# Role of Microtubules in the Organization and Localization of the Golgi Apparatus

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ABSTRACT Normal interphase $PtK_2$ and A549 cells display long microtubules radiating from the microtubule-organizing center (MTOC) to the plasma membrane. Both MTOC and Golgi apparatus are contained in the same perinuclear area. Treatment of cells with 1 $\mu$M colcemid for 2 h results in microtubule depolymerization and fragmentation of the Golgi apparatus into elements scattered throughout the cytoplasm. Both normal microtubules and the Golgi apparatus assemble again following removal of colcemid. Injection of the $\alpha,\beta$-nonhydrolyzable GTP analog, guanosine 5'($\alpha,\beta$-methylene)diphosphate [pp($CH_2$)pG], into interphase cells growing in normal medium results in the formation of microtubule bundles resistant to colcemid and prevents the fragmentation of the Golgi apparatus. Injection of pp($CH_2$)pG into cells incubated with colcemid results in substitution of tubulin ribbons for microtubules and has no effect on the Golgi-derived elements scattered throughout the cytoplasm. Removal of colcemid 1 h after the injection of pp($CH_2$)pG results in polymerization of large numbers of short, single randomly oriented microtubules, whereas the Golgi apparatus remains fragmented. Treatment of cells with 10 $\mu$M taxol for 3 h results both in polymerization of microtubule bundles without relation to the MTOC in the cell periphery and fragmentation of the Golgi apparatus. The Golgi-derived fragments are present exclusively in regions of the peripheral cytoplasm enriched in microtubules. The codistribution of microtubules and Golgi elements can be reversed in taxol-treated cells by injection of a monoclonal (YL 1/2) antibody reacting specifically with the tyrosylated form of $\alpha$-tubulin. Cells incubated with colcemid after treatment with taxol have large numbers of Golgi-derived elements in close association with colcemid-resistant microtubule bundles. Incubation of cells with 50 $\mu$M vinblastine for 90 min results in microtubule disassembly, formation of tubulin paracrystals, and fragmentation of the Golgi apparatus into elements without relation to the tubulin paracrystals.

Many interphase cells have the Golgi apparatus located in the area of the cytoplasm that contains the microtubule-organizing center (MTOC) (5). Moreover, migrating cells show coordinated movements of both MTOC and Golgi apparatus (5). Also, during the disassembly of microtubules preceeding the cell division (17) or resulting from treatment of cells with colcemid (6, 8), the Golgi apparatus is fragmented into elements that are randomly scattered throughout the cytoplasm. This suggests that microtubules play a role in determining the location of the Golgi apparatus in the vicinity of the nucleus and in maintaining its organization.

Here we report the effect of changes in the organization of cytoplasmic microtubules on the location of the Golgi apparatus in cells in interphase.

## MATERIALS AND METHODS

$PtK_2$ (kidney potorous tridactylis) and NRK-49F (normal rat kidney fibroblast) cells were grown in Ham's F12 medium with 5% fetal calf serum. A549 cells

