein and guinea pig antibodies to vimentin. All primary affinity-purified antibodies were used at a concentration of 10 μg/ml. After labeling with the primary antibodies, cells were rinsed three times in PBS/0.2 M glycine and then finally reacted with a mixture of rhodamine-conjugated goat antibodies to rabbit IgG and fluorescein-conjugated goat antibodies to guinea pig IgG at a concentration of 10–20 μg/ml. Microscopy was performed with a Zeiss Photomicroscope III (Carl Zeiss, Inc., New York) equipped for epi-illumination fluorescence with appropriate rhodamine and fluorescein filters and differential interference-contrast microscopy with a ×63 Planapochromat objective (Carl Zeiss, Inc.).

The use of the temperature shift-down protocol with O-45-infected NRK cells allowed the Golgi complex to be visualized by immunolabeling for the G protein (Fig. 1) (5). In other fluorescent labeling experiments, use of the lectin wheat germ agglutinin (35), or a polyclonal antibody directed to a Golgi-specific 135,000 mol wt antigen described by Louvard et al. (19) and used for GA labeling by Kupfer et al. (16), gave closely similar staining patterns for the GA as did the labeling for the G protein (Rogalski, A., G.-A. Keller, and S. J. Singer, unpublished observations). In interphase cells, the GA was seen as a compact punctate perinuclear complex that overall was superimposed on the MTOC (Fig. 1, B and C), as revealed by double-immunofluorescence labeling (16).

When the cytoplasmic microtubules in interphase cells were completely depolymerized by a 90-min treatment with 30 μM nocodazole (Fig. 2 A'), the GA was dispersed into a set of discrete elements that became labeled for the G protein in the periphery of the cell (Fig. 2 A). Under less than optimal conditions for microtubule depolymerization (as for example, the same treatment with 30 μM nocodazole for only 30 min instead of 90 min), when only a fraction of the cytoplasmic microtubules were still present, the GA remained compact and in the usual perinuclear location. In the completely dispersed condition, we observed between 20 and 40 labeled GA elements of various sizes per cell. An interconnecting structure between the well dispersed GA elements could not be detected by G protein labeling.

Removal of the nocodazole from the cells by rinsing with fresh medium led to a progressive reassembly of the cytoplasmic microtubules emanating from the MTOC (Figs. 2 B' and 3 B) which was completed by ~30 min after drug removal. This was accompanied by an apparent directional movement of the dispersed GA elements, and their progressive recompaction back to the normal perinuclear location of the GA during the same time span (Figs. 2 B and 3 A). In such doubly labeled specimens (corresponding arrows in Fig. 2, B and B'; Fig. 3, A and C), the positions of individual GA elements in the process of recompaction appeared to be closely associated with the positions of individual microtubules or arrays of microtubules.

If the treatment with nocodazole that led to complete microtubule disassembly was followed by a brief rinse and then immediate treatment with taxol, enough reassembly occurred at the MTOC to render it visible by immunolabeling (Fig. 2 C') as described previously (12), but such reassembly was limited, and the bulk of the tubulin reassembled into short bundles of free microtubules dispersed in the cell periphery. Under these circumstances, the elements of the GA that had been dispersed into the cell periphery upon nocodazole treatment remained so distributed (Fig. 2 C); i.e., they underwent no recompaction.

