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Microtubules Are Critical for Radial Glial Morphology: Possible Regulation by MAPs and MARKs

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ABSTRACT   Radial glia are a polarized cell type that in most neural regions appear only transiently during development. They have long been recognized as glia or glial progenitors that support neuronal migration. Recent evidence indicates that radial glia also give rise to neurons and appear to be a major population of dividing precursor cells in the embryonic cortical ventricular zone. Radial glia extend long processes from the ventricular zone to the pial surface that provide guides for neuronal migration. We reasoned that the unique morphology of radial glia is due to the composition and organization of their cytoskeleton. In this present study, we have used C6-R, a radial glial-like cell line and isolated perinatal cerebellar radial glia to ask what are the critical cytoskeletal elements in radial glial cells and how they are regulated. Treatments with nocodazole and cytochalasin D showed that microtubules, but not microfilaments, are critical for the polarized morphology of radial glia. In addition, quantitative real-time PCR indicated that certain mRNAs specific for microtubule-associated proteins (MAPs) are selectively expressed in radial glia. These results together with the known ability of microtubule affinity-regulating kinases to regulate microtubule organization suggest that microtubules and MAPs are critical for the morphology of radial glia. © 2003 Wiley-Liss, Inc.

INTRODUCTION

Radial glia appear during the earliest stages of neurogenesis spanning developing neuroepthelia, including the cortex (Rakic, 1990). As cortical development proceeds, radial glia become very long, extending from the ventricles to the pial surface, and many persist for a while after birth in mammals. These radial processes provide guides for radial migration of neurons that are generated near the ventricular region and migrate to more superficial locations in the cortex. Recent evidence indicates that radial glia are neural stem cells that initially generate neurons and later develop into astroglia (Noctor et al., 2001; Gotz et al., 2002; Gregg et al., 2002). Most radial glia do not persist into the adult nervous system except in the cerebellum as Bergmann glia and in the retina as Müller cells (Gotz et al., 2002).

The intermediate filament protein nestin (Hockfield and McKay, 1985) is expressed by neural stem cells, including radial glia, and is lost as cells differentiate into neurons that express neurofilaments or into glia that express glial fibrillary acidic protein (GFAP) (Gotz et al., 2002; Gregg et al., 2002). Nestin does not appear to polymerize by itself; rather, it copolymerizes with other intermediate filament proteins such as vimentin (Marvin et al., 1998). At later stages of development, radial glia can coexpress nestin and GFAP, and these

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intermediate filament proteins may form copolymers. While expression of intermediate filament proteins is useful for distinguishing different types of cells during neural development, the functions of nestin are not well understood and it is unlikely that nestin is critical for determining the shapes of radial glia (Marvin et al., 1998). Rather, previous studies of polarized cells indicate critical roles for microfilaments or microtubules (Small et al., 2002), both of which are expressed in radial glia.

Studies of radial glia in vitro as well as in vivo have been difficult because of the transient nature of these cells. Hatten (1999) has pioneered isolation of radial glia from cerebella in vitro and found that their rapid differentiation into astrocytes can be slowed by interaction with granule neurons. Given the transient nature of radial glia, it has been very difficult to isolate cell lines as models of radial glia. However, we have isolated a cell line called C6-R that resembles radial glia in that it has a long asymmetric morphology, can support migration of granule neurons, and aligns with native radial glia in the developing brain (Friedlander et al., 1998). In the present study, we used C6-R and radial glia isolated from perinatal cerebella to analyze what filament systems are responsible for radial morphology. The results clearly show a critical role for microtubules and indicate selective expression of certain microtubule regulatory proteins, including specific microtubule-associated proteins (MAPs) and microtubule affinity-regulating kinases (MARKs) in radial glia.

MATERIALS AND METHODS
Drug Treatment on Cultured Cells

C6-R cells were cultured at 37°C in DMEM medium containing 10% fetal bovine serum, glutamine, and gentamycin. Radial glia were maintained in serum-free DMEM/F12, including basic fibroblast growth factor (bFGF; 10 ng/ml; BD Biosciences) and $1 \times$ B27 (Invitrogen, Carlsbad, CA) as a supplement containing many critical factors. For cytoskeletal studies, cells were plated on glass coverslips (laminin-coated for radial glia) in 24-well plates at 4×10^4 cells per well and cultured overnight before drug treatment. For most drug treatment experiments, C6-R and radial glia were treated with one or more of the following: 5 μg/ml nocodazole (NCD), 10 μM cytochalasin D (Cyto D), 10 μM taxol, 5 μM phenylarsine oxide, and 10 μM Staurosporine, although a range of different concentrations was used for dose-response studies (all drugs were purchased from Sigma). Dimethylsulfoxide (DMSO) was used as a solvent control for all drug experiments. Cells were treated for 2 h (overnight in the case of taxol) and then fixed in 4% paraformaldehyde. For reversibility studies, drug-containing medium was removed and the cells were washed and maintained in fresh culture medium for 2 days.

