# A Novel Pool of Protein Phosphatase 2A Is Associated with Microtubules and Is Regulated during the Cell Cycle

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*Abstract.* Immunofluorescence microscopy revealed the presence of protein phosphatase 2A (PP2A) on microtubules in neuronal and nonneuronal cells. Interphase and mitotic spindle microtubules, as well as centrosomes, were all labeled with antibodies against individual PP2A subunits, showing that the ABαC holoenzyme is associated with microtubules. Biochemical analysis showed that PP2A could be reversibly bound to microtubules in vitro and that ~75% of the PP2A in cytosolic extracts could interact with microtubules. The activity of microtubule-associated PP2A was differentially regulated during the cell cycle. Enzymatic activity was high during S phase and intermediate during G1, while the activity in G2 and M was 20-fold lower than during S phase. The amount of microtubule-bound PP2A remained constant throughout the cell cycle, implying that cell cycle regulation of its enzymatic activity involves factors other than microtubules. These results raise the possibility that PP2A regulates cell cycle–dependent microtubule functions, such as karyokinesis and membrane transport.

THE reversible phosphorylation of proteins is a ubiquitous biochemical mechanism involved in the regulation of cellular behavior, including structural and functional dynamics of the cytoskeleton. The cytoskeleton undergoes a dramatic alteration during the transition from interphase to mitosis. In nonpolarized cells at interphase, microtubules are usually arrayed in a radial network emanating from the perinuclear centrosome. At the onset of mitosis, the interphase network disappears and microtubules reorganize to form the mitotic spindle. Reversible phosphorylation of component proteins is involved in regulating the stability and function of interphase microtubules, microtubule reorganization during mitosis, and the function of the mitotic spindle. The onset of mitosis is controlled by activation of the cyclin B/p34$^{cdc2}$ protein kinase at the G2/M transition (Norbury and Nurse, 1992). Treatment of *Xenopus* oocyte extracts with p34$^{cdc2}$ kinase induces a decrease in microtubule stability similar to that observed during the interphase to metaphase transition (Verde et al., 1990, 1992). The cyclin B/p34$^{cdc2}$ complex is localized to cytoplasmic microtubules and centrosomes (Ookata et al., 1993), and to the spindle during mitosis or meiosis (Bailly et al., 1989, 1992; Riabowol et al., 1989; Pines and Hunter, 1991; Ookata et al., 1992). Effects on microtubule stability similar to those caused by p34$^{cdc2}$ kinase have also been observed with mitogen-activated protein kinase (MAP kinase)[1] (Gotoh et al., 1991), which is activated at M phase, and localizes to the mitotic spindle poles and to cytoplasmic microtubule organizing centers (Verlhac et al., 1993). The serine/threonine phosphatase inhibitor okadaic acid causes depolymerization of interphase microtubules and abnormalities in the mitotic spindle in LLC-PK cells (Vandré and Wills, 1992). Okadaic acid also induces interphase to mitotic-like dynamic instability of sea urchin egg microtubules (Gliksman et al., 1992) and a loss of interphase microtubules in BHK-21 cells (Eriksson et al., 1992). These results suggest that maintenance of the interphase microtubule network requires protein phosphatase activity. Important sites of phosphorylation in the control of microtubule function and stability are likely to be microtubule-associated proteins and centrosomal proteins.

Protein phosphatase 2A (PP2A) accounts for a significant portion of the total phosphatase activity in many tissues and cell types (Cohen, 1989), and plays important roles in cell growth and transformation (Mumby and Walter, 1993). PP2A is a heterotrimeric holoenzyme composed of a common core structure bound to different regulatory subunits. The core enzyme is a complex between the catalytic (C) and structural (A) subunits. The third subunit, termed B, comprises several distinct families of regulatory subunits that

1. *Abbreviations used in this paper*: MAP, microtubule-associated protein; MAP kinase, mitogen-activated protein kinase; PP2A, protein phosphatase 2A.

generate a diversity of holoenzymes (Mumby and Walter, 1993). The B subunits bind to the heterodimeric AC form of PP2A and regulate phosphatase activity and specificity (Waelkens et al., 1987; Umi et al., 1988; Chen et al., 1989; Kamibayashi et al., 1992, 1994).

There is substantial evidence that PP2A performs functions essential for cell division. Mutations in genes encoding subunits of PP2A cause defects in cell division in fungi and *Drosophila* (Kinoshita et al., 1990, 1993; Healy et al., 1991; Mayer-Jaekel et al., 1993). To better understand the role of PP2A in regulation of cell division, we investigated its subcellular localization, expression, and activity during the cell cycle. In this paper, we report that ~50–75% of the cytosolic pool of PP2A is associated with microtubules and is localized to interphase microtubules, centrosomes, and the mitotic spindle in various cell types. Moreover, the activity of microtubule-associated PP2A is regulated in a cell cycle-dependent manner. These findings suggest that PP2A plays a critical role in controlling the phosphorylation of proteins involved in controlling microtubule functions.

## Materials and Methods

### Proteins and Antibodies

Tubulin was purified from bovine brain by multiple cycles of GTP-stimulated assembly at 37°C and cold-induced disassembly, followed by DEAE chromatography, as described previously (Bloom et al., 1988). F-actin was purified from rabbit skeletal muscle (Spudich and Watt, 1971). The antitubulin monoclonal antibody has been characterized previously (Vallee and Bloom, 1983). Antibodies against the subunits of PP2A included: a monoclonal antibody against the catalytic subunit (Mumby et al., 1985), an antiserum raised against a COOH-terminal 20–amino acid peptide (residues 290–309) of the catalytic subunit (Kamibayashi et al., 1991), a polyclonal antibody raised against the A subunit, and an antipeptide antiserum against residues 13–26 of the 55-kD Bα subunit. The specificities of these antibodies have been demonstrated previously by immunoblotting of whole-cell or tissue extracts (Mumby et al., 1985; Kamibayashi et al., 1994). Nocodazole was obtained from Aldrich (Chemical Co., Milwaukee, WI) and taxol was a gift from the National Cancer Institute (Bethesda, MD).

### Cell Culture

Monkey kidney CV-1 cells (American Type Culture Collection, Rockville, MD), rat embryo fibroblasts (REF52), CHO, bovine kidney MDBK cells, mouse 3T3 fibroblasts and N1A neuroblastoma cells were maintained at 5% $CO_2$ in DME containing 10% bovine calf serum. In some cases, microtubules were depolymerized by treating CV-1 cells for 45 min with 4 μg/ml nocodazole.

### Selective Cell Extraction

Selective extraction of cultured CV-1 cells was performed as described previously (Solomon, 1986). For immunofluorescence studies, CV-1 cells were grown overnight on glass coverslips and washed twice with phosphate-buffered saline at room temperature. The washed cells were extracted for 90 s to 5 min by addition of 0.1% NP-40 in microtubule-stabilizing buffer, PEM2G (0.1 M Pipes, 2 M glycerol, 5 mM $MgCl_2$, and 2 mM EGTA, pH 6.9). The solubilized materials were then removed by a second extraction in PEM2G without detergent. The preparations were then fixed for 3 min at −20°C in methanol and labeled as described in the subsection titled "Immunofluorescence."

For quantitation of PP2A activity in synchronized CV-1 cells, the cells were selectively extracted using the same protocol and scraped from culture dishes in PEM2G buffer. Microtubules in the cytoskeletal preparations were stabilized by the addition of 10 μM taxol to the extraction buffer and were recovered after centrifugation for 2 min at 700 g. The microtubule-containing pellets were washed extensively with PEM buffer (0.1 M Pipes, 5 mM $MgCl_2$ and 2 mM EGTA, pH 6.9) and were analyzed for protein content and phosphatase activity. Control experiments consisted of preincubating cells with 4 μg/ml nocodazole for 45 min before detergent extraction in the absence of taxol. No appreciable microtubules or PP2A were recovered in the pellet fraction of nocodazole-treated cells.

### Cell Synchronization

For synchronization to G1, 150-mm dishes of CV-1 or mouse neuroblastoma cells were grown to confluency, starved for 24 h in DME containing 0.05% calf serum, and harvested. Synchronization to S was achieved by incubating cells for 24 h in leucine-free medium containing 2% dialyzed fetal calf serum followed by an incubation in DME containing 10% calf serum and 2.5 mM thymidine for 16–24 h. Cells were then released for 1 or 2 h in DME containing 10% calf serum and harvested. Cells were synchronized to G2/M by a 24-h thymidine block, followed by incubation for 24 h in DME containing 0.26 μM nocodazole. Cells were then either harvested directly (G2/M), harvested by mitotic shake-off (M), or released for 5 h in DME containing 10% calf serum (early G1). Growing cells (G1/S) were used in some experiments. All cells were washed extensively with phosphate-buffered saline before harvesting.

The efficiency of cell synchronization was analyzed by FACS® (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Cells (1 × $10^6$) were fixed in saline glucose/95% ethanol (1:3), washed with phosphate-buffered saline, and stained with propidium iodide solution containing 30 U/ml of RNase A. After incubating for 10 min at 37°C, NaCl was added to a final concentration of 0.15 M, and the cells were filtered through nylon mesh before being analyzed by flow cytometry.