In cells treated directly with taxol at 10 μM, with no prior nocodazole incubation, a drastic reorganization of the cytoplasmic microtubules was observed leading to a variety of different arrangements of microtubule bundles in different cells (Figs. 4 and 5) (12). In such cells, the elements of the GA also exhibited a variety of intracellular locations and arrangements, but in each such cell, the GA elements were always located near the ends of individual microtubules or microtubule bundles (corresponding arrows in Fig. 4, B–D; F and G; I and J). Where the microtubule bundles appeared more regularly organized and two distinct ends of the bundles
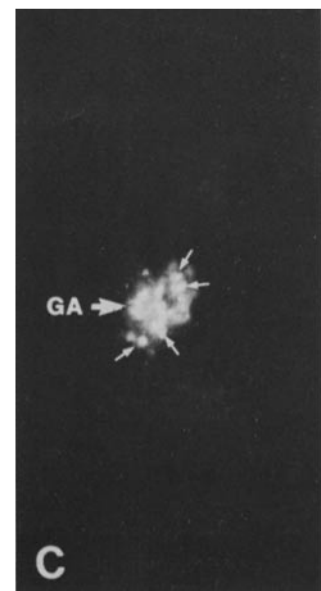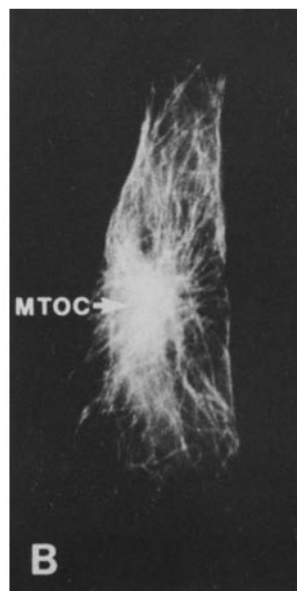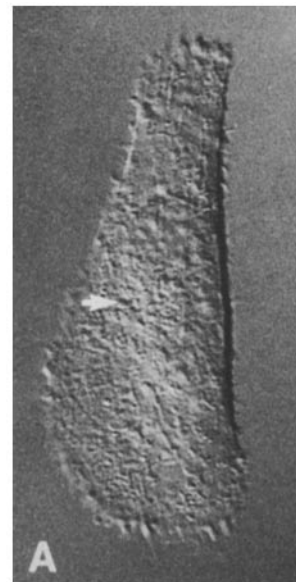

FIGURE 1 An NRK cell infected with the O-45 mutant of VSV, 13 min after the temperature shift-down to 32°C. The cell, visualized in Nomarski optics in A, was doubly immunofluorescently labeled with antibodies to tubulin in B, revealing the MTOC (arrow), and with antibodies to the G protein of VSV in C, revealing the Golgi apparatus (GA, large arrow). Note the compact perinuclear configuration of the GA, the discreteness of its elements (small arrows), and its general superposition on the MTOC. × 930.

could be discerned (Fig. 4, C and D; Fig. 5, A and B; C; D), the elements of the GA were associated with only one of the two end regions.