Isolation of Radial Glial Cells and Granule Neurons

Radial glia from cerebella were isolated according to a method modified from the protocol of Hatten (1985). Briefly, cerebella were dissected out from postnatal day 2–4 Sprague-Dawley rats, and meninges were removed. Cells were dissociated following treatment with trypsin/EDTA, and a single cell suspension was applied onto a one-step percoll gradient (35%). After centrifugation, two separate populations of cells were collected: one containing mainly radial glia from the interface layer and the pellet containing mainly granule neurons. Both radial glial and granule neuron populations were plated on separate Petri dishes for 30 min to remove adhesive cell types (e.g., macrophages), and the nonadherent cells were then plated individually on tissue culture dishes coated with laminin (20 μg/ml, overnight at 4°C) and incubated at 37°C in culture medium (DMEM/F12, $1 \times$ B27, and 10 ng/ml of bFGF) in a CO_2 incubator. After incubation for 2 h, cultures were treated as follows. For radial glial cultures, the medium was removed, and attached cells were washed three times with cold PBS and supplied with fresh culture medium. For neuronal cultures, adhesive nonneuronal cells were adsorbed during a 2-h incubation on tissue culture dishes and the medium enriched in granule neurons was then transferred onto new laminin-coated dishes. Both radial glial and neuronal-enriched cultures were maintained overnight and then they were either harvested for total RNA purification or passed onto laminin-coated coverslips for drug treatments. Radial glia were identified by staining with antinestin and anti-GFAP, and neurons were identified by staining with anti-β-III tubulin (Tu-20; Chemicon). Cerebellar astrocytes were obtained by incubating cerebellar radial glial cells in DMEM/10% fetal bovine serum for more than 3 weeks; these cells had a flat morphology typical for astrocytes.

Immunocytochemistry

Cultured cells were fixed with 4% paraformaldehyde in PBS for 20 min, washed with PBS, and then incubated with primary antibodies in PBS, 10% goat serum, and 0.3% Triton X-100 for 1 h. After washing with PBS, secondary antibodies were added and incubated for 1 h, and the cells were washed, mounted with Gel/mount (Biomeda, Foster City, CA), and examined under Zeiss Axiophot microscope (Thornwood, NY) with appropriate filters. Primary antibodies include rat monoclonal anti-tyrosinated tubulin (1:200; Chemicon, Temecula, CA), mouse anti-nestin (Developmental Studies Hybridoma Bank), rabbit anti-GFAP (Dako), rabbit anti-brain lipid-binding protein (BLBP) (Feng and Heintz, 1995), mouse anti-NeuN (Chemicon), and mouse anti-β-III tubulin (Tu-20). Secondary antibodies include Oregon-Green goat anti-rabbit IgG and Rho-

TABLE 1. *Oligonucleotide Primers Used for Q-RT-PCR*

Gene name	Forward	Reverse
β-actin	CGTAGCCATCCAGGCTGTGT	CCAGTGGTACGACCAGAGGC
MAP1a	CTCTTCAGCACTTAAACCGCCTA	CACGATACAGAGGCTCGGCT
MAP1b	CGAGCTCTCACCTTCCTTCATTA	CCAGCAAACCACTCGAGAGG
MAP2	GTGGTGTGGAAGTGCGTCTCT	GCCCATCCTAACGAAAGTGC
MAP2c	TCCATCTCTTCAGCACGACG	TGCTCTGCGAATTGGTTCTG
MAP4	GGTTCTCTGGCTTGTGGCAT	GCCCAAGCTCTCCAGGTAGC
MAP7	GAGGACACAGGAAAAGCGGA	CGTCGCAAATGTCCTCAGTG
MARK1	GTGGGAGATGGAGGTCTGCA	CGGACCCCATTGAGTGAGAG
MARK2	CCCCAGACCGAACTAATTTCC	GGAAGGTGCTTCGACTGGAC
MARK3	AGATGTGCAGCGGACAGACAT	GCTGCTTTCTTTGCCGTTCT
MARKL1	CTCAGGAGGTCGAGAGCTGG	CTGCTCAGATTCCCCTGGAG
OP18	AACAACTTCAGCAAAATGGCAG	AGCCTCCATTTTGTGGGTCA
Tau	GCCAGACCTAAAGAACGTCAGG	TTCAGGTTCTCAGTGGAGCCA
Tektin-1	GCCAAGCTACTGCAATCTCCA	AATATACCACTCGGCGGGAAG
α-tubulin	TATCCTCTCGAGCCTCGGAG	ATGTGGGTGAGGGAATGGAG

damine-Red goat antimouse IgG (1:200; Molecular Probes, Eugene, OR). Alexa Fluor 568 phalloidin (Molecular Probes) was used to identify actin fibers.

