Motile areas of leech neurites are rich in microfilaments and two actin-binding proteins: gelsolin and profilin

M. D. NEELY*† AND E. MACALUSO

Department of Pharmacology, Biozentrum of the University of Basel, Basel, Switzerland

SUMMARY

Cell motility is produced by changes in the dynamics and organization of actin filaments. The aim of the experiments described here was to test whether growing neurites contain two actin-binding proteins, gelsolin and profilin, that regulate polymerization of actin and affect non-neuronal cell motility. The distribution of gelsolin, profilin and the microfilaments was compared by immunocytochemistry of leech neurons growing in culture. We observed that microfilaments are enriched in the peripheral motile areas of the neurites. Both gelsolin and profilin are also concentrated in these regions. Gelsolin is abundant in filopodia and is associated with single identifiable microfilament bundles in lamellipodia. Profilin is not prominent in filopodia and shows a diffuse staining pattern in lamellipodia. The colocalization of gelsolin and profilin in motile, microfilament-rich areas supports the hypothesis that they synergistically regulate the actin dynamics that underlie neurite growth.

1. INTRODUCTION

The extension of a neurite requires a forward motility of the membrane at the distal end of the neurite. Such forward motility at the periphery of cells is called membrane protrusion. Two types of protrusive structures, lamellipodia and filopodia, have been observed in non-neuronal cells and the motile tips of neurons, the growth cones (Mitchison & Cramer 1996). The machinery for membrane protrusion is the cytoskeleton, in particular the microfilaments. Both the filopodia and lamellipodia contain dense arrays of actin filaments. In the filopodia they form parallel bundles, most of which are oriented with the barbed end (rapidly polymerizing end) towards the tip (Forscher & Smith 1988; Bridgman & Dailey 1989; Lewis & Bridgman 1992). In lamellipodia, actin filaments form an orthogonal network oriented at about 45° to the direction of protrusion with the rapidly polymerizing ends of the filaments directed towards the periphery (Small 1988; Small *et al*. 1995; Mitchison & Cramer 1996). Motile lamellipodia of neuronal growth cones also contain actin bundles that radiate from the centre of the growth cone, with their fast-growing ends directed towards the leading edge (Small *et al*. 1978; Lewis & Bridgman 1992).

The peripheral localization and polarity of the microfilaments suggest that actin polymerization may drive membrane protrusion, a hypothesis that has been supported by several different observations (Yamada *et al*. 1970, 1971; Marsh & Letourneau 1984; Bentley & Toroian-Raymond 1986; Okabe & Hirokawa 1991; Forscher *et al*. 1992; O'Connor & Bentley 1993; Neely & Gesemann 1994). If actin polymerization is to play a role in membrane protrusion, microfilament assembly must be controlled spatially and temporally. This regulation is likely to be mediated by microfilament-associated proteins (Cooper 1991; Hartwig & Kwiatkowski 1991; Condeelis 1993).

Gelsolin and profilin are two actin-binding proteins that affect the polymerization–depolymerization equilibrium of microfilaments, and also interact with the membrane (Yin 1988; Theriot & Mitchison 1993). Profilin primarily acts to promote actin filament growth in cells (Theriot & Mitchison 1993), while gelsolin severs microfilaments, creating actin nuclei for new polymerization (Yin 1988). The complementary effects of gelsolin and profilin, and their ability to bind to the membrane, led to the hypothesis that they synergistically regulate microfilament assembly in the submembraneous space (Yin 1988; Forscher 1989; Stossel 1989).

We show here that microfilaments, gelsolin and profilin are abundant in peripheral motile areas of leech neurites. These observations support the hypothesis that gelsolin and profilin together regulate the actin assembly that underlies neurite extension.

2. MATERIALS AND METHODS

(*a*) *Cell culture*

The techniques for the identification, isolation, and culture of neurons from leech central nervous system (CNS) have

*Author for correspondence.
(diana.neely@mcmail.vanderbilt.edu).
†Present address: Department of Pathology, Vanderbilt University, Medical Center North 1161 21st Street South, Nashville, TN 37232-2561, USA.

been described in detail (Fuchs *et al*. 1981; Dietzel *et al*. 1986; Neely 1993; Neely & Gesemann 1994). All experiments were performed with Retzius and AP cells. No differences in motility or cytoskeletal organization were observed in these two types of neurons.

(*b*) *Time-lapse video-microscopy*

Neurites of cells cultured in ConA-coated microwell dishes were observed with phase-contrast optics using an inverted microscope (Leitz-Labovert FS) and a 32 × objective (Leitz, Phaco1 32/0.40). Images were acquired every 4 min by a MTI CCD72 video camera (DAGE-MTI, Inc., Michigan City, IN, USA).