Because the labeled GA elements could be distinguished by their location and morphology from the microtubule bundles in such cells, the two structures could be immunofluorescently labeled with the same fluorophore, and additional immunolabeling of the vimentin intermediate filaments carried out with a second fluorophore. In such triply labeled cells (Fig. 5, C and C'; 5, D and D') the positions of the GA elements were correlated with the end regions of microtubule bundles, but were not correlated with the distributions of the intermediate filaments.
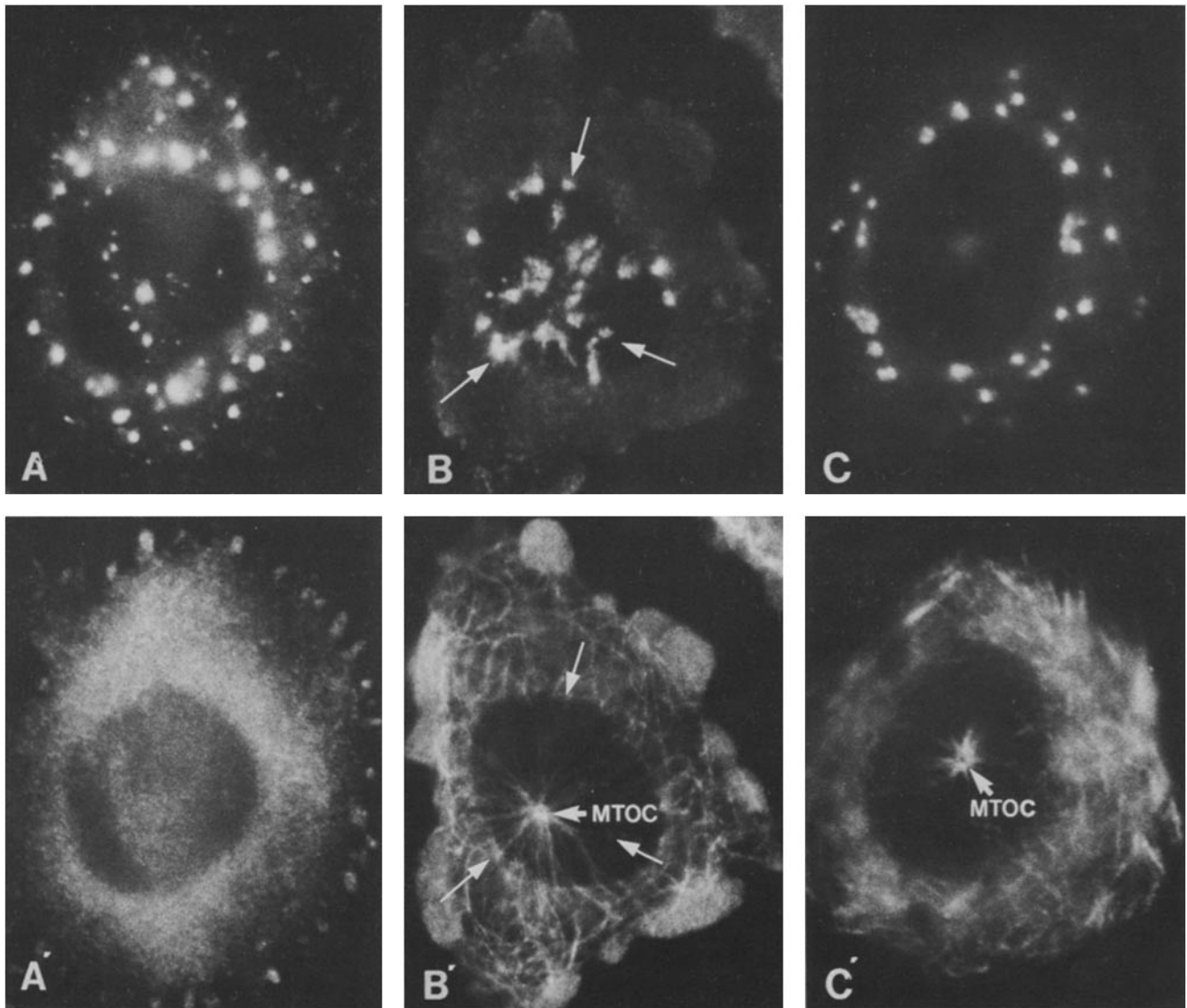
FIGURE 2 Similar specimens as in Fig. 1, except that before the temperature shift down, the cell in A/A' was treated with nocodazole to completely disassemble the cytoplasmic microtubules (A'), which led to the dispersion of the G protein-labeled GA elements into the cell periphery (A); the cell in B/B' was washed free of nocodazole, and by 20 min later had partially reassembled its microtubules (B'), which led to the partial recompaction of the GA elements (B); and the cell in C/C' was treated with taxol after nocodazole, leading to the assembly of bundles of microtubules free of the MTOC (C') with the GA elements remaining dispersed in the cell periphery (C). The blunt arrows in B' and C' designate the MTOC. In B/B', corresponding thin arrows indicate examples of GA elements that are closely associated laterally with microtubules emanating from the MTOC. × 1,725.

DISCUSSION

In these experiments, we have explored the structural inter-relationships of the Golgi complex and microtubules by immunofluorescence observations, using as a marker for the GA the G protein of VSV. It was shown earlier (5) that in cells infected with the O-45 temperature-sensitive mutant of VSV, a shift from 39.9°C to 32°C resulted 13 min later in the synchronous filling of the GA with the G protein on its way to the plasma membrane. The reason for using this novel marker for the GA was that we were studying the effect of manipulations of microtubule organization upon the intracellular transport and processing of the G protein, as described in the following article (29), and the present immunofluorescence experiments were then carried out with the same sys-