Quantitative RT-PCR

We analyzed gene expression of microtubule-associated proteins by quantitative (real-time) reverse transcription-PCR (Q-RT-PCR) using selected gene-specific primer pairs (Table 1). All primers used were designed to meet the standard Q-RT-PCR parameters by using PrimerExpress software. Beta-actin was used to normalize the expression levels of each sample. Total RNA was purified from cultured cells using RNeasy kit (Qiagen, Valencia, CA) and quantified by spectrophotometry. Template cDNAs were reverse-transcribed from 1 μg of total RNA using oligo-dT primer and SuperScript II reverse transcriptase (Invitrogen). The PCR reactions were performed in 10 μl using 1% of the reverse transcription (RT) reaction, 50 nM of each primer, and SYBR Green master mix (Applied Biosystems). The PCR reactions were carried out on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Standard curves were generated for each gene using serial dilution of a control cDNA (the pool of all the sample cDNAs). A final dissociation step was performed for each reaction to confirm the purity of amplified products. Expression levels of β-actin were used to normalize differences among individual cDNA templates.

RESULTS
Microtubules Are Critical to Polarized Morphology of Radial Glial Cells

Gene expression comparison using Affymetrix U34 microarrays between the closely related cell lines C6 and radial-like C6-R revealed regulation of several MAPs in C6-R cells, suggesting that microtubules might be important for C6-R radial morphology. For example, tau and MAP2 are higher in C6, and light

chains for MAP1 were higher in C6-R (data not shown). To test the potential role of microtubules in radial glia, we treated C6-R cells with drugs that specifically disrupt either microtubules or actin microfilaments. The microtubule-disrupting drug, nocodazole (NCD; 5 μg/ml), had a dramatic effect on C6-R cells, altering their bipolar morphology into a flat, polygonal shape within 2 h, in contrast to the control DMSO treatment (Fig. 1). However, the actin-disrupting drug, cytochalasin D (CytoD, 10 μM), did not alter the radial extent of the processes of C6-R cells; rather, it tended to collapse the processes around the fibers and nuclei. This result indicates that, indeed, microtubules are very important for C6-R radial morphology. These effects on C6-R were also observed when cells were treated overnight with concentrations of NCD as low as 0.05 μg/ml (data not shown). We also tested another microtubule-disrupting drug, taxol, which binds to and stabilizes microtubules. Our results show that taxol also converts C6-R cells into a flat polygonal morphology and is effective at concentrations as low as 0.01 μM in overnight treatment (data not shown). The effects of taxol are slower, consistent with its ability to bind to polymerized tubulin. However, only short microtubules were detected in a disorganized manner, suggesting that taxol may affect the binding affinity of MAPs to microtubules and disrupt their organization (Gruber et al., 2001), leading to loss of radial morphology for radial glia.

To verify the specificity of the drug treatments, we performed staining to visualize actin and tubulin. The results confirmed that microtubules and actin microfilaments are disrupted by treatment with NCD and CytoD, respectively (Fig. 2). In both control and CytoD-treated cells, we observed filamentous patterns of microtubules in the C6-R processes (Fig. 2). In NCD-treated cells, no microtubule filaments were seen; instead, the tubulin staining was more diffuse in the cytoplasm (Fig. 2). Actin often forms stress fibers where the cell surface meets the substratum (Small et al., 2002) that one can see in the NCD-treated C6-R cells, but the microfilaments were disrupted upon
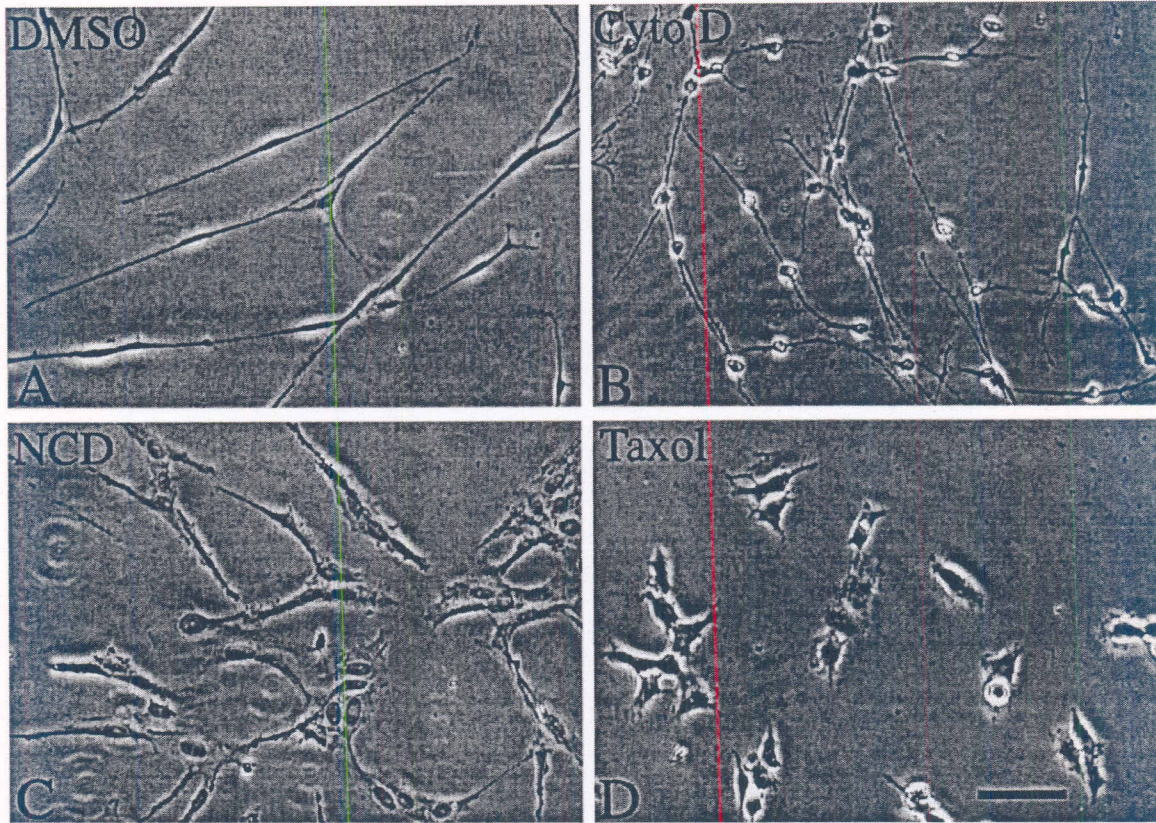
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Fig. 1. Microtubules are important for radial morphology of C6-R cells. Cells were treated for 2 h with DMSO alone or in the presence of cytochalasin D (CytoD; 10 μM), nocodazole (NCD; 5 μg/ml), or taxol (10 μM). The microtubule-disrupting drugs NCD (**C**) and taxol (**D**) converted the C6-R cells into a flat, polygonal shape, which dramati-cally contrasts to the control treatment (DMSO; **A**). In contrast, CytoD (**B**) only induced the collapse of the cytoskeletal fibers around the nucleus, leaving the radial processes of C6-R cells intact. Bar, 20 μm.