(*c*) *Immunocytochemistry*

Cells were cultured on glass Lab-Tek culture dishes overnight. They were then incubated with cytochalasin D (7.5 μM) for 30 min. Control cells were incubated with an equivalent volume of DMSO (final concentration 0.01%). Immediately after treatment they were fixed with 4% paraformaldehyde in PB buffer (0.12 M of phosphate, pH 7.4) for 30 min at room temperature and permeabilized with 1% Triton X-100 in PBS for 30 min at room temperature. The cells were incubated with primary antibodies for 3–4 h at room temperature or overnight at 4 °C. The following primary antibodies, diluted in PBS with 1% BSA, 1% FCS, 1% Triton X-100, were used: monoclonal anti-β-tubulin antibody (Boehringer, at 0.25 μg ml^{-1}), polyclonal anti-rat gelsolin antibody (donated by C. Chaponnier, University of Geneva, Switzerland; dilution 1 : 50), monoclonal anti-human gelsolin antibody (donated by C. Chaponnier (see Chaponnier *et al*. (1986)), also available from Sigma; dilution 1 : 100), polyclonal anti-sea urchin profilin antibody (donated by L. C. Smith, California Institute of Technology, Pasadena, CA (Smith *et al*. 1992); dilution 1 : 200). Secondary antibodies (FITC-coupled goat anti-rabbit IgG, Cappel #55662; FITC-coupled goat anti-mouse IgG, Cappel #55514, rhodamine-coupled goat anti-mouse IgG, Cappel #55539) were applied at a dilution of 1 : 200 in PBS with 1% Triton X-100 for 1–2 h at room temperature. The cells were mounted in PBS containing 50% glycerol, 0.5% n-propyl-gallate and 2.5% NaI. In double-labelling experiments, the two primary-antibodies were applied simultaneously, followed by a mixture of the two secondary antibodies. Microfilaments were stained with rhodamine-conjugated phalloidin (0.66 μM, Molecular Probes, Eugene, OR) in PBS with 1% Triton X-100 for 30 min at room temperature. In cells that were double-labelled for microfilaments and another cytoskeletal protein, rhodamine phalloidin and the secondary antibody were applied together. In control experiments, primary antibodies were omitted or the cells were incubated with a 100-fold excess of unlabelled phalloidin (Sigma). No staining was observed in the neurites of these cells. Neurons were observed with a Leitz DMRB microscope using 40× and 100× PL Fluotar objectives.

Because of the limited number of neurons that can be cultured in one experiment we did not attempt to analyse the reactivity of the antibodies by Western blotting extracts of cultured leech neurons. We were unable to detect immunoreactivity on Western blots of extracts of adult CNS or whole leech embryos with either the anti-gelsolin or the anti-profilin antibodies. The reason for this is unclear. Although we were unable to analyse these antibodies on Western blots, the reactivity of the antibodies appears to be specific for the following reasons: (i) all the antibodies we used have previously been characterized (personal communication and cited references above); (ii) the pattern of immunoreactivity of two different anti-gelsolin antibodies was the same; (iii) the labelling patterns of the profilin- and gelsolin antibodies in leech neurons and cell types from other species (Buss *et al*. 1992; Finkel *et al*. 1994; Tanaka *et al*. 1993) are very similar.

3. RESULTS

(*a*) *The peripheral motile regions of leech neurites are rich in microfilaments*

Retzius and anterior pagoda (AP) neurons were cultured on concanavalin A (ConA), on which they extend broad, veil-like neurites that allow for detailed analysis of the cytoskeleton (Chiquet & Acklin 1986; Neely 1993; Neely & Gesemann 1994). In phase contrast microscopy, phase-dark areas were often situated in the periphery of these lamellipodia and at the base of filopodia (figure 1*a* and figure 2*a*). By time-lapse video microscopy these phase-dark areas could be observed as they moved distally during lamellipodial protrusion (not shown). We analysed the cytoskeletal composition of these phase-dark regions and observed an enrichment in microfilaments (compare figure 2*a*,*b*); by contrast, microtubules are evenly distributed throughout the lamellipodia (not shown).

(*b*) *Gelsolin is enriched in motile areas and is bound to microfilaments*

We tested whether gelsolin is also enriched in motile areas of neurites. Immunocytochemical staining showed that this protein is associated with phase-dark areas of lamellipodia (compare figure 1*a*,*b*). Double labelling experiments showed that microfilaments and gelsolin are concentrated in peripheral parts of lamellipodia and in filopodia (compare figure 3*a*,*b*). In lamellipodia, gelsolin was associated with single identifiable microfilament bundles (compare figure 3*a*,*b*). Cytochalasin D induces loss of lamellipodia and filopodia in leech neurons and inhibits neurite outgrowth (Neely & Gesemann 1994). Here we show that the disruption of the microfilaments with cytochalasin D resulted in a general loss of microfilaments and randomly dispersed 'foci' of actin aggregates (figure 3*c*). Gelsolin was associated with these actin 'foci' (figure 3*d*), an observation that provides additional evidence that it is bound to microfilaments.