tem. Furthermore, as employed in these experiments, the G protein provided a GA marker of great sensitivity compared with other GA markers that we have used. This use of the G protein, however, depended on the proposition that, irrespective of the nature of the treatment of the O-45 cells before the temperature shift, 13 min after the shift to 32°C the G protein would be in the GA. This proposition was fully justified in two ways: (a) other GA markers, such as the polyclonal anti-GA antibody of Louvard et al. (19) (Rogalski, A., G.-A. Keller, and S. J. Singer, unpublished observation) or wheat germ agglutinin (35), showed very similar GA distributions, as did the G protein marker after manipulations of microtubule organization; and (b) such microtubule manipulations had no detectable effect on the rate or extent of oligosaccharide processing of the G protein, or the rate of surface expression
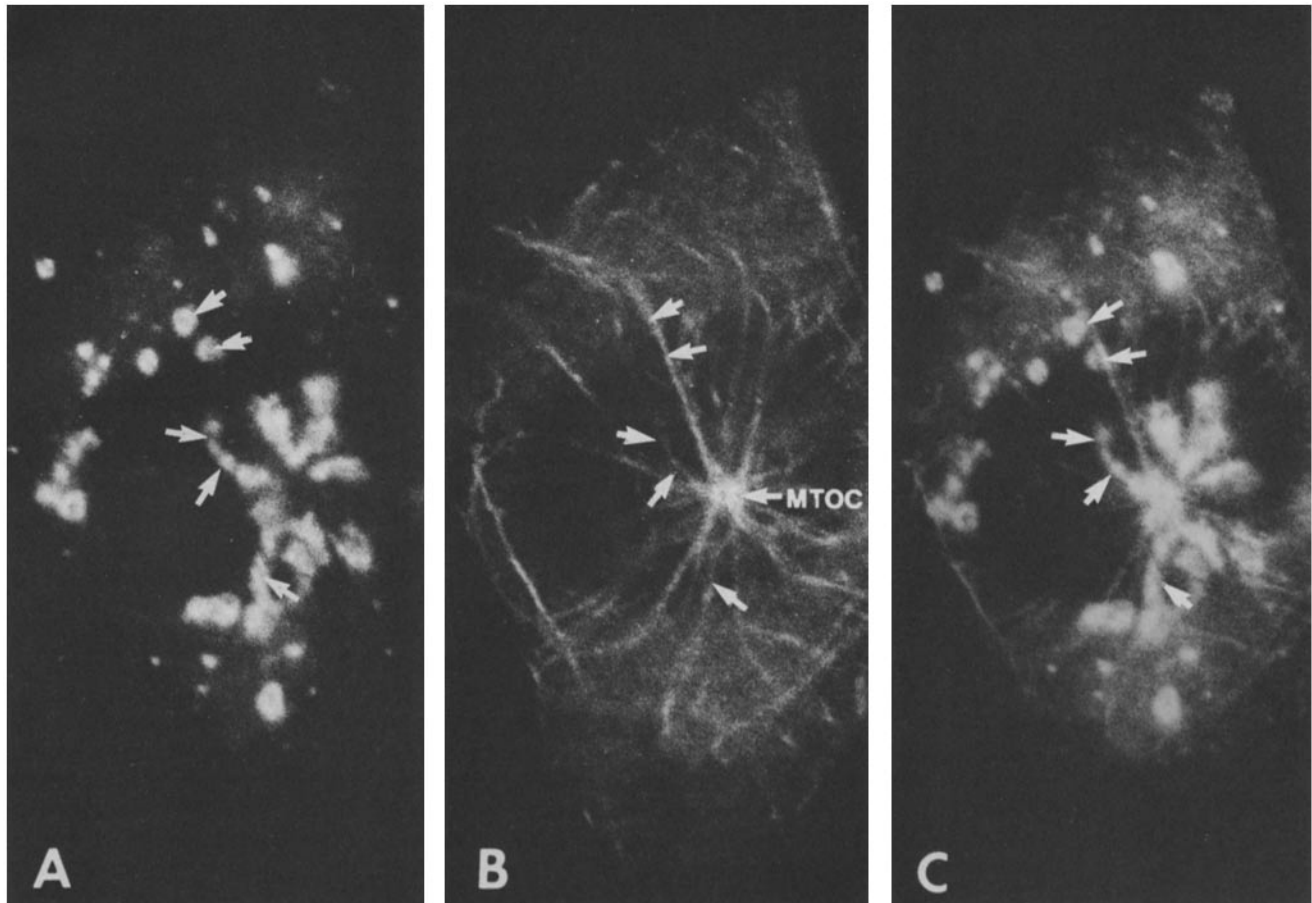
FIGURE 3 Another specimen treated as in Fig. 2, B/B', in which partial reassembly of microtubules has occurred after the washout of nocodazole (B), and the GA elements labeled with the G protein (in A) are undergoing recompaction. The photographic superposition of A and B is shown in C, where the arrows point to examples of GA elements that are closely associated with microtubules. (Corresponding arrows are shown in A and B.) × 2,425.

of the G protein (29).

