Crown of Microfilaments in the Extending Cytoplasmic Processes of Medulloblastoma Glial Progenitors

Bernard L. Maria, Robert Cumming and Loretta Sukhu

ABSTRACT: Microfilaments and microtubules play a part in the extension of neuronal processes but their roles in the formation of glial processes have not yet been determined. The objectives of this study were to determine the organization of microfilaments in differentiating glial progenitors (RB2 cells) and to study the effects of microfilament or microtubule disruption on process extension. Intense F-actin staining (crown of microfilaments) was observed at the leading edge of a small extending conical tip in differentiating RB2 cells, but was absent in process-bearing TE671 rhabdomyosarcoma cells. No significant difference was noted in the mean number of TE671 cells with processes treated with a microfilament disrupter from that of similarly treated controls. In contrast, a significant difference was noted in the mean number of RB2 cells with processes after microfilament disruption treatment from that of similarly treated controls. Microtubule disruption arrested extension and caused process retraction in both cell types. The results of this study demonstrate that microtubules play an equally important part in the extension and stabilization of the RB2 and TE671 processes. Moreover, the crown of microfilaments concentrated in the glial RB2 process (and not in the TE671 process) may be critical to its extension during differentiation.

RÉSUMÉ: Couronne de microfilaments dans les prolongements cytoplasmiques en extension des cellules progénitrices gliales du médulloblastome. Les microfilaments et les microtubules jouent un rôle dans l'extension des prolongements neuronaux, mais leur rôle dans la formation des prolongements gliaux n'a pas encore été déterminé. Les objectifs de cette étude étaient de déterminer l'organisation des microfilaments dans les cellules progénitrices gliales pendant la différenciation (RB2) et d'étudier les effets de la perturbation des microfilaments ou des microtubules sur l'extension des prolongements. Une fluorescence intense après coloration à l'actine F (couronne de microfilaments) a été observée à l'extrémité cônique en extension de cellules RB2 pendant la différenciation, mais non dans les prolongements de cellules de rhabdomyosarcome TE671. Aucune différence significative n'a été notée dans le nombre moyen de cellules TE671 avec des prolongements traités avec un agent perturbant les microfilaments, par rapport à des contrôles ayant subi le même traitement. Par contre, une différence significative a été notée dans le nombre moyen de cellules RB2 avec prolongements après perturbation des microfilaments, par rapport à des contrôles ayant subi le même traitement. La perturbation des microtubules arrête l'extension et cause la rétraction des prolongements dans ces deux types de cellules. Les résultats de cette étude montrent que les microtubules jouent un rôle tout aussi important dans l'extension et la stabilisation des prolongements des cellules RB2 et TE671. De plus, la couronne de microfilaments concentrée dans le prolongement glial RB2 (et non dans le prolongement TE671) peut être critique à son extension pendant la différenciation.

Can. J. Neurol. Sci. 1992; 19: 23-33

Neural cells are derived from neuroectodermal progenitors located in the ventricular and subventricular regions of the developing brain.[1] The progenitor cells will differentiate into mature neurons and glia and will form cytoplasmic processes. The cytoplasmic processes of cultured neurons (neurites) form by active extension.[2] The cytoplasmic processes of cultured glia form by cavitation of a fan-like expanse of cytoplasm or, in the case of cerebellar Bergmann glia, by active extension.[3,4]

The cytoskeleton of neural cells, consisting of microfilaments, microtubules, and intermediate filaments, is involved in cell migration, changes in cellular shape, and a number of important intracellular processes. In neurons, microfilaments (4-

From the Division of Neurology (Department of Pediatrics), University of Florida College of Medicine, Gainesville, Florida

Received May 6, 1991. Accepted in final form August 9, 1991

Reprint requests to: Dr. B.L. Maria, Division of Pediatric Neurology, University of Florida College of Medicine, Gainesville, Florida, U.S.A. 32610-0296

5 nm actin-containing filaments) form a cortical shell in the neuritic axoplasm and tight bundles in the surface protrusions and body of the extending neuronal growth cone.[5] The selective chemical disruption of microfilaments or microtubules can inhibit further extension of the neurite.[6] However, it is not known what role(s) the microfilaments and microtubules may have in glial cell migration or in the formation and stabilization of glial processes.

Glial progenitor cells cultured from mouse neopallium and treated with dibutyryl cyclic adenosine monophosphate (dBcAMP) form cytoplasmic processes by cavitation of a fan-like expanse of cytoplasm.[7] The maturation of these glial progenitors is accompanied by a reorganization of microfilaments in cells at various stages during the transformation of astroblasts into reactive astrocytes.[8] Moreover, prominent bundles of microfilaments radiate from the cell body into the process and may be important to its development.

Cerebellar Bergmann-like glia which are co-cultured with granular neurons form processes by active extension rather than by cavitation of their cytoplasm.[4] Glial-process extension may occur independently of neurons in maturing cerebellar medulloblastoma cells (RB2 cells) treated with dBcAMP.[9,10] The great majority of untreated RB2 cells (93%) are small and polygonal in shape, whereas a few are either large and flat (3%) or polygonal with processes (4%). RB2 cells form cytoplasmic processes in 3 ways as demonstrated by time-lapse video microscopy (unpublished data, 1990). First, the motile polygonal RB2 cells have processes that trail behind as the cells migrate in the opposite direction. As the cells continue to migrate, they periodically retract their stretched trailing process. Second, the dBcAMP-treated polygonal RB2 cells are stationary and form processes by active extension. Third, the flat dBcAMP-treated RB2 cells form processes initially by cavitation of a fan-like expanse of cytoplasm followed by process extension. Since motile glial progenitors become stationary, develop their processes, and differentiate, the RB2 model represents a unique tool for the study of the role(s) of microfilaments and microtubules.

DBcAMP has been shown to induce the differentiation of a number of cultured neoplasms and to accelerate process formation in normal and malignant cells.[11,12] The cell line TE671 has been widely used as a model to study the biology of medulloblastoma cells with neuronal features.[13-15] The addition of dBcAMP to cultured TE671 cells induces process formation similar to that of neuritic extension.[16] However, recent studies have demonstrated that the properties of the TE671 cell line should be ascribed to rhabdomyosarcoma rather than medulloblastoma.[17] Nevertheless, in addition to the RB2 cells, the TE671 cells are useful in the determination of whether the synthesis and accumulation of cytoskeletal actin filaments and microtubules are required for the cellular shape change and process extension induced by dBcAMP.

In this paper, we examine the organization of microfilaments in RB2 cells and TE671 cells as they form their cytoplasmic processes. We focus on the effects of a microfilament-disrupting agent (cytochalasin B) and a microtubule-disrupting agent (colchicine) on the formation of processes. The results of this study demonstrate that microtubules play an equally important part in the extension and stabilization of the RB2 and TE671 processes. Moreover, the crown of microfilaments concentrated in the glial RB2 process (and not in the TE671 process) may be critical to its extension during differentiation.

Materials and Methods

Cell Cultures

Tissue was obtained from an 8-month-old boy who was diagnosed with a posterior fossa cerebellar medulloblastoma after he developed symptoms and signs of increased intracranial pressure. A gross total resection was performed. Postoperatively, he was treated with nitrogen mustard, vincristine, prednisone, and procarbazine, but 2 months later the tumor recurred locally and a tumor-debulking operation was performed.

Tissue from the recurrent tumor was transferred to culture medium containing 20% fetal bovine serum (FBS). The specimen was finely minced and passed through a 20-gauge needle 3 to 5 times to make a single-cell suspension. The cell suspension was placed in 100-mm Petri dishes with Dulbecco modified Eagles/Ham's F12 medium (DMEM/F12) in a 1:1 ratio, without antibiotics but enriched with 20% FBS. The cells were incubated at 37°C in 5% CO_2 and grown until they formed a semiconfluent monolayer culture, at which time they were subcultured (RB2 cells). In addition, TE671 (American Type Culture Collection Rockville, MD) and N2Ab-1 cell lines (a gift of Dr. M. Notter, Rochester, NY) were obtained for comparative studies. The N2Ab-1 cell line was selected since culture on Cell Tak substratum (see below) spontaneously induces process outgrowth of these cells. RB2 cells from the first 20 cultured passages, TE671 cells, and N2Ab-1 cells were stored at -150°C. Frozen cells were quickly thawed and cultured in DMEM/F12 medium enriched with 10% FBS.