CytoD treatment, showing a punctate and discontinuous staining pattern (Fig. 2).

We then tested whether microtubules are also critical for the morphology of authentic radial glia. Neonatal cerebellar radial glia have been shown in culture to exhibit a bipolar morphology and to support neuronal migration (Hatten, 1985). Postnatal day 2–4 rat cerebellar radial glia were isolated according to Hatten's protocol with modifications. Treatment of these cells with NCD (Fig. 3) and taxol (data not shown) disrupted the radial morphology similar to treatment of C6-R cells. Interestingly, the cerebellar radial glia in culture were more sensitive to NCD treatment than C6-R cells, insofar as they retracted their processes much more rapidly, i.e., within 30 min upon addition of 5 μg/ml NCD. The effects of NCD on the radial glia were reversible insofar as the cells regained their radial morphology 1 day following a 1-hr treatment with NCD (data not shown). These results confirm the importance of microtubules for radial morphology. In contrast, the actin microfilaments (Figs. 1–3) and intermediate fila-ments (GFAP and nestin) (Marvin et al., 1998) in these cells did not appear to be critical for the radial morphology.

MAPs and MARKs That Are Associated With Radial Glia

The pharmacological observations indicated that microtubules are critical for the cytoskeleton of radial glial cells. Microtubules are composed primarily of assemblies of α/β-tubulin dimers and analyses of α-tubulin subunits indicated only moderate elevation of tubulin in radial glia (Fig. 4). Thus, since we did not detect dramatic differences in tubulin, we decided to examine whether differences in MAPs among cells may be important for organizing microtubule filaments into long processes within radial glia. As there is little published information on the expression of MAPs in developing radial glia, we decided to screen quantitatively for expression of known rat MAPs by Q-RT-PCR. Radial glia