We have made several different observations that suggest that the state of dispersion of elements of the GA as well as their locations inside cells are directly related to microtubule organization. The discussion of these observations follows.

Dispersal of the GA upon Disassembly of Cytoplasmic Microtubules

It has previously been observed by thin-section electron microscopy (13, 21, 27) that microtubule disassembly disrupts the compact perinuclear disposition of the GA, with stacks of GA saccules now found dispersed throughout the cytoplasm. More recently, immunofluorescence experiments that use antibodies to what are apparently structural components of the GA, such as a polyclonal antibody directed to a 135,000

mol wt protein (19), and a monoclonal antibody directed to a 110,000 mol wt protein (17) have shown that microtubule disassembly leads to a dispersion of GA elements into the cell periphery, as observed at the light microscopic level. Our immunofluorescence results have extended the latter observations in that the GA elements were recognized not structurally but rather functionally, by virtue of the labeling for the G protein that was arrested during its passage through the GA. This methodology revealed that upon microtubule disassembly, the G protein-labeled GA elements are discrete units spread out in the periphery of the NRK cell, separated by quite large distances from one another. This discontinuous G-protein labeling of the GA seems surprising in view of the results of high-voltage electron microscopy of thick sections of specimens stained for thiamine pyrophosphatase activity (22) or of osmium-stained specimens (26) that suggest that

