A Rat Monoclonal Antibody Reacting Specifically with the Tyrosylated Form of α-Tubulin. II. Effects on Cell Movement, Organization of Microtubules, and Intermediate Filaments, and Arrangement of Golgi Elements

JÜRGEN WEHLAND and MARK C. WILLINGHAM
Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205. Dr. Wehland's present address is Max-Planck-Institute for Biophysical Chemistry, D-3400 Goettingen, Federal Republic of Germany

ABSTRACT   A rat monoclonal antibody against yeast α-tubulin (clone YL 1/2; Kilmartin, J. V., B. Wright, and C. Milstein, 1982, J. Cell Biol., 93:576–582) that reacts specifically with the tyrosylated form of α-tubulin and readily binds to tubulin in microtubules when injected into cultured cells (see Wehland, J., M. C. Willingham, and I. V. Sandoval, 1983, J. Cell Biol., 97:1467–1475) was used to study microtubule organization and function in living cells. Depending on the concentration of YL 1/2 that was injected the following striking effects were observed: (a) When injected at a low concentration (2 mg IgG/ml in the injection solution), where microtubules were decorated without changing their distribution, intracellular movement of cell organelles (saltatory movement) and cell translocation were not affected. Intermediate concentrations (6 mg IgG/ml) that induced bundling but no perinuclear aggregation of microtubules abolished saltatory movement and cell translocation, and high concentrations (>12 mg IgG/ml) that induced perinuclear aggregation of microtubules showed the same effect. (b) YL 1/2, when injected at intermediate and high concentrations, arrested cells in mitosis. Such cells showed no normal spindle structures. (c) Injection of an intermediate concentration of YL 1/2 that stopped saltatory movement caused little or no aggregation of intermediate filaments and no dispersion of the Golgi complex. After injection of high concentrations, resulting in perinuclear aggregation of microtubules, intermediate filaments formed perinuclear bundles and the Golgi complex became dispersed analogous to results obtained after treatment of cells with colcemid. (d) When rhodamine-conjugated YL 1/2 was injected at concentrations that stopped saltatory movement and arrested cells in mitosis, microtubule structures could be visualized and followed for several hours in living cells by video image intensification microscopy. They showed little or no change in distribution and organization during observation, even though these microtubule structures appeared not to be stabilized by injected YL 1/2 since they were readily depolymerized by colcemid or cold treatment and repolymerized upon drug removal or rewarming to 37°C, respectively. These results are discussed in terms of the participation of microtubules in cellular activities such as cell movement and cytoplasmic organization and in terms of the specificity of YL 1/2 for the tyrosylated form of α-tubulin.

As a cytoskeletal component of interphase cells microtubules are involved in the determination of cell shape, in cell locomotion, intracellular movement, and cytoplasmic organiza-tion (for recent reviews see references 13, 21, 52). Evidence for the involvement of microtubules in these cellular activities has mainly derived from the application of drugs that specif-

ically interfere with the organization of microtubules in vivo and in vitro. Two classes of drugs are commonly used: (a) those that disassemble microtubules such as colchicine (47, 59) and (b), more recently, taxol, a drug that promotes assembly of microtubules and stabilizes them (54, 55). Another approach to study the function of defined proteins in living cells is the use of microinjection of specific antibodies, assuming that the injected antibody blocks the function of its antigen. This technique has successfully been applied first by Mabuchi and Okuno (41) to inhibit cytokinesis in starfish blastomers by injection of antimyosin serum and recently by others using antibodies against proteins of intermediate filaments to study the function of this type of filaments in tissue culture cells (18, 34, 35, 39).

With respect to microtubules, two classes of monoclonal antibodies would be favorable for a similar approach: class A antibodies would interfere with the polymerization of tubulin into microtubules by recognizing an antigenic site on the tubulin dimer that is necessary for polymerization. Class B antibodies would recognize a site on the tubulin dimer that is exposed on the surface of microtubules after polymerization; after binding to tubulin, such an antibody ideally should not interfere with polymerization and therefore should leave microtubules intact.

Injection of class A antibodies might induce depolymerization of microtubules and the results should be comparable with those derived from the application of microtubule-depolymerizing drugs such as colchicine. Class B antibodies could interfere with the functions of microtubules—without disassembling them—as follows: (a) by recognizing specific binding sites of microtubule-associated proteins (MAP)[1] and thereby displacing them, (b) by masking specific sites that are important for other cellular structures or proteins to interact with tubulin in microtubules, and (c) by sterically preventing interaction of other cellular structures with microtubules.

Previously, we have characterized a monoclonal antibody that specifically recognizes the tyrosylated carboxyterminus of α-tubulin. These studies have shown (a) that the antibody, although able to bind to tubulin, did not affect polymerization of microtubules and did not displace MAP in vitro and (b) that the antibody, when injected into cultured fibroblasts, readily binds to tubulin in microtubules and induces in a concentration-dependent manner a reorganization of microtubules (66). Here, we studied the effect of this antibody after microinjection into tissue culture cells on cellular functions such as intracellular movement, cell translocation, and the organization of intermediate filaments and Golgi elements. Portions of this work have been presented in abstract form (64).

MATERIALS AND METHODS

Cell Culture: Swiss 3T3 mouse fibroblasts and A549 (human lung carcinoma) cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. PtK₂ (rat kangaroo) and CHO (Chinese hamster ovary) cells were grown in HAM's F12 medium containing 5% fetal calf serum. Microinjection and immunofluorescence procedures have been previously described (63). Stock solutions of colcemid (Gibco Laboratories, Grand Island, NY) nocodazole (Sigma Chemical Co., St. Louis, MO), and taxol (National Cancer Institute) were made in DMSO. For electron microscopy, cells were grown in plastic dishes, fixed and embedded in situ, and processed following a

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[1] *Abbreviations used in this paper:* CHO, chinese hamster ovary; MES, 2-(N-morpholino)ethanesulfonic acid; and MAPs, microtubule-associated proteins.

method previously described (67).

Antibodies: Rat monoclonal antibodies to yeast α-tubulin (clones YL 1/2 and YOL 1/34) were a gift of Dr. J. V. Kilmartin (Medical Research Council, Cambridge) (33); affinity-purified rabbit human galactosyltransferase polyclonal antibodies were a gift of Dr. E. G. Berger (University of Berne, Switzerland) (7). Preparation and purification of rabbit polyclonal antibodies to tubulin from rat brain and to vimentin, isolated from CHO cells, have been previously described (10, 69). Direct labeling of YL 1/2 antibody with rhodamine and purification of rhodamine- and fluorescein-conjugated goat anti-rabbit IgG and goat anti-rat IgG were performed as previously described (66). Preparation of colloidal gold (5 nm in diameter) and adsorption to antibodies was performed using standard procedures (12, 15, 19). Gold-antibody complexes, prepared by Dr. D. J. P. FitzGerald (National Institutes of Health), were stored at 4°C in PBS containing 1% bovine serum albumin (Miles Laboratories, Inc., Elkhart, IN). Prior to injection, gold-antibody complexes were pelleted in a Beckman Airfuge (Beckman Instruments, Inc., Palo Alto, CA) for 10 min at 20 psi (~100,000 g) and resuspended in injection buffer (0.14 M KCl, 2 mM MES, pH 7.2). This procedure was repeated two times and injection solutions were finally centrifuged for 2 min at 5 psi (~20,000 g) to remove large aggregates. Concentrations of antibodies on gold were based on the assumption that 90% of initially added antibody was adsorbed to gold particles. In control experiments, purified rat IgGs (Miles) adsorbed to colloidal gold were used. Cells were normally fixed and permeabilized with cold methanol (−20°C) for 2 min. For preparation of cytoskeletons, cells were washed with buffer A (0.1 M MES, 1 mM EGTA, 1 mM MgCl₂, 4% polyethylene glycol 6000, pH 6.9) and then extracted for 30 s with buffer A containing 0.2% Triton X-100. Cytoskeletons were fixed in cold methanol (−20°C).

Cell Synchronization: CHO cells were grown in plastic tissue culture dishes and synchronized as described by Zieve et al. (75). Tissue culture dishes (35 mm) were treated with poly-L-lysine at 1 mg/ml (Sigma Chemical Co.). 1 d after plating, cells were incubated with thymidine at 2.5 mM for 15 h, then kept for 5 h in normal medium before nocodazole (0.04 μg/ml) was added for 4 h. About 80% of CHO cells were normally arrested in prometaphase by this method. The arrested cells divided within 1 h after removal of the drug. In microinjection experiments, CHO cells were injected with rhodamine-conjugated YL 1/2 antibody about 2 h before nocodazole was added. In control experiments, cells were injected with purified rhodamine-conjugated IgG at the same concentration. For better visualization of microtubule structures, cells injected with YL 1/2 antibody were extracted with detergent. About 60% of all cells microinjected with YL 1/2 antibody were arrested by treatment with nocodazole, although some mitotic cells were probably lost during washing and extraction procedures.

Analysis of Cell Movements and Intensification Microscopy: Cells grown on glass coverslips were microinjected and transferred into a modified Dvorak chamber. Saltatory movements and cell translocations were observed and recorded as previously described (68) using video intensification microscopy consisting of a SIT video camera (RCA TC1030H) coupled to a video monitor and a time-lapse video recorder (Panasonic NV-8030) (see also reference 60). Movements were normally recorded at a time-lapse ratio of 1:72. The use of a Zeiss ICM 405 microscope allowed the recording of phase-contrast micrographs directly on 35mm film during time-lapse video recording. For examining distribution of labeled antibody after microinjection in living cells, photographs were taken directly from the fluorescent screen of an EMI intensifier tube. This intensification system was recently described in detail (70).