Cell Attachment

To enhance cell attachment during treatment of cells with the microfilament or microtubule disrupter, the culture surfaces were adsorbed with Cell Tak (muscle adhesion protein from *Mytilus edulis*, 50 µg/m (Collaborative Research Inc., Bedford, MA). Approximately 100 µl of Cell Tak was adsorbed for 30 min to each 35-mm tissue-culture well. To induce process formation in RB2 and TE671 cells, we treated the cultures with dBcAMP (2mM, Sigma, St. Louis, MO) for a period of up to 48 hr. Because Cell Tak can spontaneously induce process formation in N2Ab-1 cells, we determined if Cell Tak spontaneously induced process formation in plated RB2 and TE671 cells before treating them with dBcAMP, as previously described.[18]

Organization of Microfilaments

For visualization of microfilaments, untreated and dBcAMP-treated (for 4 hr, 7 h, and 24 h) cells were fixed for 10 min with 3.7% formaldehyde in phosphate-buffered saline (PBS) and then extracted for 5 min with a solution containing 5 mM EGTA, 25 mM KCl, 10 mM piperazine-*N,N'*-bis (2-ethane sulfonic acid), 10% glycerol, 4% polyethylene glycol 6000, and 0.1% Triton X-100. After being washed with PBS, the cells were treated with 0.3 mM NBD-phallacidin (Molecular Probes Inc., Plano, Texas) for 30 min at 21°C.[8] After a brief wash with PBS, cells were mounted in 50% glycerol in PBS, pH 7.8. A Leitz Photomicroscope II equipped with epifluorescence optics and a standard fluorescein isothiocyanate filter set was used to observe and photograph the cells. Photomicrographs were recorded on Kodak film.

Disruption of Microfilaments and Microtubules

Preparation of cytochalasin b and colchicine. RB2 cells and TE671 cells were cultured on the Cell Tak substratum and treated with a microfilament disrupter (cytochalasin B, Sigma, St. Louis, MO) or a microtubule disrupter (colchicine, Sigma, St. Louis, MO) in concentrations comparable to those used in studies of neurite extension.[5,19] Cytochalasin B was made up as a stock solution of 50 µg/ml diluted in PBS, stored at 4°C for less than 3 months and used at concentrations ranging from 0.1 µg/ml to 10 µg/ml. The final concentration of 2 µg/ml was selected since higher concentrations caused cell detachment with the highly adhesive substratum Cell Tak. Colchicine was made up as a stock solution of 50 µg/ml in DMEM/F12, stored at 4°C for less than 3 months, further diluted to 10 µg/ml, and used at concentrations ranging from 0.01 µg/ml to 10 µg/ml. The final concentration of 0.01 µg/ml was selected, again since higher concentrations caused cell detachment with the highly adhesive substratum Cell Tak.

Process Extension

RB2 cells and TE671 cells were treated for 2 hr with either cytochalasin B or colchicine before induction of process formation with dBcAMP (at 2 mM for 24 hr). We quantified the process formation in triplicate experiments of both cell types (and appropriate control cultures) by counting at random the number of cells out of 200 that had cytoplasmic processes measuring more than twice the cell body's greatest diameter in length.

Process Stabilization

RB2 cells and TE671 cells were treated with dBcAMP for 48 hr. Cytochalasin B or colchicine was added for a period of 24 hr to the cultures that had already formed processes with dBcAMP treatment. Quantification of processes and of the number of cells in which processes had retracted was determined as described for process extensions.

Cell Migration

Since RB2 cells that have not been treated with dBcAMP form cytoplasmic processes that trail behind as the cells migrate in the opposite direction, we examined the effects of cytochalasin B or colchicine on cell migration by treating plated RB2 cells with these agents for a period of 24 hr. The quantification of cytoplasmic processes was determined as described for process extensions.

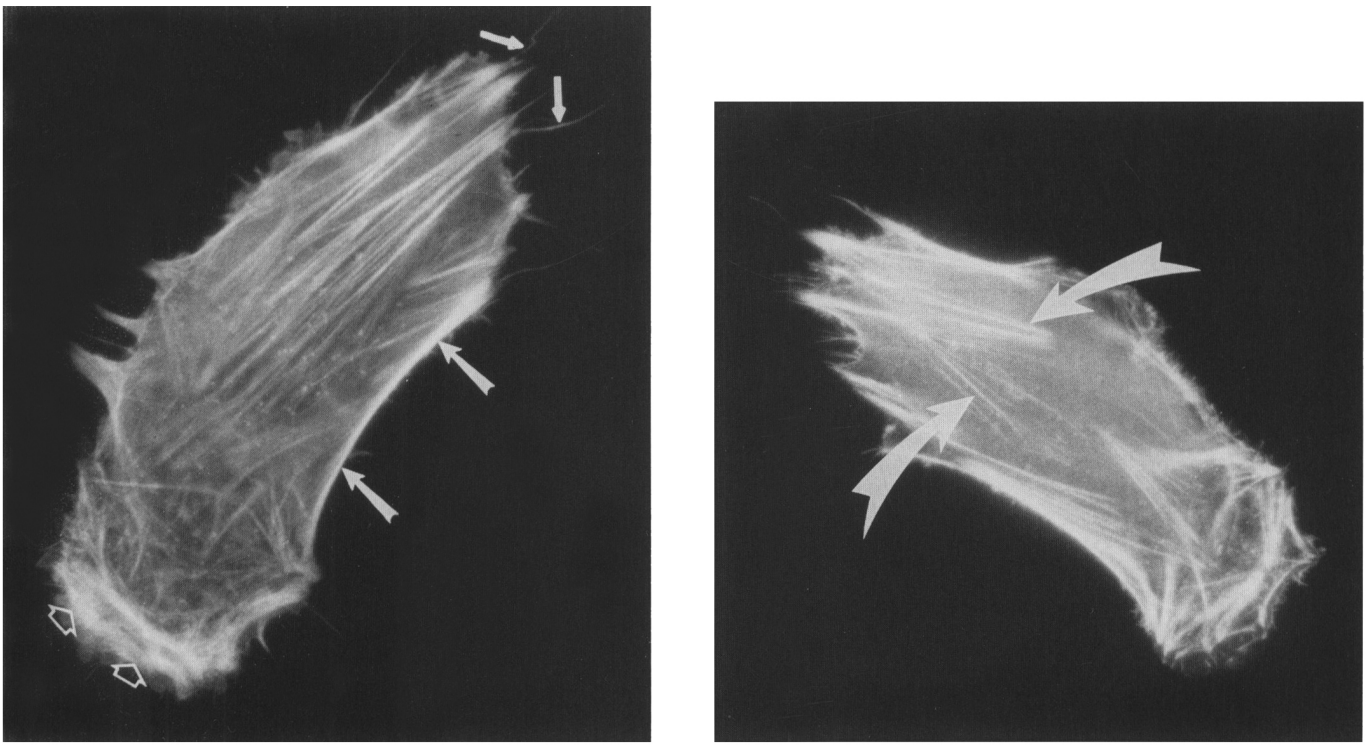
Statistical Analysis

We determined the significance of the differences between multiple independent means by using a one-factor analysis of variance and Fisher's protective least significant different test.