(*c*) *Distribution of profilin in leech neurites*

Profilin is abundant in leech neurites growing on ConA, and like gelsolin is localized to phase-dark areas rich in microfilaments (figures 1*c*, 2*c*). In contrast to gelsolin, profilin was not associated with single identifiable microfilament bundles (compare figure 2*b*,*c*), except on a few occasions where we observed colocalization with radially oriented microfilament bundles projecting into filopodia. The absence of an association between profilin and microfilament bundles is further demonstrated by the lack of association of profilin with cytochalasin-induced microfilament fragments (figure 4*a*,*b*).

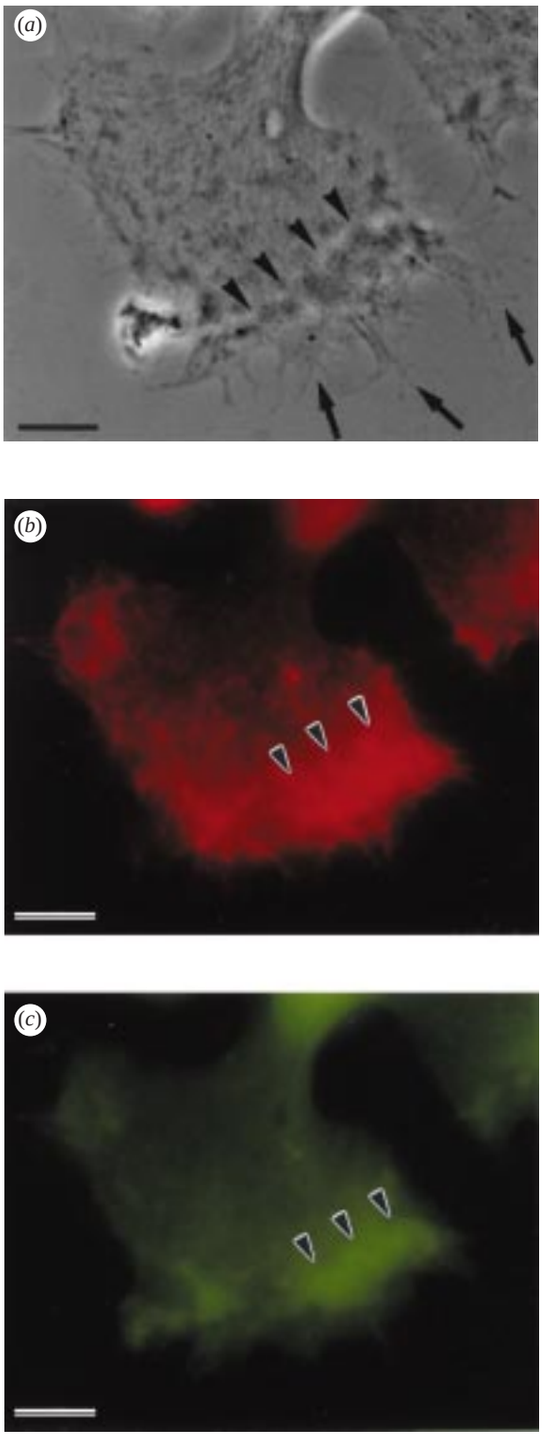

Figure 1. Neurite regions with high motility are phase-dark and rich in gelsolin and profilin. (*a*) One large veil-like neurite of a Retzius cell. A phase-dark front (arrow heads) lies just beneath an area of membrane extending many branches and filopodia (arrows). The other half of the veil is not associated with phase-dark areas and no membrane protrusions are obvious except for a few filopodia. Double staining with a monoclonal antibody against gelsolin (*b*) and a polyclonal antibody against profilin (*c*) shows that both proteins localize to the phase-dark area just beneath the area of membrane protrusion. The labelling seen at the middle upper edges of panels B and C is due to autofluorescence of the cell body and stump of the neuron. Scale bar, 10 µm.