RESULTS

Intracellular Movement and Cell Translocation

Saltatory organelle movements and cell translocation were followed by time-lapse video intensification microscopy (68) after microinjection of rhodamine-conjugated YL 1/2 antibody. Injection of labeled antibody allowed rapid identification of injected cells by video intensification microscopy and, when better resolution was required, enabled us to fix individual cells, immediately after recording, to examine the distribution of injected antibody by fluorescence microscopy. Injection of a low concentration of YL 1/2 (2 mg IgG/ml in the injection solution) that labeled microtubules without inducing their redistribution (66) did not affect saltatory movement or cell translocation. Higher concentrations that produced microtubule bundles (6 mg IgG/ml) or aggregated microtubules
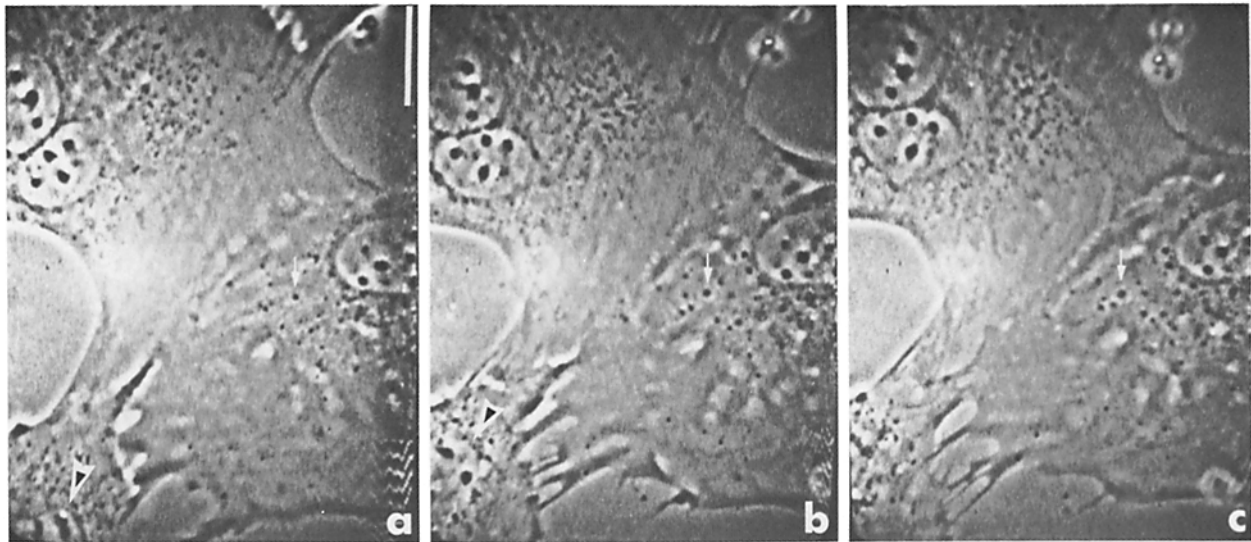
FIGURE 1 Saltatory organelle movements and cell locomotion of Swiss 3T3 cells after microinjection of YL 1/2 antibody. 1 h after injection (6 mg IgG/ml in the injection solution), video recording was started; all panels were made by photographing the video monitor display of stopped images from the time-lapse video record. The injected cell is located on the right side of the photographs. a was photographed 2 h after injection; 20 min (b) and 40 min (c) later uninjected cells on the left showed translocation in contrast to injected cell. Arrowheads in a and b indicate the nucleus in uninjected cell. Phase-dark vesicles in the injected cell barely changed position during observation; arrows in (a–c) indicate the same vesicle. Note the movement of uninjected cells by the altered positions of nuclei; the injected cell did not move. Bar, 20 μm. × 650.

around the nucleus (>12 mg IgG/ml in the injection solution) stopped saltatory movement and cell translocation. Although it is difficult to demonstrate saltatory movements in a fixed-time image (such as Fig. 1), intracellular phase-dense lysosmes barely changed their position in a cell injected with an intermediate concentration (6 mg IgG/ml) (Fig. 1). An adjacent uninjected cell showed intense saltatory movements of lysosomes when viewed by video time-lapse. The time difference between single exposures in Fig. 1 was ~20 min. Therefore, during 40 min of observation, uninjected cells showed translocation, recognized by altered position of nuclei, but the injected cell showed neither saltatory movements nor cell locomotion. Even 10 h after injection YL 1/2 could be detected along microtubule bundles. During this time some cells entered mitosis but did not progress normally through mitosis, i.e., they did not divide (see below).

We wanted to examine whether other experimental conditions that reorganize microtubules in cultured cells effect saltatory movement. One of us has recently described the effect of a nonhydrolyzable GTP-analog, guanosine 5'-[α,β-methylene] triphosphate (pp[CH₂]pG), on microtubule organization, cell locomotion, and saltatory movement after in-

jection into cultured cells (65). This GTP-analog induced formation of microtubule bundles resistant to colcemid, and, in the presence of the drug, saltatory movement was restricted to cytoplasmic areas that contained microtubule bundles (65).

Taxol has a similar effect on microtubule reorganization (55), i.e., it induces formation of microtubule bundles that are resistant to cold treatment and other microtubule-depolymerizing drugs (55). Previous reports showed that cell translocation was abolished and that cell organelles were redistributed to the perinuclear area (24, 55); also, saltatory movement was reported to be inhibited (24). For most of these studies cells were treated with taxol for ~20 h resulting in drastic reorganization of microtubules (see Fig. 2, c and c'). We rexamined the effect of taxol with respect to saltatory movement especially after shorter drug treatment.

The time course of taxol-induced stabilization of microtubules could easily be examined by colcemid treatments of cells previously incubated with taxol. Colcemid alone (1 μM) depolymerized microtubules within 1 h (Fig. 2a). When cells were pretreated with taxol (2 μM for 30 min) and then incubated further with taxol (2 μM) and colcemid (1 μM) for 1 h, microtubules remained intact and normally distributed

FIGURE 2 Microinjection of YL 1/2 antibody into Swiss 3T3 cells before or after treatment with taxol. (a–c') Indirect immunofluorescence microscopy using YL 1/2 antibody. (a) 3T3 cells after treatment with colcemid at 1 μM for 1 h, cytoplasmic microtubules were destroyed. (b) 3T3 cell treated with taxol at 2 μM for 30 min and further with taxol (2 μM) and colcemid (1 μM) for 1 h, cytoplasmic microtubules were stabilized by taxol. (c) A 3T3 cell treated with taxol at 2 μM for 24 h reveals numerous microtubule bundles; note multinucleation in corresponding phase-contrast micrograph (c'). (d–h') Localization of YL 1/2 antibody injected into 3T3 cells; at given times cells were fixed, permeabilized, and labeled with rhodamine-conjugated goat anti-rat IgG. (d and d') 2 h after injection with antibody (12 mg/ml), 3T3 cells were treated with taxol at 2 μM for 3 h. (e) 3T3 cell 5 h after injection of antibody (2 mg/ml). (f and f') 2 h after antibody injection (2 mg/ml), 3T3 cells were treated with taxol at 2 μM for 3 h. (g, h, and h') 3T3 cells treated with taxol at 2 μM for 1 (g) or 24 h (h and h') were injected with antibody (12 mg/ml) and fixed 2 h later. Note that taxol treatment did not reorganize microtubules previously aggregated by injection of antibody (d and d') and that injection of antibody was ineffective to aggregate microtubules in cells which were pretreated with taxol (g, h, and h'). Bars, 20 μm. (a, b, e, and h) × 450. (c, d, f, and g) × 600.
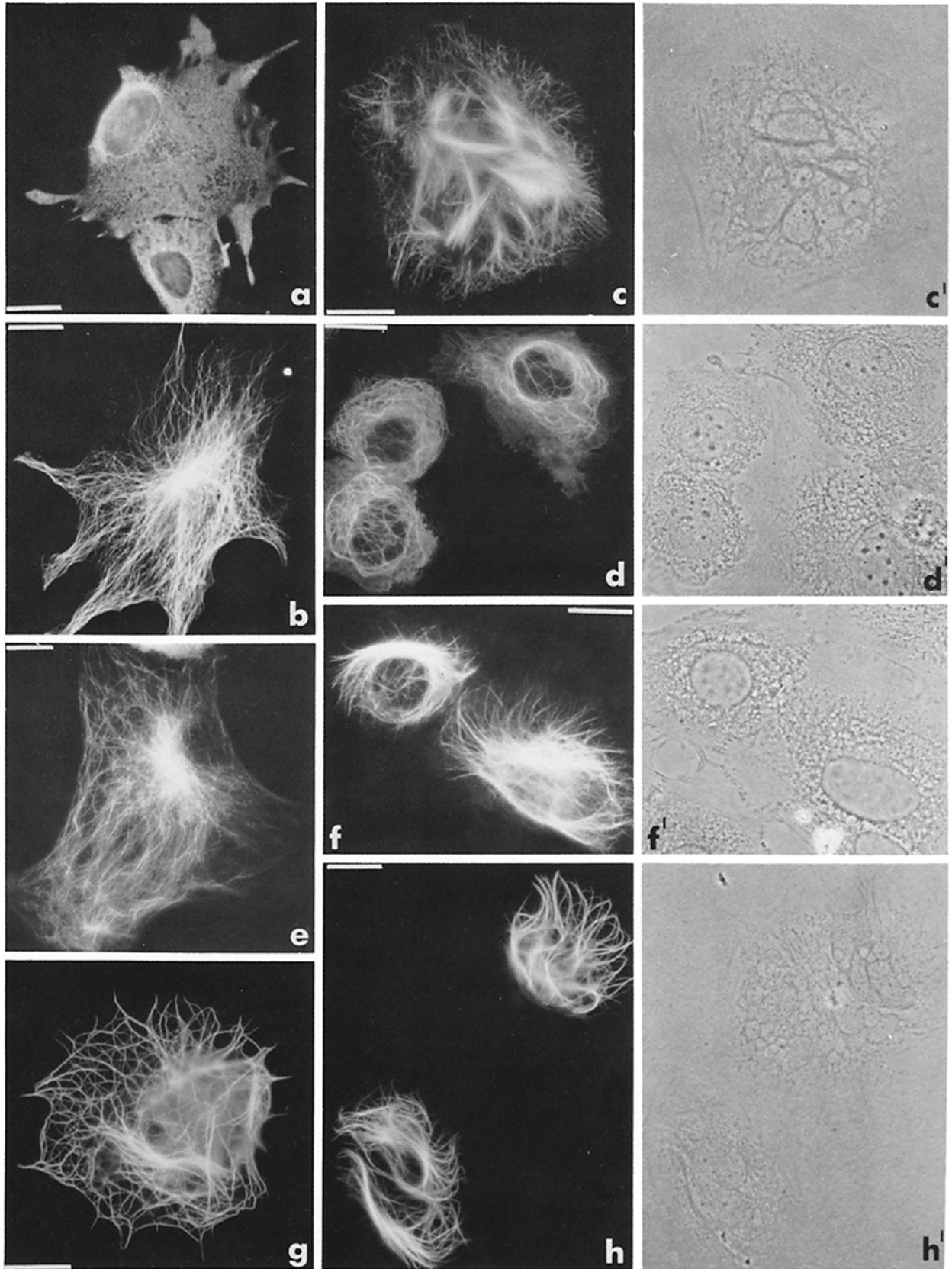
(Fig. 2 b). This indicates that treatment of cells with taxol for 30 min is sufficient to stabilize microtubules (see also reference 55). Cells treated with taxol alone for 90 min or with taxol and then colcemid as described above for Fig. 2 b, revealed normal saltatory movement but no cell translocation, whereas cells treated with taxol for 24 h showed impeded saltatory movement restricted to cytoplasmic areas containing microtubule bundles (data not shown).

Next we were interested whether injected YL 1/2 could effect the distribution of microtubules that were stabilized and reorganized by previous taxol treatment or whether taxol could reorganize microtubules in cells previously injected with YL 1/2. When microtubules were aggregated by high concentrations of injected antibody, further taxol treatment was ineffective in reorganizing microtubules (Fig. 2, d and d') in contrast to cells previously injected with low concentrations of antibody (Fig. 2, e, f, and f'). When fibroblasts were preincubated with taxol for 30 min (Fig. 2 g) or 24 h (Fig. 2 h and h') and then injected with high concentrations of antibody, YL 1/2 altered the distribution of taxol-stabilized microtubules (in Fig. 2, compare g with b) and taxol-induced microtubule bundles (in Fig. 2, compare h with c). Cells treated with taxol (30 min) and then injected with YL 1/2 as in Fig. 2 g showed no saltatory movement by video time-lapse recording. Such cells revealed microtubule bundles spanning the whole cytoplasm (Fig. 2 g). Again, injection of YL 1/2 at low concentration that did not alter microtubule organization in normal cells (see Fig. 2 e) did not affect saltatory movement in taxol-treated cells. Together with the effect of a microinjected GTP-analog that induced microtubule bundles and restricted saltatory movement along such microtubule structures (65), and similar to results of extended treatment of cells with taxol (see above), these results suggest that the antibody stopped saltatory movement in a concentration-dependent manner by binding to the surface of microtubules rather than by inducing their bundling and aggregation.

