A Microtubule-associated Protein Antigen Unique to Mitotic Spindle Microtubules in PtK₁ Cells

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ABSTRACT Microtubule-associated proteins (MAPs) that copurify with tubulin through multiple cycles of in vitro assembly have been implicated as regulatory factors and effectors in the in vivo activity of microtubules. As an approach to the analysis of the functions of these molecules, a collection of lymphocyte hybridoma monoclonal antibodies has been generated using MAPs from HeLa cell microtubule protein as antigen. Two of the hybridoma clones secrete IgGs that bind to distinct sites on what appears to be a 200,000-dalton polypeptide. Both immunoglobulin preparations stain interphase and mitotic apparatus microtubules in cultured human cells. One of the clones (N-3B4.3.10) secretes antibody that reacts only with cells of human origin, while antibody from the other hybridoma (N-2B5.11.2) cross-reacts with BSC and PtK₁ cells, but not with 3T3 cells. In PtK₁ cells the N-2B5 antigen is associated with the microtubules of the mitotic apparatus, but there is no staining of the interphase microtubule array; rather, the antibody stains an ill-defined juxtanuclear structure. Further, neither antibody stains vinblastine crystals in either human or marsupial cells at any stage of the cell cycle. N-2B5 antibody microinjected into living PtK₁ cells binds to the mitotic spindle, but does not cause a rapid dissolution of either mitotic or interphase microtubule structures. When injected before the onset of anaphase, however, the N-2B5 antibody inhibits proper chromosome partition in mitotic PtK₁ cells. N-2B5 antibody injected into interphase cells causes a redistribution of MAP antigen onto the microtubule network.

Microtubules purified by cell fractionation or by cycles of assembly/disassembly contain, in addition to tubulin, a heterogeneous population of accessory proteins called microtubule-associated proteins (MAPs). Many of the MAPs that have been isolated are capable of stimulating microtubule assembly in vitro (6, 26, 29, 31, 34). Immunocytochemistry has shown that MAPs from brain tissue associate with microtubules in situ (8, 9, 13, 28), consistent with the hypothesis that MAPs have tubulin modulating function(s) in living cells as well as promoting tubulin polymerization in vitro. MAP cofactors may therefore mediate the cytoskeletal and motility functions of microtubules in vivo, and this has fostered our interest in the identification and characterization of those MAPs that are involved in the formation and function of the mitotic apparatus.

Brain is commonly used for the preparation of microtubule protein because it contains a high density of microtubules competent to polymerize in vitro as well as in vivo. Brain is, however, a poor source of material for the investigation of mitotic spindle components because after neurogenesis the tissue has a low mitotic index. Thus any mitotic apparatus-specific factors would be expected to be present at low concentrations. Microtubule protein prepared from cultured cells contains a different complement of assembly MAPs than neurotubule protein prepared from brain tissue (4, 27, 30, 32, 36). As first suggested by Nagle and colleagues (27), this may indicate that some brain MAPs have neuron-specific functions. Indeed, MAPs from brain either do not appear to be associated with the microtubules of nonneuronal cells (13, 24) or are found in association with the interphase microtubule network as well as the mitotic apparatus and thus are not spindle-specific components (8). There are many polypeptides that associate with HeLa cell microtubules purified by cycles of assembly/disassembly (4, 30). Such material is potentially a plentiful source of mitotic apparatus MAPs. We are producing a catalogue of specific lymphocyte hybridoma monoclonal.
antibodies against the mixture of HeLa MAPs to serve as biochemical, cytological, and pharmaceutical probes for MAP function.

This report presents observations on two of the hybridoma clones that produce IgGs directed against determinants on high molecular weight MAPs in HeLa cells, only one of the antibodies cross-reacts with rat kangaroo MAP; cells in which the staining is restricted to mitotic spindle microtubules. Microinjection of this antibody into living PK cells interferes with normal mitosis, suggesting that this MAP contributes to the formation and/or function of the mitotic spindle.