FIGURE 4 Specimens similar to that in Fig. 1, except that they were treated with taxol before the temperature shift-down. The cell visualized by Nomarski optics in A, is in the terminal stage of cell division, and exhibits its G protein-labeled GA elements (B) in close juxtaposition to the end regions of taxol-induced microtubule bundles (C). The corresponding arrows in B and C, and in the photographic superposition of B and C shown in D, indicate this spatial relationship. Two taxol-treated interphase cells seen in Nomarski optics in E and H similarly show their G protein-labeled GA elements (in F and I, respectively) in association with end regions of microtubules (in G and J, respectively), as pointed out by corresponding arrows in F, G, and I and J. The general dispositions of the GA elements and microtubules in the two cells shown are, however, grossly different. × 1,389.
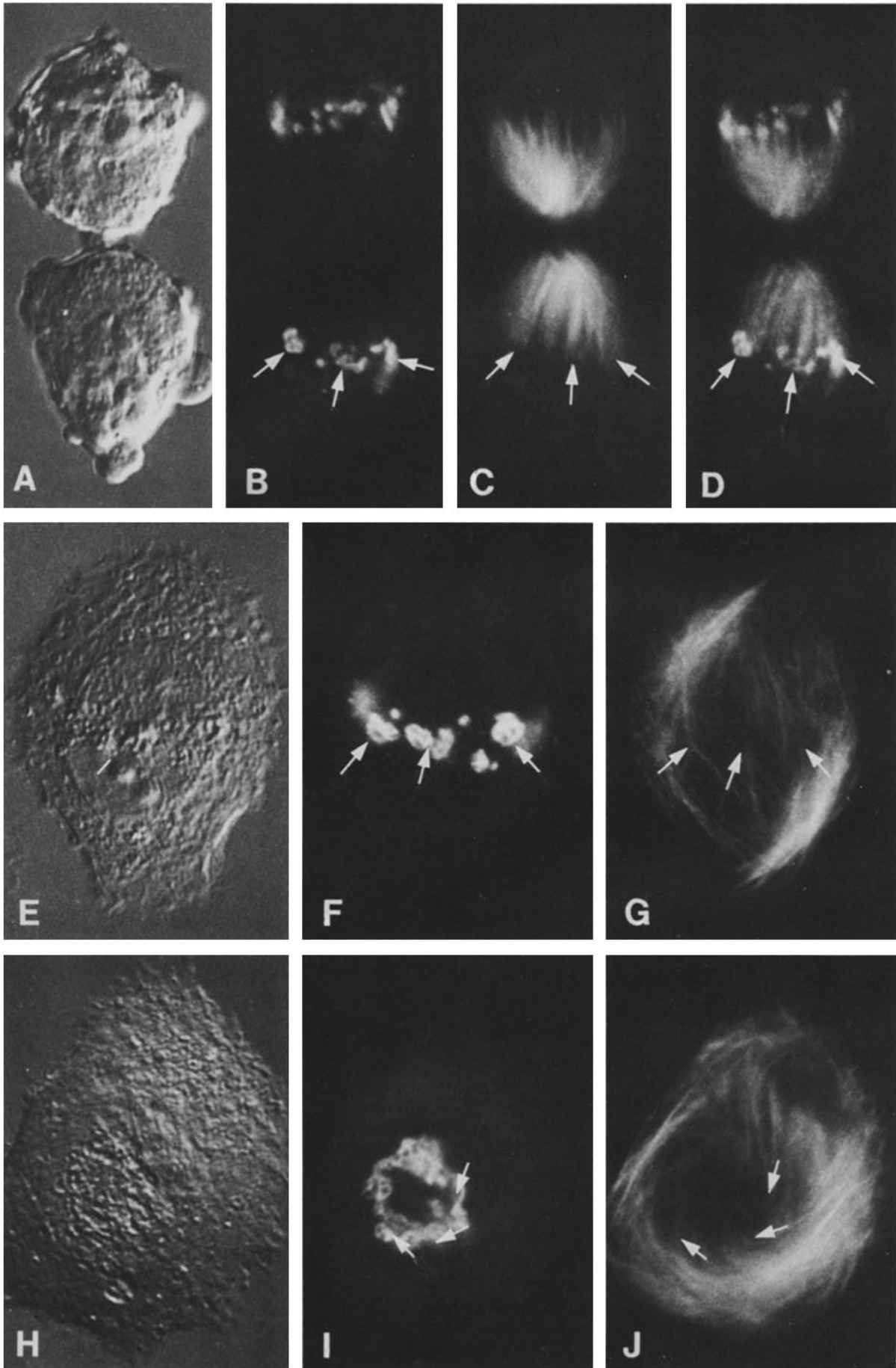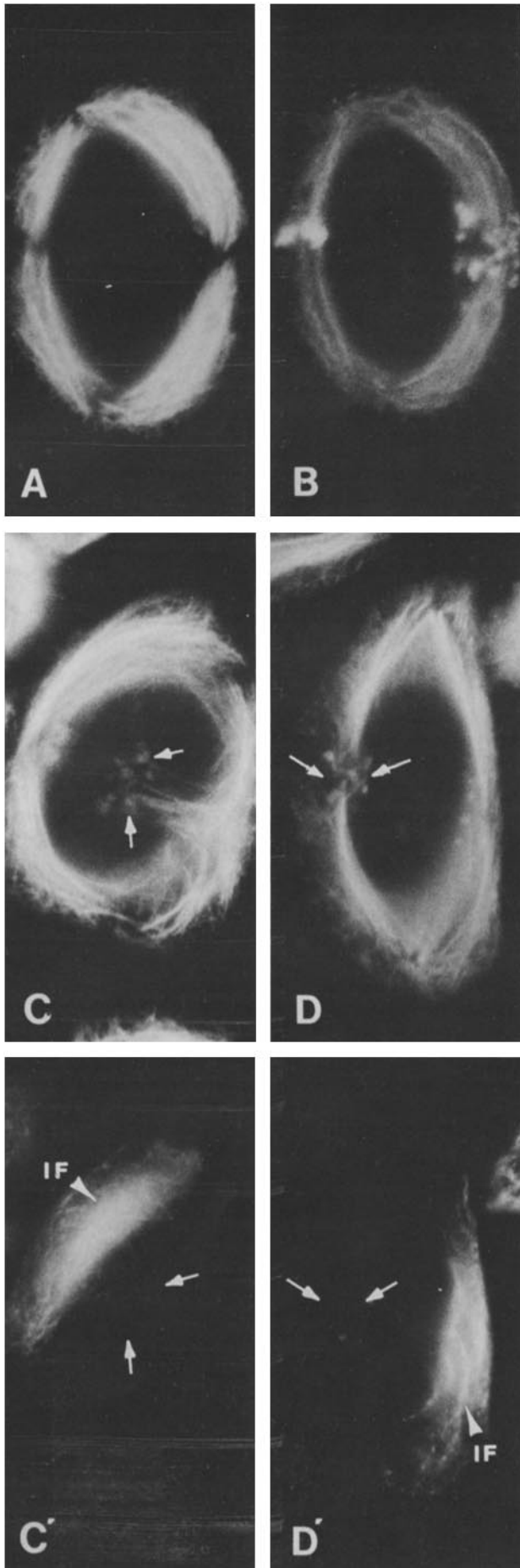
the GA is a single continuous structure within any one cell, with stacks of saccules interconnected by similarly stained but morphologically distinguishable membranous elements that were designated "intersaccular connecting regions." The two sets of results may be reconciled, however, if it is suggested that, during its passage through the GA, the G protein is largely concentrated in the stacks of saccules, and is either absent or present at very low concentrations in the intersaccular connecting regions, whereas certain enzymes and structural proteins of the GA may be present in both the saccules and the intersaccular connecting regions.