RESULTS

Organization of Microfilaments in RB2 and TE671 Cells

The microfilaments in the motile polygonal RB2 cell (93% of cell population) formed a cortical shell and were more condensed in some parts than others (Figure 1). Stress fibers that



A **B**

*Figure 1 — Microfilament organization in polygonal RB2 cell. (**A**) Note the cortical shell of microfilaments that delineates the cell body (large arrows). Abundant microfilaments form a crown at the leading edge of the migrating RB2 cell (open arrows). Trailing processes of migrating RB2 cells are rich in microfilaments (small arrows). Magnification × 256. (**B**) Curved arrows indicate stress fibers that contain microfilaments. Magnification × 161.*

https://doi.org/10.1017/S0317167100042499 Published online by Cambridge University Press

contained microfilaments ran parallel to the longitudinal axis of the migrating cells and were detected in the trailing processes. Abundant microfilaments formed a crown at the leading edge of the migrating RB2 cell. The microfilaments in the large adhesive flat RB2 cell (3% of cell population) were organized in complex patterns (Figure 2). The microfilaments formed a cortical shell around the edge of the flat cell and an underlying finer concentric array (Figure 2A). Microfilaments were found in stress fibers that extended to the surface of the cell and in fine processes that touched neighboring cells. In addition, microfilaments were found in lattice-like structures in the cytoplasm (Figure 2B).

The microfilaments in the TE671 cells formed a cortical shell around the edge of the cell (Figure 3A) but lacked the crown of microfilaments observed at the leading edge of the migrating polygonal RB2 cell. Moreover, many more microfilament-containing filopodia and fewer stress fibers were detected in TE671 cells than in RB2 cells. As in RB2 cells, microfilaments were abundant in the fine processes that bridged neighboring TE671 cells (Figure 3B).

Organization of Microfilaments in RB2 and TE671 Cells Forming Processes

Intense F-actin staining was observed within 4 hr of dBcAMP treatment at the leading edge of a small (5-μm) extending conical tip in stationary polygonal RB2 cells (Figure 4A). After 7 hr of dBcAMP treatment, the process contained a crown of microfilaments at its tip (Figure 4B). A prominent cor-

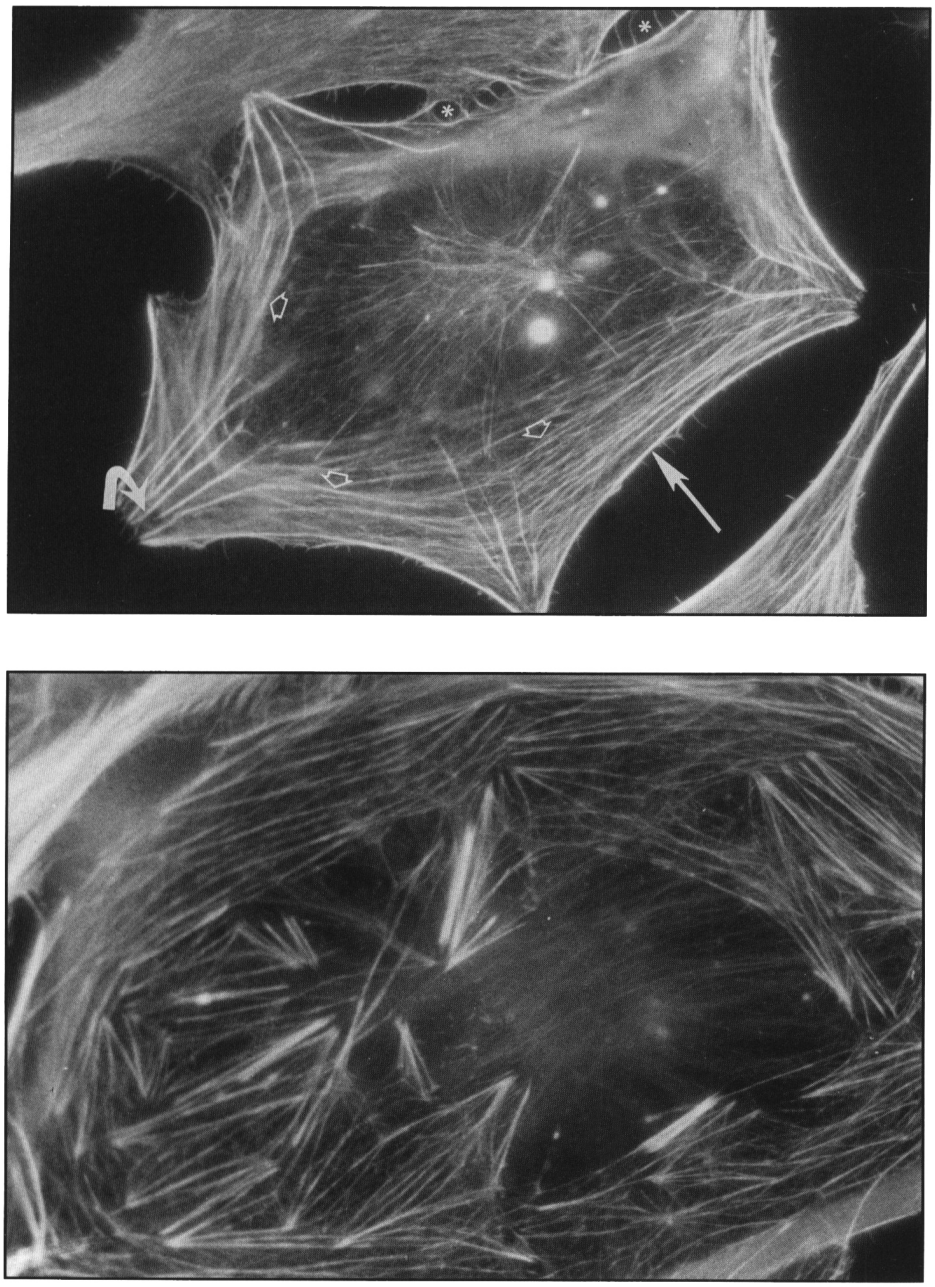


*Figure 2 — Microfilament organization in large adherent flat RB2 cells. **(A)** Note the cortical shell of microfilaments that surrounds the cell body (large arrow) and the underlying finer concentric array (small open arrows). Microfilaments found in stress fibers extend to the surface of the cell (curved arrow) and in fine processes that bridge neighboring cells (asterisks). Magnification ×161. **(B)** Microfilaments are arranged in lattice-like structures in the cytoplasm. Magnification ×256.*

https://doi.org/10.1017/S0317167100042499 Published online by Cambridge University Press

tical shell of actin filaments was present at the different time points, including at 24 hr when the process had extended further (Figure 4C). Microfilament-containing stress fibers and filopodia that protruded from the surface of the cell were observed at 24 hr. In the few large RB2 cells treated with dBcAMP and the microfilament disrupter cytochalasin B (Figure 2C), the cell body was no longer well-delineated by a ring of microfilaments. In addition, the finer bundles of microfilaments in the fan-like expanse of cytoplasm were no longer present and the cell processes had lost their linear configuration and their prominently stained microfilament bundles.

TE671 cells treated with dBcAMP for 4 hr contained a microfilament-containing cortical shell (Figure 5A), stress fibers, cytoplasmic peppering of polymerized actin, and numerous filopodia. In contrast to the process of the RB2 cell, the extending process of the TE671 cell did not contain a crown of microfilaments at its tip. In addition, the process of the TE671 cell contained bead-like dilatations (Figure 5B) not seen in the glial RB2 cells.

Process Extension in RB2 and TE671 Cells Treated with Cytochalasin B or Colchicine

Of the 200 RB2 cells treated with dBcAMP for 24 hr and counted at random in each of 3 experiments, a mean of 187 ± 14 had cytoplasmic processes (Table 1, Figure 6). When RB2 cells were treated with dBcAMP and cytochalasin B (Figure 4D), 23 ± 20 had processes ($p \leq 0.01$). No RB2 cells had processes when treated with dBcAMP and colchicine. When TE671 cells were treated with dBcAMP for 24 hr, 181 ± 9 had cytoplasmic processes. When TE671 cells were treated with dBcAMP and cytochalasin B, 183 ± 6 had processes (not significant). No TE671 cells treated with dBcAMP and colchicine had processes.

