The Role of Cytoskeleton in Organizing Growth Cones: a Microfilament-associated Growth Cone Component Depends Upon Microtubules for Its Localization

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Abstract. We are interested in the relationship between the cytoskeleton and the organization of polarized cell morphology. We show here that the growth cones of hippocampal neurons in culture are specifically stained by a monoclonal antibody called 13H9. In other systems, the antigen recognized by 13H9 is associated with marginal bands of chicken erythrocytes and shows properties of both microtubule- and microfilament-associated proteins (Birgbauer, E., and F. Solomon. 1989. J. Cell Biol. 109:1609–1620). This dual nature is manifest in hippocampal neurons as well. At early stages after plating, the antibody stains the circumferential lamellipodia that mediate initial cell spreading. As processes emerge, 13H9 staining is heavily concentrated in the distal regions of growth cones, particularly in lamellipodial fans. In these cells, the 13H9 staining is complementary to the localization of assembled microtubules. It colocalizes partially, but not entirely, with phalloidin staining of assembled actin. Incubation with nocodazole rapidly induces microtubule depolymerization, which proceeds in the distal-to-proximal direction in the processes. At the same time, a rapid and dramatic redistribution of the 13H9 staining occurs; it delocalizes along the axon shaft, becoming clearly distinct from the phalloidin staining and always remaining distal to the receding front of assembled microtubules. After longer times without assembled microtubules, no staining of 13H9 can be detected. Removal of the nocodazole allows the microtubules to reform, in an ordered proximal-to-distal fashion. The 13H9 immunoreactivity also reappears, but only in the growth cones, not in any intermediate positions along the axon, and only after the reformation of microtubules is complete. The results indicate that the antigen recognized by 13H9 is highly concentrated in growth cones, closely associated with polymerized actin, and that its proper localization depends upon intact microtubules.

The ability of neurons to develop stereotyped morphologies, suitable for the functions of each individual cell, depends upon the activities of their growth cones. All of the motility of nerve cells is confined to these structures at the tips of growing axons and dendrites. Although in migratory cells the position of the leading edge can change frequently, the position of the growth cones persists once they are specified. There have been many analyses of the behavior and properties of growth cones, and of the cues that guide growth cones to their appropriate destinations (for reviews, see Landis, 1983; Lockerbie, 1987; Bray and Hollenbeck, 1988). However, rather less is known about the endogenous determinants of growth cone formation and organization. How does the cell specify the number and position of growth cones? How is growth cone motility coupled to the function of cytoskeletal elements within the growing fiber?

Arguably, the unique properties of growth cones might arise not only from the presence of unique constituents but from unique organization of elements also found elsewhere in the cell (Bray and Hollenbeck, 1988). Such subtleties may be extremely difficult to identify and analyze, and so considerable attention has been directed toward identifying proteins restricted in growth cones. These include actin and actin-related proteins involved in motility (Bray and Hollenbeck, 1988), as well as the membrane-associated protein GAP-43, which has been hypothesized to play a role in regulating neurite growth (Skene, 1989). The latter is concentrated in axonal but not dendritic growth cones (Goslin et al., 1988).

To analyze the mechanism of growth cone formation and migration, we are studying primary neurons in culture. The cells we use, from the embryonic rat hippocampus, express a morphology in culture closely related to their morphology in vivo. In particular, they extend a single axon and several dendrites, each with a clearly defined growth cone (Dotti et al., 1988). We describe here the staining of these cells by monoclonal antibody 13H9. The antibody is characterized in detail in the accompanying manuscript (Birgbauer and Solomon, 1989). Briefly, the antigen it recognizes is a marginal band associated protein, and shows properties of both...
microtubule- and microfilament-associated proteins. Antibody 13H9 stains almost exclusively the growth cones of the processes in these cells, with very little staining elsewhere. The pattern of 13H9 immunoreactivity is similar but not identical to that of phalloidin staining of assembled actin. Exposure to a microtubule disassembly drug causes a dramatic disruption of the 13H9 staining: loss of typical structure in the growth cone, delocalization proximally along the process, and eventually disappearance of all staining. Microtubule depolymerization also induces a clearer distinction between phalloidin and 13H9 staining. Upon reversal of microtubule assembly, the 13H9 reactivity reappears in growth cones, but only after the microtubules are completely assembled in the process. We conclude that 13H9 antigen is a growth cone component that is closely associated with microfilaments, but that interacts, directly or indirectly, with microtubules.