### Immunofluorescence

Cells grown overnight on glass coverslips were fixed for 20 min in 4% paraformaldehyde-PBS and permeabilized for 10 min in PBS containing 0.1% Triton X-100. Alternatively, cells or detergent-resistant cytoskeletons (see "Selective Cell Extraction") were fixed/permeabilized by incubation for 3 min at −20°C in absolute methanol. Cells were then washed twice each with DME and PBS. Subsequent antibody incubations and washing steps were performed in PBS containing 1% bovine serum albumin. Cells were incubated for 45 min to 1 h with the following primary antibodies: purified monoclonal anti-PP2A catalytic subunit antibody (12.6 μg/ml), polyclonal anti-C subunit antiserum (1:200), polyclonal anti-A subunit antiserum (1:100), polyclonal anti-Bα subunit antiserum (1:1,000), or antitubulin IgM monoclonal antibody tissue culture supernatant (1:2). After washing, cells were incubated for 30 min with the following secondary antibodies: affinity-purified FITC-conjugated goat anti-mouse or Texas red–conjugated goat anti-rabbit antibodies (Fisher Scientific, Pittsburgh, PA) at 6.7 μg/ml for double labeling experiments. For single labeling experiments, Cy3-conjugated affinity-purified goat anti-rabbit antibody (Jackson ImmunoResearch Labs, West Grove, PA) and Texas red–conjugated affinity-purified goat anti-mouse IgG (Fisher) were used at 2.6 and 40 μg/ml, respectively. After extensive washing, the coverslips were mounted with Fluoromount (Fisher) and examined with a confocal microscope (63× objective; Carl Zeiss, Inc., Thornwood, NY). Control experiments for immunofluorescence were performed in which the first or second antibodies were omitted. Primary antibody specificity was also tested by preadsorbing antisera with the homologous antigens (purified peptides or proteins). For double labeling experiments, samples were scanned simultaneously for Texas red and FITC by laser confocal microscopy.

### Purification of Microtubules and Microtubule-binding Proteins

150-mm dishes (four to eight) of CV-1 or neuroblastoma cells were washed with ice-cold PBS and dounced homogenized in 500 μl of PEM buffer containing protease inhibitors (1 mM phenylmethylsulfonylfluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 0.1 mg/ml soybean trypsin inhibitor). Cells were centrifuged for 10 min in a microcentrifuge to remove insoluble material, and the resulting cell extract was centrifuged at 4°C for 1 h at 120,000 g.

For purification of endogenous microtubules, the cytosolic fractions from neuroblastoma cells were normalized for protein concentration and incubated at room temperature for 15 min in the presence of 20 μM taxol and 1 mM GTP. Microtubules were then pelleted by centrifugation for 30 min at 20°C at 30,000 g. The microtubule pellets were washed twice with PEM buffer. The remaining supernatant was again treated with taxol and GTP, as described above, to increase the yield of polymerized microtubules. Microtubules obtained from the second centrifugation were combined with the first microtubule pellet. The final microtubule pellet was resuspended in 100 μl of PEM buffer (one fifth of initial volume).

Microtubule-binding proteins (including structural microtubule-associated proteins [MAPs]) were also isolated from cytosolic extracts of cultured cells using purified exogenous microtubules (Bloom et al., 1985). For these experiments, the soluble cell extracts (500 μl) were incubated for 15 min on ice with 0.5 mg/ml purified bovine brain tubulin preassembled with taxol. The microtubules and associated proteins were then collected by centrifugation at 4°C for 30 min at 30,000 g. In some experiments (see Table I), the postmicrotubule supernatants were supplemented with microtubules and centrifuged again, a process that was repeated multiple times. As a control for exogenous microtubules, cell extracts were incubated in an identical fashion, but with 0.5 mg/ml purified F-actin instead of polymerized tubulin.

Microtubules and microtubule-binding proteins were also prepared from rat brain using a taxol-dependent procedure (Vallee, 1982), modified such that the microtubule assembly step was performed at room temperature, instead of at 37°C. To maximize yield, the rat brain extract (1 ml) was treated twice with taxol and GTP. The washed microtubule pellets were resuspended in 200 μl of PEM buffer (one fifth of initial volume). Microtubule-binding proteins were dissociated from microtubules with high salt (Vallee, 1982). Salt-extracted microtubule-associated proteins were dialyzed against PEM buffer. To determine the maximum amount of brain PP2A activity that could associate with microtubules, rat brain microtubule-binding proteins were isolated by cosedimentation with multiple successive aliquots of exogenous microtubules, as described for cultured cells.

## Phosphatase Assay

Aliquots of cytosol, isolated microtubules, and other cytosol-derived fractions were assayed for protein phosphatase activity for 5 min at 30°C using phosphorylated myosin light chain as a substrate (Mumby et al., 1987). In parallel experiments, the fractions were preincubated with 1–5 nM okadaic acid before assay for phosphatase activity. PP2A was defined as the myosin light chain phosphatase activity that was sensitive to 1 or 5 nM okadaic acid (Cohen et al., 1989). 1 mU of phosphatase activity is that amount which released 1 nmol of $^{32}$P per minute. Protein concentrations were determined using the Bradford assay and bovine serum albumin as standard.

## Electrophoresis and Immunoblotting

The samples obtained during isolation of microtubules and microtubule-binding proteins were diluted with 3× SDS sample buffer. Equal volumes or equivalent amounts of protein were resolved on 8% SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and blotted with antibodies against the C, Bα, and A subunits of PP2A. SDS-PAGE was carried out in 0.75-mm-thick slab gels. Immunoblots were developed using the ECL chemiluminescence detection method (Amersham Corp., Arlington Heights, IL).

## Results

### Immunofluorescence Localization of PP2A on Microtubules

The subcellular localization of PP2A in intact CV-1 cells was examined by immunofluorescence with polyclonal and monoclonal antibodies raised against individual PP2A subunits or subunit-specific peptides. Our efforts were focused in particular on the ABαC form of the enzyme, the predominant heterotrimeric species of PP2A in CV-1 cells (Sontag et al., 1993). Fig. 1, A and B, show that the A and C subunits of PP2A were colocalized in the cytoplasm. The most intense staining of both subunit polypeptides was observed in the perinuclear region. The nonperinuclear cytoplasmic staining of the A and C subunits was faint and had a somewhat filamentous appearance. Similar results were obtained using either monoclonal or polyclonal antipeptide antibodies against the catalytic subunit (Fig. 1, B and C). Immunofluorescent staining of the Bα subunit showed a strikingly filamentous pattern (Fig. 1 D). Double labeling with antitubulin monoclonal antibodies showed that Bα was localized to the centrosome and the interphase microtubule network (Fig. 1, D and E). A perinuclear staining of Bα was also observed

using confocal microscopy. Perinuclear Bα staining was distinct from the microtubule network and was similar to the staining with anti-A and anti-C antibodies (Fig. 1 D). Some nuclear staining was also observed with all anti-PP2A subunit antibodies using confocal microscopy (results not shown).

Control experiments were performed to assess the specificity of all antibodies by preadsorbing antisera with homologous antigens (purified peptides or proteins). The staining of all three subunits was specifically blocked by the corresponding antigen (data not shown). In particular, the microtubule labeling observed with anti-Bα antibody was abolished by preincubating the antiserum with an excess of purified Bα peptide. The same distribution of Bα was observed using either methanol or paraformaldehyde as fixatives. Nocodazole treatment of cells before fixation resulted in the disappearance of microtubule staining with both antitubulin (results not shown) and anti-Bα antibodies (Fig. 1 F). Finally, immunoblotting analysis showed that anti-Bα antiserum did not cross-react with purified tubulin or any protein other than Bα in CV-1 cell extracts (results not shown).

The strong microtubule staining with anti-Bα antibodies, but not with anti-A or anti-C antibodies, suggested the possibility that the heterodimeric (AC) form of PP2A was not colocalized on microtubules with Bα. Alternatively, the absence of obvious microtubule labeling with anti-A and anti-C antibodies might have resulted from masking of the antigenic sites by microtubules or a high background of non–microtubule-associated subunits. Although anti-A and anti-C subunit antibodies primarily stained the perinuclear region, labeling of the centrosome with the monoclonal anti-C subunit antibody was observed using confocal microscopy (Fig. 1 B, arrow). Centrosome staining suggested that the C subunit might also be localized to microtubule-associated structures. Further evidence for the interaction of PP2A with microtubules came from experiments in which cells were selectively extracted with nonionic detergent before fixation and labeling. This type of extraction can solubilize ≤80% of the total cellular protein (Brown et al., 1976), but when appropriate buffers are used, most cytoskeletal elements, including microtubules, remain intact (Osborn and Weber, 1977). The presence of intact microtubules in the extracted cells was verified by immunofluorescence using antitubulin monoclonal antibody (Fig. 2 B). Extraction of CV-1 cells removed the bulk of the perinuclear staining observed with all the PP2A subunit antibodies (Fig. 2, A, C, and D). The microtubule labeling with anti-Bα antibody in extracted cells was unchanged relative to intact cells but its relative intensity was enhanced due to elimination of background perinuclear and cytoplasmic staining (Fig. 2 A). Strong staining of interphase microtubules with antibodies against the A and C subunits was also observed in extracted cells (Fig. 2, C and D). Specificity of the labeling obtained with all anti-PP2A subunits antibodies in extracted cells was verified in parallel control experiments (results not shown).