Process Retraction in Process-Bearing RB2 and TE671 Cells Treated with Cytochalasin B or Colchicine

Of the 200 RB2 cells treated with dBcAMP for 48 hr and counted at random in each of 3 experiments, a mean of 183 ± 6 had cytoplasmic processes (Table 1, Figure 7). When RB2 cells were first treated with dBcAMP and then with cytochalasin B, 22 ± 12 had processes ($p < 0.01$). Process retraction occurred in a mean of 95% of RB2 cells treated with colchicine. When TE671 cells were treated with dBcAMP for 48 hr, 187 ± 5 had processes. When TE671 cells were first treated with dBcAMP and then with cytochalasin B, 187 ± 6 had processes (not significant). Process retraction occurred in a mean of 97% of TE671 cells treated with colchicine.

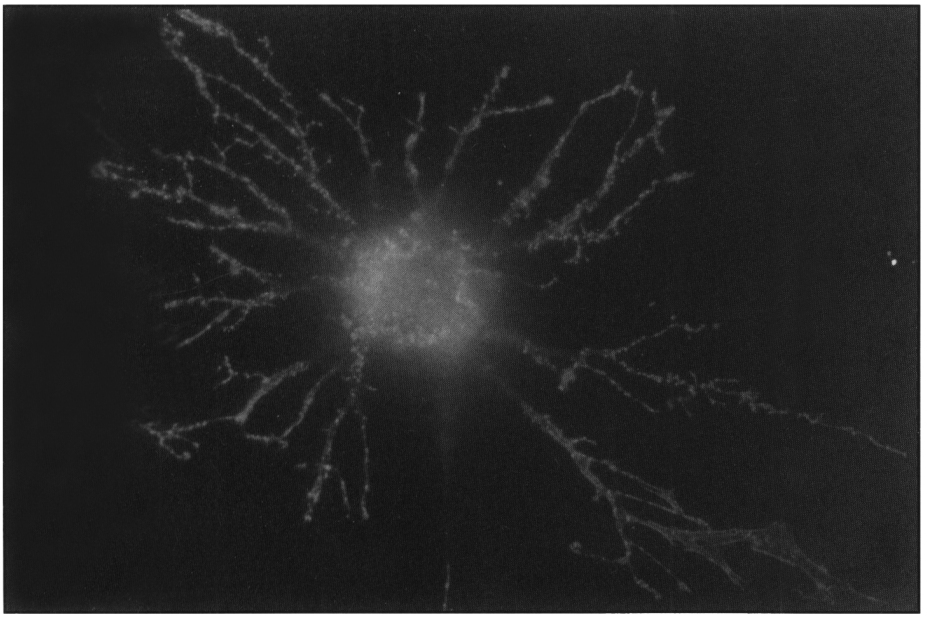
Cell Migration in Cultured RB2 Cells Treated with Cytochalasin B or Colchicine

Of the 200 untreated RB2 cells counted in each of 3 separate experiments, a mean of 46 ± 8 had trailing cytoplasmic processes that extended a distance of at least twice the cell body's greatest diameter. When RB2 cells were treated with cytochalasin B at the time of plating, 4 ± 4 had trailing processes (Table 2, Figure 8, $p < 0.01$). When RB2 cells were treated with colchicine at the time of plating, 2 ± 3 had trailing processes ($p < 0.01$).

Discussion

The motile tip of the neurite is commonly referred to as the growth cone. Its form in tissue culture ranges from a simple bulbous club to an array of surface protrusions (such as filopodia and lamelliopodia). Just before the initiation of axogenesis in neurons, actin filaments accumulate at the proximal cell pole.[20] Moreover, the growth cone and its surface protrusions contain abundant microfilaments that presumably participate in establishing the motile leading edge of the neurite. The organization of growth-cone actin has been studied by light and electron microscopy, and has been correlated with growth-cone morphology.[21] The actin filaments in the leading edge are important to growth-cone elongation whereas microfilament-containing filopodia have a role in correctly steering the growth cone *in vitro* and *in vivo*.[22]

A characteristic organization of actin filaments occurs in glial progenitor cells that transform into reactive astrocytes after



C

*Figure 2 — **(C)** Large flat RB2 cell treated with dBcAMP and cytochalasin B. The cell body is no longer encompassed by a ring of microfilaments. Finer bundles of microfilaments have disintegrated and cell processes have lost their linear configuration and their prominently stained microfilament bundles. Magnification ×161.*

https://doi.org/10.1017/S0317167100042499 Published online by Cambridge University Press

the addition of dBcAMP.[23] Astroblasts contain a thick, prominent, strongly stained ring of microfilaments that delineates the cell body. Electron microscopy has revealed that this ring is composed of many finer bundles of microfilaments arranged around the circumference. In addition, bundles of filaments radiate from the cell body into the processes. Based on these observations, Fedoroff et al.[8] suggested that the contractile properties of the prominent microfilament ring and the bundles of microfilaments located more towards the periphery play an important role in the development of cell processes.

In our study, the organization of actin filaments in the few large adhesive flat RB2 cells before treatment with dBcAMP was similar to that which has been described for astroblasts treated with dBcAMP during the early stages of transformation into reactive astrocytes. When these large RB2 cells were treated with dBcAMP and the microfilament disrupter cytocha-