Mitosis

Because 3T3 fibroblasts injected with YL 1/2 in interphase were able to enter mitosis but did not divide, the effect of different antibody concentrations on mitosis was examined. Low antibody concentration (2 mg IgG/ml) that did not alter microtubule organization in interphase cells did not affect mitosis. In contrast, intermediate concentration (6 mg IgG/ml) that bundled and high concentration (>12 mg IgG/ml) that aggregated microtubules in interphase cells did affect mitosis. The experiments were as follows:

(a) PtK₂ cells were injected in prophase with YL 1/2

antibody or control rat IgG (12 mg/ml in injection solution). 2 h later, cells were fixed and injected antibodies were localized. All cells injected with control IgG had divided and were still connected by the midbody (not shown), whereas 80% of cells injected with YL 1/2 antibody did not divide. Many of such cells arrested in mitosis showed fluorescent bundles (Fig. 3, b and b') that did not resemble normal spindle structures (Fig. 3 a).

(b) After treatment with 2.5 mM thymidine for 15 h, CHO cells were incubated for 5 h in normal medium, and then nocodazole (0.04 μg/ml) was added for 4 h (75). About 80% of cells were arrested in prometaphase. 1 h after removal of nocodazole, all cells had divided. Cells were injected with rhodamine-conjugated Y1 1/2 antibody or control rat IgG (both at 4 mg/ml) ~2 h before nocodazole was added. Fig. 3 c shows the distribution of microtubules in a CHO cell which was previously treated with thymidine. A CHO cell injected with rhodamine-conjugated YL 1/2 antibody (4 mg/ml) after incubation with thymidine revealed typical microtubule bundles (Fig. 3, d and d'). For better visualization of injected antibody along microtubule structures, cells were extracted with microtubule-stabilizing buffer containing 0.2% Triton X-100 and then fixed. In control experiments, synchronized CHO cells showed spindle structures typical of cells in metaphase 10 min after removal of nocodazole (Fig. 3, e and e'). At 45 min after removal of the drug, cells had divided but were still connected by the midbody (Fig. 3, f and f').

About 60% of injected cells were arrested in prometaphase by nocodazole. In Fig. 3, g and g', three of the four cells were injected with rhodamine-conjugated YL 1/2 antibody 2 h before incubation with nocodazole, and the cells were extracted and fixed 25 min after removal of nocodazole. The uninjected cell (Fig. 3 g') has already started to divide, whereas the injected cells retained their round shape and showed disoriented bundles of microtubules. At higher magnification (Fig. 3, h and h') these bundles resembled those observed after injection of YL 1/2 antibody in interphase cells (compare with Fig. 3 d). Uninjected cells divided normally (Fig. 3, i and i') in contrast to cells injected with YL 1/2; even 2.5 h after removal of the drug, injected cells could easily be identified by their round shape (Fig. 3, k and k'). Cells injected with control rat IgG divided normally.

Intermediate Filaments

Treatment of fibroblasts with colcemid induces a redistribution of intermediate filaments not directly correlated with the disassembly of microtubules (8, 20, 32). Typical perinuclear bundles of the vimentin-type filaments were observed

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FIGURE 3 Mitotic arrest of cells microinjected with YL 1/2 antibody. (a, b, and b') PtK₂ cells fixed with formaldehyde, then permeabilized. (c–k') cytoskeletons of CHO cells. (a, c, e, and f) indirect immunofluorescence microscopy using YL 1/2 antibody followed by rhodamine-conjugated goat anti-rat IgG. (a) spindle microtubules of a PtK₂ cell in metaphase. (b and b') a PtK₂ cell was injected in early prophase with YL 1/2 antibody, 2 h later fixed, permeabilized, and labeled with fluorescent antibody. (c) distribution of microtubules in a CHO cell in interphase. (d, g, h, i, and k) fluorescent micrographs of cytoskeletons prepared from CHO cells which were previously injected with rhodamine-conjugated YL 1/2 antibody. (d) A CHO cell was injected with fluorescent YL 1/2 antibody, extracted with detergent 2 h later and fixed. (e–k') CHO cells were synchronized with thymidine and arrested in prometaphase with nocodazole. (e and e') 10 min and (f and f') 45 min after removal of drug. (g–k') after treatment with thymidine, CHO cells were injected with rhodamine-conjugated YL 1/2 antibody, then incubated for 4 h with nocodazole and extracted with detergent 20 min (g and g' and h and h'), 40 min (i and i') and 2.5 h (k and k') after removal of the drug. Note normal spindle formation in (e and e'), synchronized cell division in (f and f'), but absence of spindle structures (g and h) and inhibited cell division (i–k') in cells previously injected with YL 1/2 antibody. Bars, 20 μm. (a and b) × 1,100. (c and d) × 1,000. (e and g) × 750. (f) × 200. (h) × 1,250. (i) × 250. (k) × 100.
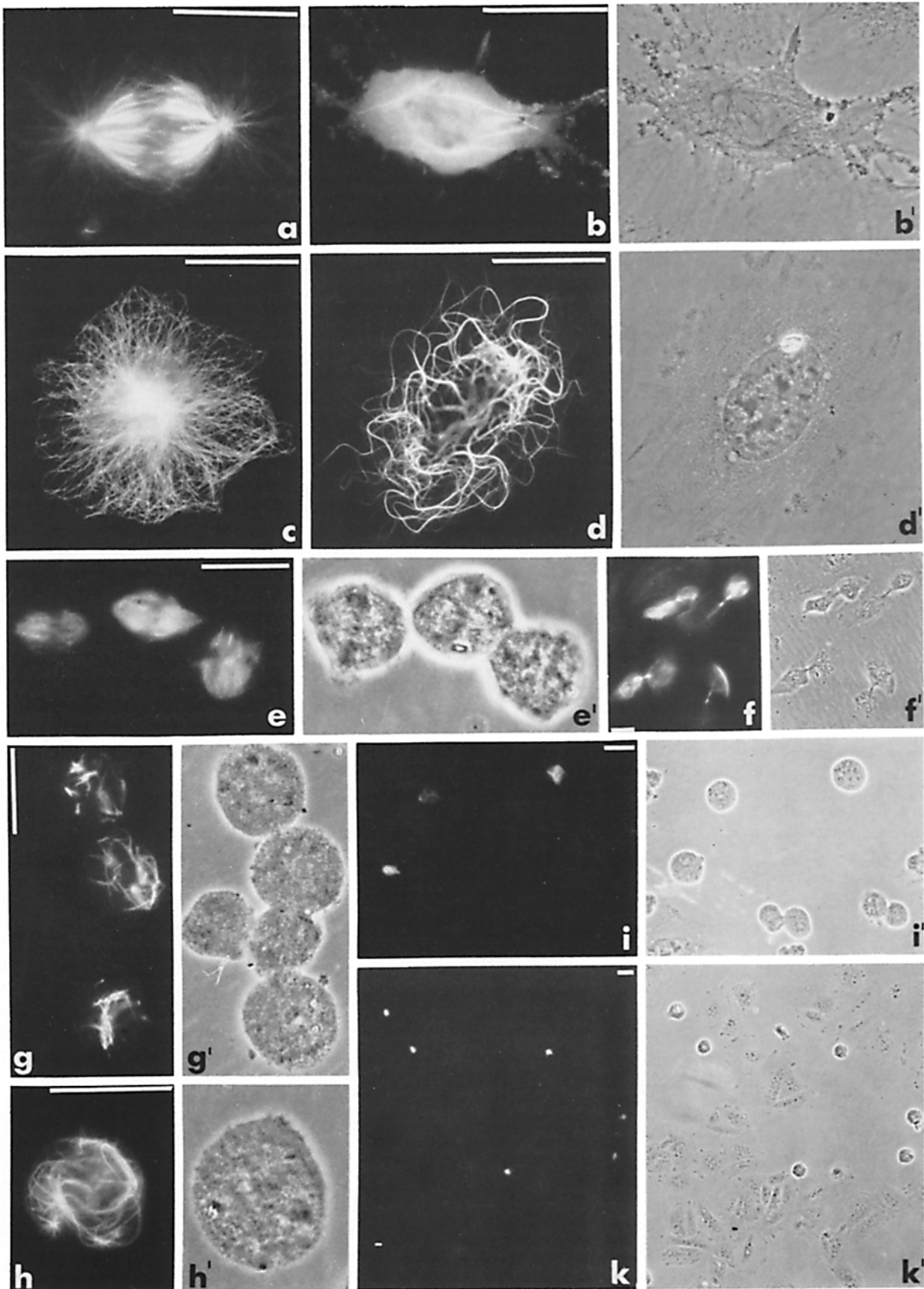
when Swiss 3T3 cells had been incubated with colcemid (1 μM) for ~8 h (Fig. 4c). A similar result was obtained when YL 1/2 antibody was injected into fibroblasts at a concentration (12 mg IgG/ml) that extensively aggregated microtubules in the perinuclear area (Fig. 4, d, e, and f). Again, the vimentin filaments did not redistribute immediately in response to the reorganization and aggregation of microtubules. In this respect Blose et al. (9) were also able to induce the collapse of intermediate filaments in cultured cells by microinjection of tubulin antibodies.

3T3 cells injected with YL 1/2 antibody at concentrations (6 mg IgG/ml) that did not aggregate microtubules but stopped saltatory movements (compare with Fig. 1) did not show such a drastic redistribution of intermediate filaments (i.e., perinuclear bundles); instead, quite often a codistribution with microtubule structures was observed (Fig. 4, g, h, and i). Nevertheless, it could not be excluded that the redistribution of intermediate filaments was dependent on changes in cell morphology induced by the injected antibody (Figs. 4, g–i).

Golgi Structures

For localization of Golgi structures by indirect immunofluorescence microscopy, we used antibodies against human β-galactosyltransferase (7). Recent studies of tissue culture cells have shown the localization of this enzyme in the perinuclear Golgi area by immunofluorescence microscopy (7, 29) and the limited distribution to trans-cisternae of the Golgi complex by electron microscopy (53).

A549 cells in interphase displayed the Golgi complex in the juxtanuclear area that also contains the microtubule-organizing center (Fig. 5, a and b). Incubation of A549 cells with 1 μM colcemid for 2-h depolymerized cytoplasmic microtubules (Fig. 5c) and induced a rearrangement of galactosyltransferase (Fig. 5d). The enzyme was found dispersed in small vesicles throughout the cytoplasm. Such an effect of antimitotic drugs on Golgi morphology and eventually Golgi function(s) is well documented (29, 40, 43, 51, 62).