### PP2A Associates with Microtubules In Vitro

The results obtained by immunofluorescence indicated that PP2A was associated with microtubules in vivo. To verify this association at the biochemical level, microtubules and microtubule-binding proteins were purified from high speed
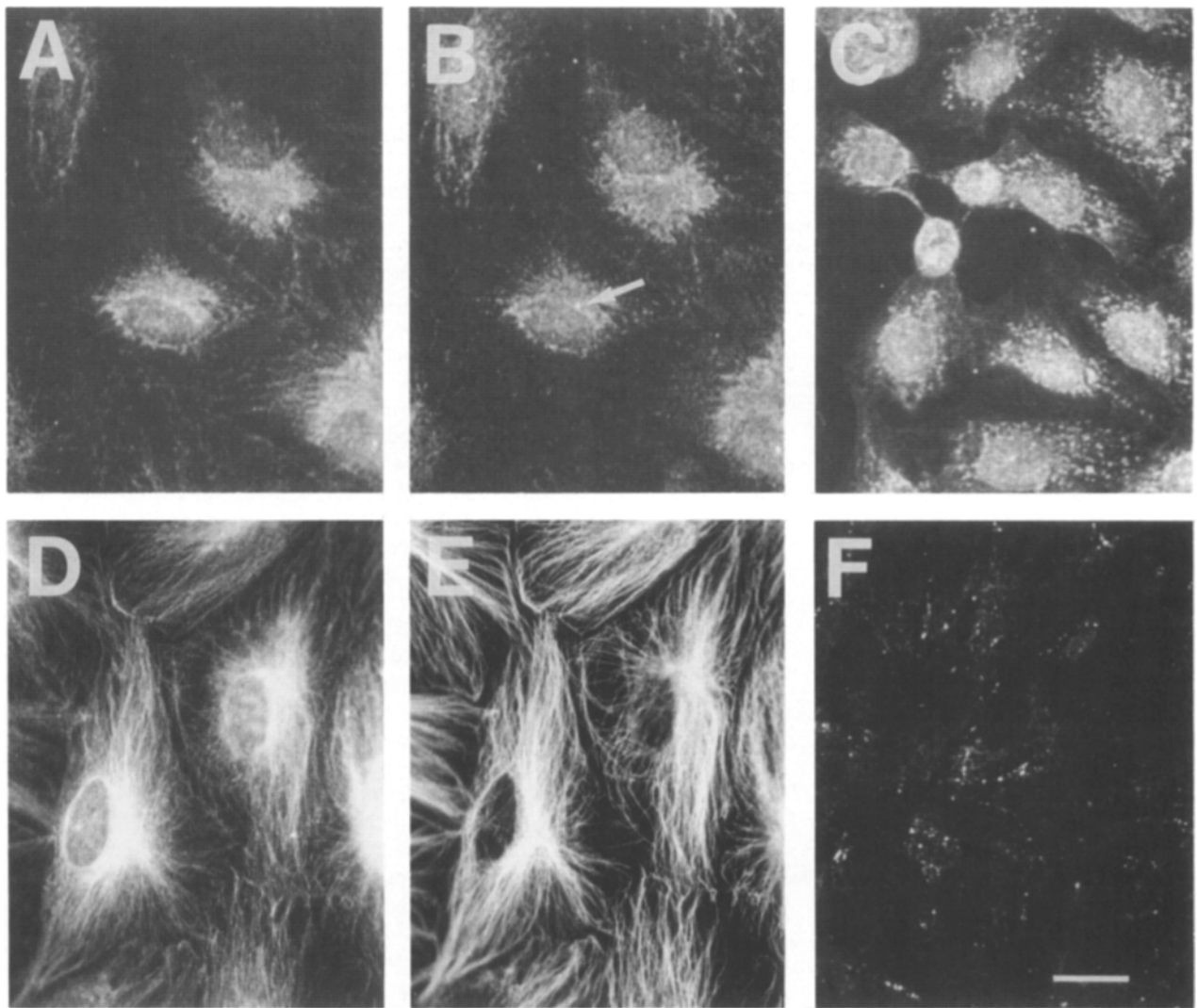
*Figure 1.* Analysis of PP2A distribution in CV-1 cells by indirect immunofluorescence. Growing CV-1 cells were fixed/permeabilized with 4% paraformaldehyde/0.1% Triton X-100 and labeled with the following primary antibodies: (*A* and *B*) Cells were double stained with PP2A polyclonal anti-A and monoclonal anti-C subunit antibodies, respectively. (*C*) Cells were labeled with anti-C subunit peptide antiserum. (*D* and *E*) Cells were doubled stained with PP2A anti-Bα and monoclonal antitubulin antibodies, respectively. (*F*) Cells were pretreated with 4 μg/ml nocodazole for 45 min before fixation and subsequent labeling with PP2A and anti-Bα antiserum. Bar in F, 25 μm for *A–C* and *F*, and 12 μm for *D* and *E*.

extracts of cultured mammalian cells and rat brain using either of two standard methods. Isolation of microtubule-binding proteins by the first method was achieved by cosedimentation with microtubules assembled from endogenous tubulin with the aid of taxol (Vallee, 1982). The microtubule assembly step in this case required a 15–30-min incubation of cytosol at a temperature of 20°C or higher. The second method made use of exogenous microtubules that were preassembled from purified brain tubulin with taxol and, when added to cytosol, associated with endogenous microtubule-binding proteins (Bloom et al., 1985). This latter technique allows cytosol to be maintained at 0–4°C throughout isolation of microtubule-binding proteins, and is especially useful for situations in which prolonged exposure of such proteins to elevated temperature compromises their structural and functional integrity (Bloom et al., 1985).

Fig. 3 documents experiments in which PP2A was de-

tected in microtubule pellets that had been sedimented out of CV-1 cell or rat brain cytosol. Exogenous microtubules were used for CV-1 cells because the 37°C incubation of cytosol that was required for assembly of endogenous tubulin nearly abolished the enzymatic and microtubule-binding activities of CV-1 cell PP2A (data not shown). This problem was circumvented by using exogenous microtubules and maintaining CV-1 cytosol at 0–4°C. In the case of the rat brain, microtubules were efficiently assembled from endogenous tubulin at room temperature, which did not adversely affect the functional properties of the endogenous cytosolic PP2A.

Two distinct types of assays indicated that approximately half of the PP2A present in high speed extracts of both CV-1 cells and rat brain could be recovered in microtubule pellets that were collected by one cycle of centrifugation. Microtubule-bound PP2A was detected by Western blotting using
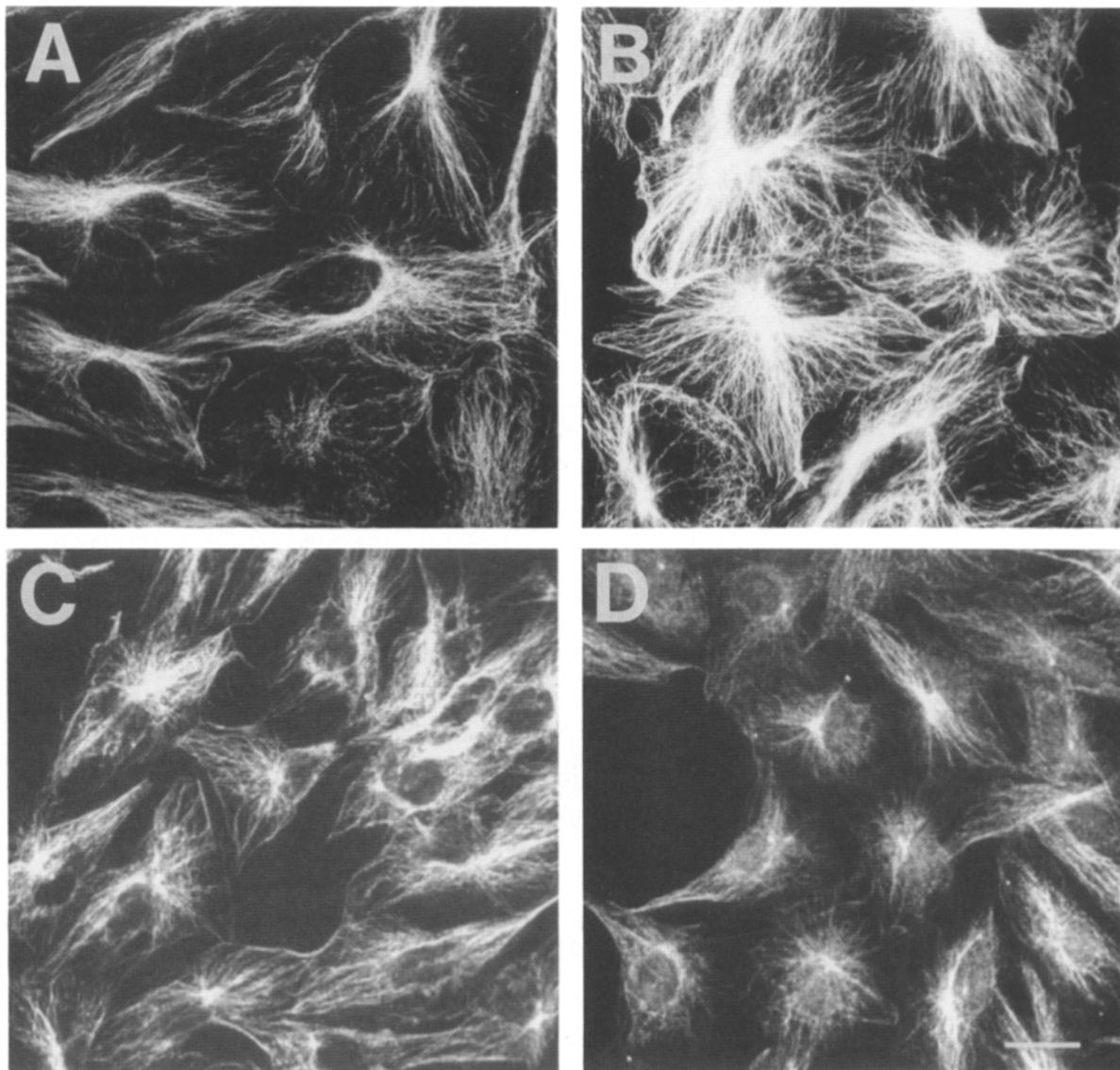
*Figure 2.* Immunofluorescent localization of PP2A in extracted CV-1 cells. CV-1 cells were extracted for 1.5 min with a detergent-containing, microtubule-stabilizing buffer before fixation. The cells were then fixed in methanol and stained with anti-Bα (*A*), antitubulin (*B*), anti-A (*C*), or monoclonal anti-C (*D*) antibodies. Bar in *D*, 12 μm for *A* and *B*, and 25 μm for *C* and *D*.