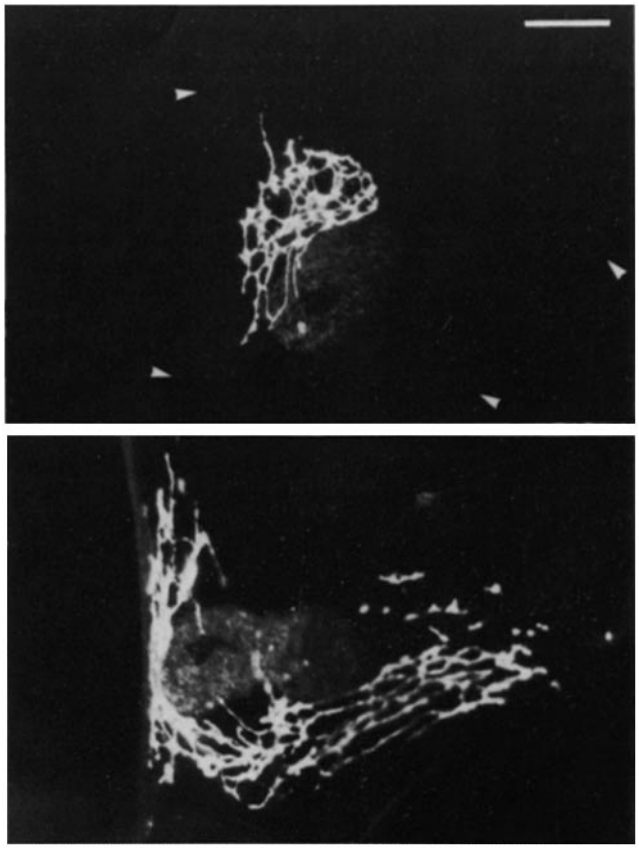

FIGURE 1 Immunofluorescence microscopy study of the Golgi apparatus of A549 cells in interphase. The Golgi apparatus was localized with a rabbit monospecific antibody raised against the Golgi enzyme β-galactosyltransferase. Rhodamine-conjugated goat anti-rabbit IgG was used as second antibody. Observe the perinuclear location and reticular structure of the Golgi apparatus. Arrowheads mark the cell edges. Bar, 10 μm.

(human lung carcinoma cells) were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum. 0.1 M solutions of pp($CH_2$)pG, GTP, and p($CH_2$)ppG were prepared in 0.1 M 2-(*N*-morpholino)ethane sulfonic acid (MES) (pH 7.2).[1] Solutions of 20 mg/ml of YL ½ and YOL 1/34 antitubulin antibodies were prepared in phosphate-buffered saline (pH 7.0). Both antibodies were provided by Dr. J. V. Kilmartin. Stock solutions of taxol (National Cancer Institute), colcemid (Grand Island Biological Co.) and vinblastine (Sigma Chemical Co.) were prepared in dimethyl sulfoxide and diluted 1:1,000 when added to the culture medium. The organization and distribution of both microtubules and the Golgi apparatus were studied by dual immunofluorescence microscopy. Cells were permeabilized by immersion in cold methanol (−20°C) for 2 min. The Golgi apparatus was studied by using a rabbit monospecific antibody raised by Dr. E. G. Berger against the Golgi enzyme β-galactosyltransferase (Fig. 1). Microtubules were studied by using the rat monoclonal antibody YL ½. Rhodamine-conjugated goat anti-rabbit IgG and fluorescein-conjugated goat anti-rat IgG were purified with, respectively, columns of rabbit and rat IgG coupled to Sepharose 4B and used as second antibodies in indirect immunofluorescence microscopy studies. For electron microscopy, cell monolayers were fixed in 2.5% glutaraldehyde in 0.125 M cacodylate buffer (pH 7.4) at room temperature, postfixed in $OsO_4$, stained in situ with uranyl acetate, dehydrated in ethanol, and embedded in Epon. After polymerization of the Epon, serial sections parallel to the plane of the monolayer were cut and examined so that the distribution of microtubules and Golgi elements could be evaluated thoroughly. Golgi membranes were prepared by the method of Howell and Palade (3). Microtubule-associated proteins (MAPs) were prepared by chromatography of microtubular protein, purified by two cycles of temperature-dependent microtubule polymerization-depolymerization, over a phosphocellulose column equilibrated and washed with 20 mM MES, 1 mM $MgCl_2$, 0.1 mM EGTA (pH 6.75) (buffer A) and then eluted with buffer A containing 0.8 M NaCl. Both Golgi proteins and MAPs were prepared to 1 mg protein/ml in 0.25 M sucrose and buffer A without EGTA, respectively, and used as immunogens. On days 1 and 15, 4-wk-old female BALB mice were injected subcutaneously with 200 μg of immunogenic protein mixed with 200 μl of complete Freund's adjuvant. On day 27, 200 μg of immunogen alone was injected intraperitoneally and 3 d later the mouse spleen cells were fused with SP 2/0 myeloma cells. Before the fusion the mice were bled to obtain immune serum. Clones were screened for production of antibodies with specificity for the Golgi apparatus and MAPs by immunofluorescence microscopy using freshly prepared rat hepatocytes and $PtK_2$ cells, respectively.