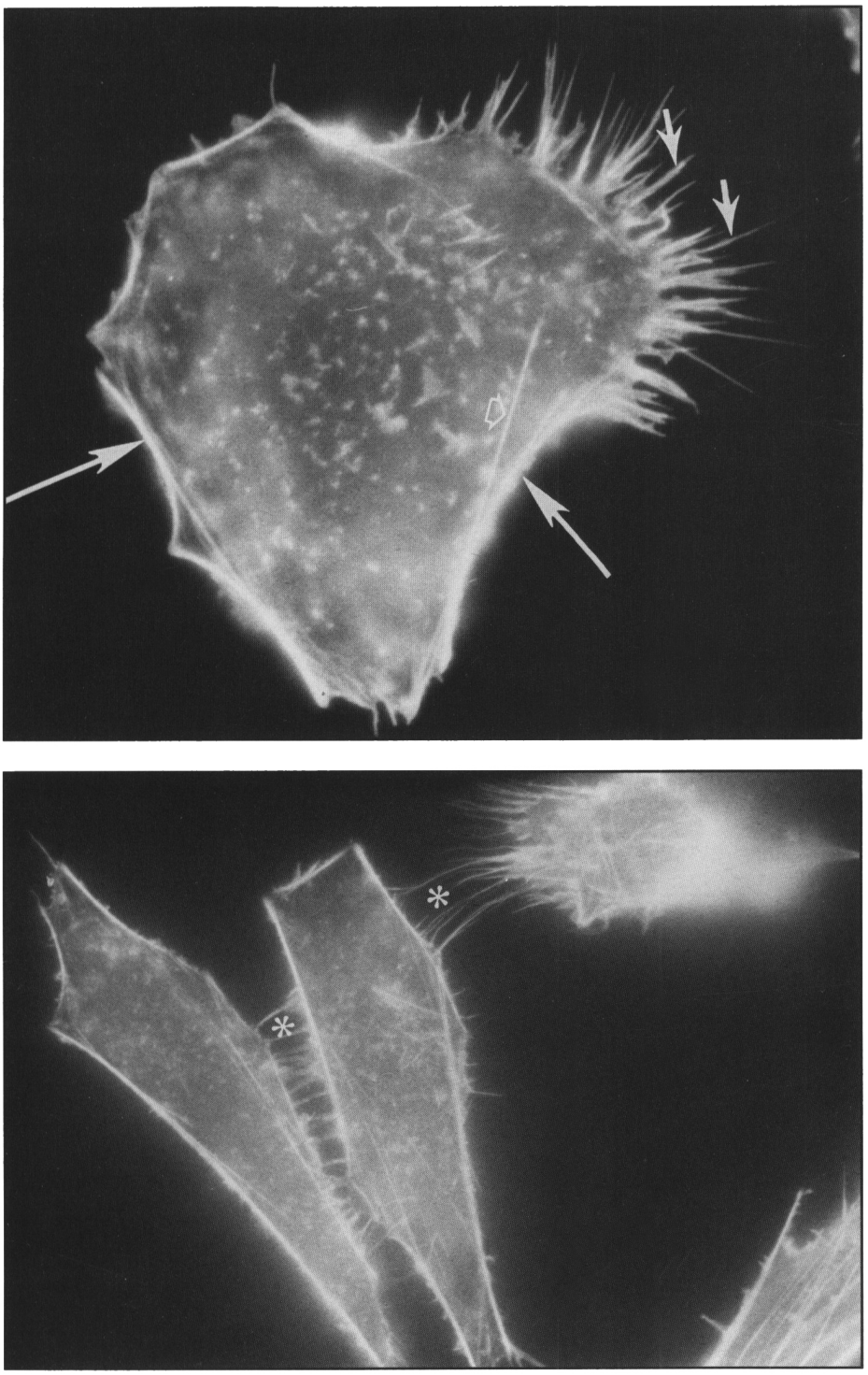


*Figure 3 — Organization of microfilaments in TE671 cell. **(A)** Large arrows indicate the cortical shell around the edge of the cell. Note the numerous microfilament-containing filopodia (small arrows) and few stress fibers (open arrow). Magnification × 256. **(B)** Many fine processes abundant in microfilaments bridge neighboring TE671 cells (asterisks). Magnification × 256.*

https://doi.org/10.1017/S0317167100042499 Published online by Cambridge University Press

lasin B, the cell body was no longer well-delineated by a ring of microfilaments. In addition, the finer bundles of microfilaments in the fan-like expanse of cytoplasm were no longer present and the cell processes had lost their linear configuration and their prominently stained microfilament bundles. Based on these observations, we believe that the microfilaments play an important role in the development of normal-appearing glial processes in dBcAMP-treated astroblasts.

The distribution of microfilaments in the great majority of motile polygonal RB2 cells and in stationary polygonal RB2 cells forming cytoplasmic processes revealed a unique crown formation of microfilaments at the leading edge of migration and process extension respectively. The most striking difference between the patterns of microfilaments in RB2 cells and TE671 cells was the complete absence of a crown in TE671 cells before dBcAMP treatment and at all time points after treatment. This observation led us to postulate that the crown of microfilaments in polygonal RB2 cells may play an important part in cell migration and in the extension of cell processes. Exposure of RB2 cells to the actin microfilament-disrupting agent cytochalasin B inhibited cell migration. Moreover, cytochalasin B prevented the extension of the RB2 glial process. TE671 cells treated with dBcAMP demonstrated a microfilament-containing cortical shell, stress fibers, cytoplasmic peppering of polymerized actin, and numerous surface protrusions. Nevertheless, cytochalasin B did not inhibit the extension of the TE671 processes in response to dBcAMP. Taken together, these results suggest that the crown of microfilaments in the polygonal RB2 process may be critical to its extension during differentiation.

We examined what role(s) microfilaments may have in the stabilization of extended processes in RB2 cells and TE671 cells by treating process-bearing cells with cytochalasin B.

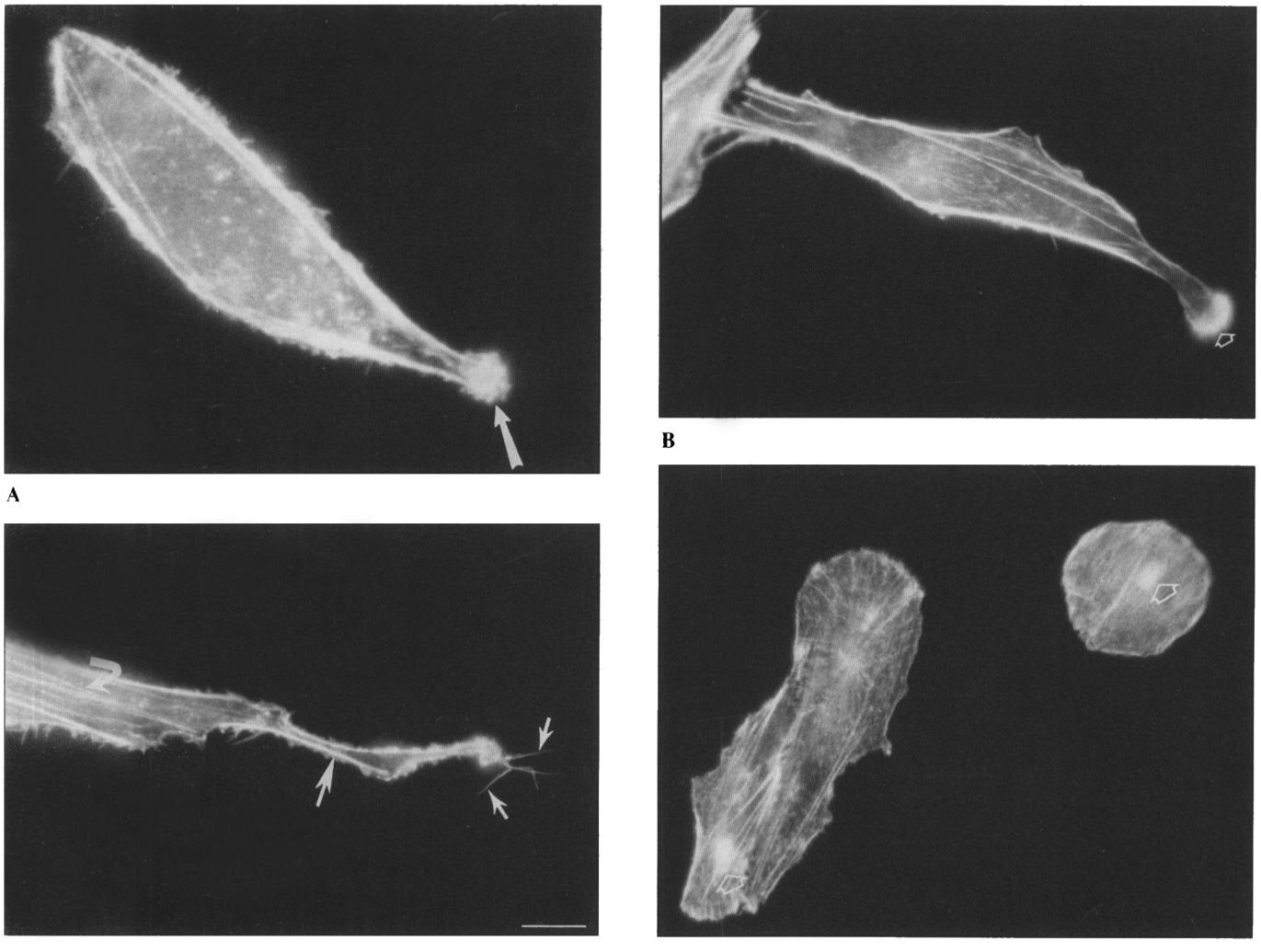


Figure 4 — Organization of microfilaments in dBcAMP-treated polygonal RB2 cells ***(A)*** *Four hr after dBcAMP treatment. Note the intense F-actin staining observed at the leading edge of a small (5 μm) extending conical tip (arrow). Magnification x 256.* ***(B)*** *Seven hr after dBcAMP treatment. RB2 cell process contains a crown of microfilaments (open arrow). Magnification × 256.* ***(C)*** *Twenty-four hr after dBcAMP treatment. RB2 cell process has extended further. A prominent cortical shell of actin filaments delineates the cell (large arrow). Microfilament-containing filopodia protrude from the cell surface (small arrows). Stress fibers are abundant (curved arrow). Bar denotes 10 μm. Magnification × 256.* ***(D)*** *RB2 cells treated with dBcAMP and cytochalasin B. Note that cytoplasmic processes are absent and prominently stained aggregates of actin filaments are apparent (open arrows). Magnification × 161.*

https://doi.org/10.1017/S0317167100042499 Published online by Cambridge University Press