antibodies to the A, Bα, or C subunits of the protein (Fig. 3 *B*), as well as by enzymatic assays (Fig. 3 *C*). When microtubules were washed with a high salt buffer, most of the bound PP2A was solubilized, demonstrating that ionic interactions are important for association of the enzyme with microtubules (Fig. 3 *B*). About 80% of the PP2A in the salt wash fraction was able to rebind to purified microtubules after dialysis against salt-free buffer (data not shown). Nearly all of the protein phosphatase activity detectable at all stages of the experiments shown in Fig. 3 was inhibited by 1 nM okadaic acid. Sensitivity to low concentrations of OA indicated, as reported earlier (Sontag et al., 1993), that PP2A was the major serine/threonine protein phosphatase present in the cell and tissue extracts used for these experiments (Fig. 3 *C*). As was the case for CV-1 cells, the brain microtubule-associated phosphatase activity was extremely sensitive to OA (Fig. 3 *C, right panel*). Because of the abundance of the

PP2A in brain samples, a concentration of 5 nM OA was necessary to fully inhibit microtubule-bound PP2A activity.

When exogenous actin filaments, instead of microtubules, were added to cytosol and centrifuged, the resulting pellets contained nearly all of the added actin, but they lacked detectable PP2A, as judged by both Western blotting and enzymatic assays (data not shown). The actin filament experiments emphasize that the association of PP2A with microtubules is specific, and does not merely reflect adventitious binding of PP2A to acidic, fibrous protein polymers.

To estimate the maximal amount of cytosolic PP2A that could associate with microtubules, supernatants, obtained after cosedimentation of cytosol with exogenous microtubules, were supplemented with additional microtubules, incubated for another 15 min, and centrifuged. The resulting fractions were then assayed for PP2A activity. This process was repeated until no additional PP2A activity could be de-
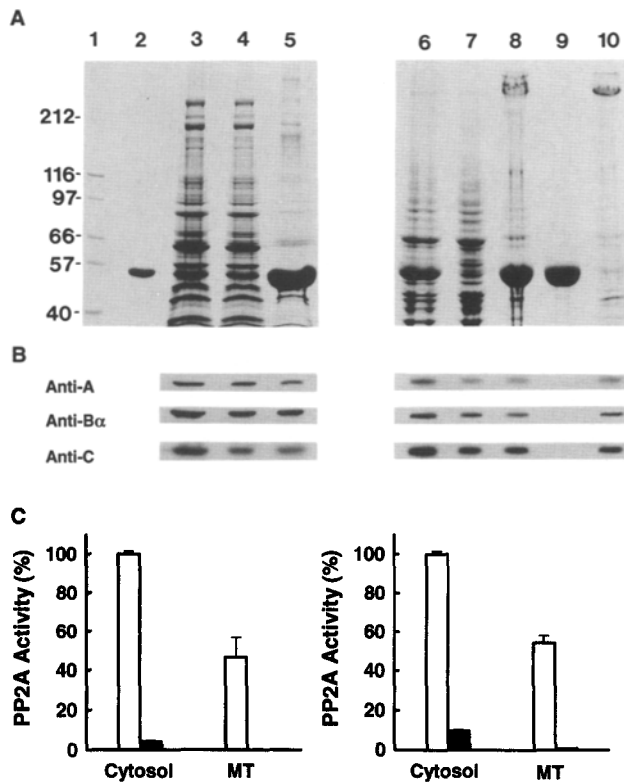
A



B

C

*Figure 3.* Binding of PP2A to exogenous microtubules in CV-1 cell extracts (*left portion*) and to endogenous rat brain microtubules (*right portion*). (*A*) Coomassie blue–stained gel of the different stages of microtubule purification. Microtubule-binding proteins were purified from a CV-1 cell extract by cosedimentation with exogenous bovine brain tubulin preassembled with taxol (*left portion*), as described in Materials and Methods. Lane *1*, high molecular weight markers; lane *2*, purified tubulin standard; lane *3*, cytosolic extract; lane *4*, postmicrotubule supernatant; lane *5*, microtubule pellet obtained after one cycle of centrifugation and extensive PEM washing. The microtubule pellet was resuspended in one fifth of the initial extract volume, and all lanes contained 50 μl of each fraction. Endogenous microtubules and microtubule-binding proteins were isolated from rat brain using one cycle of taxol-assisted assembly (*right portion*), as described in Materials and Methods. Lane *6*, cytosolic extract; lane *7*, postmicrotubule supernatant; lane *8*, microtubule pellet obtained after one cycle of centrifugation and PEM washing; lane *9*, microtubule pellet obtained after salt extraction; lane *10*, proteins eluted from microtubules by salt extraction. The microtubule pellets were resuspended in one fifth of the initial volume, and all lanes contained 20 μl of each fraction. Thus, for both CV-1 cells and rat brain, the MT pellets were concentrated five times relative to cytosol to emphasize the presence of microtubule-binding proteins. (*B*) Chemiluminescent immunodetection of samples obtained during the microtubule purification in CV-1 cells (*left portion*) or rat brain (*right portion*) with antibodies directed against the A (*anti-A*), Bα (*anti-Bα*), and C (*anti-C*) subunits of PP2A. All lanes contained 10 μl of each fraction. To facilitate direct comparison of the PP2A subunit levels in each fraction, the microtubule pellets were resuspended to the original volume of the cell extracts from which they were derived. Note that about half of the immunoreactive subunits were recovered in the pellets. (*C*) Phosphatase activity was determined in the total cytosolic extract (*Cytosol*) and in the microtubule fraction (*MT*) in the presence (*solid bars*) or absence (*open bars*) of 1 nM okadaic acid. Values are expressed as the percentage of the total PP2A activity in the cytosolic extract, and they represent the mean ± SD of duplicate assays. The specific activity in the cytosolic extract was 2 ± 0.3 mU/mg for CV-1 cells (*left portion*). Similar results were

*Table I. Binding of PP2A to Exogenous Microtubules*

| | Phosphatase activity | |
| --- | --- | --- |
| Treatment | Supernatant | Microtubule pellet |
| | *mU* | |
| 1 | 2.06 | 2.10 |
| 2 | 0.98 | 1.06 |
| 3 | 0.87 | 0.10 |
| 4 | 0.86 | 0 |

A CV-1 cell extract was supplemented with purified brain microtubules and then incubated for 15 min on ice as described in Materials and Methods. Microtubules were collected by centrifugation, and total PP2A activity remaining in both the postmicrotubule supernatant and the microtubule pellet was then measured (Treatment 1). This entire process was repeated three more times by substituting the postmicrotubule supernatant from each successive round for the original CV-1 cell extract (Treatments 2–4). The specific activity of PP2A in the starting cytosol was 1.7 mU/mg, and the total activity was 4.2 mU.

tected in the microtubule pellets. Table I summarizes results from a typical experiment using CV-1 cells. After one round of microtubule sedimentation, 2.1 mU of PP2A activity remained in the postmicrotubule supernatant, and 2.1 mU was present in the microtubule pellet. After the next round, the supernatant and pellet contained 1 and 1.1 mU of activity, respectively. Little, if any, PP2A activity was detected in subsequent microtubule pellets. Taken together, these data demonstrate that ≤75% of the PP2A activity in CV-1 cytosol was able to associate with microtubules in vitro. Nearly identical results were obtained using cytosol isolated from rat brain and mouse neuroblastoma cells (data not shown).