A coherent interpretation of these results and those in the following article (29) is that the dispersion of GA elements upon microtubule disassembly does not involve a fragmentation of the Golgi complex, but rather a lateral unfolding and extension of the structure, probably largely at the intersaccular connecting regions, without extensive structural (27) or functional impairment of the stacks of saccules themselves. Such a lateral unfolding without fragmentation can also most readily account for the rapid recovery of the normal compact configuration of the GA upon allowing the microtubules to reassemble, as discussed next. If fragmentation of the GA occurred during dispersal, a large number of specific membrane fusion events would have to occur upon recompaction, which seems unlikely.

Microtubule Involvement in the Recompaction of the GA

After complete disassembly of cytoplasmic microtubules by nocodazole, washout of the drug results in reassembly of microtubules from the MTOC as the region of origin (8, 23). As microtubule polymerization progressed, labeled elements of the GA were observed to undergo a progressive inward movement towards the nucleus, ultimately collecting into a compact perinuclear configuration superimposed upon the MTOC. At stages during this inward movement, individual GA elements were often closely associated with labeled microtubules (Fig. 2, B and B'; Fig. 3). Despite the low resolution of immunofluorescence microscopy, it may be inferred from such an entirely nonrandom association that some type(s) of direct or indirect interactions exist between structures of the Golgi complex and microtubules in these interphase cells. Similar double immunofluorescence observations have likewise provided evidence for direct or indirect interactions along the length of microtubules with other membrane-bound intracellular organelles, including mitochondria (2), lysosomes (9), and secretory granules (33). Such interactions between Golgi elements and microtubules may be involved in the movement of these elements into the compact conformation of the GA that is associated with the MTOC.

FIGURE 5   Specimens similar to those in Fig. 4, treated with taxol before the temperature shift-down, showing the polar association of G protein-labeled elements of the GA with one of the two end regions of microtubule bundles. In the pair A and B, optical leakage of the intense rhodamine-fluorescent labeling of microtubules (A) into the fluorescein labeling of GA elements (B) shows the localization of the GA elements at the microtubule end regions. In the pairs, C/C' and D/D' the GA elements (arrows) and the microtubules were specifically immunofluorescently labeled with fluorescein (C and D), and the vimentin intermediate filaments (IF, arrowhead) in the same cells (C' and D', respectively) were labeled with rhodamine. These experiments show that the positions of the GA elements (arrows in C' and D') are not correlated with the intermediate filaments as they are with the microtubules (C and D). (A and B) × 1,351; (C and C' and D and D') × 1,225.

That microtubules are involved in the recompaction of the GA is supported by the results of experiments with cells that were first treated with nocodazole, followed by taxol. As has been observed before (12) under similar conditions, the kinetics of tubulin reassembly at different cytoplasmic sites were such that a small MTOC was formed with relatively short microtubules emanating from it (Fig. 2 C'), but the bulk of the reassembled tubulin formed many bundles of short microtubules dispersed in the cell periphery, not in association with the MTOC. The microtubules in such bundles are apparently free at both ends (12). In such cells, the GA elements remained dispersed in the cell periphery where the bundles of free microtubules were located (Fig. 2 C). This indicates that the presence of an MTOC is not in itself sufficient to induce the recompaction of the Golgi elements, but that the bulk of the tubulin must be assembled in long microtubules associated with the MTOC for recompaction of the GA to occur.

The Polar Association of Golgi Elements with Microtubules

Interphase cells treated directly with taxol, i.e., without a prior incubation with nocodazole, also exhibit many bundles of microtubules free in the cytoplasm (12), not in association with an MTOC. The microtubules in these bundles are generally much longer than in the cells treated with nocodazole/taxol, and these bundles show a wide variety of configurations inside different cells (Figs. 4 and 5). In cells where the ends of such microtubule bundles could be discerned, G protein-labeled elements of the GA were generally found localized to one of the two end regions of the bundles (Fig. 4, B–D; Fig. 5, A and B; C; and D) In order to interpret these results, we consider first what is known about the mechanism of taxol action.