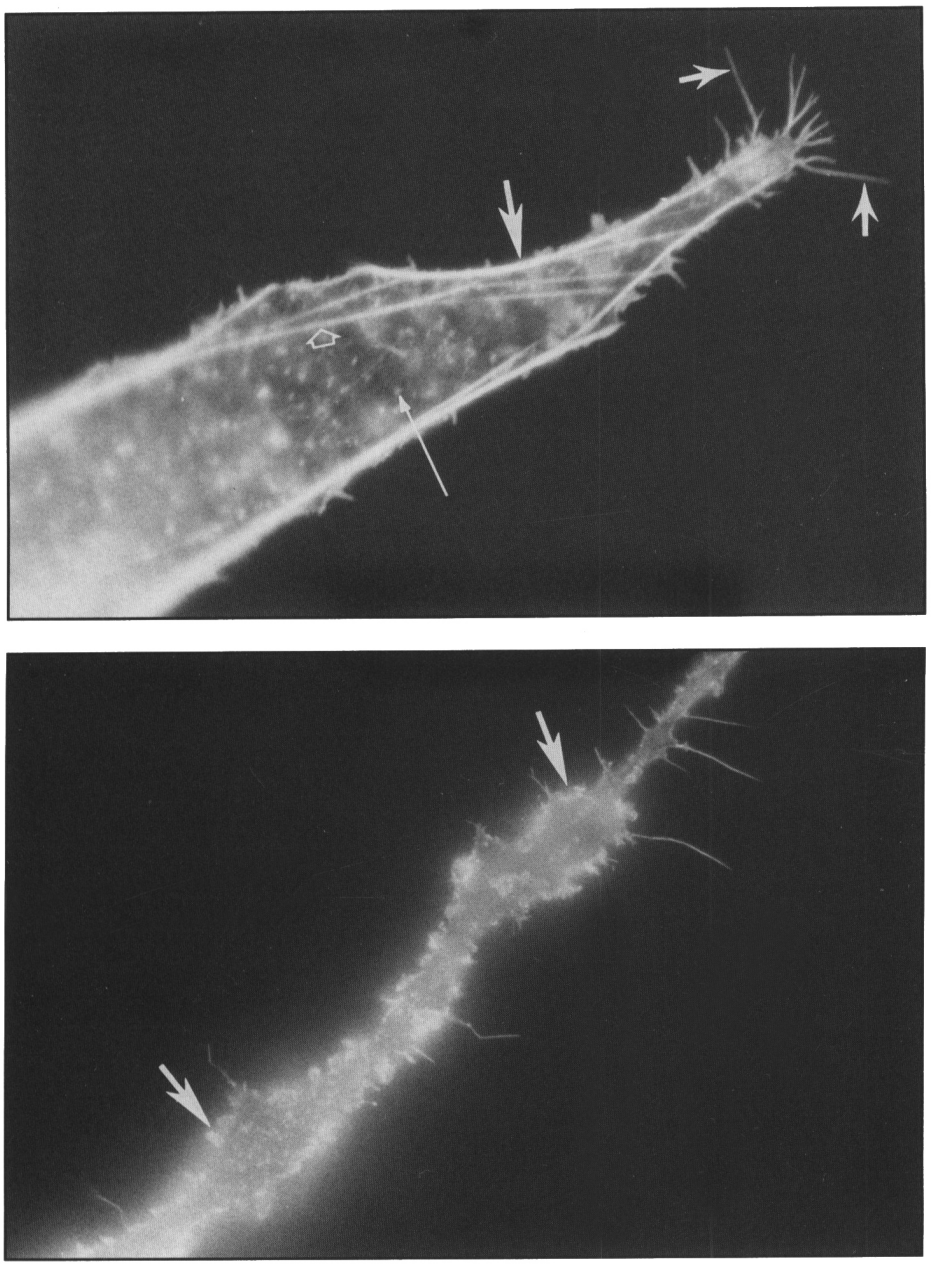


*Figure 5 — Organization of microfilaments in dBcAMP-treated TE671 cells. **(A)** Four hr after dBcAMP treatment. Note a microfilament-containing cortical shell (large arrow), stress fibers (open arrow), cytoplasmic peppering of polymerized actin (thin arrow), and abundant filopodia (small arrows). Magnification × 256. **(B)** Arrows indicate bead-like dilatations observed in the process of the TE671 cell. Magnification × 256.*

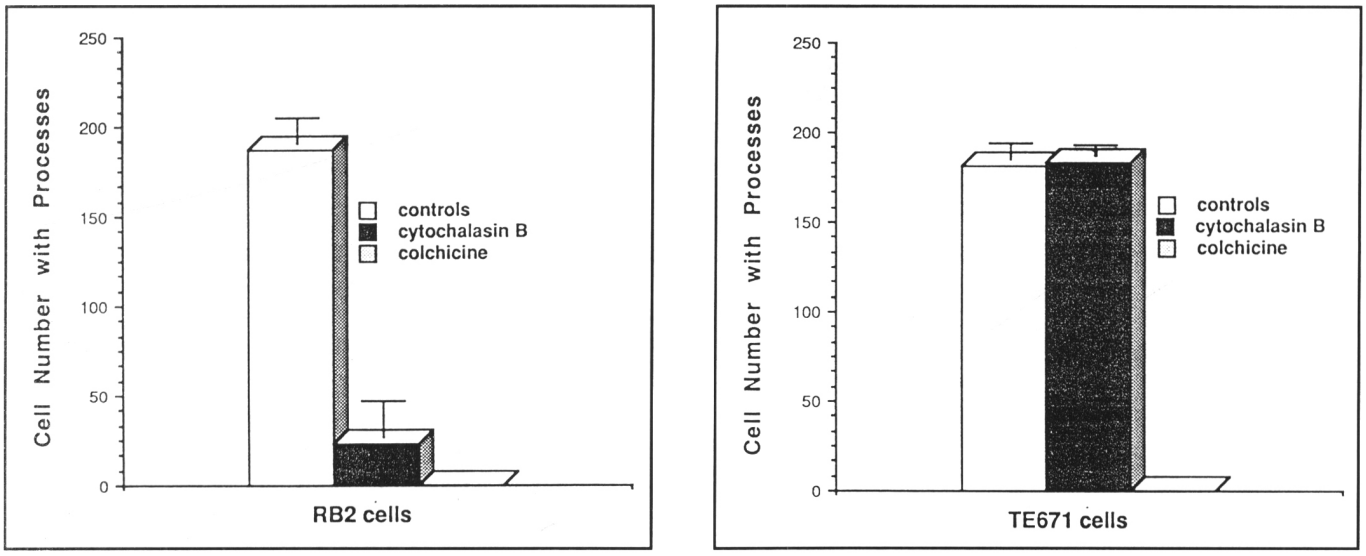
Table 1: Effects of Cytochalasin B (CB) or Colchicine (COL) on Process Extension or Stabilization of RB2 Cells and TE671 Cells

Cells	Number of cells with processes (Mean ± standard deviation)	
	Process extension	Process stabilization
RB2		
+ dBcAMP	187 ± 14	183 ± 6
+ dBcAMP + CB	23 ± 20	22 ± 12
+ dBcAMP + COL	0	16 ± 6
TE671		
+ dBcAMP	181 ± 9	187 ± 5
+ dBcAMP + CB	183 ± 6	187 ± 6
+ dBcAMP + COL	0	6 ± 6

Interestingly, although the organization of actin filaments is similar in both cell types, with the exception of the crown, cytochalasin B produced process retraction in RB2 cells and not in TE671 cells. The microfilaments in RB2 cells may be more sensitive to the cytochalasin B than are the actin filaments in TE671 cells. However, we believe it is more likely that the crown of microfilaments is important to the stabilization of the extended RB2 process.