## PP2A Is Associated with Microtubules during Mitosis

The results of the immunofluorescence and biochemical analyses showed an association of PP2A with microtubules in interphase cells. To determine if PP2A was also associated with microtubules during mitosis, the localization of PP2A during the cell cycle was investigated. Dividing cells at various stages of mitosis were either fixed directly or extracted with nonionic detergent before fixation. Cells were then processed for immunofluorescence using antibodies against all three subunits of PP2A. Although labeling of the centrosome was visible with all of the antibodies in interphase cells, a particularly pronounced staining of the duplicated centrosome was observed with the anti-C subunit monoclonal antibody after detergent extraction of dividing cells (Fig. 4 *A*). Intense staining of microtubule arrays emanating from the duplicated centrosomes was observed with anti-Bα antibody in intact prophase cells (Fig. 4 *B*). The mitotic spindle was labeled with antibodies against all three subunits. An example of the spindle localization in detergent-extracted cells is shown for the A subunit (Fig. 4 *C*). Localization of PP2A on the mitotic spindle was confirmed by double labeling experiments, as shown for the Bα subunit (Fig. 4, *D* and *E*). The localization of the Bα subunit at different stages of mitosis is shown in Fig. 4 *F*. Immunofluorescence demonstrated prominent localization of Bα on centrosomal arrays of microtubules during prophase (*thick arrow*), and on the mitotic

obtained in five separate experiments. Brain PP2A specific activity was 12.9 ± 1.9 mU/mg in the microtubule pellet (*right portion*). Similar results were obtained in two separate experiments.
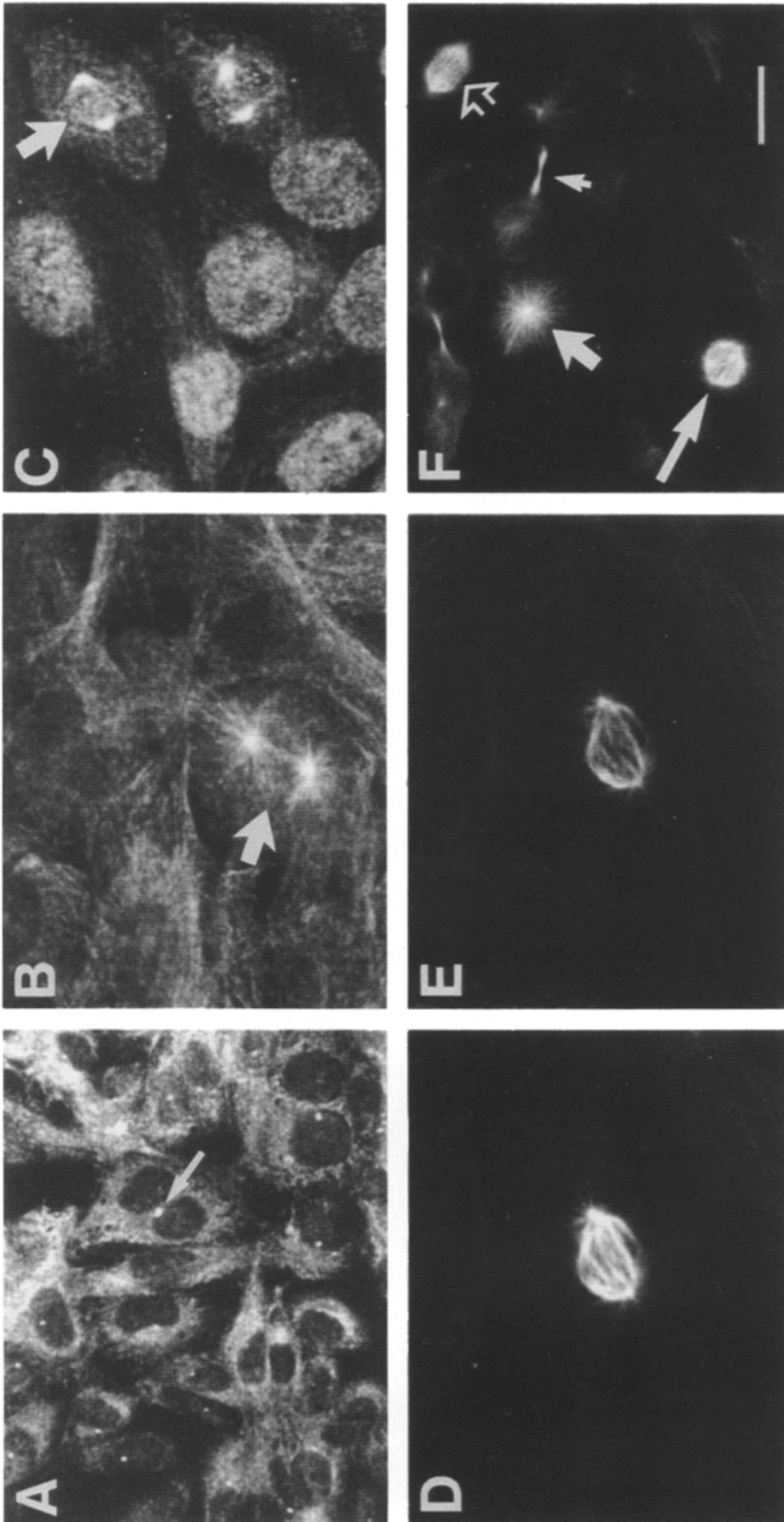
*Figure 4.* PP2A is associated with microtubules during mitosis. CV-1 cells were synchronized to G2/M as described in Materials and Methods. Intact or detergent-extracted cells were then fixed with methanol, and then processed for immunofluorescence using anti-A, anti-A, anti-Bα, or anti-C subunit antibodies. (*A*) Duplicated centrosomes (*arrow*) in detergent-extracted cells stained with monoclonal anti-C antibody. (*B*) Arrays of microtubules (*arrow*) in late prophase in intact cells stained with anti-Bα antibody. (*C*) Mitotic spindles (*arrow*) labeled with anti-A antiserum in extracted cells. (*D* and *E*) A mitotic spindle in an intact cell that is double stained with anti-Bα and antitubulin antibodies, respectively. (*F*) An array of mitotic cells stained with anti-Bα antiserum. This photograph shows a prominent centrosomal array of microtubules at prophase (*thick arrow*), a metaphase mitotic spindle (*long arrow*), an anaphase spindle (*open arrow*), and a pair of cells in late telophase/early interphase, when a bright staining of the midbody can be seen (*small arrow*). Bar in F, 25 μm for *A*, *C*, and *F*, 12 μm for *D* and *E*, and 8 μm for *B*.

spindle during metaphase (*long arrow*) and anaphase (*open arrow*). Bα was also localized to the midbody of telophase cells (*small arrow*). The labeling of the interphase microtubule network, duplicated centrosomes, and mitotic spindles with antibodies against all three subunits indicates that PP2A remains associated with microtubules throughout the cell cycle.

## PP2A Is Localized to Microtubules in Several Cell Types

To determine if localization on microtubules was a general phenomena, the distribution of PP2A in other cell types was examined. Asynchronous cultures of four cell lines were grown on coverslips and processed for immunofluorescence using the anti-Bα antiserum. Although the intensity of microtubule labeling varied with the cell type, the Bα subunit was associated with microtubules in each cell type. Fig. 5 shows some examples of the different patterns of