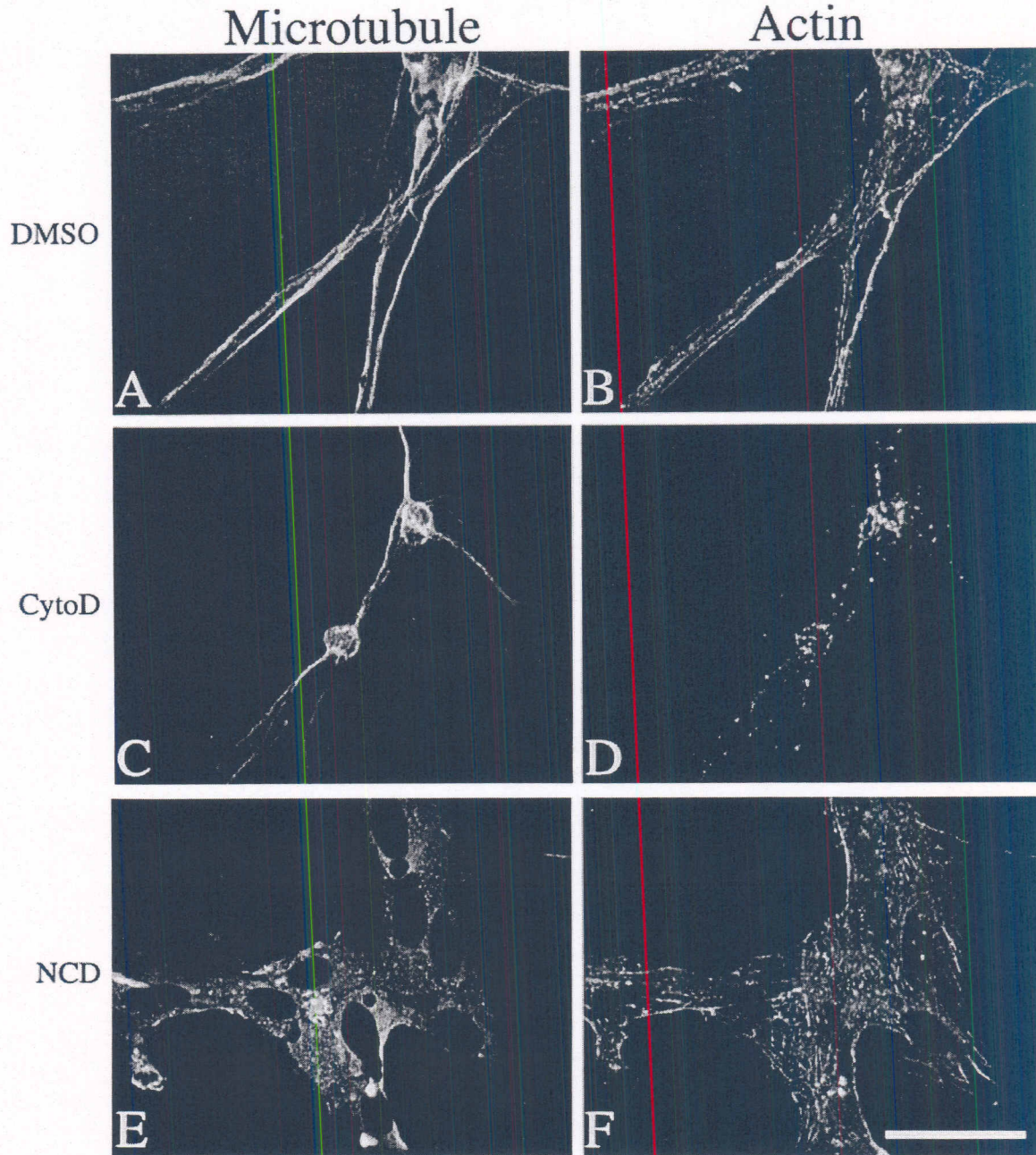
Microtubule   Actin

Fig. 2. Microtubules and microfilaments in drug-treated C6-R cells. C6-R cells were treated with DMSO alone (A and B), CytoD (10 μM; C and D), or NCD (5 μg/ml; E and F) for 2 h, and the cells were then fixed with 4% paraformaldehyde and stained with anti-tubulin (A, C, and E) or phalloidin (B, D, and F). In the DMSO control, both the microtubules and the microfilaments were polarized, reflecting the cell morphology. Following treatment with CytoD, the actin microfilaments were disrupted but the microtubules remained polarized in the thin radial processes of the cells. In contrast, following treatment with NCD, the microtubules were disorganized within the cells that exhibited dramatic flattening with microfilaments organized in bundles resembling stress fibers (F). Bar, 20 μm.

and granule neurons were isolated following percoll gradient centrifugation and differential adhesion on laminin, and the resulting cultures were characterized for purity by immunostaining with markers to identify radial glia (BLBP) and neurons (NeuN). The radial glial cultures were enriched in BLBP$^+$ cells (72.5% ±
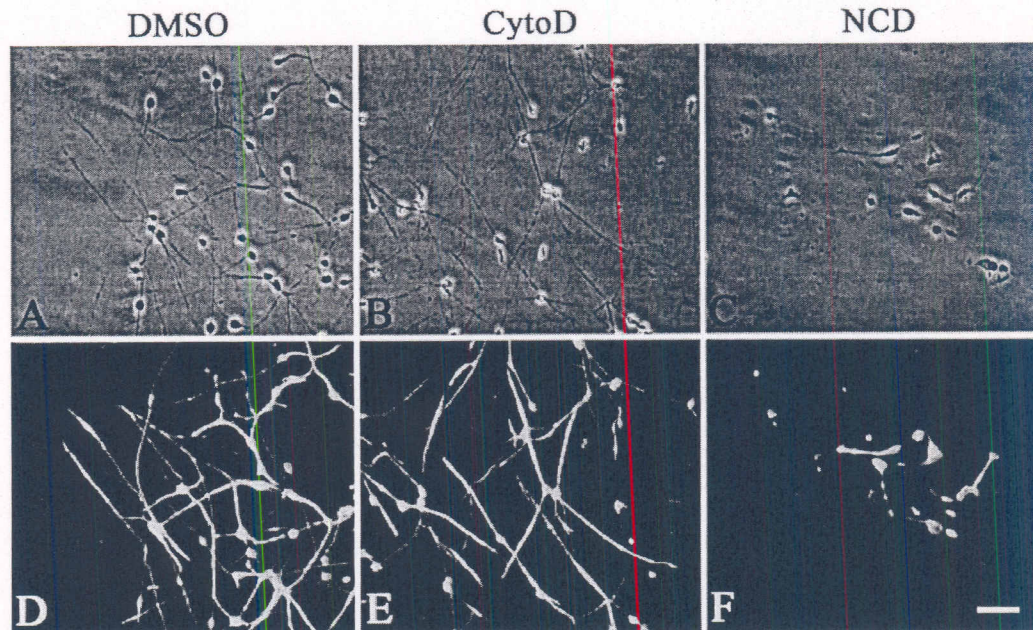
Fig. 3. Microtubules are important for radial morphology of cerebellar radial glial cells. Postnatal day 3 radial glial cells were isolated and plated on laminin-coated coverslips. Cells were treated with DMSO alone (**A** and **D**), CytoD (10 μM; **B** and **E**), or NCD (5 μg/ml; **C** and **F**) for 2 h, and the cells were then fixed with 4% paraformalde- hyde and stained with anti-GFAP. Cells and their processes were visualized by phase contrast microscopy (A–C) and by GFAP immu- nostaining (D–F). Radial glial cells retract their long bipolar processes upon NCD treatment, but CytoD had little effect on their morphology. Bar, 20 μm.