MATERIALS AND METHODS

Antigens and Antibodies: HeLa cells were grown in suspension culture in modified Eagle's medium (MEM) supplemented with 10% fetal calf serum. Cells were collected by centrifugation, washed in 100 mM PIPES, pH 6.9, 1.0 mM MgCl₂, 1.0 mM EGTA (PME buffer) and lysed by sonication at 0°C. Microtubules were pelleted and purified by cycles of assembly/disassembly as described in detail elsewhere (30). The microtubule-associated protein (MAP) fraction was isolated by ion exchange chromatography (31).

BALB/c mice were inoculated intraperitoneally with 100 µg of HeLa MAPs in complete Freund's adjuvant and boosted intravenously 21 d later with 50 µg of HeLa MAPs in phosphate-buffered saline (PBS). On day twenty-four, the mice were killed, their spleens excised, and 1.25 x 10⁶ spleen lymphocytes were fused to 1.25 x 10⁶ spleen lymphocytes from N-2B5.11.2 and N-3B4.3.10 that secreted antibody that bound to the HeLa MAP mixture in solid phase radioimmunoassay using the original HeLa MAP antigen as ligand, and antibody reacting with the immunogen was detected with [125I]agonist protein A (SAP) labeled with diiodinated Bolton-Hunter reagent (New England Nuclear, Boston, MA). Two of the hybrids (N-2B5.11.2 and N-3B4.3.10) that secreted antibody that bound to the HeLa MAP mixture in solid phase radioimmunoassay and stained HeLa cell microtubules by immunofluorescence microscopy were cloned twice for use in subsequent experiments. Antibacterial antiserum was the generous gift of Dr. Jordan Olmsted (University of Rochester).

Immunochromatographic Characterization: The specificity of the antibodies from the resultant clones was tested by staining gel slices containing electrophoretically separated cell proteins with [35S]methionine-labeled antibody (7) 3°-monoclonal antibody was prepared by growing the hybridomas overnight in MEM lacking methionine (Flow Laboratories, Rockville, MD) supplemented with pyruvate, glutamine, nonessential amino acids (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), and 10 µg/ml [35S]methionine (New England Nuclear). Labeled antibody was harvested by ammonium sulfate precipitation and purified by chromatography on SAP-Sepharose A-50 (Pharmacia) and then spraying the cells into a 10-fold excess of acetone precipitate was collected by centrifugation at 3,000 g for 10 min at 4°C. The antibody-stained cell preparations were observed on a Zeiss microscopie III equipped with epi-fluorescence optics. Images were recorded on Plus-X film and developed in HC-100.

Microinjection: For microinjection experiments the PK cells, cultured on 12-mm glass coverslips for 2 d and mounted in 35-mm diameter observation chambers with 3 ml of warm medium. During prolonged observation fresh 59% O₂-equilibrated medium was circulated through the chamber at the rate of 10 ml/h. Temperature was maintained with an air curtain incubator (Sage Instruments, Cambridge, MA) and monitored with a needle probe thermometer placed directly in the culture medium (Yellow Springs Instrument Co., Yellow Springs, OH). Cells were injected with glass microneedles (W-P Instruments, Inc., New Haven, CT) with an outside tip diameter of 0.25 µm (as determined by scanning electron microscopy) pulled on a P-77 Brown-Flaming micropipet puller (Sutter Instruments, San Francisco, CA). Needles were backfilled by capillary action and then inserted into the microtocol of a Leitz micromanipulator (E. Leitz, Inc., Rockleigh, NJ).

Monoclonal antibody at a concentration of 1.0-2.5 µg/ml containing 0.5 µg/ml of fluorescein-isothiocyanate-labeled BSA was dialyzed into the injection buffer 1.0 M glutamic acid, 0.09 M citric acid, 0.14 M NaOH, 0.1 mM dithiothreitol, pH 7.0 before use. Antibody was injected by initiating a gentle flow of antibody using air pressure developed by a 50-ml disposable syringe connected to the needle by silastic tubing and then briefly touching the needle to the cell. Injection volume was ~1 x 10⁻⁹ ml as determined by the injection of [125I]-BSA (Zavortink, M., and J. R. McIntosh, unpublished results) or by calculation using the size of the vesicle that forms transiently during microinjection (Iran, J., and J. R. McIntosh, unpublished observations).