microtubule staining observed in these cell types with anti-Bα antibody. In rat embryo fibroblasts and bovine MDBK cells, which have abundant microtubules, prominent labeling of the interphase microtubule network, mitotic spindles, and centrosomes was observed (Fig. 5, *A* and *C*). Similar observations were made in 3T3 and CHO cells, as illustrated in Fig. 5, *B* and *D*. The interphase microtubules in these latter micrographs were not prominent because they were printed in a manner that highlighted the mitotic spindle staining. The Bα subunit also localized to centrosomes and microtubules in mouse neuroblastoma cells (results not shown). In all cases, the anti-Bα antiserum staining of microtubules colocalized with antitubulin monoclonal antibody and was blocked by preincubation with the Bα antigenic peptide (results not shown). The localization of Bα in these cell types indicates that the association of PP2A with microtubules occurs in epithelial cells, fibroblasts, and neurons, and that it is likely to be a general property of the ABαC heterotrimeric form of PP2A.
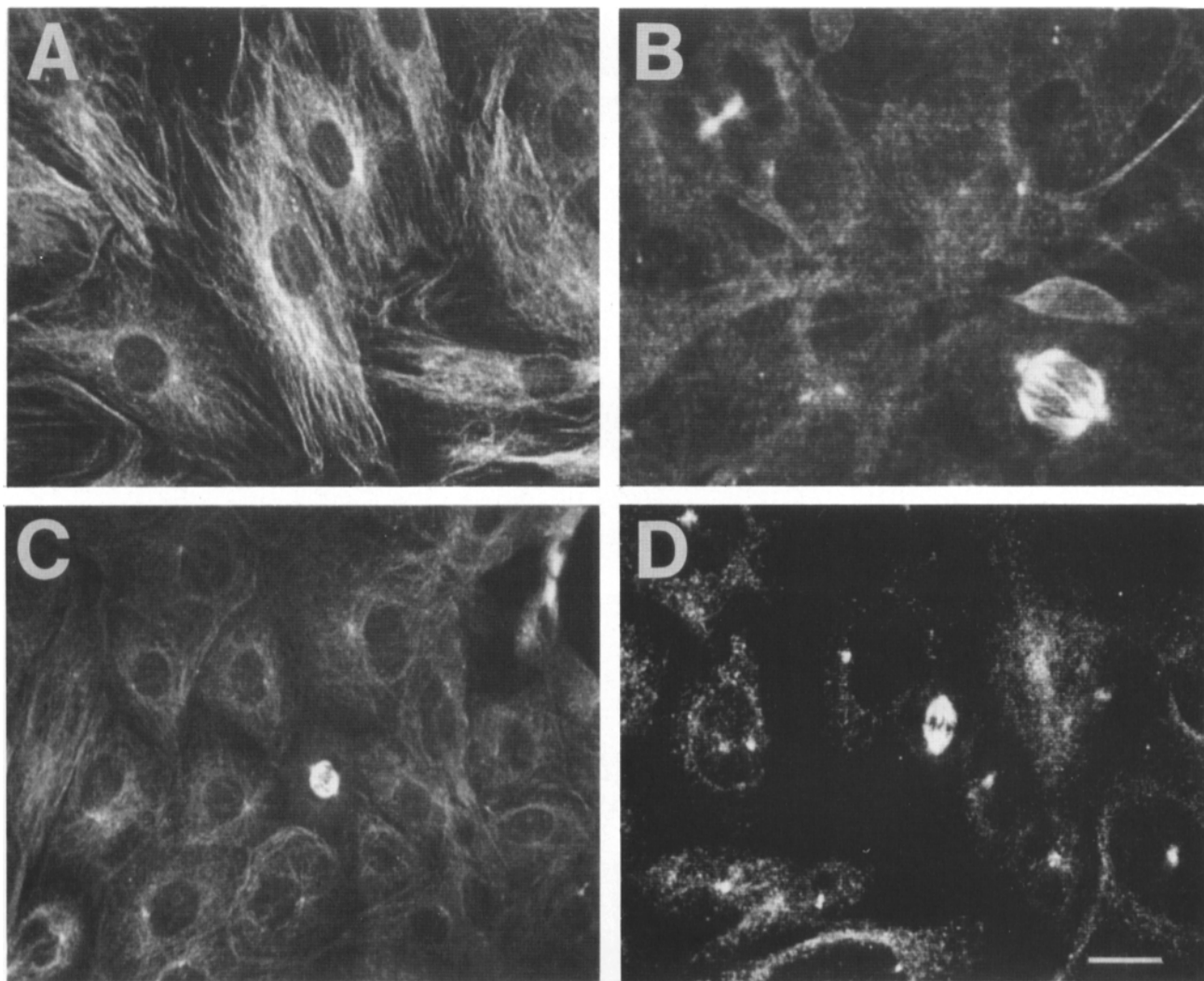


*Figure 5.* PP2A is localized with microtubules in several cell lines. Cells were grown on coverslips, fixed with methanol, and stained with anti-Bα antiserum, as described in Materials and Methods. (*A*) Rat embryo fibroblasts, (*B*) mouse 3T3 cells, (*C*) bovine MDBK cells, and (*D*) Chinese hamster ovary cells. Bar, 25 μm in panels *A* and *C*, and 12 μm for *B* and *D*.
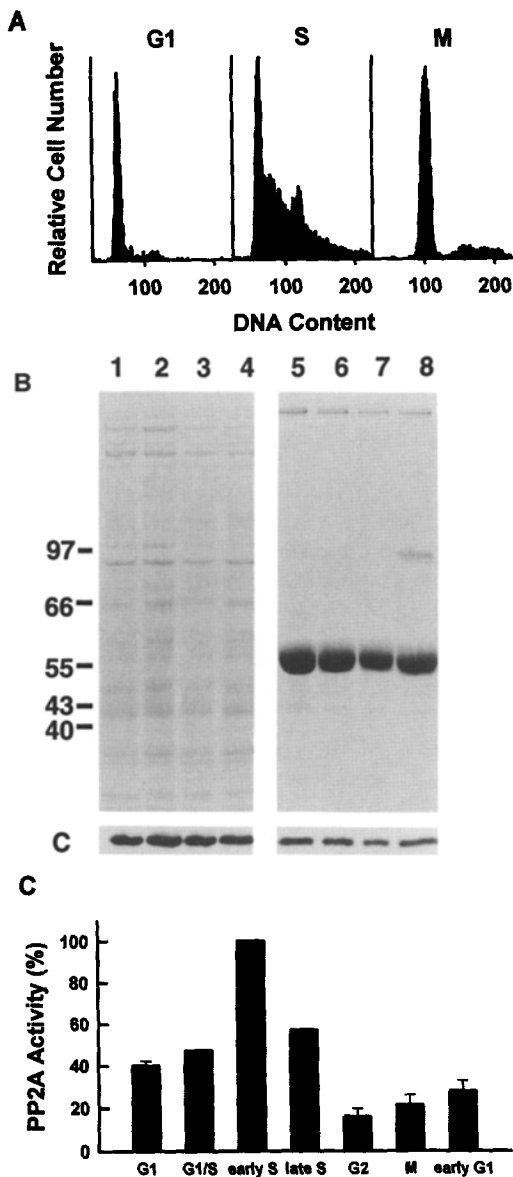
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## The Activity of Microtubule-associated PP2A Is Regulated during the Cell Cycle

To define the role of microtubule-associated PP2A in the regulation of microtubule function, we investigated the possibilities that its microtubule-binding and enzymatic activity are altered during the cell cycle. CV-1 cells were synchronized to various stages of the cell cycle and analyzed for total and microtubule-bound PP2A. The efficiency of the cell synchrony was determined by flow cytometry (Fig. 6 *A*). Microtubule-binding proteins were isolated from the synchronized cells using an excess of exogenous brain microtubules (Fig. 6 *B*, *upper portion*, lanes *5–8*), since endogenous microtubules could not be polymerized efficiently from the small number of cells available. The amount of PP2A in microtubule pellets was determined by immunoblot analysis, and the activity of the bound PP2A was determined by phosphatase assay.

As reported previously (Virshup et al., 1989; Kinoshita et al., 1990; Ruediger et al., 1991), the level of PP2A in the CV-1 extracts was constant during the cell cycle (Fig. 6 *B*, *Western blot*, lanes *1–4*). The amount of PP2A that associated with exogenous microtubules were also constant throughout the cell cycle (Fig. 6 *B*, *Western blot*, lanes *5–8*). Although only data for the C subunit are shown, immunoblotting with anti-A and anti-Bα antibodies showed that the levels of these subunits associated with microtubules were also constant during the cell cycle. In contrast, the phosphatase activity associated with the microtubule pellets fluctuated significantly during the cell cycle. A peak of activity was observed in early S phase, and it was followed by a progressive decline in late S, G2, and M (Fig. 6 *C*). PP2A activity was significantly lower in G2 and M than in interphase cells. When cells were released from M phase, phosphatase activity gradually increased (early G1) until it reached the level present in resting cells. In all cases, the microtubule-associated phosphatase activity was completely inhibited by 1 nM okadaic acid, showing that dephosphorylation of the substrate was caused by PP2A (results not shown). The changes in PP2A activity in the microtubule pellets were not the result of a change in PP2A binding since the amount of PP2A catalytic subunit in the microtubule pellets was constant.

To provide further evidence for cell cycle–dependent changes in PP2A activity, the levels and activity of microtubule-associated PP2A were determined in detergent-resistant cytoskeletons prepared from synchronized CV-1 cells using the same method of detergent extraction that was used for some of the immunofluorescence experiments. As shown by immunofluorescence in Figs. 2 and 4, microtubules and microtubule-bound PP2A remain associated with cytoskeletons prepared in this manner. The amount of the PP2A catalytic subunit in the cytoskeletal preparations was invariant throughout the cell cycle, as detected by immunoblotting (Fig. 7, *top right-hand portion*). As controls for these experi-

*Figure 6.* Binding of PP2A to microtubules at different stages of the cell cycle. CV-1 cells were synchronized to G1, S, G2, M, and in some cases, to intermediate stages of the cell cycle. For each cell population, a soluble extract was prepared and incubated at 0–4°C with exogenous bovine brain tubulin that had been preassembled with taxol. Microtubules and microtubule-binding proteins (microtubule pellet) were isolated as described in Materials and Methods. (*A*) DNA profiles obtained after FACS® analysis of cells synchronized to G1 (85%), S (75%), and M (93%). (*B*), upper portion: Coomassie blue–stained SDS gel of total soluble extracts (lanes *1–4*) or microtubule pellets (lanes *5–8*), which were resuspended to the same volumes as the extracts from which they were derived. All lanes contained 5 μl of each fraction. The mobility of molecular mass marker proteins, expressed in kilodaltons, is indicated on the left. Cells were synchronized to G1 (lanes *1* and *5*), S (lanes *2* and *6*), G2 (lanes *3* and *7*), and M (lanes *4* and *8*). (*B*, *lower portion*) The same samples shown in the upper portion were analyzed by chemiluminescent immunodetection with a monoclonal anti-C subunit antibody. The portion of the immunoblot corresponding to the migration of the C subunit (*C*) is shown. Note that approximately half of the immunoreactive protein in each extract was recovered in the corresponding microtubule pellets. (*C*) Microtubule-associated PP2A activity was assayed in the microtubule pellets