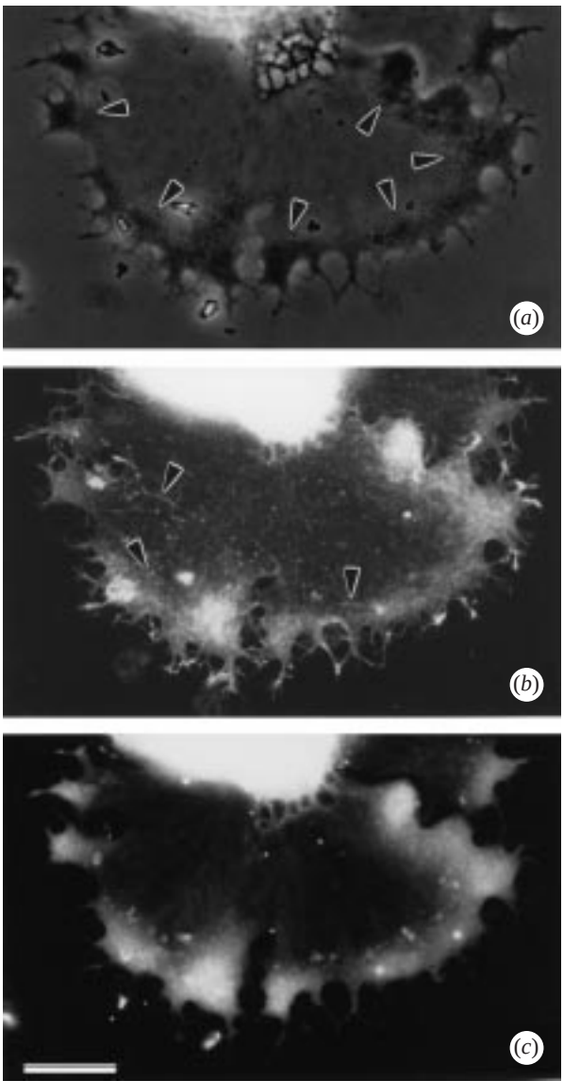

Figure 2. Phase-dark areas are rich in microfilaments and profilin. This Retzius neuron was double labelled with rhodamine phalloidin to visualize the microfilaments (*b*) and with an antibody against profilin (*c*). Most of the periphery of this single large lamellipodium appears phase-dark (*a*, arrow heads) and displays many filopodia-like extensions. The phase-dark areas are rich in microfilaments (*b*). Some microfilament bundles can be identified (*b*, arrow heads). Profilin is also concentrated in phase-dark areas rich in microfilaments, but a direct association with microfilament bundles was not observed (*c*; note lack of staining of microfilament bundles labelled in *b*). Scale bar, 25 µm.

4. DISCUSSION

We show here that motile areas of leech neurites contain high concentrations of microfilament, gelsolin, and profilin. Microfilament distribution has also been analysed in growing pioneer neurons in the grasshopper limb, and motile growth cones of *Aplysia* neurons. In these neurons, an accumulation of actin in filopodia and extending growth cone branches was observed, while reduction of microfilaments was characteristic for retracting areas (O'Connor & Bentley 1993; Forscher *et al*. 1992). Actin assembly has also been correlated spatially and temporally with