The cells were observed with a 40x lens immersed in the tissue culture medium on a Zeiss Universal microscope equipped with phase, epi-fluorescence, differential interference contrast (DIC), and polarization optics. Images were captured with a Venus DV2/9002 video intensification camera and recorded on an NEC WV-C507 video tape recorder using a Panasonic WV-J810 time-date generator. This produced high quality images at low incident light intensities. Individual photographic records were recorded on Pan-X film from a Panasonic WV-3635U monochrome.

RESULTS

The HeLa MAP antigen mixture is composed of many polypeptides. Three of the predominant proteins have electrophoretic mobilities corresponding to 200,000, 125,000, and 68,000 daltons as shown in Fig. 1. Hybridoma ciones N-2B5 and N-3B4 bind to the original antigen in solid phase radioimmunossays (data not shown), and each antibody binds to a HeLa cell polypeptide with an apparent molecular weight of ~200,000 daltons (Fig. 1). No binding to rodent cell homologues has been observed. Like the neurotubule-associated pro-
The antibodies recognize different antigenic determinants. The CNBr disk assay result that confirms the same structure and suggests that the antibodies bind to the bound antibody or omitting antigen, is low for the N-3B4 resin. This indicates that the antibodies bind to the antigen immobilized on CNBr paper disks and in vivo. In the immunochemical procedures used to characterize the antigens, precautions to inhibit proteolysis were necessary to prevent the generation of lower molecular weight fragments to which the monoclonal antibodies also bind.

In order to study the relationship between the N-2B5 and N-3B4 antigens, competition experiments were performed using antigen immobilized on CNBr paper disks and in vivo labeled monoclonal antibody (Fig. 2). Unlabeled N-2B5 antibody is significantly more effective than unlabeled N-3B4 at competing with 35S-labeled N-2B5 for binding to the HeLa MAP antigens, suggesting that the immunoglobulins produced by the two clones recognize different antigenic determinants. The reciprocal experiment using 35S-N-3B4 confirms the lack of direct competition for antigenic determinants (Fig. 2). To determine if the two antigenic determinants recognized by the antibodies are on the same or different proteins, another competition experiment using antibody directly coupled to dextran beads was designed. As diagrammed in Fig. 3, N-3B4 antibody covalently bound to the resin should be able to retain the complementary 35S-labeled N-2B5 antibody if and only if the two antigenic determinants are on the same MAP molecule. Nonspecific association, as determined by adding excess unbound antibody or omitting antigen, is low for the N-3B4 resin. The N-3B4-resin is able to bind 35S-labeled N-2B5 antibody in an antigen-dependent fashion, and unbound N-3B4 successfully competes with N-3B4 resin for the 35S-N-2B5-antigen complex (Fig. 3). This indicates that the antibodies bind to the same structure and confirms the CNBr disk assay result that the antibodies recognize different antigenic determinants.

In immunofluorescence microscopy both of the monoclonal antibodies stain interphase and mitotic spindle microtubules in all human lines tested (Figs. 4 and 5 and Table I). N-3B4 stains only cells of human origin, whereas the N-2B5 antibody stains human, nonhuman primate, and marsupial cell lines. The N-2B5 antibody does not stain 3T3 cells. The immunofluorescence images of the N-2B5 antibody in PtK1 cells is especially intriguing since only the microtubules of the mitotic apparatus are especially sensitive to endogenous protease activity (12, 29).
are stained (Fig. 4). During interphase the N-2B5 antibody stains an amorphous, frequently squiggly mass at the edge of the nucleus. It is difficult to determine if this image corresponds to the position of the cell center. During prophase the enlarging spindle pole asters stain brightly, with no staining of the residual interphase microtubules. Similarly, during telophase there is some staining of the midbody, but no staining of the new array of microtubules forming in each daughter cell. Thus, the N-2B5 antibody can clearly discriminate between the microtubules of the mitotic apparatus and the cytoplasmic microtubules. The lack of interphase microtubule staining is not due to inadequate fixation of the interphase microtubule arrays, since antitubulin antisera staining and the absorption experiments described below demonstrate the presence of a normal