Microinjection of YL 1/2 antibody at a concentration which affected saltatory movements, but did not aggregate microtubules (6 mg/ml in injection solution), did not change the Golgi pattern (Fig. 5, e and f). Increasing the concentration of antibody in the injection solution to 12 mg/ml destabilized Golgi structures which appeared more vesicularized than in adjacent uninjected cells, but sometimes still remained in an asymmetric juxtanuclear position (Figs, 5, g and h). More often, because microtubules were aggregated, Golgi-derived vesicles were randomly distributed throughout the cytoplasm (Fig. 5, i and j) similar to results obtained after treatment of cells with colcemid (Fig. 5, c and d).

Effects of Colcemid on Cells Previously Injected with Antibody

YL 1/2 antibody was injected into Swiss 3T3 cells at concentrations (6 mg/ml) that induced bundling of microtubules without obvious aggregation (Fig. 6a). After incubation with 1 μM colcemid for 1 h, the injected antibody was diffusely distributed in the cytoplasm (Fig. 6b); no microtubules could be detected in these cells by double immunofluorescence microscopy using polyclonal tubulin antibody (not shown). Removal of colcemid allowed repolymerization of microtubules in the presence of injected antibody. Microtu-

bule bundles were formed again (Fig. 6c). Repolymerization was not random but started from organizing centers (48), quite often two per cell (Fig. 6d). Repolymerization of microtubules starting from organizing centers in the presence of injected antibody could easily be detected when cells were allowed to recover in colcemid-free medium for a short time (Fig. 6, e and f). Microtubule bundles in injected cells could also be depolymerized by cold treatment (4°C) and repolymerization occurred upon warming up injected cells to 37°C (not shown).

Image Intensification Microscopy

Rhodamine-conjugated YL 1/2 antibody (6 mg/ml) was injected into Swiss 3T3 cells and the distribution of the injected antibody in the living cells was followed by image intensification microscopy (for details see Materials and Methods). Photographs were taken every 15 min from the intensifier tube and changes in the distribution of injected antibody could be followed and documented over several hours. Fig. 7 shows single photographs of the same 3T3 cell starting 2 h after injection (Fig. 7a). The antibody induced bundling of microtubules. The cell showed no saltatory movements but still had disoriented ruffle activity when examined by time-lapse video microscopy using phase-contrast optics. At 30 min (Fig. 7b) and 60 min (Fig. 7c) later, some microtubule bundles have changed their orientation, but still showed a good correlation to previous positions in Fig. 7a or b, respectively. Most of the bundles seen in Fig. 7a can be traced in Fig. 7c. As this cell was active with respect to ruffle activity during 1 h of observation, alterations of cell morphology might have indriectly changed the position of microtubule bundles.

Microinjection of Antibodies Adsorbed to Colloidal Gold

As an approach to examine the distribution of injected antibody at the electron microscopic level, both antibodies (YL 1/2 and YOL 1/34) were adsorbed to 5 nm colloidal gold. Uncomplexed antibody and conjugated gold particles could easily be separated by centrifugation before starting microinjection. After injection of gold-antibody complexes, the injected cells were first processed for indirect immunofluorescence (Fig. 8). 2 h after microinjection of 2 mg IgG/ml on colloidal gold, the injected YL 1/2 antibody could be localized along microtubule structures (Fig. 8, a and b). When injected at higher concentrations (6 mg IgG/ml), typical bundles could be detected in injected cells (Fig. 8c) visible as phase-dense structures (Fig. 8d), indicating the accumulation of gold particles along these bundles. To examine the stability of antibody-gold complexes after microinjection, YL 1/2 antibody on gold could be detected along microtubule structures (Fig. 8, e–h) 24 h after injection. In Fig. 9, cells were fixed different times after injection of antibody-gold complexes and processed for electron microscopy. Colloidal gold particles adsorbed to YL 1/2 were found specifically accumulated along microtubules excluding other filamentous structures (Fig. 9, A–C). Colloidal gold adsorbed to YOL 1/34 antibodies remained diffusely distributed after microinjection into the cytoplasm (Fig. 9D). Even 24 h after injection, colloidal gold adsorbed to YL 1/2 antibodies could be detected along microtubules (not shown).
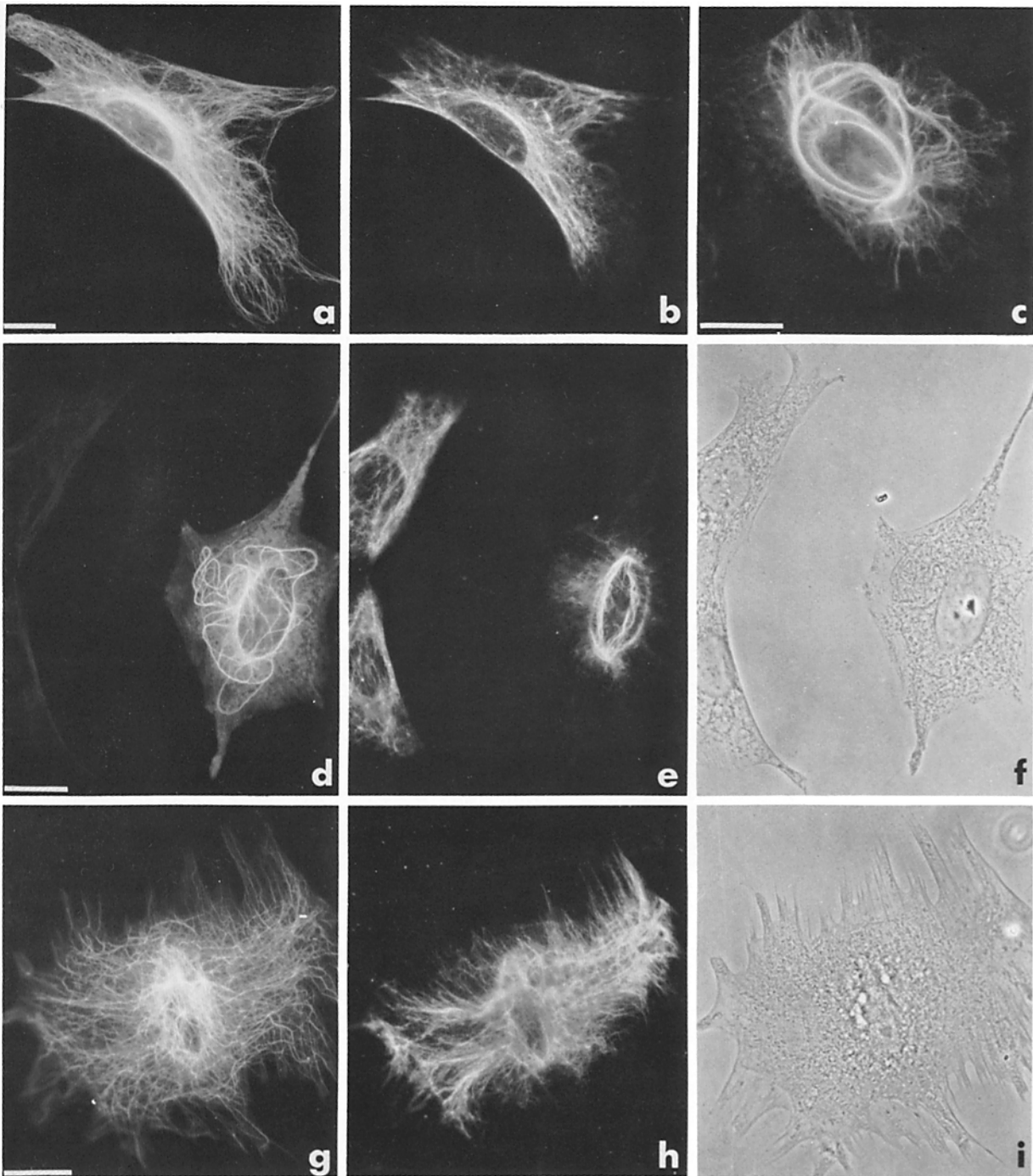
FIGURE 4 Distribution of intermediate filaments in Swiss 3T3 cells microinjected with YL 1/2 antibody. (a and b) indirect double immunofluorescence microscopy to reveal distribution of microtubules (a) and intermediate filaments (b) in the same cell using YL 1/2 antibody and rabbit vimentin antibody followed by fluorescein-conjugated goat anti-rat IgG and rhodamine-conjugated goat anti-rabbit IgG. (c) Distribution of intermediate filaments in a 3T3 cell treated with colcemid at 1 μM for 8 h. (d–i) Double immunofluorescence of injected 3T3 cells. Cells were microinjected with YL 1/2 antibody at 12 mg/ml (d, e, and f) or 6 mg/ml (g, h, and i), fixed and permeabilized 8 h after injection and labeled with rabbit vimentin antibody followed by a mixture of second antibodies. Cells in a, d, and g were viewed by optics selective for fluorescein to visualize microtubules (a) or injected antibody (d and g). The same fields were viewed with optics selective for rhodamine to visualize distribution of intermediate filaments (b, e, and h). Note collapse of intermediate filaments when microtubules were aggregated after injection of antibody (d and e). Bars, 20 μm. (a) × 400. (c) × 650. (d and g) × 500.