**Materials and Methods**

**Hippocampal Cell Cultures**

Cell cultures were prepared from hippocampi of 18-d-old fetal rats, as described previously (Banker and Cowan, 1977; Bartlett and Banker, 1984). In brief, cell suspensions were obtained by trypsin treatment (0.25% for 15 min at 37°C) and trituration through a fire-polished Pasteur pipette. The cells, at a density of ~600/cm², were plated onto poly-L-lysine-treated glass coverslips in MEM containing 10% horse serum. After 3-4 h, when the cells had attached, the coverslips were transferred to dishes containing an astroglial monolayer (Booher and Sensenbrenner, 1972), so that they faced, but did not contact, the glia. Previous work has shown that this coculture technique enhances neuronal survival (Banker, 1980). The cells were maintained in MEM containing the N2 supplements of Bottenstein and Sato (1979) plus ovalbumin (0.1%) and pyruvate (0.1 mM).

**Antibodies**

Antibody 13H9 is characterized in the preceding paper (Birgbauer and Solomon, 1989). It is a mouse monoclonal of the IgM class. Tubulin was visualized with antibody 429 (Kim et al., 1987), a rabbit polyclonal antibody raised against a peptide representing the 12 carboxy-terminal amino acids of chicken beta-2 tubulin. It reacts with tubulins from many sources.

**Immunofluorescence Microscopy**

Cultures were fixed for 30 min with 4% formaldehyde in PBS containing 0.12 M sucrose, then permeabilized in 0.5% Triton X-100 in PBS, and then rinsed in PBS. They were blocked with 10% BSA for 30 min at 37°C. Antibodies 13H9 (1:100) and 429 (1:4,000) were diluted in 1% BSA in PBS. The cultures were then rinsed with PBS and incubated for 30 min at 37°C with 1:400 dilutions of either FITC-labeled goat anti-mouse IgM or rhodamine-labeled goat anti-rabbit IgG (both second antibodies obtained from Cappel Laboratories, Malvern, PA). Coverslips were mounted in PBS/glycerol (1:1) containing 0.2% p-phenylene-diamine (Johnson et al., 1981). In some experiments, rhodamine-labeled phalloidin (Molecular Probes Inc., Eugene, OR) was included with the secondary antibody to visualize filamentous actin. Cells were photographed using 63× (1.4 NA) or 100× (1.3 NA) objectives (Carl Zeiss, Inc., Thornwood, NY), using film (Tri-X; Eastman Kodak Co., Rochester, NY) developed in Diafine.

**Nocodazole Treatment**

Nocodazole (Altrich Chemical Co., Milwaukee, WI) was added to cultures at a final concentration of 10 μg/ml (from a stock solution of 1 mg/ml in DMSO). Cultures were incubated with nocodazole for 10-30 min, rinsed in PBS, and fixed for immunofluorescence as above. For reversal experiments, cultures exposed to nocodazole for 30 min were rinsed 2-3 times over 10 min, and then returned to the incubator for 30-120 min. In some experiments, the axons of 1-d-old cells were transected (Goslin and Banker, 1989) before treatment with nocodazole.

**Results**

**Localization of 13H9 Staining in Cultured Hippocampal Neurons**

Shortly after the neurons attach to the substratum, phase-contrast microscopy reveals extensive lamellipodia in a circumferential apron. These structures are observed in other cells freshly plated on culture substrata; characteristically, they display radial striations and are known to be highly dynamic (Albrecht-Buehler, 1976). Antibody 13H9 stains these lamellipodia, with highest intensity in the radial phase-dense striations (Fig. 1, a and b). The cytoplasm of the cell body shows diffuse staining.

After 24 h in culture, almost all neurons have extended a single axon and several minor processes that eventually become dendrites (Dotti et al., 1988). At this stage, the antibody 13H9 staining is heavily concentrated at the growth cones of both types of processes (Fig. 1, e and f). In the cell bodies, the antibody diffusely stains the cortical cytoplasm. Although in these images the processes themselves appear unstained, longer exposures do reveal some staining along their lengths (Fig. 3).