[1] *Abbreviations used in this paper:* GTP, guanosine 5′-triphosphate; MAPs, microtubule-associated proteins; MES, 2-(*N*-morpholino)ethane sulfonic acid; MTOC, microtubule-organizing center; pp($CH_2$)pG, guanosine 5′-(α,β-methylene)diphosphonate; p($CH_2$)ppG, guanosine 5′-(α,β-methylene) triphosphate.

## RESULTS AND DISCUSSION

### *Manipulation of Microtubule Polymerization and Organization in Cells in Interphase*

The microtubules of cells in interphase are initiated and organized by the MTOC (1, 7). The activity of the MTOC results from its capacity to induce the polymerization of microtubules at a concentration of tubulin lower than that required for polymerization in other areas of the cytoplasm (4). It follows that any increase in the ability of tubulin to polymerize below the concentration required for the MTOC would result in assembly of microtubules off of the MTOC, disruption of microtubule organization, and, as a consequence, alteration of the normal microtubule function. Both the α,β-nonhydrolyzable GTP analog pp($CH_2$)pG (9, 11) and the drug taxol (12) produce dramatic decreases in the critical tubulin concentration and can be used to manipulate the polymerization of microtubules in cells in interphase.

As shown in Fig. 2, *D* and *E*, $PtK_2$ cells first treated with colcemid to depolymerize microtubules and then injected with pp($CH_2$)pG assembled a large number of short, single randomly oriented microtubules after removal of colcemid. Thus, normal and pp($CH_2$)pG-injected cells showed dramatic differences in the number, length, and organization of their microtubules. Moreover, microtubules polymerized by pp($CH_2$)pG in vivo displayed high resistance to concentrations of calcium (Fig. 2*K*) and colcemid (Fig. 2*B*) that readily depolymerized microtubules from normal cells. We further manipulated the polymerization of tubulin in vivo inducing the substitution of tubulin ribbons (10) for microtubules, by injecting pp($CH_2$)pG into cells that were cultured continuously in the presence of colcemid (Fig. 2, *F* and *G*). Cells injected with pp($CH_2$)pG displayed normal stress fibers and intermediate filaments. Control cells injected with either GTP or p($CH_2$)ppG displayed normal microtubules.

Cells in interphase treated with taxol showed a peculiar organization of colcemid-resistant microtubules into bundles that were located in the cell periphery (Fig. 3*E*). The microtubule bundles showed no connection with the MTOC, which remained apparently intact in the vicinity of the nucleus (2).

### *Effect of Changes in Microtubule Polymerization and Organization on the Location and Organization of the Golgi Apparatus in Interphase Cells*

The Golgi apparatus of A549 cells in interphase was located in the vicinity of the MTOC (Fig. 1). From the MTOC,