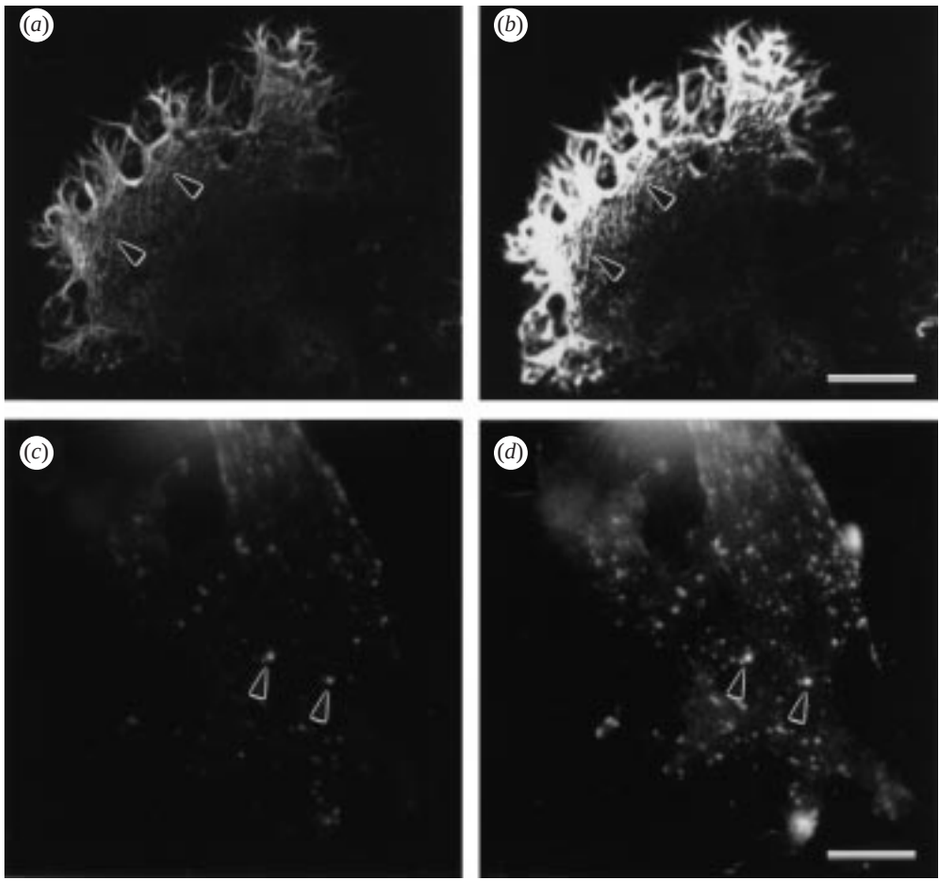

Figure 3. Gelsolin colocalizes with microfilaments. These pictures show two Retzius neurons that were double-stained with rhodamine phalloidin (*a* and *c*) and a monoclonal antibody against gelsolin (*b* and *d*). Gelsolin is enriched in the peripheral zones of the neurites and in the filopodia (*b*), regions that are rich in microfilaments (*a*). Gelsolin (arrow heads in *b*) colocalizes with single identifiable microfilament bundles (arrow heads in *a*). After treatment with cytochalasin D, the microfilaments appear in dot-like aggregates in this Retzius cell lamellipodium (*c*, arrow heads). Gelsolin colocalizes with these microfilament aggregates (*d*, arrow heads). Scale bars: (*a*) and (*b*), 25 μm; (*c*) and (*d*), 10 μm.

membrane protrusion in neutrophils, *Dictyostelium* amoebae, platelets, and malignant tumour cells. In these cells actin polymerization usually precedes lamellipodial extension, and the newly assembled actin is localized in the motile lamellipodia (Omann *et al*. 1987; Hall *et al*. 1988; Jones *et al*. 1991; Coates *et al*. 1992; Hartwig 1992).

Areas in leech neurites that are rich in microfilaments also contain high concentrations of profilin. This protein is not directly associated with microfilament bundles except for rare colocalization with radially oriented microfilament bundles extending into filopodia. Profilin has been observed in cultured cortical neurons where it shows a punctate staining pattern in the neurites and the core of the growth cones (Faivre-Sarrailh *et al*. 1993). In fibroblasts, profilin is enriched in motile lamellipodia and pseudopodial lobes, and colocalizes with centripetally oriented microfilament bundles. As in leech neurites, no association was observed with circumferentially oriented microfilament bundles (Buss *et al*. 1992). In Chinese hamster ovary cells, profilin is present in high concentrations in areas of membrane ruffling (Finkel *et al*. 1993). The presence of this protein in areas of cell motility in several different cell types suggests that it is involved in the regulation of actin dynamics. This hypothesis is supported by experiments aimed at analysing the role of profilin in living cells. Profilin has been shown to cause polymerization/stabilization or destabilization of microfilaments in different cell culture systems (Haarer *et al*. 1990; Cao *et al*. 1992; Cooley *et al*. 1992; Balasubramanian *et al*. 1994; Finkel *et al*. 1994; Giuliano & Taylor 1994). No studies addressing the role of profilin in neurons have been performed, yet its localization to regions of high motility (this study) suggests that profilin may also play a role in neuronal cell motility.

Gelsolin, like profilin, was present in regions of leech neurites that showed high motility and were rich in microfilaments. In contrast to profilin, gelsolin was directly associated with the microfilament bundles and was abundant in the filopodia. Colocalization of gelsolin and microfilaments has been observed in growth cones of PC12 cells, but is less clear in dorsal root ganglion neurons (Tanaka *et al*. 1993). Gelsolin is also present in neurites and growth cones of cultured sympathetic neurons (Petrucci *et al*. 1983). This