DISCUSSION

The main purposes of this study were to investigate the following: (a) whether in vivo decoration of microtubules by injected antibody and possible reorganization (but not depolymerization) would interfere with microtubule functions; (b) whether aggregation of microtubules induced by injection of high concentrations of antibody would have effects similar to
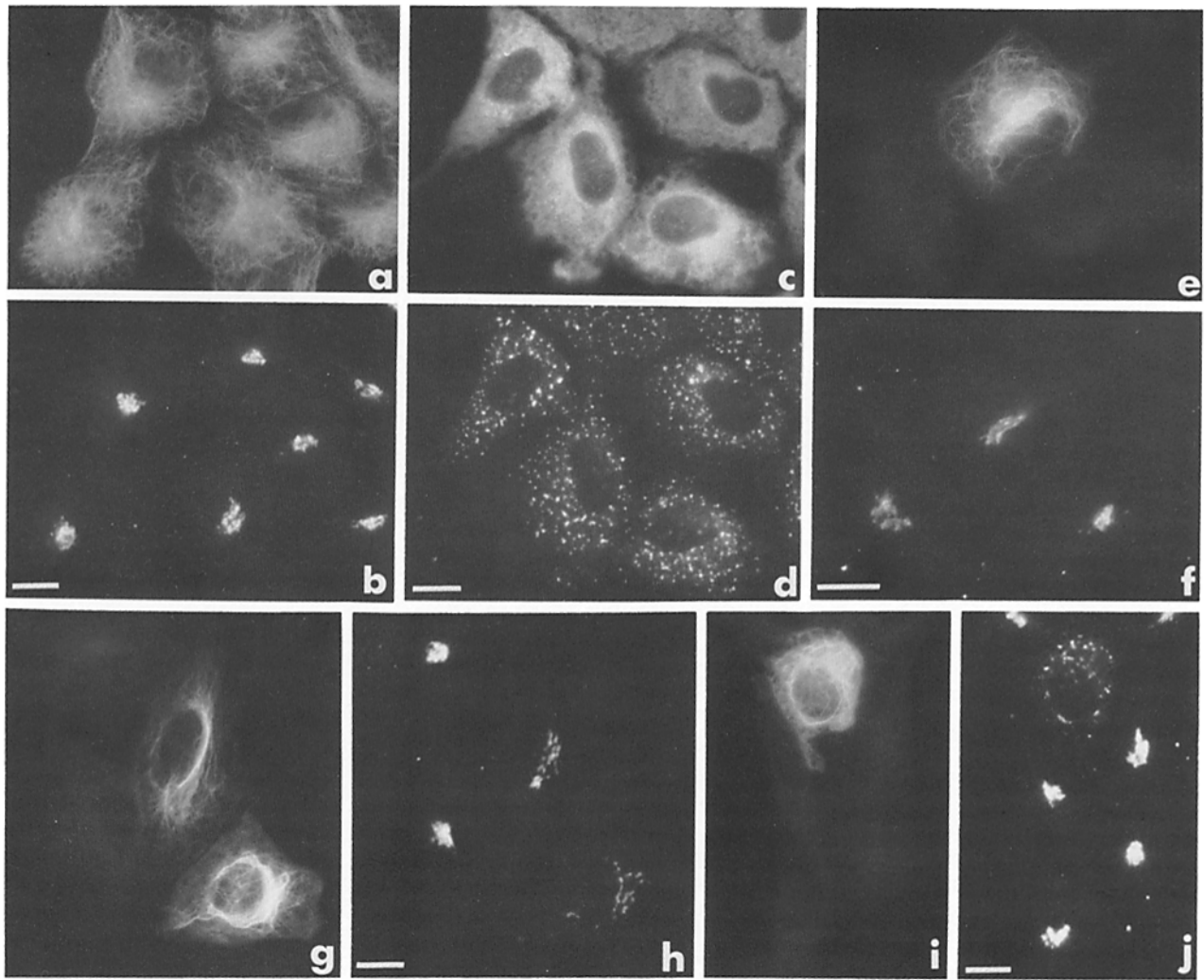
FIGURE 5 Distribution of Golgi elements in A549 cells microinjected with YL 1/2 antibody. (a–d) indirect double immunofluorescence of A549 cells. Normal cells (a and b) or cells treated with colcemid at 1 μM for 2 h (c and d) were labeled with YL 1/2 antibody and rabbit galactosyltransferase antibody followed by fluorescein-conjugated goat anti-rat IgG and rhodamine-conjugated goat anti-rabbit IgG. (e–j) double immunofluorescence of injected A549 cells. Cells were injected with YL 1/2 antibody at 6 mg/ml (e and f) or 12 mg/ml (g–j), labeled 2 h after injection with rabbit galactosyltransferase antibody followed by a mixture of second antibodies. Cells in (a, c, e, g, and i) were viewed by optics selective for fluorescein to visualize distribution of microtubules (a and c) or of injected antibody (e, g, and i). The same fields were viewed with optics selective for rhodamine to visualize distribution of galactosyltransferase (b, d, f, h, and j). Note specific reorganization of Golgi elements dependent on amount of injected antibody and dispersion of Golgi in d and j. Bars, 20 μm. (a and i) × 300. (c and g)× 350. (e) × 450.

the treatment of cells with microtubule-depolymerizing agents; and (c) whether the antibody would interfere with the (de)polymerization of microtubules in vivo by binding to the tyrosylated carboxyterminus of α-tubulin. For c, recent studies have suggested that the reversible posttranslational modification of tubulin by removal and addition of a tyrosine residue to the carboxyterminal glutamate of the α-chain might influence microtubule assembly in vivo (14, 37, 46; see also reference 61).

Saltatory Movements and Cell Locomotion

Recent studies on the cytoskeletal organization of axons have shown that microtubules are connected to membranous organelles by fine cross-bridges, indicating the involvement of microtubules in active organelle transport (13, 27, 53, 54). The nature of cross-bridges is not clear; either organelles

possess projections on their surface that interact with microtubule walls, or MAP form projections (see reference 31). Nevertheless, these findings support the hypothesis that microtubules serve as rails or tracks for movement of membranous organelles and that cross-linkers could play an active role in this process (17, 22). Another model originally proposed by Ochs (46) suggests that vesicles and organelles could be bound to a transport filament like actin. These filaments would move by interacting with myosin-like cross-bridges attached to microtubules. In this respect, our results could indicate that injected antitubulin antibody might sterically interfere with the formation of such cross-bridges by preventing interaction of either organelle-derived projections with microtubule walls or of MAP-projections with the surface of organelles, or by preventing the formation of cross-bridges in the actin-like filament transport model. On the other hand, the antibody, when injected into cells, might directly interfere
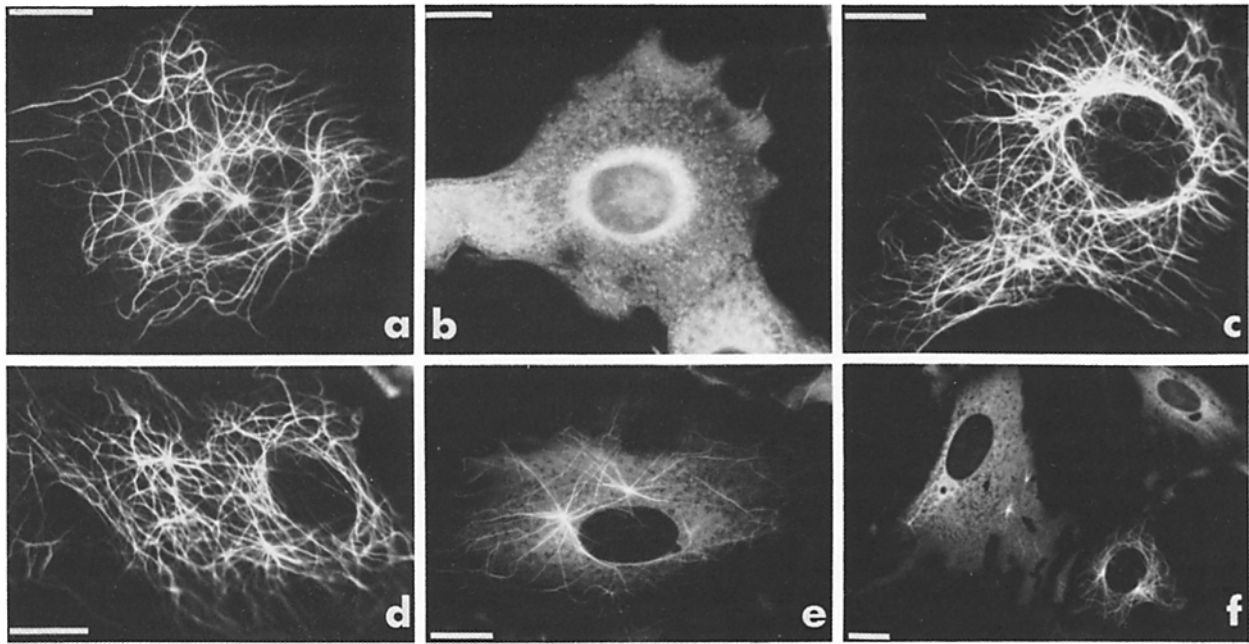
FIGURE 6   Effects of colcemid on microtubule structures in Swiss 3T3 cells microinjected with YL 1/2 antibody. (a) A 3T3 cell was injected with YL 1/2 antibody (6 mg/ml), fixed 2 h later and labeled with rhodamine-conjugated goat anti-rat IgG. (b) As in a, but the cell was incubated with colcemid at 1 µM for 1 h. (c–f) As in b, but cells were allowed to recover in medium without drug for 1 h (c and d) or 30 min (e and f). Note recovery of microtubules in presence of injected antibody; some cells contain two microtubule organizing centers. Bars, 20 µm. (a, c, and d) × 550. (b and e) × 400. (f) × 300.

with binding of either a yet unknown MAP or other cellular components to the tyrosylated carboxyterminus of α-tubulin.

It is also possible that cross-bridges between microtubules and membranous organelles have some enzyme activity that generates the force for sliding of these organelles on microtubules (6, 11, 16, 58). Without directly preventing formation of cross-bridges between organelles and microtubules, the antibody might affect the flexibility or conformation of these cross-bridges and, thereby, inhibit "saltations" of organelles along microtubules. After we had submitted the present study, Hayden et al. (26) were able to show that in living keratocytes of *Rana pipiens* cell organelles and vesicles moved along microtubules in a saltatory fashion, and the authors suggested a modification of the actin-like filament transport model. Thereafter microtubules would serve as passive tracks rather than being involved as a force-generating system and soluble myosin would be responsible for providing the force independent of microtubules (26). Although we cannot exclude that the continuous motion of very small particles observed by Allen et al. (2) still takes place under conditions where we were able to stop saltatory movement of larger cell organelles and vesicles by injection of antibody, we would favor the hypothesis that microtubules are actively involved in saltatory movement. This does not exclude that the actual motive force can be provided by another yet unidentified system.

Effects on the Cytoplasmic Organization of Microtubules

High-voltage electron microscopic studies of whole cells have revealed an extensive three-dimensional lattice of filamentous structures or microtrabeculae (14, 70, 71) interconnecting membranous organelles, microtubules, and plasma membrane. Axoplasm, when studied by quick-freeze, deep-etching methods, also revealed an extensive system of cross-

links between microtubules, neurofilaments, and membranous organelles (30, 56). In addition, it has been suggested that MAP could form cross-bridge-like structures (25, 27). As a result, the tubulin antibody, when injected into cultured cells, could prevent the formation of cross-links to other cellular components and thereby disrupt the highly organized spatial distribution of microtubules without depolymerization. Thus, the bundling of microtubules and finally their collapse in antibody-injected cells can be a secondary effect rather than a direct one mediated by cross-linking of microtubules by injected antibody.

Two problems arise from the use of the YL 1/2 antibody. First, the parental line of the YL 1/2 clone secretes a light chain, resulting in mixtures of IgG molecules that contain the myeloma and antibody light chains in different combinations (33). Second, the concentration of antibody that is injected is critical. The total tubulin concentration of cultured nonneuronal cells in interphase is ~2–3 mg/ml with some 50% in the depolymerized state (28). Microinjection introduces a volume of ~5–10% of the total cellular volume (23) and ~15% of the total cellular α-tubulin purified from brain is tyrosylated (61). Therefore, using 2 mg IgG/ml in the injection solution should be sufficient to saturate up to 20% of the cellular tyrosylated tubulin with the antibody that shows in vitro a dissociation constant of 5×10^{-7}M (33) and therefore should be fully tubulin-bound. If YL 1/2 is used at concentrations of 6–12 mg IgG/ml, between half and all of the cellular tyrosylated tubulin could be saturated, assuming that brain and cells in culture have equal percentages of tyrosylated tubulin.