A more detailed view of the antibody 13H9 staining pattern in the growth cone is provided by the high magnification images in Fig. 1, c and d. The antibody labeling clearly is concentrated at the more peripheral portion of the growth cone, which resembles a lamellipodium. As in early spreading cells, the more intense antibody staining frequently is coincident with radial striations visible by phase-contrast microscopy. This pattern of staining is seen in virtually every growth cone identified by phase-contrast microscopy. The staining patterns of growth cones on axons and on minor processes are indistinguishable from one another. In addition, in older cultures, when the minor processes have become dendrites and are elongating, the staining patterns are the same as those shown here.

**The Staining Patterns of Antibody 13H9 and Phalloidin Are Similar but Distinguishable**

In chicken erythrocytes, antibody 13H9 staining colocalizes with antitubulin staining, and with the most intense staining of filamentous actin. In fibroblasts, it does not colocalize precisely with either cytoskeletal structure (Birgbauer and Solomon, 1989). The situation in hippocampal neurons is quite different. There is little antibody 13H9 staining in the processes, which contain many microtubules (Fig. 4). However, there is a close correspondence at all stages between antibody 13H9 staining and the distribution of polymerized actin. Fig. 2 shows phase-contrast and immunofluorescence micrographs of cells double stained with antibody 13H9 and phalloidin. Cells in the early stages of spreading show staining with both reagents in the lamellipodia, again concentrated in the radial striations (Fig. 2, a-c). Typically, the phalloidin staining appears more diffuse than the 13H9 staining. After the cells have extended processes, both the antibody 13H9 staining and the phalloidin staining are concentrated in growth cones of both axons and minor processes, and in the lamellipodia still associated with the cell body (Fig. 2, d-f). As the processes elongate further, and the somatic lamellipodia disappear, the 13H9 and phalloidin staining are essentially confined to growth cones.
Figure 1. The distribution of 13H9 immunoreactivity in cultured hippocampal neurons. Phase contrast and immunofluorescence micrographs of hippocampal neurons at different stages of development, stained with antibody 13H9. (a and b) A freshly plated cell, showing 13H9 staining of the lamellipodia, with highest intensity in the phase-dense striations; c-f, Cell (e, f) and high magnification view of a growth cone (c, d) after 1 d in culture. The cell (e, f) displays a single axon and several minor processes. The 13H9 immunoreactivity is heavily concentrated in all growth cones, with some diffuse staining of the cortical cytoplasm of the soma. Little staining is detected along the processes. (c and d) Higher magnification view of a growth cone, showing 13H9 staining in the lamellipodia of the growth cone, again with more intense staining coincident with the phase-dense striations. Bars, (a and c) 5 μm; (e) 10 μm.

Although both reagents stain the same parts of the cell, there are clear differences in the two staining patterns, illustrated by the higher magnification views of growth cones shown in Fig. 3 (a-c and d-f). Comparisons of these images show that the localizations of the two reagents overlap in detail; e.g., in the striated structures of growth cone lamellipodia. Near those structures are domains that contain only F-actin or 13H9 fluorescence. In general, there is 13H9 staining in a position slightly distal to the region of overlap, while the phalloidin staining extends proximally into the central portion of the growth cone. Another difference is in the cell bodies, where the cortical regions apparently are stained more intensely with the antibody than with phalloidin.

Although the major proportion of both 13H9 and phalloidin staining is in growth cones, faint immunoreactivity can be detected along the lengths of the processes themselves. This staining, which appears as streaks and spots along both axons and minor processes, is shown by the longer exposures used to produce the micrographs in Fig. 3, j and k. Again, the distribution of 13H9 immunoreactivity colocalizes closely but not precisely with the distribution of polymerized actin. The staining of both extends into filopodia.

The close correlation between phalloidin and 13H9 staining is unique to neurons. In particular, in the glial cells found in the same culture as the hippocampal neurons, the two patterns are markedly different (Fig. 3, g-i). In these cells, as
in fibroblasts (Birgbauer and Solomon, 1989), phalloidin stains primarily cytoplasmic actin filaments and, less intensely, the edges of cells, while 13H9 staining is largely confined to the periphery of the cells and stains the cytoplasmic filaments relatively faintly. In neuroblastoma cells, both 13H9 and phalloidin stain processes, but the former is at the edges of the processes and in filopodia, while the latter is concentrated in the core of the processes (E. Birgbauer, unpublished results).