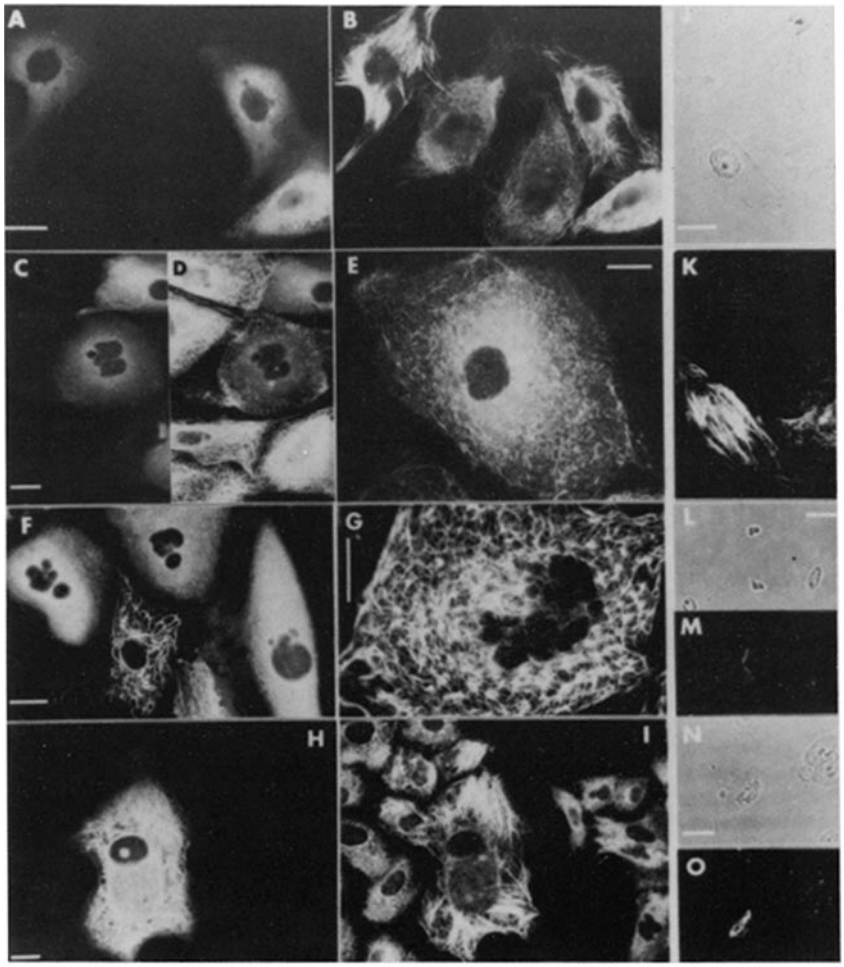


FIGURE 2 Regulation of microtubule polymerization in pp($CH_2$)pG-injected $PtK_2$ and A549 cells. (*A* and *B*) $PtK_2$ cells growing in normal medium were injected with pp($CH_2$)pG and the marker rhodamine-labeled goat anti-guinea pig IgG. The three cells injected with pp($CH_2$)pG (*A*, rhodamine channel) showed bundles of microtubules (*B*, fluorescein channel), whereas the uninjected cells displayed normal microtubules. (*C–E*) The two $PtK_2$ cells showing rhodamine fluorescence (C) were injected with pp($CH_2$)pG after 2 h of incubation with 1 μM colcemid. 1 h later, the colcemid was removed and the cells were incubated in colcemid-free medium for 2 h. Observe the large number of short, single randomly oriented microtubules polymerized in the pp($CH_2$)pG-injected cells (*D* and *E*). (*F* and *G*) As in *C–E*, but colcemid was not removed from the culture medium after injection of pp($CH_2$)pG. Observe the presence of wavy polymers of tubulin in the cells injected with pp($CH_2$)pG and the absence of tubulin polymers in the uninjected cells. (*H* and *I*) As in *A* and *B*, except that 2 h after injection of pp($CH_2$)pG the cells were treated with 1 μM colcemid for 2 h. Observe (*H*, rhodamine channel; *I*, fluorescein channel) that uninjected cells having close membrane contact with pp($CH_2$)pG-injected cells also display colcemid-resistant microtubules. (*J* and *K*) Cytoskeletons of normal and pp($CH_2$)pG-injected cells obtained in the presence of 2 mM $CaCl_2$. The two cells shown at the bottom of the phase-contrast picture (*J*) were injected with pp($CH_2$)pG while being incubated in normal medium. Observe the resistance of cytoplasmic microtubules to calcium in the pp($CH_2$)pG-injected cells. (*L–O*) Resistance of midbody (*L* and *M*) and spindle (*N* and *O*) microtubules to calcium in cytoskeletons of uninjected $PtK_2$ cells. Bars, 20 μm. (Reprinted, with permission, from reference 13 [*Proc. Natl. Acad. Sci. USA.* 1983. 80:1938–1941.].