obtained from cells synchronized to different stages of the cell cycle. Values shown are expressed as the percent of maximal PP2A activity (early S phase), and they represent the mean ± SD of duplicate assays performed on samples from four separate experiments. Standard deviations for G1/S, early S, and late S were <1%.
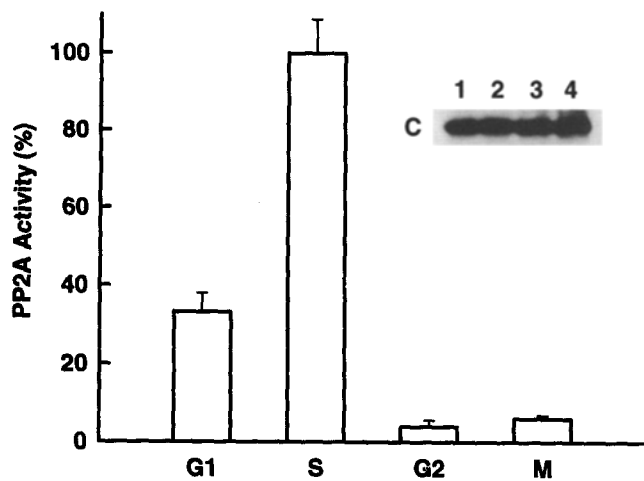
Figure 7. Analysis of microtubule-associated PP2A activity in synchronized CV-1 cells. CV-1 cells were synchronized to G1, S, G2, and M, and then extracted in a microtubule-stabilizing buffer containing 0.1% NP-40, as described in Materials and Methods. In parallel, duplicate dishes of synchronized cells were incubated on ice with 4 μg/ml nocodazole to depolymerize microtubules before extraction. Phosphatase activity was measured in the total cytoskeleton prepared from each set of cells. Phosphatase activity resulting from PP2A was defined as the activity inhibited by 1 nM okadaic acid. The microtubule-associated PP2A was calculated by subtracting the activity in nocodazole pretreated cells from the PP2A activity in untreated cells synchronized to the same stage. Values are expressed as the percent of maximal PP2A activity (obtained in S phase cells), and they represent the mean ± SD of duplicate assays performed on samples from three separate experiments. The inset at the top right-hand portion of the figure shows the amount of PP2A catalytic subunit (C) detected by immunoblotting in the cytoskeletal preparations from extracted cells synchronized to G1, S, G2, and M (lanes 1-4, respectively).

ments, we assayed cytoskeletons that were isolated from cells which, before detergent extraction, had been incubated with nocodazole to depolymerize their microtubules. The amount of PP2A activity remaining in the nocodazole-treated cytoskeletal preparations was only 2–4% of that found in preparations isolated from untreated cells. These data show that microtubule-associated PP2A accounted for nearly all of the okadaic acid–sensitive phosphatase activity in the cytoskeletal preparations that contained microtubules.

Further use of this method allowed determination of the level of microtubule-associated PP2A activity in synchronized cells (Fig. 7). The cell cycle profile of microtubule-associated PP2A activity in cytoskeletal preparations was qualitatively similar to those determined by sedimentation of microtubule-associated proteins with exogenous microtubules (Fig. 6). There was a peak of phosphatase activity in S phase, followed by a pronounced decrease in activity during G2 and M. The microtubule-associated PP2A activity in G1 was 35% of the S phase activity, while the activity in G2 and M was only 5–7% of the S phase activity.

Cell cycle changes in microtubule-associated PP2A activity were also determined in synchronized neuroblastoma cells (Fig. 8). Neuronal cell lines are especially rich sources of microtubules, and isolation of microtubule-binding proteins was achieved by cosedimentation with endogenous microtubules polymerized with the aid of taxol. Yields of
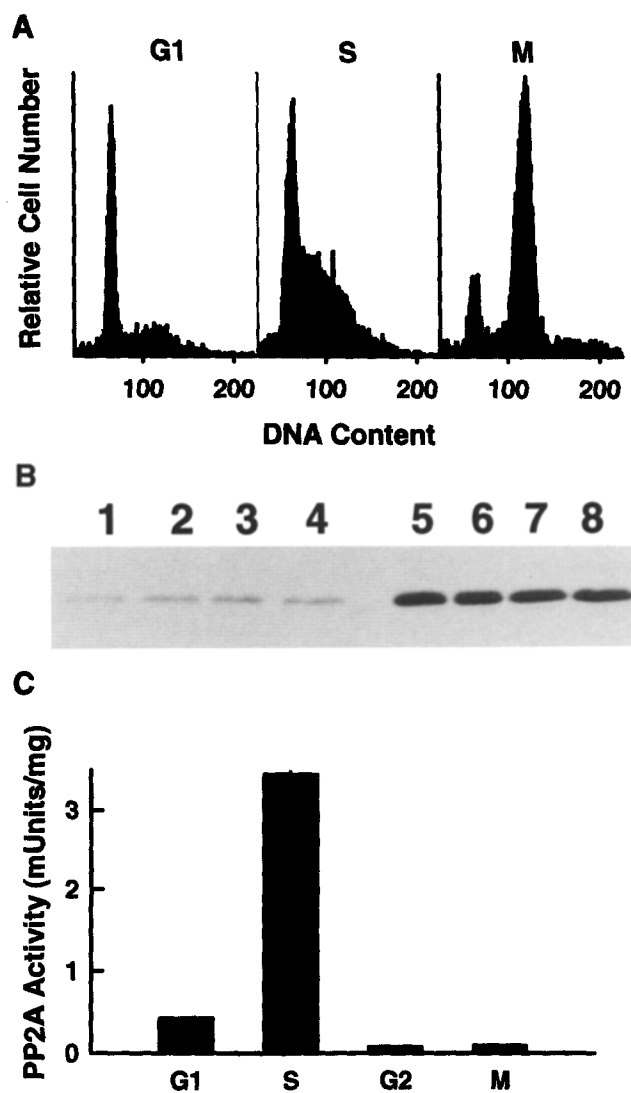


Figure 8. Microtubule-associated PP2A in synchronized mouse neuroblastoma cells. Mouse neuroblastoma cells were synchronized to G1, S, G2, and M, and endogenous taxol-stabilized microtubules were isolated as described in Materials and Methods. (A) DNA profiles of neuroblastoma cell populations synchronized to G1 (78%), S (65%), and M (75%). (B) Immunoblot showing the amount of PP2A catalytic subunit present in the total soluble extract (lanes 1-4) and in the microtubule pellets (lanes 5-8). The pellets were resuspended in one fifth of the original volume of the cell extract. Each lane contained 5 μg of protein from cells synchronized to G1 (lanes 1 and 5), S (lanes 2 and 6), G2 (lanes 3 and 7), and M (lanes 4 and 8). (C) Microtubule-associated PP2A activity during the cell cycle. PP2A was defined as the activity sensitive to 1 nM okadaic acid. Results are expressed as the mean ± SD of duplicate assays. Microtubule-associated PP2A activity represented 56% of total activity present in S-phase cell extracts. Similar results were obtained in a separate experiment.

microtubules isolated in this manner from synchronized neuroblastoma cells remained constant throughout the cell cycle (results not shown). PP2A was enriched in the microtubule pellet relative to the cytosol (Fig. 8 B, lanes 1-4 vs lanes 5-8). As observed in CV-1 cells, the levels of total PP2A catalytic subunit were constant during the cell cycle (Fig. 8 B, lanes 1-4). Moreover, the ability of PP2A to bind to en-

dogenous microtubules was not affected by cell division since the amount of microtubule-associated PP2A catalytic subunit was the same in G1, S, G2, and M (Fig. 8 B, lanes 5–8). In contrast, the activity of microtubule-associated PP2A varied significantly. PP2A activity was maximum in S phase, very low in G2 and M (<5% of S phase), and ~15% of the S phase activity in G1 (Fig. 8 C). The fluctuations in PP2A activity in synchronized neuroblastoma cells were consistent with the changes in microtubule-associated PP2A activity in CV-1 cells. These results indicate that microtubule-bound PP2A activity is regulated in both neuronal and nonneuronal cell lines in a cell cycle–dependent fashion. Furthermore, the cell cycle regulation of microtubule-associated PP2A does not involve a change in the amount of PP2A that is bound to microtubules.