Microtubule Depolymerizing Drugs Significantly Disrupt 13H9 Staining

In chicken erythrocytes, the 13H9 staining is confined to the position of the marginal band, where all the cellular microtubules and most but not all of the assembled actin is localized (Birgbauer and Solomon, 1989). In hippocampal neurons, however, the distribution of 13H9 staining is coincident only with that of F-actin, and not with microtubules (Fig. 4, a–c). Assembled tubulin is concentrated in the cell body and along the axons and minor processes. The majority of the tubulin staining terminates at the base of the growth cone. At the level of light microscopy, the distal edge of the antitubulin staining and the proximal edge of the 13H9 staining appear closely apposed, although at this resolution it is not possible to determine if they overlap.

The pattern of 13H9 staining is significantly disrupted by incubation with the microtubule depolymerizing drug nocodazole. The effect of this drug on microtubule assembly in these cells is rapid and readily observable, since the protocol used for fixation and permeabilization apparently permits the release of unassembled tubulin. This conclusion is supported by the staining of flat, nonneuronal cells in the same cultures (data not shown) and by the total absence of tubulin staining in neurons after long incubations in nocodazole (see below). In the cell shown in Fig. 4, d–f, fixed after 10 min in nocodazole, there is no antitubulin staining in the distal portion of the axon. In the same cell, the radial pattern of 13H9 staining typical of normal growth cones is disrupted, as is the morphology of the growth cone itself. The 13H9 reactivity appears in randomly oriented bars, but is still confined to the distal tip of the cell.

With increasing time in nocodazole, the disruptions of microtubules and of 13H9 localization become more extreme. No antitubulin staining is found in distal regions of the axons, and the 13H9 staining is displaced from the growth cone and instead is present as streaks in the distal axon. The changes in these two staining patterns appear to be coordinated (Fig. 4, g–i, j–l). In particular, from images of many cells, we could detect a close spatial relationship between the distal edge of the receding tubulin staining and the
proximal edge of the 13H9 staining. In every case, the 13H9 staining was confined to the region distal to the remaining microtubules. In some cells, incubated for 30 min or longer, no 13H9 staining is detected in the axon (Fig. 4, m–o). A similar sequence of events is observed in the minor processes. This relationship between microtubule assembly and 13H9 staining is unique to growth cones. In freshly plated cells, like those shown in Fig. 1, a and b, as well as in chicken embryo fibroblasts (data not shown) or chicken erythrocytes (Birgbauer and Solomon, 1989), the localization of the 13H9 antigen is not substantially affected by depolymerization of microtubules.

The appearance of 13H9 staining in proximal regions of the axon upon microtubule depolymerization could arise from delocalization of the material ordinarily associated with the growth cone. Alternatively, it could represent accumulation of new 13H9 antigen transported from the cell body that failed to localize properly. To distinguish between these possibilities, we transected axons, and then incubated the cells with nocodazole for 10–30 min before fixation. The 13H9 staining shifted from the growth cone to fill the distal portion of the transected axon. However, no staining was observed in the axonal stump proximal to the site of transection (data not shown). We conclude that microtubule depolymerization causes delocalization of the 13H9 antigen from the growth cone.