1.06%; n = 216) with few NeuN$^+$ cells (12.2% ± 0.49%; n = 166), while granule neuron cultures contained few BLBP$^+$ cells (11% ± 3.8%; n = 240) and mostly NeuN$^+$ cells (78.7% ± 4.6%; n = 387). Q-RT-PCR analyses indicated that MAP-1a, MAP-4, MAP-7, and tektin mRNAs are expressed at relatively higher levels in radial glia by comparison to granule neurons (Fig. 4). As expected, the expression levels of known neuronal MAPs including MAP2, Tau, and OP18/stathmin mRNAs (Kreis and Vale, 1999; Diaz et al., 2002) are higher in granule neurons (Fig. 4), consistent with the relative purity of these cultures. In contrast, much lower levels of MAPs were detected in astrocytes except for MAP4, which has been reported in astrocytes (Bern- hardt et al., 1985).

We also compared the expression patterns of MAPs between C6-R radial glia and the C6 cells from which they were derived. Q-RT-PCR results also showed rel- atively higher expression of MAP1a and MAP4 in C6-R by comparison to C6 cells (Fig. 4). Other MAPs are not expressed at relatively higher levels in C6-R cells than in C6 cells. However, Q-RT-PCR indicated that levels of α-tubulin mRNAs were not more than a third higher in C6-R than in C6 and in cerebellar radial glia than in granule neurons (Fig. 4). The combined results suggest that differences both in tubulin and MAPs may con- tribute to the increased organization of microtubules in radial glia. It has been recognized that the function of MAPs depends on their phosphorylation state (Drewes

et al., 1998). Several MAPs, including tau, MAP2, and MAP4, have been found to dissociate from microtu- bules when they are phosphorylated on specific serine residues in their microtubule-binding sites. This phos- phorylation can be promoted by several MARKs, two of which have been reported to be expressed in the brain (Drewes et al., 1998). Interestingly, RT-PCR analyses indicated that mRNAs specific for several MARKs are present in radial glial cells and C6-R cells, as well as in granule neurons (Fig. 4), consistent with the idea that phosphorylation of MAPs can regulate their function by altering their interactions with and organization of microtubules in glia and neurons.

The levels of MARK3 and MARKL1 were higher in radial glia than in neurons (Fig. 4). To analyze the potential role of MARKs in radial glia, we tested phe- nylarsine oxide, a drug that reacts with vicinal thiols and can activate MARKs, which can in turn phosphor- ylate MAPs (Jenkins and Johnson, 2000). When we treated either C6-R or primary radial glial cells in culture with phenylarsine oxide, the cells retracted their long radial processes and eventually rounded up (Fig. 5 and data not shown). However, pretreatment of radial glia for 30 min with staurosporin, which inhibits protein kinase activity, eliminated the effect of pheny- larsine oxide (Fig. 5). The results suggest that the effects of phenylarsine oxide on radial glia are specific and may involve disassembly of microtubules by acti- vation of MARKs and phosphorylation of MAPs.
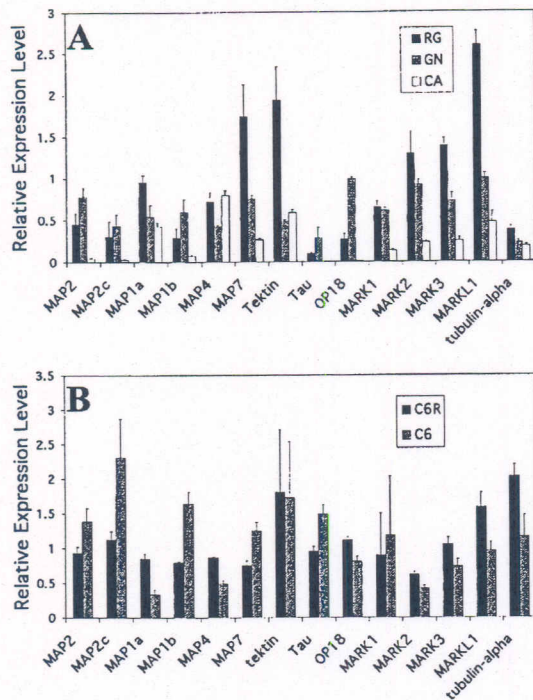
Fig. 4. Real-time RT-PCR analysis of MAPs and MARKs on total RNAs from cerebellar radial glia (**A**) and C6-R cells (**B**). Cerebellar radial glia and granule neurons were isolated following percoll gradient centrifugation (Hatten, 1985) and differential adhesion, and the resulting cultures were characterized for purity by immunostaining with markers to identify radial glia (BLBP) and neurons (NeuN; see text). RT-PCR analyses indicated that MAP-1a, MAP-4, MAP-7, and tektin are present and expressed at higher levels in cerebellar radial glia by comparison to granule neurons. RG, cerebellar radial glia; GN, granule cells neurons; CA, cerebellar astrocytes. Samples were analyzed in triplicate and the standard deviations are indicated.