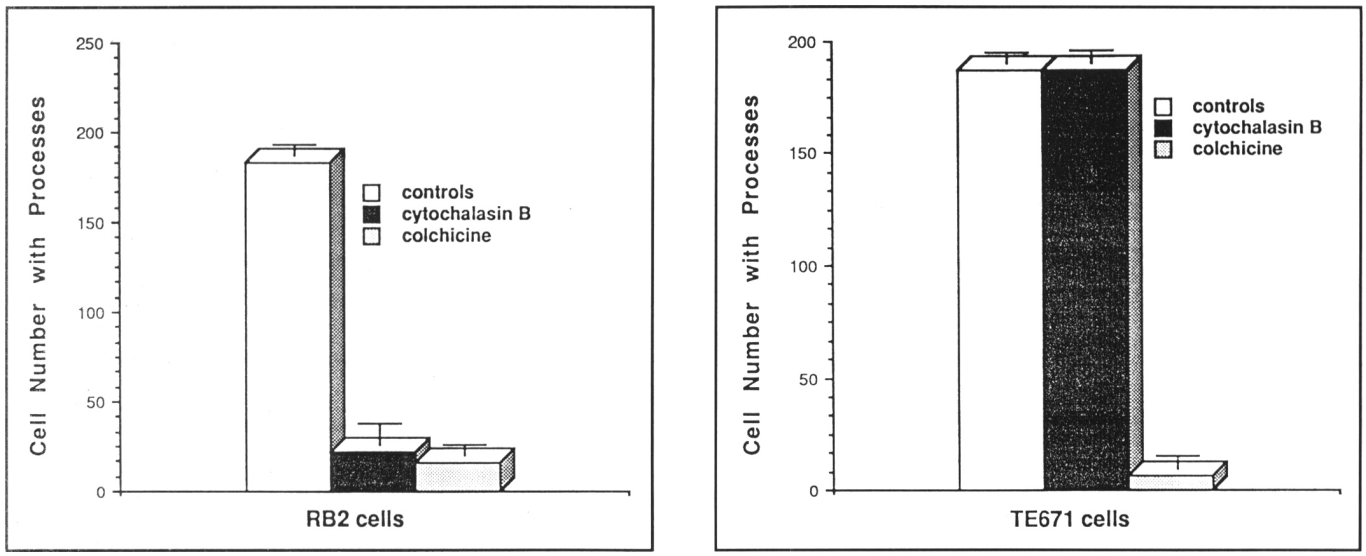
Neuronal growth cones can still elongate, although relatively slowly and in a disoriented fashion, under conditions in which most of the actin has been depolymerized by cytochalasin B.[24] Evidence has accumulated that microtubules are important in the generation and maintenance of neuronal processes.[25,26] When colchicine is applied to depolymerize the microtubules in either neuroblastoma cells or primary neurons in culture, the

https://doi.org/10.1017/S0317167100042499 Published online by Cambridge University Press



A B

Figure 6 — Process extension in RB2 and TE671 cells. ***(A)*** *Note that cytochalasin B treatment causes a significant decrease in the number of process-bearing RB2 cells ($P \leq 0.01$, one-way ANOVA, Fisher's PLSD).* ***(B)*** *No significant difference is seen between treated and untreated TE671 cells. Colchicine completely inhibits process extension in both cell types. Error bars denote standard deviation.*



A B

Figure 7 — Process stabilization in RB2 and TE671 cells. ***(A)*** *Note the significant decrease in the average number cells with processes when cytochalasin B is added to process-bearing RB2 cells ($P < 0.01$), one way ANOVA, Fisher's PLSD).* ***(B)*** *In contrast, no significant difference is noted in the number of process-bearing TE671 cells before and after cytochalasin B treatment. Colchicine causes process retraction in both cell types. Error bars denote standard deviation.*

neurites retract into the cell body.[27] Therefore, actin-independent neurite elongation and stabilization may be microtubule-based.

We examined what role(s) microtubules might have in the extension and stabilization of processes in RB2 cells and TE671 cells by depolymerizing the microtubules. When colchicine was applied to either RB2 cells or TE671 cells before or after processes had formed, the inevitable consequence was a retraction of processes into the soma. In addition, colchicine inhibited the migration of RB2 cells. Evidently, then, assembled microtubules are essential to the migration of RB2 cells and the integrity of the cytoplasmic processes of RB2 and TE671 cells. As in neurite outgrowth, the process extension in other cell types probably involves a number of mechanisms operating simultaneously. In this study, we did not examine the organization of microtubules in RB2 cells and TE671 cells before and after process extension. Future experiments should be designed to determine if and what microtubule modifications take place in differentiating glial progenitors.

The glial processes of RB2 cells have features that are similar to those of neurites: the vast majority of cultured RB2 cells treated with dBcAMP develop cytoplasmic processes by active extension; and, a highly motile tip is present at the leading edges

https://doi.org/10.1017/S0317167100042499 Published online by Cambridge University Press

of the RB2 processes (unpublished data, 1990). The results of the present study demonstrate that the tip of the extending RB2 process, as is the case with the neuronal growth cone, contains abundant microfilaments and an array of surface protrusions such as filopodia; the extended processes of RB2 cells, like the neurites, contain a cortical shell of actin filaments. We found that microfilaments and microtubules have a major role in the formation of RB2 processes as they do in the formation of dendrites and axons.[24,25] Since microfilaments provide an underlying motile force in the extension of RB2 processes and neurites and since growth factors (e.g., nerve-growth factor, basic fibroblast-growth factor, glial-derived nexin) stimulate the extension, branching, and orientation of neurites,[28-37] we hypothesize that one or more of these regulatory factors will also promote the extension and guidance of RB2 processes.

Active process extension is observed in the radial glia during the development of the cerebellum and process outgrowth represents an early marker of differentiation of RB2 cells derived from a cerebellar medulloblastoma.[38,39] Although growth factors are important to neuronal and glial differentiation, their role or roles in glial-process outgrowth are unknown. The RB2 model represents a unique and powerful tool for studying the modes of action of growth factors in cells of the glial lineage as they stop dividing, develop processes and differentiate. Prior studies have shown that cell division is arrested when RB2 cells develop processes.[9,10] Observed changes in microfilament organization during differentiation occur at early stages following growth arrest and are therefore probably germane to the regulation of differentiation. Since growth factors may operate in the development of malignant brain tumors, an understanding of their modes of action in the regulation of the differentiated state of glial cells, including cytoskeletal assembly may have future clinical applications and lead to the development of new therapies to reduce the mortality and morbidity associated with childhood medulloblastoma.

Table 2: Effects of Cytochalasin B (CB) on Colchicine (COL) or RB2 Cell Migration

Cells	Number of cells with trailing processes (Mean ± standard deviation)
RB2 controls	46 ± 8
+ CB	4 ± 4
+ COL	2 ± 3

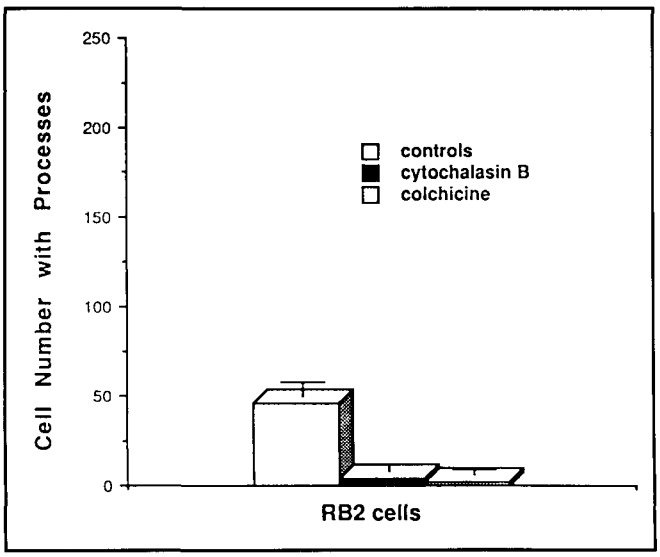


Figure 8 — RB2 cell migration. Both cytochalasin B and colchicine inhibit the migration of RB2 cells. Note the significant difference in the number of RB2 cells with trailing processes before and after microfilament or microtubule disruption ($P < 0.01$, one-way ANOVA, Fischer's PLSD). Error bars denote standard deviation.