## Discussion

PP2A is one of the predominant protein serine/threonine phosphatases in most cells and tissues, but relatively little is known about its subcellular distribution. Numerous biochemical studies have suggested that PP2A is largely a soluble cytosolic protein (Cohen, 1989; Shenolikar and Nairn, 1991; Mumby and Walter, 1993). Small but significant amounts of PP2A are also present in the nucleus (Jakes et al., 1986; Kuret et al., 1986). We provide evidence in this report that PP2A is also targeted to the cytoskeleton through association with microtubules. PP2A is associated with the interphase microtubule network, the centrosome, and the mitotic spindle in several cell types, including fibroblasts, epithelial cells, and neurons, as demonstrated by immunofluorescence analysis with antibodies against three different PP2A subunits. Cosedimentation studies showed that 50–75% of the PP2A in extracts of CV-1 cells, neuroblastoma cells, and brain tissue was capable of binding to microtubules. Immunoblotting of microtubule-associated PP2A indicated that all three subunits were associated with microtubules, in the same abundance relative to each other as in cytosol. These results imply that the heterotrimeric (AB$\alpha$C) species of PP2A, and not the catalytic subunit or AC complex, is the form bound to microtubules. The association may involve direct interaction of one or more subunits with polymerized tubulin, or it may be mediated by MAPs. Indirect immunofluorescence analysis showed that the A, B$\alpha$, and C subunits are also localized in the perinuclear region in addition to microtubules. The perinuclear fluorescence had a reticular pattern suggesting association with the endoplasmic reticulum. Preliminary studies using subcellular fractionation and immunofluorescence have shown that significant amounts of PP2A are localized in the endoplasmic reticulum, the Golgi, and in the nucleus (Sontag, E., unpublished results). We found little evidence for a diffuse pattern of immunostaining associated with a soluble cytoplasmic protein. Thus, in contrast to the previous consensus that PP2A is a cytoplasmic protein, our data indicate that this protein serine/threonine phosphatase is highly localized in cells. The microtubule localization of PP2A may have been overlooked in previous work because of the fact that microtubules depolymerize, and their constituent proteins are thereby solubilized, under conditions normally used for tissue homogenization.

Despite the prevalence of PP2A, its intracellular sub-strates are largely unknown. The localization of PP2A described in this report indicates that phosphoproteins associated with microtubules, microtubule organizing centers, and the mitotic spindle are likely to be targets of PP2A. A dramatic reorganization of the microtubule cytoskeleton occurs during the transition from interphase to mitosis. Mitotic spindle microtubules, assembled after dissolution of the interphase network, are highly dynamic (Wynford-Thomas et al., 1989). Current evidence suggests that microtubules are destabilized during mitosis as a result of increased phosphorylation of microtubule-associated proteins. Phosphoproteins are also components of mitotic microtubule organizing centers (Vandré et al., 1984). The increased phosphorylation of proteins associated with microtubules and microtubule organizing centers is likely to be at least partly due to the activation of the cyclin B/p34[cdc2] protein kinase. This kinase is associated with microtubules (Hill et al., 1989; O'Callahan et al., 1988), as well as with centrosomes during G2 and M (Alfa et al., 1989; Bailly et al., 1989; Riabowol et al., 1989; Maldonado-Codina and Glover, 1992; Gallant and Nigg, 1992). The data presented in this report indicate that the M phase increase in protein phosphorylation may also be caused in part by the decrease in microtubule-associated PP2A activity during G2 and M. Okadaic acid activates the cyclin B/p34[cdc2] protein kinase activity and induces a premature mitosis-like state (Yamashita et al., 1990). Studies in *Xenopus* oocytes (Fèlix et al., 1990; Lee et al., 1991) and yeast (Kinoshita et al., 1993) have suggested that PP2A can act as a negative regulator of cyclin B/p34[cdc2] kinase activation. Localization of PP2A on microtubules and centrosomes, as well as the decrease in PP2A activity in G2 and M, are consistent with a role of PP2A in regulation of microtubule-associated cyclin B/p34[cdc2] activity and/or dephosphorylation of cyclin B/p34[cdc2] substrates. A role of microtubule-associated PP2A in dephosphorylation of cyclin B/p34[cdc2] substrates is supported by studies showing that only the AB$\alpha$C form of PP2A, and not the free catalytic subunit, the AC heterodimer, or other heterotrimeric forms of PP2A, has high activity toward proteins phosphorylated by cyclin B/p34[cdc2] (Ferrigno et al., 1993; Kamibayashi et al., 1994). The B$\alpha$ subunit may play a role in targeting PP2A to microtubules and stimulating dephosphorylation of substrates of cyclin-dependent protein kinase.

The MAP kinase ERK2 (p42[mapk]) is another enzyme that is activated at metaphase and is associated with microtubule-organizing centers in oocytes (Verlhac et al., 1993) and in CV-1 cells (Sontag, E., unpublished observation). In a previous report, we showed that PP2A is involved in the normal dephosphorylation and inactivation of MAP kinase and MAP kinase kinase (Sontag et al., 1993). The distribution of PP2A on centrosomes and its low activity in M phase, when MAP kinase is activated, indicate that PP2A could also regulate microtubule-associated MAP kinase by preventing inactivation during G2 and M.

Although it is clear that PP2A plays an important role in the cell cycle, there has been little evidence until now that its activity is regulated during the cell cycle. Several studies have shown that both the expression and activity of PP2A are constant throughout the cell cycle in mammalian cells and in yeast (Virshup et al., 1989; Kinoshita et al., 1990; Ruediger et al., 1991). However, a study using SV-40 large T antigen as substrate suggested that a PP2A-like activity is

elevated during S phase in CV-1 cells (Ludlow, 1992). The changes in microtubule-associated PP2A activity also suggest that PP2A activity is elevated during S phase. Changes in the activity of microtubule-associated PP2A were not caused by changes in binding of the enzyme to microtubules, since equivalent amounts of PP2A could bind to microtubules in each phase of the cell cycle. This indicates that the activity of microtubule-associated PP2A is controlled by factors other than differential binding of the catalytic subunit or loss of the B$\alpha$ regulatory subunit. It is possible that the change in the activity of microtubule-associated PP2A is caused by covalent modifications, such as phosphorylation or methylation, which are known to occur on PP2A (Chen et al., 1992; Floer and Stock, 1994; Xie and Clarke, 1994).

The association of PP2A with the centrosome suggests that protein dephosphorylation plays an important role in microtubule assembly/disassembly during the cell cycle. Centrosomes are the preferred site of microtubule nucleation during interphase (Kellog et al., 1994). Cytoskeletal integrity during interphase requires protein phosphatase activity (Eriksson et al., 1992), and PP2A has been implicated in the regulation of microtubule stability in fibroblasts (Gurland and Gundersen, 1993). Microtubule organizing centers also serve as a focal point for microtubule nucleation at the onset of mitosis (Karsenti, 1991), when phosphoproteins are major components of these structures (Vandre et al., 1984). The phosphatase inhibitor okadaic acid suppresses spindle formation without interfering with the ability of tubulin to polymerize (Alexandre et al., 1991), implicating a phosphatase in microtubule nucleation. Okadaic acid also induces dynamic instability of microtubules similar to that observed during the transition from interphase to mitosis (Gliksman et al., 1992). These previous findings, along with the association of PP2A with microtubules reported here, point to an important role of this phosphatase in regulation of the microtubule network. PPX, another protein serine/threonine protein phosphatase whose distribution is more restricted than PP2A, has also been localized on centrosomes (Brewis et al., 1993). It will be interesting to determine the substrate specificities of these related protein phosphatases.

The localization of PP2A reported here is different from that described in a previous report, which claimed that PP2A is localized in the cytoplasm, nucleus, and the nucleoli, but not on centrosomes or microtubules (Brewis et al., 1993). The previous study used an antiserum raised against bacterially expressed PP2A catalytic subunit to examine the distribution of PP2A in human MRC-5 cells. In our study, staining of PP2A on microtubules was only readily observed in intact cells using anti-B$\alpha$ antibody. The indirect immunofluorescent staining of the interphase microtubule network or the mitotic spindle with anti-A or anti-C subunit antibodies was not obvious until the cells were selectively extracted with the nonionic detergent NP-40. Detergent-based cell permeabilization has also been used to show the association of the cyclin B/p34$^{cdc2}$ complex with the centrosome and mitotic spindles (Ookata et al., 1993). Visualization of microtubule-associated A and C subunits in detergent-extracted cells appeared to result from the removal of the relatively intense perinuclear staining. Detergent extraction may also remove proteins that prevent or reduce recognition by the anti-A and anti-C subunit antibodies.

The presence of microtubule-associated PP2A in a non-

dividing tissue, such as the brain, suggests that microtubule-associated PP2A is likely to have a role in addition to cell cycle regulation. Recent findings indicate that PP2A is able to dephosphorylate specific phosphorylation sites in tau, a major brain-specific MAP (Drewes et al., 1993), which assembles into the paired helical filaments of Alzheimer's neurofibrillary tangles (Goedert, 1993). One of the sites dephosphorylated by PP2A in vitro has been implicated in regulation of tau binding to microtubules.

Another potential role for microtubule-associated PP2A is in regulation of membrane-bounded organelle transport along microtubules. For example, the elaboration of Golgi-derived membrane tubule networks along microtubules in vitro occurs readily in the presence of interphase, but not mitotic cytosol (Allan and Vale, 1991). Perhaps this difference reflects the cell cycle–dependent fluctuations reported here for the enzymatic activity of microtubule-bound PP2A. In addition, okadaic acid has been reported to stimulate microtubule-based membrane transport in live cells and cell extracts (Hamm-Alvarez et al., 1993; McIlvain et al., 1994), presumably as a result of inhibiting PP2A.

In conclusion, data obtained by immunocytochemistry and biochemical analyses strongly support the idea that PP2A is associated with microtubules in neuronal tissue and a variety of nonneuronal cells. The microtubule localization of PP2A in both dividing and nondividing cells suggests potential roles for PP2A in regulation of microtubule dynamics and microtubule-based motility. The relative abundance of PP2A on microtubules indicate that it is likely to play a major role in controlling the phosphorylation state of structural MAPs, such as tau, and of other microtubule-binding proteins.

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