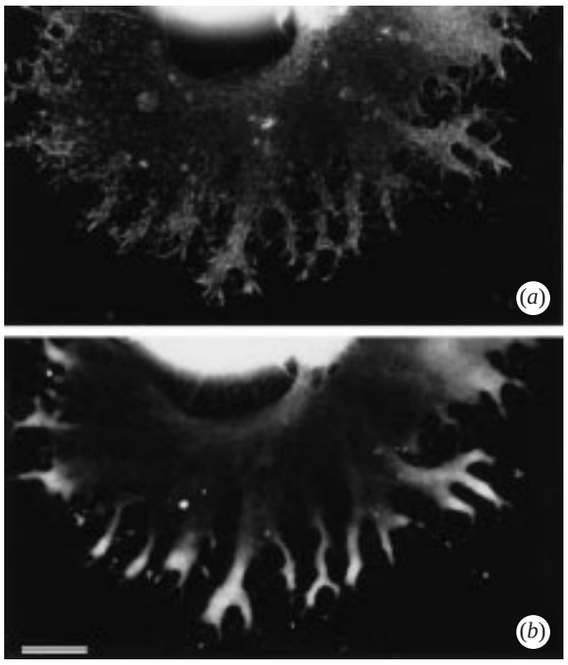

Figure 4. Profilin is not associated with microfilament bundles. This Retzius cell was treated with cytochalasin, which resulted in the disruption of microfilaments and the appearance of dispersed microfilament aggregates (*a*). Profilin did not colocalize with these aggregates (*b*). Scale bar, 25 μm.

abundance of gelsolin in growing cultured neurons and its transient *in vivo* expression in Purkinje cells (Legrand *et al*. 1986) and retinal ganglion neurons (Legrand *et al*. 1991) in the developing rabbit suggest an involvement of gelsolin in cytoskeletal changes that control neurite extension. This hypothesis is supported by the role gelsolin plays in the motility of non-neuronal cells (Jockusch *et al*. 1985; Weeds *et al*. 1985; Cooper *et al*. 1987; Sanger *et al*. 1987; Huckriede *et al*. 1990; Cunningham *et al*. 1991).

To our knowledge this is the first comparison of the subcellular distribution of profilin and gelsolin in the same cell, and the first detailed analysis of the distribution of these two proteins in neurites. Our observation that gelsolin and profilin colocalize with the microfilament-rich motile areas of neurites supports the hypothesis that gelsolin and profilin coordinately regulate microfilament dynamics underlying neurite extension. The organization of the microfilaments in neurites is influenced by extracellular cues eliciting transmembrane signals (Lankford & Letourneau 1989; Fan *et al*. 1993; Lin & Forscher 1993; O'Connor & Bentley 1993; Neely & Gesemann 1994). The sensitivity of gelsolin and profilin to calcium and polyphosphoinositides, and their ability to bind to the plasma membrane (Yin 1988; Forscher 1989; Stossel 1989), make these proteins likely candidates for messengers carrying extracellular signals to the cytoskeleton of the neurite. The abundance of gelsolin in the filopodia of leech neurites is intriguing, since this organelle is likely to be involved in the reception, integration, and transmission of extracellular signals.

We thank our colleagues Dr John G. Nicholls for his unfailing support throughout the work and for his crucial suggestions for improving the manuscript, and Dr W. Adams for his skilful help with the video microscopy. This work was supported by grant no. 3127814.89 from the Swiss Nationalfonds to Dr John G. Nicholls.

REFERENCES

Balasubramanian, M. K., Hirani, B. R., Burk, J. D. & Gould, K. L. 1994 The *Schizosaccharomyces pombe cdc3*$^{+}$ gene encodes a profilin essential for cytokinesis. *J. Cell Biol*. **125**, 1289–1301.

Bentley, D. & Toroian-Raymond, A. 1986 Disoriented path-finding by pioneer neurone growth cones deprived of filopodia by cytochalasin treatment. *Nature* **323**, 712–715.

Bridgman, P. C. & Dailey, M. E. 1989 The organization of myosin and actin in rapid frozen nerve growth cones. *J. Cell Biol*. **108**, 95–109.

Buss, F., Temm-Grove, C., Henning, S. & Jockusch, B. M. 1992 Distribution of profilin in fibroblasts correlates with the presence of highly dynamic actin filaments. *Cell. Motil. Cytoskeleton* **22**, 51–61.

Cao, L.-G., Babcock, G. G., Rubenstein, P. A. & Wang, Y.-L. 1992 Effects of profilin and profilactin on actin structure and function in living cells. *J. Cell Biol*. **117**, 1023–1029.

Chaponnier, C., Janmey, P. A. & Yin, H. L. 1986 The actin filament-severing domain of plasma gelsolin. *J. Cell Biol*. **103**, 1473–1481.

Chiquet, M. & Acklin, S. E. 1986 Attachment to ConA or extracellular matrix initiates rapid sprouting by cultured leech neurons. *Proc. Natn. Acad. Sci. USA* **83**, 6188–6192.

Coates, T. D., Watts, R. G., Hartman, R. & Howard, T. H. 1992 Relationship of F-actin distribution to development of polar shape in human polymorphonuclear neutrophils. *J. Cell Biol*. **117,** 765–774.