FIGURE 4  Indirect immunofluorescence microscopy with N-2B5 anti-MAP antibody. The N-2B5 antibody stains interphase and mitotic spindle microtubules in Va90 (a) and HeLa (b) cells. In PtK2 cells (c and d) an ill-defined juxtanuclear structure is stained during interphase (c; arrows) and mitotic spindle fibers stain brightly (d). There is no staining of 3T3 cells (e) by this antibody. The last panel (f) shows HeLa cells stained with the secondary fluorochrome-labeled antibody alone. Bar, 10 μm. X 1,250.
complement of intact microtubules in the interphase cells. As with antitubulin and other anti-MAP antisera (2, 10, 33), both the monoclonal anti-MAP antibodies and the antigen-antibody complex fail to stain the center of the spindle poles and the midbody of telophase cells.

To examine the fate of the N-2B5 and N-3B4 MAPs during microtubule depolymerization, dual fluorochrome labeling of MAP and tubulin antigens was performed in HeLa and PtK₁ cells treated with the microtubule inhibitors colchicine and vinblastine. Both cell lines and both MAP antibodies gave results identical to those documented in Fig. 6. Vinblastine treatment results in a diffuse cytoplasmic distribution of tubulin and both the MAP antigens (Fig. 6). Colchicine treatment promotes the formation of crystals of microtubule fragments (1) that stain intensely with antitubulin antibody. However, there is no detectable N-2B5 or N-3B4 staining associated with the paracrystals in either HeLa or PtK₁ cells (Fig. 6), suggesting that there is little if any association of these MAPs with vinblastine-induced crystals.

As a test for the specificity of the N-2B5 antibody, diluted preparations of the N-2B5 antibody were absorbed with purified MAP2 (17), phosphocellulose-purified tubulin (34), or the original HeLa MAP antigen. While MAP2 and tubulin have no effect on the staining pattern of the N-2B5 antibody, preparations of the N-2B5 antibody absorbed with HeLa MAPs stain both interphase and mitotic spindle microtubules with unusual brilliance in PtK₁ (Fig. 7) as well as HeLa cells (data not shown). Since the N-2B5 antibody alone does not stain interphase microtubules in PtK₁ cells, the N-2B5 antibody absorbed with HeLa MAPs must be binding to fixed interphase microtubules in a MAP-dependent fashion. Consistent with this interpretation, absorption of the N-3B4 antibody with HeLa MAPs confers on this non-cross-reacting antibody the capacity to stain microtubules in PtK₁ cells (Fig. 7). The microtubule staining by either antigen-antibody complex can be eliminated by preincubating the cells in excess HeLa MAPs. These results also indicate that the paraformaldehyde/glutaraldehyde fixation protocol used preserves the interphase as well as mitotic apparatus microtubules in PtK₁ cells.

The N-2B5 antibody was microinjected into PtK₁ cells to investigate the function of this spindle-specific antigen during mitosis. Unlike most cultured cell lines, PtK₁ cells remain flat during cell division which facilitates microinjection and observation. Fluorescein-labeled BSA was included with the antibody to allow confirmation that a cell had been injected (an absolute necessity for mitotic cells) and to permit scoring of daughter cell pairs. Injection of the non-cross-reacting N-3B4 antibody provides a valuable control for the specificity of the localization and the effect of microinjected N-2B5 antibody since both immunoglobulin preparations bind to MAPs, but only the N-2B5 reacts with PtK₁ cells.

To determine whether the microinjected antibody had any effect on mitosis and cytokinesis, metaphase and prometaphase PtK₁ cells were injected with antibody and incubated 3 h before fixation and examination. Cells that appeared to have been damaged by the injection were not scored. As shown in Table II, the fraction of cells able to complete mitosis successfully within the incubation period was lowered from 70% for cells injected with the control N-3B4 antibody (which is comparable to that found when buffer alone is injected), to 14.6% for cells injected with the N-2B5 antibody. The introduction of N-2B5 immunoglobulin into living PtK₁ cells produces a distinct inhibition of normal mitosis.