DISCUSSION

This study provides the first evidence for a critical role of microtubules in determining the characteristic morphology of radial glia. Microfilaments are also found organized in these cells along their long processes but they do not appear to regulate cell shape, although they may be linked to microtubules by MAPs (Kreis and Vale, 1999). Intermediate (100 nm) filaments have not been found in general to be major modulators of cell shape, and nestin does not appear to regulate the morphology of radial glia (Marvin et al., 1998). Insofar as C6-R radial cells have thicker processes than primary radial glia in culture, they may utilize more microtubules as well as different combinations of MAPs to organize their microtubules. This may explain why they were somewhat less sensitive than the primary radial glia to treatment with NCD and taxol. Nevertheless, the use of these drugs indicates their radial shape was completely dependent on microtubules and not on actin microfilaments.

Previous studies have shown that microtubules play a critical role in the polarized structure of neurons as well as their directed migration along radial glia (Rivas and Hatten, 1995). While slightly elevated levels of α-tubulin may contribute to increased numbers of microtubules in radial glia, it is important to explore how these filaments are organized. Therefore, it was of interest to determine whether the MAPs as regulators of microtubules are similar in these cells and we have correlated these data with previous observations of localization of MAPs in radial glia and neurons.

Comparisons of expression levels confirmed that MAP1b, MAP2, and tau are selectively expressed in neurons as expected (Hyams and Lloyd, 1994; Kreis and Vale, 1999). In contrast, these MAPs are expressed at relatively lower levels in radial glia, and MAP1a, MAP4, and MAP7 are expressed at relatively higher levels in radial glia. Tau expression was relatively low in the neurons analyzed possibly because they are relatively young. Given the fact that tau is mainly associated with axons and that in our overnight culture conditions most granule neurons have not developed long neuronal processes, we were not surprised to observe a low level of tau expression. Indeed, we found higher levels when we cultured neurons for longer times in vitro (e.g., 3 days; data not shown). MAP1b expression early in neuronal development complements MAP1a expression later when neurons become more highly polarized, consistent with our observations that relatively lower levels of MAP1a was found in neurons than in radial glia (Mei et al., 2000). The ability of MAP1a to stimulate microtubule polymerization and stability (Pedrotti and Islam, 1994) suggests that it may contribute to generate stable microtubules in radial glial cells. Interestingly, astrocytes, which are less polarized than radial glia, had lower expression of all MAPs tested except MAP4, which has been reported to be expressed in various nonneuronal cells, including astrocytes (Parysek et al., 1984; Bernhardt et al., 1985).