individual microtubules stretched radially toward the plasma membrane (Figs. 2*B* and 3*A*). Incubation of cells with colcemid resulted in both microtubule disassembly (Fig. 3*B*) and fragmentation of the Golgi apparatus into elements that were randomly dispersed throughout the cytoplasm (Fig. 3*B'*). The fragmentation of the Golgi apparatus was observed not only in cells without microtubules but also in cells with changes in the organization of microtubules. Thus, cells induced by pp($CH_2$)pG to assemble large numbers of short, randomly oriented microtubules showed fragmented Golgi apparatus (Fig. 4, *G* and *H*). A similar fragmentation of the Golgi apparatus was observed in cells treated with taxol containing long bundles of microtubules in the cell periphery (Fig. 3, *E* and *E'*). Interestingly, in taxol-treated cells the Golgi elements were conspicuously present in areas of the cytoplasm rich in microtubules and absent from areas poor in microtubules, as shown by immunofluorescence (Fig. 3*E'*) and electron microscopy (data not shown). Among the areas of cytoplasm poor in both microtubules and Golgi elements was that containing the MTOC. The codistribution of microtubules and Golgi elements in the cytoplasm of the cells treated with taxol suggested some sort of interaction between these two structures. In other, similar experiments we observed the failure of colcemid to fragment the Golgi apparatus of cells containing