Cells were also fixed at 5, 30, and 120 min after injection and stained with rhodamine-labeled rabbit anti-mouse immu-
FIGURE 6 Dual fluorochrome labeling of tubulin and N-2B5 antigens in colchicine and vinblastine-treated cells. PtK1 cells were treated with $10^{-6}$ M colchicine (a and b) or with $10^{-6}$ M vinblastine sulfate (c and d) for 2 h before fixation and then stained with rabbit antitubulin and mouse monoclonal N-2B5 antibody. Anti-tubulin was detected with fluorescein-conjugated goat anti-rabbit immunoglobulin and the N-2B5 antibody was detected with tetramethyl-rhodamine-conjugated goat anti-mouse immunoglobulin. Colchicine produced diffuse distributions of both antigens (a and b). Images recorded using fluorescein excitation and barrier epifluorescence filters (a and c) document the presence of tubulin paracrystals in vinblastine-treated cells. Micrographs recorded with rhodamine filters (b and d) show that both in interphase and mitotic cells the vinblastine-induced tubulin paracrystals do not contain detectable N-2B5 MAP. Bar, 10 μm. × 1,250.

DISCUSSION

The present results suggest that hybridoma clones N-2B5 and N-3B4 secrete IgGs that bind to distinct sites on a HeLa MAP of ~200,000 daltons. This MAP binds to microtubules during cycles of in vitro assembly (31) and is associated with interphase and mitotic spindle microtubules in human cells. For reasons that remain enigmatic, antibody staining of polyacrylamide...
FIGURE 7 Staining of PtK₁ cells with absorbed MAP antibodies. PtK₁ cells were stained with N-2B5 anti-MAP antibody absorbed with HeLa MAP antigen (a and b), with N-3B4 absorbed with HeLa MAPs (c) or with N-2B5 antibody absorbed with purified MAP2 (d). The antigen-antibody complex stains both interphase and mitotic spindle microtubules, whereas absorption with MAP2 or tubulin produces images similar to the N-2B5 antibody alone. Bar, 10 μm. × 1,250.

TABLE I

| Antibody | Cells* scored | Daughter pairs | Binucleate cells | Unsuccessful karyokinesis£ |
|----------|---------------|----------------|-----------------|---------------------------|
| N-3B4.3.10 | 109 | 65 (60%) | 11 (10%) | 33 (30%) |
| N-2B5.11.2 | 226 | 21 (9.3%) | 12 (5.3%) | 193 (85%) |

* Cells were scored 3 h after injection
£ Includes mitotic arrest and mononuclear cells

A rabbit antiserum to a 210,000-dalton HeLa assembly MAP has been prepared (5). Although this antiserum reacts with a HeLa MAP of a molecular weight similar to that of the N-2B5 and N-3B4 antigen, the cross-reactivity of the antiserum is different from the cross-reactivities of both N-2B5 and N-3B4. Whereas the antiserum reacts with human and several primate cell lines, the N-3B4 antibody appears to be human-specific. In contrast to both, the N-2B5 antibody shows cross-reactivity in both primates and marsupials. It is possible that the rabbit antiserum reacts with only a subset of the 200,000-dalton HeLa MAPs, and thus does not stain cells lacking those particular MAPs. Alternatively (or in addition), the N-2B5 antibody may bind a MAP antigenic determinant that is common between HeLa and PtK₁ cells but that is not recognized by the antiserum.

The experiments with antigen immobilized on CNBr cellulose disks and those with antibody bound to chromatography resin both indicate that the N-2B5 and N-3B4 antibodies bind...
to distinct, nonoverlapping sites on HeLa MAPs. The ability of immobilized N-3B4 antibody to bind 35S-labeled N-2B5 antibody in an antigen-dependent fashion suggests that the two immunoglobulins bind to a single polypeptide. However, we cannot unequivocally exclude the possibility that the two antibodies recognize two distinct 200,000-dalton MAPs that bind tightly to one another. Indeed, gel filtration studies demonstrate that native high molecular weight HeLa assembly MAPs exist as dimers in solution (6). If N-3B4 and N-2B5 bind distinct polypeptides, then the current data suggest that the high molecular weight HeLa MAP exists as a heterodimer of N-2B5 and N-3B4 antigen subunits in vivo.