Although we do not know how much of the tyrosylated tubulin is in the polymerized state, it is interesting that the degree of saturation of tyrosylated tubulin within microtubules can be correlated with the effect of saltatory movement and rearrangements in the distribution of cytoplasmic micro-
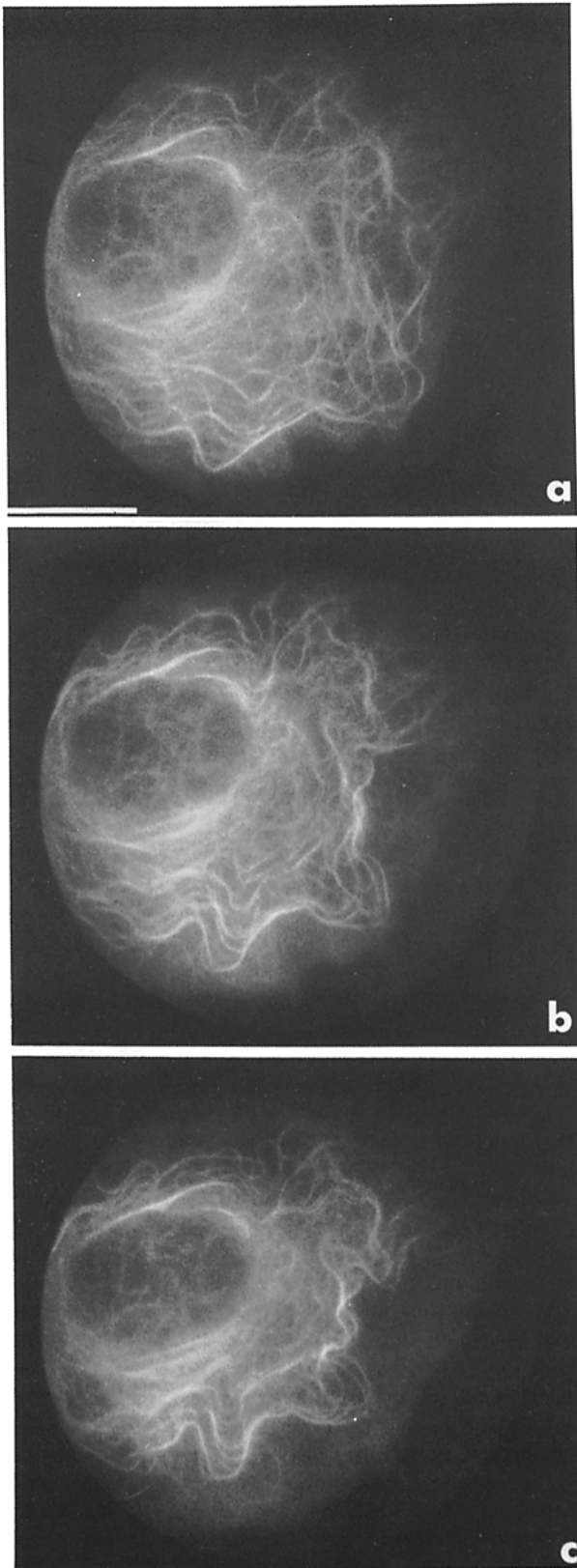
Figure 7 Fluorescence intensification microscopy of living Swiss 3T3 cells microinjected with rhodamine-conjugated YL 1/2 antibody. Photographs were taken at intervals of 30 min directly from the EMI intensifier tube. Fluorescent bundles seen in a can still be traced in c. Bars, 20 μm. × 900.

tubules after injection of different amounts of antibody. Perhaps in interphase cells cross-linkers between microtubules, cell organelles, and other cellular components are necessary

for saltatory movement and the spatial organization of microtubules.

Alterations in the Organization of Intermediate Filaments and Golgi Elements

A characteristic property of intermediate filaments of the vimentin type is their aggregation into cytoplasmic or perinuclear filament bundles in cells exposed to colcemid or colchicine. These observations have supported the assumption that microtubules and certain intermediate filaments are interconnected, even though the alteration in the organization of intermediate filaments occurs much slower than the disruption of microtubules caused by the drug (8, 20, 32). The distribution of intermediate filaments can be specifically altered: microinjection of some antibodies to vimentin or vimentin-associated proteins induces a rapid and reversible aggregation of intermediate filaments. Under these conditions, the distribution of actin filament bundles and microtubules remains unaltered. Cellular activities such as cell locomotion and saltatory organelle movements were unaffected and injected cells even divided (18, 34, 39). Interestingly our results show that intermediate filaments of the vimentin type collapse when microtubules were aggregated by injection of tubulin antibody. Thus, the aggregation of intermediate filaments in cells treated with colcemid or colchicine is not necessarily attributable to a direct effect of these drugs. The nature of a possible interaction between microtubules and intermediate filaments still remains unclear.

Several observations have led to the assumption that microtubules are involved in the location and organization of Golgi elements. In interphase cells, the Golgi complex and the microtubule-organizing center are often found nearby (13, 38). The breakdown of interphase cytoplasmic microtubules for establishing the mitotic spindle is correlated with the loss of polarization and often with the fragmentation of the Golgi complex and is thought to guarantee an equal distribution of Golgi elements to the daughter cells (29; for further references, see reference 74). Changes in organelle character and distribution in mitotic cells are strikingly similar to changes in nonmitotic cells treated with antimitotic drugs. Colcemid and colchicine for example are known to induce fragmentation and dislocation of Golgi elements (29, 40, 43, 51). Nevertheless, disintegration of Golgi elements by colchicine was often considered to be produced by binding of the drug to (Golgi) membranes (42, 73). The redistribution of galactosyltransferase as a Golgi marker upon injection of tubulin antibody demonstrates that the organization and location of the Golgi complex is directly dependent on the integrity of microtubules. In agreement with these results one of us has recently shown that microtubule reorganization, induced by injection of a nonhydrolyzable GTP analog, also affected the organization of Golgi elements (65).