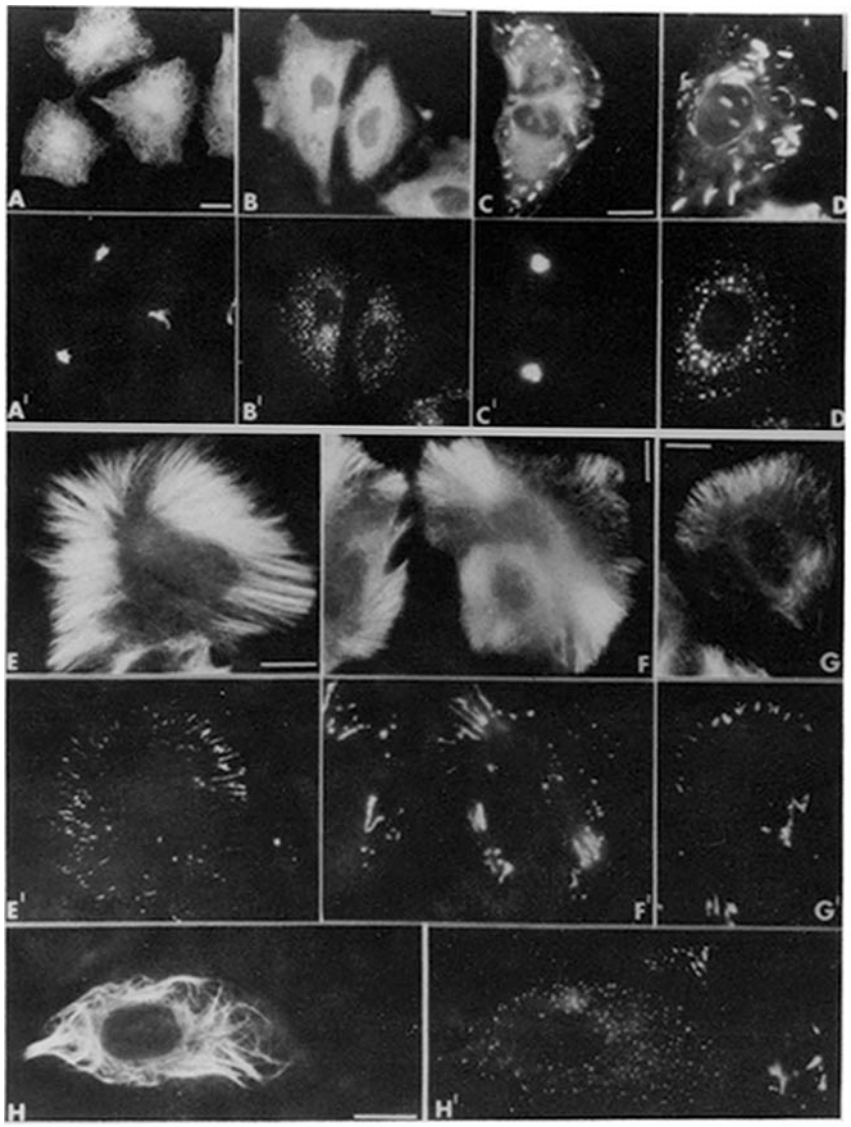


FIGURE 3 Effect of microtubule depolymerization by colcemid and vinblastine and of taxol-controlled polymerization on the integrity and location of the Golgi complex in A549 cells. (*A* and *A'*) Microtubules and Golgi apparatus as displayed by A549 cells growing in normal medium. Observe the long microtubules stretching radially from the vicinity of the nucleus to the plasma membrane (*A*) and the perinuclear location of the Golgi apparatus in the area of intense fluorescence containing the MTOC (*A'*). (*B* and *B'*) Microtubule depolymerization and fragmentation and dispersion of the Golgi apparatus in A549 cells treated with colcemid. Cells were incubated with 1 μM colcemid for 2 h. Note the complete disappearance of microtubules (*B*) and the fragmentation of the Golgi apparatus into elements distributed uniformly throughout the cytoplasm (*B'*). (*C*, *C'*, *D*, and *D'*) Microtubule depolymerization, formation of tubulin paracrystals, and fragmentation and dispersion of the Golgi apparatus in A549 cells incubated with 50 μM vinblastine for 15 (C and C') and 90 min (*D* and *D'*). Observe the fast substitution of tubulin paracrystals for microtubules (C) and the slower fragmentation of the Golgi apparatus (C') after 15 min of incubation with vinblastine. Note the different distribution of tubulin paracrystals and Golgi-derived elements in the cytoplasm after the 90-min incubation of the cells with vinblastine (cf. *D* and *D'*). (*E*, *E'*, *F*, and *F'*) Effects of taxol on the integrity of the Golgi apparatus and the distribution of Golgi-derived elements and microtubules in the cytoplasm of A549 cells. Cells were incubated with 10 μM taxol for 3 h. Observe the predominant location of microtubules in the periphery of the cells (*E* and *F*) and the fragmentation of the Golgi apparatus (*E'* and *F'*) into elements that were located in areas of the cytoplasm rich in microtubules. (G and G') Codistribution of Golgi-derived elements with colcemid-resistant microtubules in the cytoplasm of taxol-treated A549 cells. Cells were treated with 10 μM taxol for 3 h and then incubated with 1 μM colcemid for 2 h. Observe the conspicuous accumulation of Golgi-derived elements (G') in areas of the cytoplasm containing colcemid-resistant microtubules. (*H* and *H'*) Effect of the monoclonal YL ½ anti-tyrosylated α-tubulin antibody on the distribution of microtubules and Golgi-derived elements in the cytoplasm of A549 taxol-treated cells. Cells treated with 10 μM taxol for 3 h were injected with ~1.2 amol of fluorescein-conjugated YL ½ antibody per cell; 2 h later the cells were fixed. Microtubules were studied by direct immunofluorescence microscopy, and the Golgi elements were studied by indirect immunofluorescence microscopy. Observe the changes in microtubule organization (cf. *H* with *E* and *F*) and distribution of Golgi elements (cf. *H'* with *E'* and *F'*) produced by YL ½ in taxol-treated cells. The patches of fluorescence shown at the right in *H'* correspond to the Golgi-derived elements of a taxol-treated cell not injected with antibody. Bars, 20 μm. (Reprinted, with permission, from reference 14 [*Proc. Natl. Acad. Sci. USA.* 1983. 80:4286–4290.].