The observations on the coincident localization of the HeLa MAP antigens and microtubules in immunofluorescence experiments support the theory that polypeptides that copurify with tubulin in vitro also interact directly with microtubules in vivo. These observations are consistent with the hypothesis that MAPs play a direct role in the modulation of tubulin assembly and/or function in living cells. Furthermore, the current results indicate that in PtK1 cells the N-2B5 antigen is associated specifically with the mitotic spindle. The N-2B5/PtK1 antigen is a novel MAP in its capacity to discriminate between different classes of cytoplasmic microtubules in cultured cells. Although immunocytochemical studies have previously shown that calmodulin (35), cyclic nucleotide-dependent kinase (3), and NuMA (21) also associate specifically with mitotic spindle microtubules, no other accessory proteins from cycled brain or HeLa microtubule protein have been found in unique association with mitotic apparatus microtubules (5, 8, 9, 10, 13, 28). Our collection of anti-MAP hybridomas includes one clone (O-2D5) that produces IgG that preferentially stains microtubules in the mitotic spindle of human cells, while in interphase cells it stains the interphase microtubules faintly and produces a distinctive punctate nuclear staining pattern (15, 16). Whereas the O-2D5 MAP antigen can be sparsely detected along interphase microtubules, the N-2B5 MAP antigen associates exclusively with mitotic spindle microtubules in PtK1 cells and cannot be detected on interphase PtK1 microtubules at all. The only precedent for the interaction of an assembly-promoting MAP with a specific subset of cellular microtubules is the brain polypeptide MAP2 which has been localized to dendritic processes in situ (24).

The 200,000-dalton MAP (or MAPs) and tubulin are not always found associated with each other as evidenced by the vinblastine-induced tubulin paracrystals that do not contain detectable amounts of either the N-2B5 or N-3B4 antigen. While consistent with the in vitro results of Luduena and coworkers (20), our images are in contrast to observations made by other workers (10, 11) who found that a 210,000-dalton HeLa MAP antigen was associated with vinblastine-induced

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**FIGURE 8** Distribution of microinjected N-2B5 anti-MAP antibody in PtK1 cells. Mitotic (a and b) and interphase (c and d) PtK1 cells were microinjected with N-2B5 antibody, incubated for 5 min (a and c) to 30 min (b and d) and then fixed and stained with fluorochrome-labeled secondary antibody. The N-2B5 antibody binds readily to mitotic spindles in living as well as fixed PtK1 cells and promotes a gradual disorganization of the spindle. When injected into interphase cells the N-2B5 antibody promotes a redistribution of antigen onto the interphase microtubule array (d). Bar, 10 μm. X, 1,250.
tubulin aggregates. The reasons for this discrepancy are not obvious. It may be that the determinants recognized by the N-2B5 and N-3B4 antibodies are masked in the crystals, or perhaps the antiserum used in other studies contains a heterogeneous group of antibodies against several high molecular weight HeLa polypeptides, one of which does bind to vinblastine crystals.

The serendipitous absorption experiment indicates that preincubation of antibody with HeLa MAPs allows the N-2B5 and the N-3B4 immunoglobulins to bind to interphase PtK₁ cell microtubules via the HeLa MAPs. The soluble antigen-antibody complex is in fact an excellent probe for microtubule distribution. The antigenic determinants recognized by the N-2B5 and the N-3B4 antibodies do not appear to be part of the microtubule binding site on the MAP molecule, since the soluble antigen-antibody complex binds readily and specifically to microtubules in situ. The results also demonstrate that chemically fixed microtubules can be specifically recognized by microtubule-associated proteins. This supports the notion that chemically stabilized microtubules are valuable affinity