Reorganization of Microtubules in Interphase and Mitotic Cells

In in vitro polymerization experiments, YL 1/2 antibody does not interfere with polymerization of microtubules (66). Microtubule bundles, induced by injection of antibody into cultured cells, were readily depolymerized by treatment of injected cells with cold or colcemid, and repolymerized in the presence of antibody. On the other hand, the results obtained from living cells by intensification microscopy or from synchronized cells indicate that injected cells were unable to
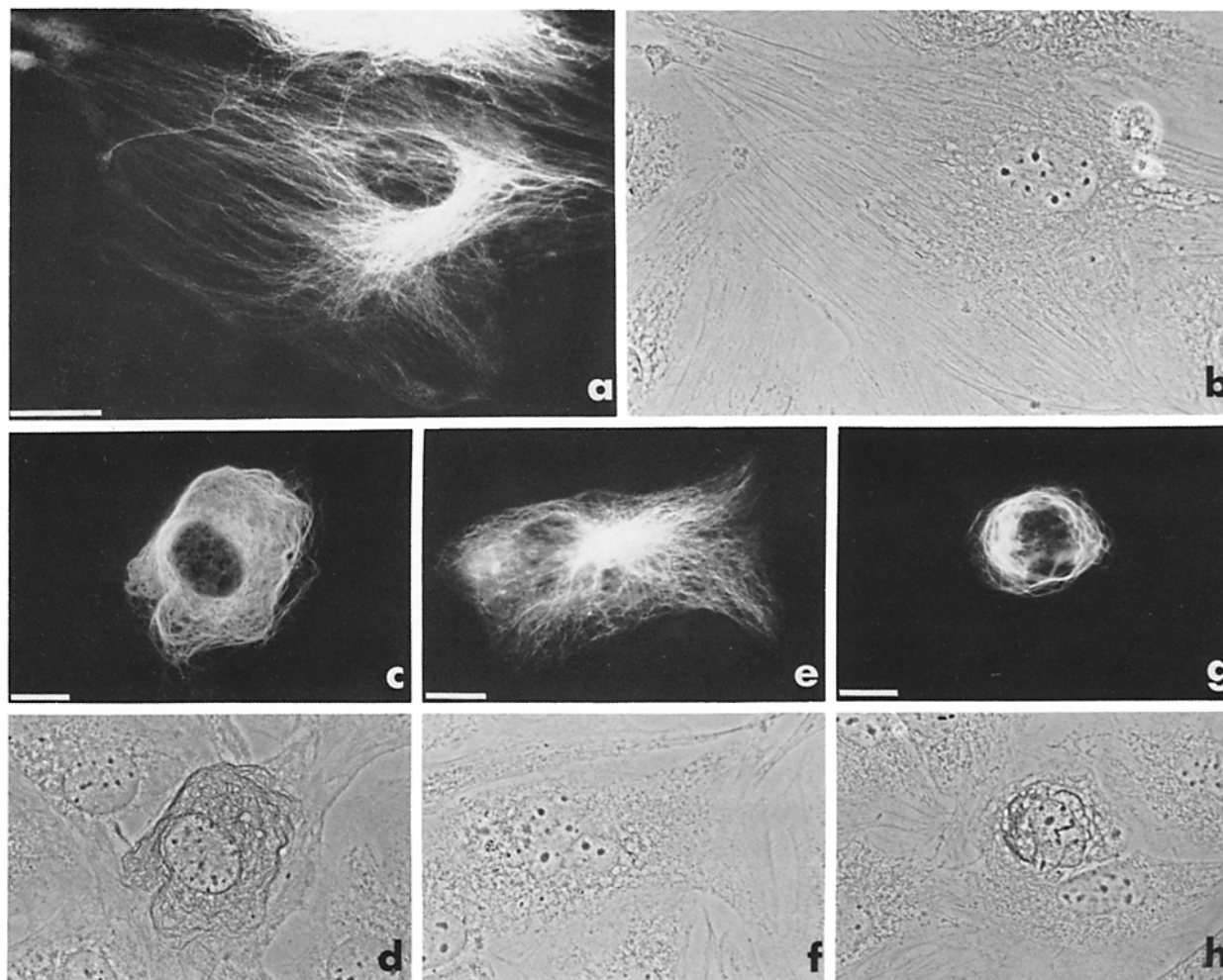
FIGURE 8 Indirect immunofluorescence of Swiss 3T3 fibroblasts injected with YL 1/2 antibody adsorbed to colloidal gold. Cells in a and b and e and f were injected with 2 mg IgG/ml, cells in c and d and g and h with 6 mg IgG/ml. 3 h after injection (a–d) or 24 h after injection (e–h), cells were fixed, permeabilized, and labeled with rhodamine-conjugated goat anti-rat IgG. Note phase-dense bundles in d and h indicating accumulation of injected colloidal gold. Bars, 20 μm. (a) × 600. (c, e, and g) × 400.
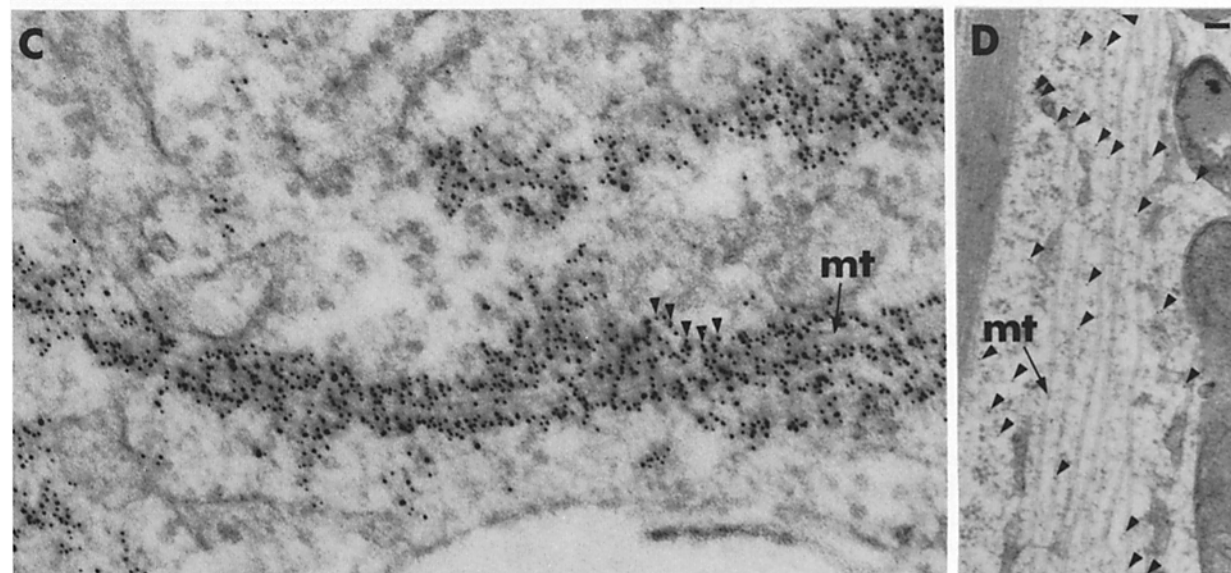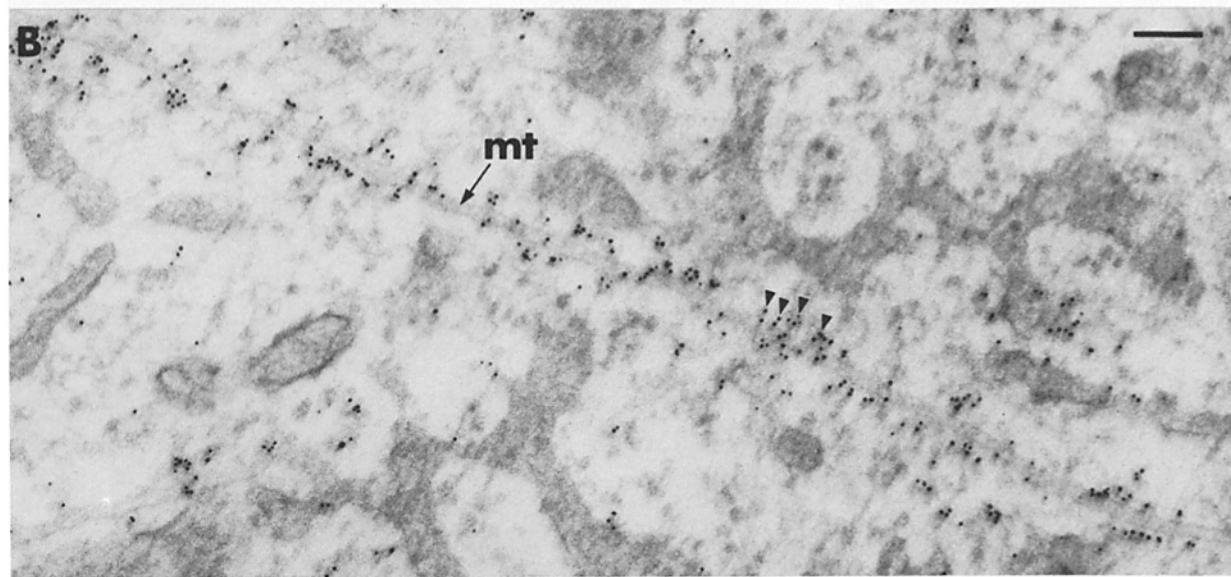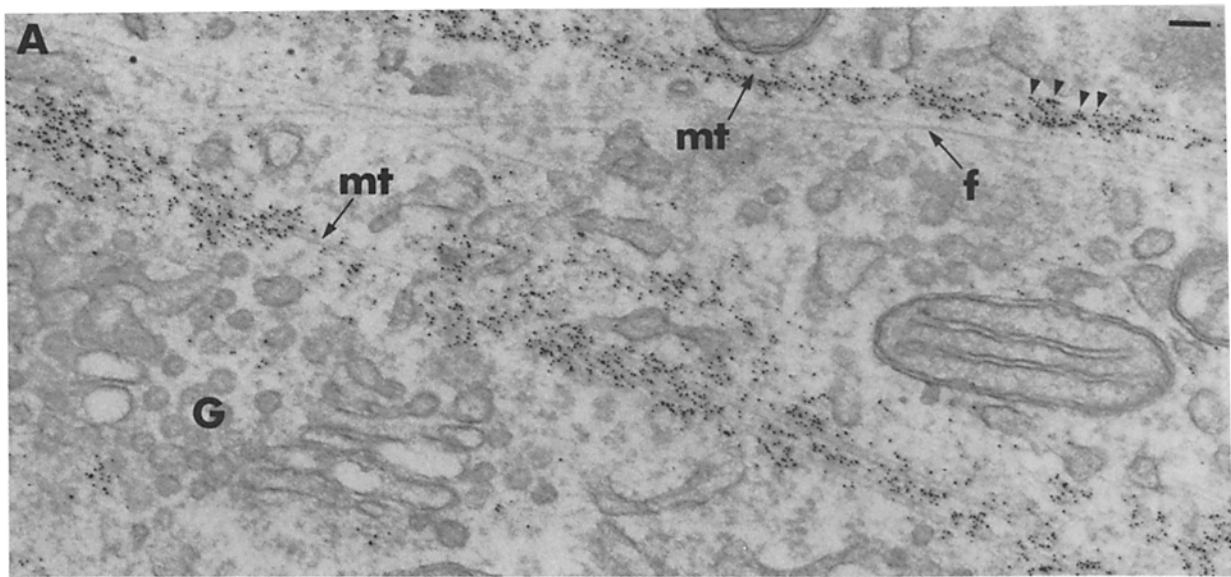
reorganize their microtubules when these structures were highly decorated by the injected antibody. In interphase cells, antibody-decorated microtubule structures revealed a static appearance and obviously did not disassemble when such cells entered mitosis. As a result, these cells never formed spindle microtubules. Decoration of microtubules by injected antibody might interfere with the ability of the cell to reorganize its microtubules in two ways: the injected antibody might block the binding of a regulatory protein to microtubules that could induce their depolymerization or, because of the specificity of the antibody, prevent the removal of the carboxyterminal tyrosine. The latter mechanism would be in agreement with recent findings on the reversible posttranslational modification of tubulin, whereby a tyrosine residue is removed and added to the carboxyterminal glutamate of the α-chain. The enzyme that catalyzes addition of tyrosine, tubulin-tyrosine ligase, has been detected in both vertebrate (3, 5, 44, 49, 50) and invertebrate tissues (36). Also, another distinctly different enzyme, tubulin-tyrosine carboxypeptidase, which is believed to be responsible for the removal of carboxyterminal tyrosine of α-tubulin, has been detected and characterized (1, 37). The existence of these two enzymes

makes it possible that there is probably a cyclic process of tyrosylation and detyrosylation that might be important in modulating the activity of tubulin in cells. So far the functional significance of tyrosylation and detyrosylation remains unclear and further studies are necessary to confirm such a mechanism with respect to possible regulation of microtubule polymerization in vivo.

REFERENCES

1. Agarana, C. E., H. S. Barra, and R. Caputto. 1980. Tubulin-tyrosine carboxypeptidase from chicken brain: properties and partial purification. *J. Neurochem.* 34:114–118.
2. Allen, R. D., I. Iasaki, S. T. Brady, and S. P. Gilbert. 1982. Fast axonal transport in squid giant axon. *Science (Wash. DC).* 218:1127–1129.
3. Arce, C. A., H. S. Barra, J. A. Rodriguez, and R. Caputto. 1975. Tentative identification of the amino acid that binds tyrosine as a single unit into a soluble brain protein. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 50:5–7.
4. Arce, C. A., M. E. Hallak, J. A. Rodriguez, H. S. Barra, and R. Caputto. 1978. Capability of tubulin and microtubules to incorporate and to release tyrosine and phenylalanine and the effect of the incorporation of these amino acids on tubulin assembly. *J. Neurochem.* 31:205–210.
5. Barra, H. S., C. A. Arce, J. A. Rodriguez, and R. Caputto. 1974. Some common properties of the protein that incorporates tyrosine as a single unit and the microtubule proteins. *Biochem. Biophys. Res. Commun.* 60:1384–1390.
6. Beckerle, M. C., and K. R. Porter. 1982. Inhibitors of dynein activity block intracellular transport in erythrophores. *Nature (Lond.).* 295:701–703.
7. Berger, E. G., T. Mandel, and U. Schilt. 1981. Immunohistochemical localization of galactosyltransferase in human fibroblasts and HeLa cells. *J. Histochem. Cytochem.* 29:364–370.
8. Blose, S. H., and S. Chacko. 1976. Rings of intermediate (100 Å) filament bundles in the perinuclear region of vascular endothelial cells. Their mobilization by colcemid and mitosis. *J. Cell Biol.* 70:459–466.
9. Blose, S. H., D. I. Meltzer, and J. R. Feramisco. 1982. 10-nm filaments induced to collapse in cells microinjected with antibodies against tubulin. *J. Cell Biol.* 95(2, Pt. 2):229a. (Abstr.)
10. Cabral, F., M. C. Willingham, and M. M. Gottesman. 1980. The localization of an insoluble 58 K protein from Chinese hamster ovary cells to 10 nm filaments in cultured fibroblasts by electron microscopic immunocytochemistry. *J. Histochem. Cytochem.* 28:653–663.
11. Clark, T. G., and J. L. Rosenbaum. 1982. Pigment particle translocation in detergent-permeabilized melanophores of *Fundus heteroclitus. Proc. Natl. Acad. Sci. USA.* 79:4655–4659.
12. De Mey, J., M. Moeremans, G. Geuens, R. Nuydens, and M. De Brabander. 1981. High resolution light and electron microscopic localization of tubulin with the IGS (immuno gold staining) method. *Cell Biol. Int. Rep.* 5:889–899.
13. Dustin, P. 1978. Microtubules. Springer-Verlag, New York.
14. Ellisman, M. H., and K. R. Porter. 1980. Microtrabecular structure of the axoplasmic matrix: visualization of cross-linking structures and their distribution. *J. Cell Biol.* 87:464–479.
15. Faulk, W. R., and G. M. Taylor. 1971. An immunocolloid method for the electron microscope. *Immunochemistry.* 8:1081–1083.
16. Forman, D. S. 1982. Vanadate inhibits saltatory organelle movement in a permeabilized cell model. *Exp. Cell Res.* 141:139–147.
17. Freed, J. J., and M. M. Lebowitz. 1970. The association of a class of saltatory movements with microtubules in culture cells. *J. Cell Biol.* 45:334–354.
18. Gawlitta, W., M. Osborn, and K. Weber. 1981. Coiling of intermediate filaments induced by microinjection of a vimentin-specific antibody does not interfere with locomotion and mitosis. *Eur. J. Cell Biol.* 26:83–90.
19. Geoghegan, W. D., and G. A. Ackerman. 1977. Adsorption of horseradish peroxidase, ovomucoid and anti-immunoglobulin to colloidal gold for the indirect detection of concanavalin A, wheat germ agglutinin and goat antihuman immunoglobulin G on cell surfaces at the electron microscopic level: a new method, theory and application. *J. Histochem. Cytochem.* 25:1187–1200.
20. Goldman, R. D. 1971. The role of three cytoplasmic fibers in BHK21 cell motility. I. Microtubules and the effects of colchicine. *J. Cell Biol.* 51:752–762.
21. Goldman, R. D., T. D. Pollard, and J. Rosenbaum, editors. 1976. Cell Motility. Cold Spring Harbor Conf. Cell Proliferation. Vol. 3 (Book A).
22. Grafstein, B., and D. S. Forman. 1980. Intracellular transport in neurons. *Physiol. Rev.* 60:1167–1283.
23. Graessmann, M., and A. Graesmann. 1976. "Early" Simian-Virus-40-specific RNA contains information for tumor antigen formation and chromatin replication. *Proc. Natl. Acad. Sci. USA.* 73:366–370.
24. Green, K., and R. D. Goldman. 1982. The effects of taxol on microtubule and intermediate filament distribution in cultured fibroblasts and epithelial cells. *J. Cell Biol.* 95(2, Pt. 2):235a. (Abstr.)
25. Griffith, L. M., and T. D. Pollard. 1978. Evidence for actin filament-microtubule interaction by microtubule-associated proteins. *J. Cell Biol.* 78:958–965.
26. Hayden, J. H., R. D. Allen, and R. D. Goldman. 1983. Cytoplasmic transport in keratocytes: direct visualization of particle translocation along microtubules. *Cell Motil.* 3:1–19.
27. Heuser, J. E., and M. Kirschner. 1980. Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. *J. Cell Biol.* 86:212–234.
28. Hiller, G., and K. Weber. 1978. Radioimmunoassay for tubulin: a quantitative comparison of tubulin content of different established tissue culture cells and tissues. *Cell.* 14:795–804.
29. Hiller, G., and K. Weber. 1982. Golgi detection in mitotic and interphase cells by antibodies to secreted galactosyltransferase. *Exp. Cell Res.* 142:85–94.
30. Hirokawa, N. 1982. Cross-linker system between neurofilaments, microtubules, and membraneous organelles in frog axons revealed by the quick-freeze, deep-etching method. *J. Cell Biol.* 94:129–142.
31. Hyams, J. S., and H. Stebbinds. 1979. Microtubule associated transport. *In* Microtubules. K. Roberts and J. S. Hyams, editors. Academic Press, Inc., New York. 487–530.