Condeelis, J. 1993 Life at the leading edge: the formation of cell protrusions. *A. Rev. Cell Biol*. **9**, 411–444.

Cooley, L., Verheyen, E. & Ayers, K. 1992 *Chickadee* encodes a profilin required for intercellular cytoplasm transport during *Drosophila* oogenesis. *Cell* **69**, 173–184.

Cooper, J. A. 1991 The role of actin polymerization in cell motility. *A. Rev. Physiol*. **53**, 585–605.

Cooper, J. A., Bryan, J., Schwab III, B., Frieden, C., Loftus, D. J. & Elson, E. L. 1987 Microinjection of gelsolin into living cells. *J. Cell Biol*. **104**, 491–501.

Cunningham, C. C., Stossel, T. P. & Kwiatkowski, D. J. 1991 Enhanced motility in NIH 3T3 fibroblasts that overexpress gelsolin. *Science* **251**, 1233–1236.

Dietzel, I. D., Drapeau, P. & Nicholls, J. G. 1986 Voltage dependence of 5-hydroxytryptamine release at a synapse between identified leech neurones in culture. *J. Physiol. (Lond.)* **372**, 191–205.

Faivre-Sarrailh, C., Lena, J.Y., Had, L., Vignes, M. & Lindberg, U. 1993 Location of profilin at presynaptic sites in the cerebellar cortex; implication for the regulation of the actin-polymerization state during axonal elongation and synaptogenesis. *J. Neurocytol*. **22**, 1060–1072.

Fan, J., Mansfield, S. G., Redmond, T., Gordon-Weeks, P. R. & Raper, J. A. 1993 The organization of F-actin and microtubules in growth cones exposed to a brain-derived collapsing factor. *J. Cell Biol*. **121**, 867–878.

Finkel, T., Theriot, J. A., Dise, K. R., Tomaselli, G. F. & Goldschmidt-Clermont, P. J. 1994 Dynamic actin structures stabilized by profilin. *Proc. Natn. Acad. Sci. USA* **91**, 1510–1514.

Forscher, P. 1989 Calcium and polyphosphoinositide control of cytoskeletal dynamics. *Trends Neurosci*. **12**, 468–474.

Forscher, P. & Smith, S. J. 1988 Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. *J. Cell Biol*. **107**, 1505–1516.

Forscher, P., Lin, C. H. & Thompson, C. 1992 Novel form of growth cone motility involving site-directed actin filament assembly. *Nature* **357**, 515–518.

Fuchs, P. A., Nicholls, J. G. & Ready, D. F. 1981 Membrane properties and selective connexions of identified leech neurones in culture. *J. Physiol. (Lond.)* **316**, 203–223.

Giuliano, K. A. & Taylor, D. L. 1994 Fluorescent actin analogs with a high affinity for profilin *in vitro* exhibit an enhanced gradient of assembly in living cells. *J. Cell Biol.* **124**, 971–983.

Haarer, B. K., Lillie, S. H., Adams, A. E. M., Magdolen, V., Bandlow, W. & Brown, S. S. 1990 Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. *J. Cell Biol.* **110**, 105–114.

Hall, A. L., Schlein, A. & Condeelis, J. 1988 Relationship of pseudopod extension to chemotactic hormone-induced actin polymerization in amoeboid cells. *J. Cell Biochem.* **37**, 285–299.

Hartwig, J. H. 1992 Mechanisms of actin rearrangements mediating platelet activation. *J. Cell Biol.* **118**, 1421–1442.

Hartwig, J. H. & Kwiatkowski, D. J. 1991 Actin-binding proteins. *Curr. Opin. Cell Biol.* **3**, 87–97.

Huckriede, A., Fuechtbauer, A., Hinssen, H., Chaponnier, C., Weeds, A. & Jockusch, B. M. 1990 Differential effects of gelsolins on tissue culture cells. *Cell. Motil. Cytoskeleton* **16**, 229–238.

Jockusch, B. M., Fuechtbauer, A., Wiegand, C. & Hoener, B. 1985 Dynamics of microfilament organization and membrane anchorage. In *Hormones and cell regulation*, vol. 9 (ed. J. E. Dumont, B. Hamprecht & J. Nunez), pp. 135–142. Amsterdam: Elsevier.

Jones, J., Segall, J. & Condeelis, J. 1991 Molecular analysis of amoeboid chemotaxis: parallel observations in amoeboid phagocytes and metastatic tumor cells. In *Cell motility factors* (ed. I. D. Goldberg), pp. 116. Basel, Switzerland: Birkhaeuser.

Lankford, K. L. & Letourneau, P. C. 1989 Evidence that calcium may control neurite outgrowth by regulating the stability of actin filaments. *J. Cell Biol.* **109**, 1229–1243.