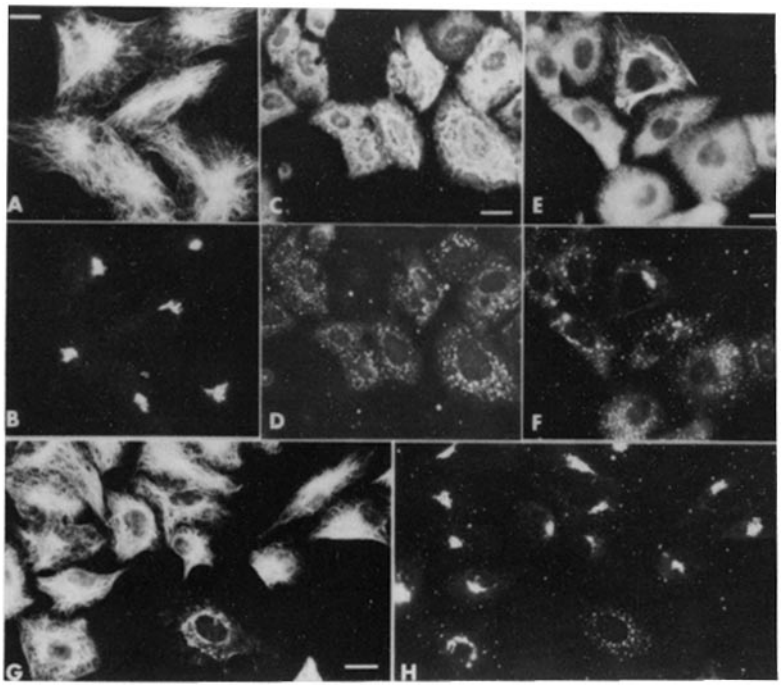


FIGURE 4 Effect of pp($CH_2$)pG-controlled polymerization of microtubules on the integrity and location of the Golgi complex in A549 cells. (*A* and *B*) Microtubules (*A*) and Golgi complexes (*B*) of A549 cells growing in normal medium. Observe that both the Golgi complex and the MTOC are located in the same perinuclear area. (*C* and *D*) Failure of tubulin ribbons polymerized by pp($CH_2$)pG in the presence of colcemid to reassemble the Golgi complex fragmented by colcemid (see description of the experiment in legend to Fig. 2, *F* and *G*). Observe the tubulin ribbons and the dispersion of the Golgi elements in cells injected with pp($CH_2$)pG and the absence of tubulin polymers and dispersion of the Golgi elements in uninjected cells. (*E* and *F*) Resistance of both microtubules and the Golgi complex to colcemid in cells injected with pp($CH_2$)pG (see description of the experiment in legend to Fig. 2, *A* and *B*). Observe the microtubule bundles (*E*) and the perinuclear location of the Golgi complex (*F*) in the pp($CH_2$)pG-injected cell and the absence of microtubules and the dispersed Golgi complex in the uninjected cells. (*G* and *H*) Reorganization of the colcemid-dispersed Golgi complex requires assembly of microtubules of the right length, proper orientation, and proper distribution (see description of the experiment in legend to Fig. 2, *C* and *D*). Observe the large numbers of short, single randomly oriented microtubules and the dispersion of the Golgi complex in the cell injected with pp($CH_2$)pG and the display of normal microtubules and Golgi complex in the uninjected cells. Bars, 20 μm. (Reprinted, with permission, from reference 13 [*Proc. Natl. Acad. Sci. USA.* 1983. 80:1938–1941.].

colcemid-resistant microtubules polymerized by pp($CH_2$)pG (Fig. 3, *E* and *F*) and to reverse the codistribution of colcemid-resistant microtubules and Golgi elements in the cytoplasm of taxol-treated cells (Figs. 3, *F* and *F'* and 3, *G* and *G'*). We also observed that neither the long ribbons assembled by pp($CH_2$)pG from colcemid tubulin complexes (Fig. 4, *C* and *D*) nor the crystals of short, curled tubulin ribbons produced by vinblastine (Fig. 3, *D* and *D'*) interacted with Golgi elements, as shown by their different distributions in the cytoplasm.