From in vitro studies of the interaction of tubulin and taxol (24, 30), it has been established that the drug lowers the critical concentration of tubulin required for assembly into microtubules. De Brabander (10, 11) has proposed a model for the function of an MTOC in normal microtubule assembly, in conjunction with which he has suggested that the mechanism of taxol action in intact cells is to so lower the critical concentration of tubulin as to bring it below the concentration required for microtubule assembly at the MTOC, so that disassembly occurs at the MTOC, and reassembly of free microtubules results elsewhere in the cell.

The association of GA elements with one end region of the taxol-induced microtubule bundles, however, suggests that each bundle has a polarity, which in turn suggests that the individual microtubules in a given bundle all have the same polarity. This inference, however, does not seem compatible with a random de novo assembly of free microtubules in these cells and their subsequent clustering into bundles. Such a process might be expected to produce bundles with a random orientation of their individual microtubules. We can account not only for the polar association of Golgi elements with taxol-induced microtubule bundles, but also the normal association of the GA with the MTOC in untreated cells, if we adopt the following two proposals:

(a) Elements of the GA form stable interactions with the end regions of microtubules, but only with the regions around the minus, or more slowly growing, ends (3, 6). All the cytoplasmic microtubules in normal interphase cells are known to have their minus ends associated with the MTOC

(14, 20, 34). Thus, a basis is provided for the GA/MTOC co-localization observed in interphase cells as well as for the dispersion of GA elements upon complete disassembly of the cytoplasmic microtubules. In the same context, the fact that when disassembly of microtubules is extensive but incomplete, the GA retains its compact perinuclear configuration, can be explained as due to the continued presence of minus ends of microtubules at the MTOC.

(b) The action of taxol involves the release of the cytoplasmic microtubules from the MTOC before the microtubules regions that are attached to the MTOC are completely disassembled. This would allow the released microtubules, all having the same polarity, to form bundles by lateral association before the individual microtubules had the chance to become reoriented. It is further proposed that, in such taxol-treated cells, de novo initiation of microtubule assembly occurs only infrequently; instead, most of the microtubules are formed by assembly of tubulin monomers onto the partially disassembled microtubules that are released from the MTOC, and that are now bundled and free. Under these conditions, the microtubules within each bundle would have the same polarity. Binding of GA elements to the minus-end regions of the bundled microtubules would then occur by the same or similar mechanism that determined the normal GA/MTOC association in proposal a.

Associations of Golgi Elements with Microtubules

Two different types of association of Golgi elements with microtubules have been postulated to account for the results presented in this paper: (a) one type (lateral) along the length of microtubules, observed during the recompaction of Golgi complex elements as microtubules are reassembled after removal of nocodazole (Fig. 2, B and B'; Fig. 3), and (B) a second type (end-on) confined to the region around the minus ends of microtubules or microtubule bundles that is seen in steady-state normal cells and in taxol-treated cells. Presumably the end-on type of association is the more stable of the two, and is therefore the predominant type in the steady state. We purposely leave vague the detailed nature of these proposed associations. Our observations (Fig. 5) suggest that intermediate filaments are not involved in the end-on type of association. Although thin-section electron micrographs have often been published in which stacks of Golgi saccules and a pair of interphase centrioles are seen in the same field, no firm conclusions about the basis for this spatial relationship have been drawn. This region of the cell contains a meshwork of microtubules and intermediate filaments, but no pattern to their arrangements vis-à-vis the Golgi elements has yet been discerned. Although we conclude that our observations at the light microscopic level of resolution suggest that these two types of interactions of Golgi elements and microtubules exist, there is little point in speculating further about the possible molecular details of such interactions at this time.

After this work was completed and published in a preliminary form (28), two relevant reports appeared, one (36) showing a co-distribution of Golgi elements with taxol-induced microtubule bundles, the other (37) that the microinjection of a monoclonal antibody reacting with the tyrosylated form of α-tubulin produces a dispersion of the Golgi apparatus much like that produced by colchicine and nocodazole.

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