Legrand, C., Ferraz, C., Clavel, M.-C. & Rabié, A. 1986 Immunocytochemical localisation of gelsolin in oligodendroglia of the developing rabbit central nervous system. *Devl Brain Res.* **30**, 231–235.

Legrand, C., Ferraz, C., Clavel, M.-C. & Rabié, A. 1991 Distribution of gelsolin in the retina of the developing rabbit. *Cell. Tissue Res.* **264:** 335–338.

Lewis, A. K. & Bridgman, P. C. 1992 Nerve growth cone lamellipodia contain two populations of actin filaments that differ in organization and polarity. *J. Cell Biol.* **119**, 1219–1243.

Lin, C.-H. & Forscher, P. 1993 Cytoskeletal remodeling during growth cone–target interactions. *J. Cell Biol.* **121**, 1369–1383.

Marsh, L. & Letourneau, P. C. 1984 Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. *J. Cell Biol.* **99**, 2041–2047.

Mitchison, T. J. & Cramer, L. P. 1996 Actin-based cell motility and cell locomotion. *Cell* **84**, 371–379.

Neely, M. D. 1993 Role of substrate and calcium in neurite retraction of leech neurons following depolarization. *J. Neurosci.* **13**, 1292–1301.

Neely, M. D. & Gesemann, M. 1994 Disruption of microfilaments in growth cones following depolarization and calcium influx. *J. Neurosci.* **14**, 7511–7520.

O'Connor, T. P. & Bentley, D. 1993 Accumulation of actin in subsets of pioneer growth cone filopodia in response to neural and epithelial guidance cues *in situ*. *J. Cell Biol.* **123**, 935–948.

Okabe, S. & Hirokawa, N. 1991 Actin dynamics in growth cones. *J. Neurosci.* **11**, 1918–1929.

Omann, G. M., Allen, R. A., Bokoch, G. M., Painter, R. G., Traynor, A. E. & Sklar, L. A. 1987 Signal transduction and cytoskeletal activation in the neutrophil. *Physiol. Rev.* **67**, 285–322.

Petrucci, T. C., Thomas, C. & Bray, D. 1983 Isolation of a Ca2+-dependent actin-fragmenting protein from brain, spinal cord, and cultured neurones. *J. Neurochem.* **40**, 1507–1516.

Sanger, J. M., Mittal, B., Wegner, A., Jockusch, B. M. & Sanger, J. W. 1987 Differential response of stress fibers and myofibrils to gelsolin. *Eur. J. Cell Biol.* **43**, 421–428.

Small, J. V. 1988 The actin cytoskeleton. *Electron Microsc. Rev.* **1**, 155–174.

Small, J. V., Herzog, M. & Anderson, K. 1995 Actin filament organization in the fish keratocyte lamellipodium. *J. Cell Biol.* **129**, 1275–1286.

Small, J. V., Isenberg, G. & Celis, J. E. 1978 Polarity of actin at the leading edge of cultured cells. *Nature* **272**, 638–639.

Smith, L. C., Britten, R. J. & Davidson, E. H. 1992 *SpCoelI*: a sea urchin profilin gene expressed specifically in coelomocytes in response to injury. *Molec. Biol. Cell* **3**, 403–414.

Stossel, T. P. 1989 From signal to pseudopod. How cells control cytoplasmic actin assembly. *J. Biol. Chem.* **264**, 18261–18264.

Tanaka, J., Kira, M. & Sobue, K. 1993 Gelsolin is localized in neuronal growth cones. *Devl Brain Res.* **76**, 268–271.

Theriot, J. A. & Mitchison, T. J. 1993 The three faces of profilin. *Cell* **75**, 835–838.

Weeds, A. G., Harris, H. E., Gooch, J. & Pope, B. 1985 Actin capping proteins. In *Hormones and cell regulation*, vol. 9 (ed. J. E. Dumont, B. Hamprecht & J. Nunez), pp. 143–165. Amsterdam: Elsevier.

Yamada, K. M., Spooner, B. S. & Wessells, N. K. 1970 Axon growth: roles of microfilaments and microtubules. *Proc. Natn. Acad. Sci. USA* **66**, 1206–1212.

Yamada, K. M., Spooner, B. S. & Wessells, N. K. 1971 Ultrastructure and function of growth cones and axons of cultured nerve cells. *J. Cell Biol.* **49**, 614–635.

Yin, H. L. 1988 Gelsolin: calcium- and polyphosphoinositide-regulated actin-modulating protein. *BioEssays* **7**, 176–179.

Received 16 June 1997; accepted 10 July 1997