32. Hynes, R. O., and A. T. Destree. 1978. 10 nm filaments in normal and transformed cells. *Cell.* 13:151–163.
33. Kilmartin, J. V., B. Wright, and C. Milstein. 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. *J. Cell Biol.* 93:576–582.
34. Klymkowsky, M. W. 1981. Intermediate filaments in 3T3 cells collapse after intracellular injection of a monoclonal anti-intermediate filament antibody. *Nature (Lond.).* 291:249–251.
35. Klymkowsky, M. W. 1982. Vimentin and keratin intermediate filament systems in cultured PtK2 epithelial cells are interrelated. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:161–165.
36. Kobayashi, T., and M. Flavin. 1981. Tubulin tyrosylation in invertebrates. *Comp. Biochem. Physiol. B Comp. Biochem.* 69B:387–392.
37. Kumar, N., and M. Flavin. 1981. Preferential action of a brain detyrosylating carboxypeptidase on polymerized tubulin. *J. Biol. Chem.* 256:7678–7686.
38. Kupfer, A., D. Louvard, and S. J. Singer. 1982. Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound. *Proc. Natl. Acad. Sci. USA.* 79:2603–2607.
39. Lin, J. J.-C., and J. R. Feramisco. 1981. Disruption of the *in vivo* distribution of the intermediate filaments in fibroblasts through the microinjection of a specific monoclonal antibody. *Cell.* 24:185–193.
40. Lin, J. J.-C., and S. A. Queally. 1982. A monoclonal antibody that recognizes Golgi-associated proteins of cultured fibroblast cells. *J. Cell Biol.* 92:108–112.
41. Mabuchi, I., and M. Okuno. 1977. The effect of myosin antibody on the division of starfish blastomeres. *J. Cell Biol.* 74:251–264.
42. Mitranic, M. M., J. M. Boggs, and M. A. Moscarello. 1981. An effect of colchicine on galactosyl- and sialytransferase of rat liver Golgi membranes. *Biochim. Biophys. Acta.* 672:57–64.
43. Moskalewski, S., J. Thyberg, S. Lohmander, and U. Friberg. 1975. Influence of colchicine and vinblastine on the Golgi complex and matrix deposition in chondrocyte aggregates. *Exp. Cell Res.* 95:440–454.
44. Nath, J., and M. Flavin. 1979. Tubulin tyrosylation *in vivo* and changes accompanying differentiation of cultured neuroblastoma-glioma hybrid cells. *J. Biol. Chem.* 254:11505–11510.
45. Nath, J., M. Flavin, and E. Schiffman. 1981. Stimulation of tubulin tyrosinolation in rabbit leukocytes evoked by the chemoattractant formyl-methionyl-leucyl-phenylalanine. *J. Cell Biol.* 91:232–239.
46. Ochs, S. 1974. Systems of material transport in nerve fibers (axoplasmic transport) related to nerve function and trophic control. *Ann. N.Y. Acad. Sci.* 228:202–223.
47. Olmsted, J. B., and G. G. Borisy. 1973. Characterization of microtubule assembly in porcine brain extracts by viscometry. *Biochemistry.* 12:4282–4289.
48. Osborn, M., and K. Weber. 1976. Cytoplasmic microtubules appear to grow from an organizing structure towards the plasma membrane. *Proc. Natl. Acad. Sci. USA.* 73:867–870.
49. Preston, S. F., G. G. Deanin, R. K. Hanson, and M. W. Gordon. 1979. The phylogenetic distribution of tubulin: tyrosine ligase. *J. Mol. Evolution.* 13:233–244.
50. Raybin, D., and M. Flavin. 1975. An enzyme tyrosylating α-tubulin and its role in microtubule assembly. *Biochem. Biophys. Res. Commun.* 65:1088–1095.
51. Robbins, E., and N. K. Gonatas. 1964. Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell. *J. Histochem. Cytochem.* 12:704–711.
52. Roberts, K., and J. S. Hyams, editors. 1979. Microtubules. Academic Press, Inc., New York.
53. 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.* 93:223–229.
54. Schiff, P. B., J. Fant, and S. B. Horwitz. 1979. Promotion of microtubule assembly *in vitro* by taxol. *Nature (Lond.).* 277:665–667.
55. Schiff, P. B., and S. B. Horwitz. 1980. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA.* 7:1561–1565.
56. Schnapp, B. J., and T. S. Reese. 1982. Cytoplasmic structure in rapid-frozen axons. *J. Cell Biol.* 94:667–679.
57. Smith, D. S. 1971. On the significance of cross-bridges between microtubules and synaptic vesicles. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 261:395–405.
58. Stearns, M. E., and R. L. Ochs. 1982. A functional in vitro model for studies of intracellular motility in digitonin-permeabilized erythrophores. *J. Cell Biol.* 94:727–739.
59. Taylor, E. W. 1965. The mechanism of colchicine inhibition of mitosis. *J. Cell Biol.* 25:145–160.
60. Taylor, D. L., and Y.-L. Wang. 1980. Fluorescently labeled molecules can probe the structure and function of living cells. *Nature (Lond.).* 284:405–410.
61. Thompson, W. C. 1982. The cyclic tyrosination/detyrosination of alpha-tubulin. *Methods Cell Biol.* 24:235–255.
62. Thyberg, J., S. Nilsson, S. Moskalewski, and A. Hinek. 1977. Effects of colchicine on the Golgi complex and lysosomal system of chondrocytes in monolayer culture. An electron microscopic study. *Cytobiologie.* 15:175–191.
63. Wehland, J., M. Osborn, and K. Weber. 1977. Phalloidin-induced actin polymerization in the cytoplasm of cultured cells interferes with cell locomotion and growth. *Proc. Natl. Acad. Sci. USA.* 74:5613–5617.
64. Wehland, J., D. J. P. FitzGerald, and M. C. Willingham. 1982. Microinjection of a monoclonal antitubulin antibody into cultured cells interferes with the organization and function of microtubules. *J. Cell Biol.* 95(2, Pt. 2):345a. (Abstr.)
65. Wehland, J., and I. V. Sandoval. 1983. Cells injected with guanosine 5'-[α,β-methylene]triphosphate, an α,β-nonhydrolyzable analog of GTP, show anomalous patterns of tubulin polymerization affecting cell translocation, intracellular movement, and the organization of Golgi elements. *Proc. Natl. Acad. Sci. USA.* 80:1938–1941.
66. Wehland, J., M. C. Willingham, and I. V. Sandoval. A rat monoclonal antibody that

FIGURE 9   Electron microscopy of Swiss 3T3 fibroblasts injected with YL 1/2 antibody adsorbed to colloidal gold. (a, b, and c) cells were injected with YL 1/2 antibody adsorbed to colloidal gold (2 mg IgG/ml), fixed 3 h after injection and processed for electron microscopy. Colloidal gold (arrowheads) is specifically accumulated along microtubules (*mt*), but not along other filamentous structures (*f*). (d) YOL 1/34 antibody adsorbed to colloidal remains diffusely distributed. G, Golgi. Bars, 0.1 µM. (a) × 52,000; (b and c) × 90,000; and (d) × 30,000.

reacts specifically with the tyrosylated form of α-tubulin. I. Biochemical characterization, effects on microtubule polymerization in vitro and microtubule polymerization and organization in vivo. *J. Cell Biol.* 97:1467-1475.

67. Willingham, M. C., and I. Pastan. 1975. Cyclic AMP and cell morphology in cultured fibroblasts. *J. Cell Biol.* 67:146-159.

68. Willingham, M. C., and I. Pastan. 1978. The visualization of fluorescent proteins in living cells by video intensification microscopy (VIM). *Cell.* 13:501-507.

69. Willingham, M. C., S. S. Yamada, and I. Pastan. 1980. Ultrastructural localization of tubulin in cultured fibroblasts. *J. Histochem. Cytochem.* 28:453-462.

70. Willingham, M. C., and I. Pastan. 1983. Intensification techniques for detection of proteins in cultured cells by fluorescence microscopy. *Methods Enzymol.* In press.

71. Wolosewick, J. J., and K. R. Porter. 1976. Stereo high-voltage electron microscopy of whole cells of human diploid line WI-38. *Am. J. Anat.* 147:303-324.

72. Wolosewick, J. J., and K. R. Porter. 1979. The microtrabecular lattice of the cytoplasmic ground substance: artifact or reality? *J. Cell. Biol.* 82:114-139.

73. Wunderlich, F., R. Muller, and V. Speth. 1973. Direct evidence for a colchicine-induced impairment in the mobility of membrane components. *Science (Wash. DC).* 182:1136-1138.

74. 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.

75. Zieve, G. W., D. Turnbull, J. M. Mullins, and J. R. McIntosh. 1980. Production of large numbers of mitotic mammalian cells by use of the reversible microtubule inhibitor nocodazole. *Exp. Cell Res.* 126:397-405.