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**FIGURE 9** Microinjection of monoclonal anti-MAP antibodies into metaphase PtK₁ cells. Cells were observed with polarization (a) or DIC optics (b–d) after the injection of N-2B5 (a–c) or N-3B4 (d) antibodies. Time shown is minutes after antibody injection. Injection of N-2B5 antibody promotes a reduction in spindle size and birefringence (a) in PtK₁ cells. Most cells do not enter anaphase (b); rather there is disorganization of the chromosome arrangement. Some PtK₁ cells injected with N-2B5 antibody initiate chromosome separation (c) but it is neither as fast nor as extensive as in cells injected with the non-cross-reacting N-3B4 antibody (d). Bar, 10 μm. × 700.
ligands for the biochemical identification of cultured MAPs (14). The staining intensity of the antigen-antibody complex is actually greater than that detected with the antibody alone, suggesting that the binding of MAPs along microtubules is not at saturation in vivo. This extends the observations of Kim and colleagues (17) on neurotubules that there are significant numbers of MAP binding sites on the tubulin polymer lattice that are normally unoccupied.

Microinjection of monoclonal antibodies provides an approach to the production of phenocopies of MAP structural gene mutations in a biological system that is not readily amenable to thorough genetic analysis (19, 22). The introduction of the N-2B5 antibody into living cells provides a novel view of MAP function in vivo. As summarized in Table II, the microinjection of N-2B5 antibody seriously impairs the ability of PtK₁ cells to complete karyokinesis successfully. Due to the intrinsic biological variability of the system and the complexity of the effects its was necessary to examine several hundred injected cells to be confident of the observations.

Immunofluorescence microscopy confirms that the N-2B5 antibody binds rapidly and specifically to the mitotic spindle in living as well as fixed PtK₁ cells. Observations with fluorescence, polarization, and DIC optics indicate that the injection of N-2B5 promotes a slow disorganization of the mitotic apparatus rather than a rapid disassembly, as is the case with calcium ion injection (Izant, J., manuscript in preparation). Thus, the antigenic determinant recognized by the N-2B5 antibody is probably not directly involved in microtubule uncoiling, but rather than a rapid disassembly, as is the case with calcium ion injection (Izant, J., manuscript in preparation). Thus, the antigenic determinant recognized by the N-2B5 antibody is probably not directly involved in microtubule uncoiling, but rather in the dynamic regulation of microtubule nucleation and polymerization. The injection of N-2B5 antibody into living cells provides a novel approach to the production of phenocopies of MAP structural gene mutations in a biological system that is not readily amenable to thorough genetic analysis (19, 22).

The rate and extent of chromosome movement in the injected cells that initiate anaphase is reduced, but nevertheless chromosome movement is still possible. Thus while mitotic apparatus motors are not completely jammed, the N-2B5 antibody may inhibit microtubule elongation in metaphase cells or act as a “monkey wrench” for a subset of microtubule interactions. The lack of effect when N-2B5 antibody is injected into ana-phase cells is potentially due to the lag period necessary for antigen-antibody binding, but it may further suggest that the N-2B5 antigenic determinant in PtK₁ cells is not the only site of mitotic force production.

Since the N-2B5 MAP antigen in PtK₁ cells clearly differentiates between interphase microtubule networks and mitotic spindle fibers, it would be valuable to determine whether the discrimination arises from the MAP binding to some other spindle-specific factor that is in turn bound to spindle microtubules or whether it is due to a cell cycle-dependent change in the PtK₁ N-2B5 MAP antigen that promotes microtubule binding only during mitosis. The current results favor the latter possibility. The redistribution of microinjected N-2B5 antibody onto microtubules in interphase cells is probably a reflection of the redistribution of the PtK₁ MAP itself, since, even when high concentration of the antibody are incubated with fixed PtK₁ cells for long periods, the N-2B5 antibody does not detect any antigen on fixed interphase microtubules. The antibody-induced redistribution of the PtK₁ MAP following microinjection demonstrates that this MAP can bind to microtubules during interphase and suggests that it is a chemical or conformational change in the N-2B5 MAP or MAP complex that promotes microtubule binding during mitosis. This binding may be physiologically significant in modulating mitotic apparatus formation in vivo.

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