ACKNOWLEDGEMENTS

We would like to thank Dr. V.I. Kalnins for his help with these studies, which were supported in part by the Research Institute, The Hospital for Sick Children and the Brain Research Fund Foundation. This paper was prepared with the assistance of Medical Publications, The Hospital for Sick Children, Toronto, Ontario.

REFERENCES

1. Fedoroff S. Prenatal ontogenesis of astrocytes. *In*: Fedoroff S, Vernadakis A, eds. Astrocytes. Development, Morphology and Regional Specialization of Astrocytes Vol 1. Orlando: Academic Press 1981: 35-37.
2. Mitchison TJ, Kirschner MW. Cytoskeletal dynamics and nerve growth. Neuron 1988; 1: 761-772.
3. Trimmer PA, Reier PJ, Oh THE, et al. An ultrastructural and immunocytochemical study of astrocytic differentiation *in vitro*: changes in the composition and distribution of the cellular cytoskeleton. J Neuroimmunol 1982; 2: 235-260.
4. Mason CA, Edmondson JC, Hatten ME. The extending astroglial process: development of glial cell shape, the growing tip, and interactions with neurons. J Neurosci 1988; 8: 3124-3134.
5. Marsh L, Letourneau PC. Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. J Cell Biol 1984; 99: 2041-2047.
6. Forscher P, Smith SJ. Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. J Cell Biol 1988; 107: 1505-1516.
7. Fedoroff S, White R, Subrahmanyan L, et al. Putative astrocytes in colony cultures of mouse neopallium. *In*: Acosta VE, Fedoroff S, eds. Glial and Neuronal Cell Biology. Proceedings of the Eleventh International Conference of Anatomy, part A; sponsored by the International Federation of the Association of Anatomists and The Mexican Society of Anatomy, Aug 17-23, 1980, Mexico City, Mexico. New York: Liss 1981: 1-19.
8. Fedoroff S, Ahmed I, Opus M, et al. Organization of microfilaments in astrocytes that form in the presence of dibutyryl cyclic AMP in cultures, and which are similar to reactive astrocytes *in vivo*. Neuroscience 1987; 22: 255-266.
9. Maria BL, Steck PA, Yung WK, et al. The modulation of astrocytic differentiation in cells derived from a medulloblastoma surgical specimen. J Neurooncol 1989; 7: 329-338.
10. Maria BL, Wong D, Kalnins VI. Dibutyryl cyclic AMP induces vimentin and GFAP expression in cultured medulloblastoma cells. Can J Neurol Sci 1990; 17: 15-20.
11. Fedoroff S, White R, Neal J, et al. Astrocyte cell lineage. II. Mouse fibrous astrocytes and reactive astrocytes in cultures have vimentin- and GFP-containing intermediate filaments. Brain Res 1983; 283: 303-315.
12. Bullard DE, Bigner SH, Bigner DD. The morphologic response of cell lines derived from human gliomas to dibutyryl adenosine 3':5'-cyclic monophosphate. J Neuropathol Exp Neurol 1981; 40: 231-246.
13. McAllister RM, Isaacs H, Rongey R, et al. Establishment of a human medulloblastoma cell line. Int J Cancer 1977; 20: 206-212.

14. Siegel HN, Lukas RJ. Nicotinic agonists regulate alpha-bungarotoxin binding sites of TE671 human medulloblastoma cells. J Neurochem 1988; 50: 1272-1278.
15. Luther MA, Schoepfer R, Whiting P, et al. A muscle acetylcholine receptor is expressed in the human cerebellar medulloblastoma cell line TE671. J Neurosci 1989; 9: 1082-1096.
16. Sasaki F, Schneider SL, Zelter PM. Dibutyryl adenosine 3':5'-cyclic monophosphate (dB-cAMP) induces growth inhibition and morphologic changes in human medulloblastoma cell line TE671. *In*: Walker MD, Thomas DGT, eds. Biology of Brain Tumor. Boston: Martinus Nijhoff 1986: 27-34.
17. Stratton MR, Darling J, Pikington GJ, Lantos, et al. Characterization of the human cell line TE671. Carcinogenesis 1989; 10: 899-905.
18. Notter MFD. Selective attachment of neural cells to specific substrates including Cell Tak, a new cellular adhesive. Exp Cell Res 1988; 177: 237-256.
19. Letourneau PC. Differences in the organization of actin in the growth cones compared with the neurites of cultured neurons from chick embryos. J Cell Biol 1983; 97: 963-973.
20. Lefcort F, Bentley D. Organization of cytoskeletal elements and organelles preceding growth cone emergence from an identified neuron *in situ*. J Cell Biol 1989; 108: 1737-1749.
21. Bridgman PC, Dailey ME. The organization of myosin and actin in rapid frozen nerve growth cones. J Cell Biol 1989; 108: 95-109.
22. Bentley D, Toroian-Raymond A. Disoriented pathfinding by pioneer neuron growth cones deprived of filopodia by cytochalasin treatment. Nature 1986; 323: 712-715.
23. Kalnins VI, Opas M, Ahmet I, et al. Astrocyte cell lineage. IV. Changes in the organization of microfilaments and adhesion patterns during astrocyte differentiation in culture. J Neurocytol 1984; 13: 867-882.
24. Letourneau PC, Shattuck TA, Ressler AH. "Pull" and "Push" in neurite elongation: observations on the effects of different concentrations of cytochalasin B and taxol. Cell Motil Cytoskeleton 1987; 8: 193-209.
25. Matus A. Microtubule-associated proteins and neuronal morphogenesis. *In*: Parnavelas JG, Stern CD, Stirling RV, eds. The Making of the Nervous System. New York: Oxford University Press 1988: 417-433.
26. Bray D, Thomas C, Shaw G. Growth cone formation in cultures of sensory neurons. Proc Natl Acad Sci USA 1978; 75: 5226-5229.
27. Seeds NW, Gilman AG, Amano T, et al. Regulation of axon formation by clonal cell lines of a normal neuronal tumor. Proc Natl Acad Sci USA 1970; 60: 160-167.
28. Hanley MR. Peptide regulatory factors in the nervous system. Lancet 1989; 1: 1373-1376.
29. Walicke P, Cowan WM, Ueno N, et al. Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension. Proc Natl Acad Sci USA 1986; 83: 3012-3016.
30. Monard D. Cell-derived proteases and protease inhibitors as regulators of neurite outgrowth. Trends Neurosci 1988; 11: 541-544.
31. Davies AM. The emerging generality of the neurotrophic hypothesis. Trends Neurosci 1988; 11: 243-244.
32. Lindsay RM, Harmar AJ. Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. Nature 1989; 337: 362-364.
33. Manthorpe M, Fagnani R, Skaper SD, et al. Astroglial cell contributions to neuronal survival and neuritic growth. *In:* Fedoroff S, Vernadakis A, eds. Astrocytes Biochemistry, Physiology and Pharmacology of Astrocytes, Vol 2. Orlando: Academic Press 1986; 315-376.
34. Gundersen RW, Barrett JN. Characterization of the turning response of dorsal root neurites toward nerve growth factor. J Cell Biol 1980; 87: 546-554.
35. Raff MC. Glial cell diversification in the rat optic nerve. Science 1989; 243: 1450-1455.
36. Rotwein P, Burgess SK, Milbrandt JD, et al. Differential expression of insulin-like growth factor genes in the rat central nervous system. Proc Natl Acad Sci USA 1988; 85: 265-269.
37. Hanley AM. The emerging generality of the neurotrophic hypothesis. Trends Neurosci 1988; 11: 243-244.
38. Tower DB. Development of knowledge about astrocytes since Virchow. *In*: Norenberg MD, Hertz L, Schousboe A, eds. The Biochemical Pathology of Astrocytes. Proceedings of a Satellite Symposium of the International Society for Neurochemistry and the American Society for Neurochemistry, Miami, Florida, May 26-29, 1987. New York: Liss 1988: 3-18.
39. Carpenter MB, Sutin J. Development and histogenesis of the nervous system. *In*: Carpenter MB, Sutin J, eds. Human Neuroanatomy, 8th edn. Baltimore: Williams and Wilkins 1983: 61-85.