MAP4 has been cloned and appears to be indistinguishable from MAP3 based on several criteria, including amino acid sequences of multiple peptides derived from each protein (Kobayashi et al., 2000). Interestingly, MAP3 was found to be expressed in early postnatal cerebellum in radial glia and persists in Bergmann glia and astrocytes (Parysek et al., 1984; Bernhardt et al., 1985). The carboxyterminus of MAP3/4 contains a conserved microtubule-binding region containing the KXGS consensus sequence with a serine that can be phosphorylated by several MARKs. This sequence was first found in tau and is shared by MAP2 and MAP4, and its phosphorylation by MARKs can be induced by treatment with phenylarsine oxide (Jenkins and Johnson, 2000). Of all the MARKs investigated, MARKL1 is expressed at relatively higher levels in cerebellar radial glia than neurons. It is interesting that an alternatively spliced form of MARKL1-S containing the highly conserved serine/threonine ki-
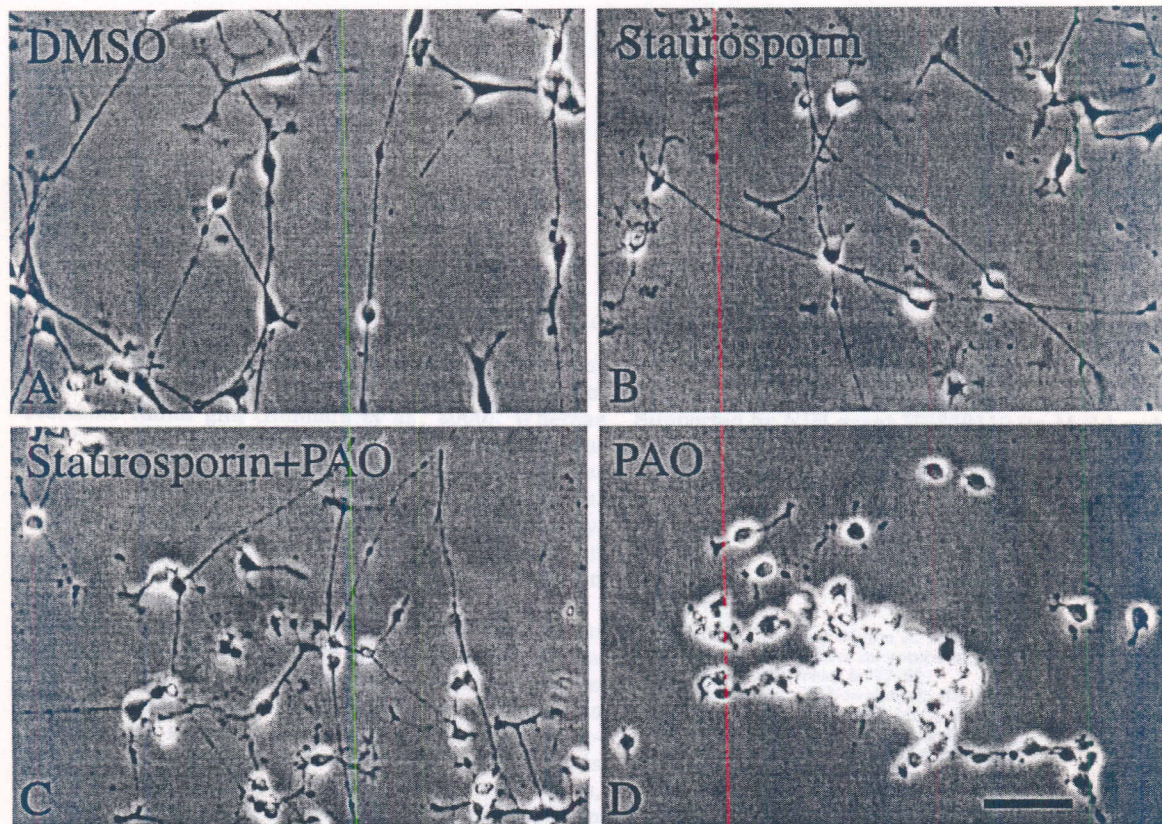
Fig. 5. Effect of phenylarsine oxide on cerebellar radial glia cells in culture. Cerebellar radial glial cells were treated for 1 h with 5 μM phenylarsine oxide (PAO), without or with pretreatment for 30 min with 10 nM staurosporin, or with 10 nM staurosporin alone. In the presence of PAO alone, cells rounded up and retracted their long radial processes but this was prevented by the pretreatment with staurosporin. Staurosporin alone had no dramatic affect on cell morphology. **A:** DMSO. **B:** Staurosporin. **C:** Staurosporin + PAO. **D:** PAO. Bar, 20 μm.

nase domain among MARKs is observed specifically in the brain (Kato et al., 2001). MARKs in radial glia including MARKL1 could be activated in response to phenylarsine oxide treatment and act on MAPs such as MAP4 to alter the organization of microtubules in radial glia. Activation of MARKs in cells has been found to destabilize microtubules, leading to cell rounding (Ebneth et al., 1999). It has been proposed that activation of MARKs in radial glia is important to destabilize microtubules when they undergo cytokinesis. Interestingly, MAP4 is expressed widely in proliferating non-neuronal cells but not in neurons, and it may be involved in disorganizing microtubules in interphase cells but it is not associated with mitotic spindle microtubules (Parysek et al., 1984; Chang et al., 2001; Iida et al., 2002). Thus, MAP4 may act in radial glia to disrupt the organization of their microtubules to facilitate cytokinesis during development. However, it has been suggested that during asymmetric division of radial glia into a neuron and another radial glia, the radial process is retained by the neuron, which migrates following collapse of its ventral process (Miyata et al., 2001). Thus, dynamic regulation of microtubules by MAPs may be involved in destabilizing microtubules as neurogenesis progresses and microtubules are reorganized with different proportions of MAPs in the processes of the newborn neurons that will support cell body migration toward the pial surface (Rivas and Hatten, 1995; Hatten, 2002).

MAP7, unlike MAP4 and several MAPs found in neurons, does not contain the KXGS consensus sequence and therefore is probably not regulated similarly by MARKs. Interestingly, MAP7 (E-MAP-115) has been found in nonneural and neural epithelia in embryonic mice and its expression correlated with increased polarization of the intestinal epithelia (Fabre-Jonca et al., 1998). Similarly, we found higher levels of MAP7 in the polarized radial glia than in astrocytes. MAP7 appears to be expressed in the developing cortex consistent with our observations of expression in radial glia (Fabre-Jonca et al., 1998). Expression has also been reported in the embryonic floor plate where MAP7

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