The interaction between microtubules and the Golgi apparatus was studied by injecting the YL ½ and YOL 1/34 monoclonal antibodies into A549 cells. The antibody YL ½ reacted specifically with the tyrosylated carboxy terminus of α-tubulin, whereas the YOL 1/34 reacted with both tyrosylated and detyrosylated α-tubulin (15). Injection of approximately 1.2 amol of YL ½ antibody into A549 cells in interphase had no effect on the organization of microtubules but produced the fragmentation of the Golgi apparatus into elements that were randomly dispersed throughout the cytoplasm (not shown; 16). In addition, injection of the YL ½ antibody into taxol-treated cells produced a marked rearrangement of the microtubule bundles in the cytoplasm (Fig. 3 *H*) and disrupted the codistribution of microtubules and Golgi elements that existed before the injection (Fig. 3 *H'*). Neither microtubules nor the Golgi apparatus of normal or taxol-treated cells were affected by injection of the YOL 1/34 antibody.

Thus, dramatic changes in the distribution of the Golgi apparatus are produced by a wide variety of manipulations of microtubules within cells. Depolymerization or abnormal polymerization of microtubules is associated with fragmentation of the Golgi apparatus throughout the cytoplasm. Redistribution of microtubules to the cell periphery by taxol results in fragmentation of the Golgi apparatus into elements that accumulate in areas of the cell rich in microtubules. This observation suggests that the microtubules themselves provide the mechanism for the localization of the Golgi apparatus. The ability of the YL ½ monoclonal antibody to disrupt microtubule-Golgi interactions suggests that microtubules possess specific binding sites that are involved in the localization of the Golgi apparatus. Whether tubulin binds to the Golgi apparatus directly or via intermediary proteins cannot be resolved by these observations.

To further characterize the molecules involved in the interaction between microtubules and the Golgi apparatus, we obtained polyclonal and monoclonal antibodies against MAPs and membrane proteins from both Golgi vesicles and cysternae. One of the monoclonal antibodies raised against MAPs reacted specifically with the MTOC and some intranuclear material (Fig. 5 *A*), whereas others reacted with microtubules.

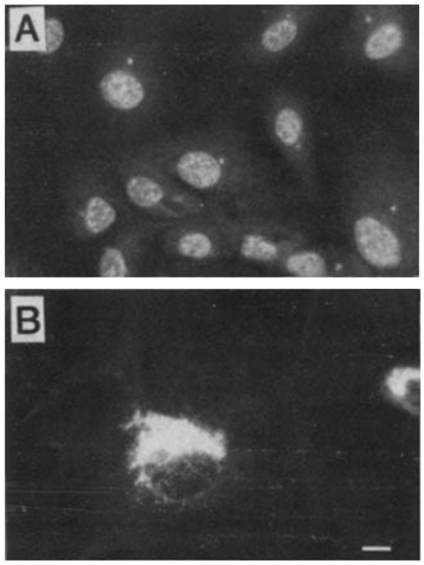


FIGURE 5 Antibodies against MTOC and Golgi apparatus. The reactivity of the antibodies was tested by indirect immunofluorescence microscopy as described in Materials and Methods. (*A*) 8D12 monoclonal antibody: the antigen reacting with the antibody was contained in a small perinuclear organelle as well as in the nucleus. The perinuclear organelle was identified as the MTOC by its ability to initiate the polymerization of microtubules in cells treated for 2 h with 1 $\mu$M colcemid and then incubated for 30 min in the absence of the drug (data not shown). (*B*) Polyclonal antibodies reacting with Golgi membrane proteins. Observe the strong specific reaction of the antibodies with the perinuclear Golgi of NRK cells. Treatment of cells with 1 $\mu$M colcemid for 2 h resulted in fragmentation of the Golgi into elements that were dispersed throughout the cytoplasm (data not shown). Bars, 20 $\mu$m.

Several polyclonal (Fig. 5 *B*) and monoclonal antibodies obtained against Golgi vesicle and cysternae membrane proteins reacted with the Golgi apparatus. We are currently studying the effect of these antibodies on the reassembly and relocation of the Golgi apparatus observed in cells treated with colcemid after removal of the drug and characterizing the proteins with which these antibodies react.

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