The pattern of phalloidin staining is also affected by microtubule disassembly, although differently from 13H9 staining. When most (Fig. 5, a and b) or all (Fig. 5, c and d) of the 13H9 staining has disappeared from the processes after nocodazole treatment, most phalloidin staining remains at the distal end of the process. In some cells, phalloidin staining can be detected along the processes as well. In most cells, however, the phalloidin staining and 13H9 staining in the distal domains of processes are decisively separated from one another after nocodazole treatment. Direct comparison of the 13H9 and phalloidin patterns is problematic, however, because 13H9 presumably recognizes the antigen regardless of its associations, while phalloidin only recognizes actin in assembled form. Also, as pointed out above, the fixation...
Figure 4. Simultaneous localization of 13H9 staining and tubulin following microtubule depolymerization. Phase-contrast micrographs (a, d, g, j, and m), 13H9 (b, e, h, k, and n) and antitubulin (c, f, i, l, and o) staining. In untreated cells (a-c), the distribution of 13H9 antigen (b) and tubulin (c) are very nearly complementary. Tubulin staining fills the axon, and terminates in the central region of the growth cone. The 13H9 labeling extends peripherally from the central region of the growth cone in the radial striations noted above. After 10 min in 10 μg/ml nocodazole (d-f), the tubulin staining in the axon (f) has receded from the growth cone, and the pattern of 13H9 staining in the growth cone is markedly disrupted (e). After 20 min in nocodazole (g-i), the tubulin staining (i) continues to recede, and the 13H9 staining (h) delocalizes proximally from its original position. The arrows indicate the proximal boundary of 13H9 staining. The arrowheads indicate a distal portion of the axon devoid of 13H9 staining but displaying lamellipodia. After 30 min in nocodazole (j-l), the antitubulin staining (l, o) is frequently undetectable in the axon and the 13H9 staining is either restricted to the most proximal portion of the axon (k) or is undetectable (n). A similar sequence of events occurs in minor processes. Bars, 10 μm.
protocol may permit partial or complete extraction of some protein. However, another growth cone component GAP-43 behaves in the same way as the F-actin; even after long incubations in nocodazole, GAP-43 usually remains discretely localized to the distal tip of the axon (data not shown).

13H9 Staining Returns to Growth Cones after Microtubule Reassembly

Microtubule depolymerization mediated by nocodazole is readily reversible simply by exchanging medium. In cultured hippocampal neurons, however, the recovery of microtubule assembly proceeds quite slowly. Even 30 min after removal of the drug, most processes are still devoid of antitubulin staining as well as 13H9 staining. Significant microtubule repolymerization does occur after 1 h of recovery (Fig. 6, a–c). From observing several intermediate stages, we concluded that the pattern of microtubule recovery is essentially the reverse of the pattern of microtubule loss during exposure to nocodazole. That is, there is a clearly demarcated front of antitubulin staining which extends from the cell body; distal to this front, the axons and minor processes are unstained. No 13H9 staining is detected in those processes before microtubule assembly is complete. In some cells, there is no detectable 13H9 staining in the growth cone even after tubulin staining has returned to the entire axon (Fig. 6, d–f).

The timing of full restoration of both tubulin and 13H9 staining is related to the length of the process. It is completely restored in the shorter, minor processes before it is restored in axons, and it appears first in the growth cone of the shorter branch of a bifurcating axon (Fig. 6, g–i). 2 h after the drug is removed, 13H9 reactivity is fully restored in all growth cones, displaying the same radial striations of the original growth cones. In the fully reversed state, the edges of the tubulin and 13H9 staining are again quite close to one another as they were before drug treatment.

These experiments suggest that the microtubule-dependent
Figure 5. Simultaneous localization of 13H9 and phalloidin staining following microtubule depolymerization. Two cells are shown, fixed after 20 min (a and b) and 30 min (c and d) in nocodazole. 13H9 staining is not detected in the distal portions of the axon after 20 min (a), although phalloidin does stain those regions (arrows). There is overlapping of the two patterns in the proximal portion of the axon, however. At the latter time, the 13H9 staining (c) is not detectable in the growth cone or axon (arrows); the phalloidin labeling (d) is somewhat disrupted by nocodazole treatment, but staining remains concentrated in the axonal growth cone. Bar, 10 μm.

Figure 6. Simultaneous localization of 13H9 staining and tubulin during recovery from nocodazole treatment. Phase-contrast (a, d, g, and j), and 13H9 (b, e, h, and k) and antitubulin (c, f, i, and l) staining of cells fixed after recovery from 30-min incubations in 10 μg/ml nocodazole. Observations of several cells indicate that tubulin repolymerization occurs in a proximal to distal direction and that 13H9 staining does not reappear in any axon or minor process until microtubule repolymerization in that process has extended to the growth cone. The cells shown here represent those findings. (a–c) A cell 1 h after removal of nocodazole. Tubulin staining has returned to the proximal portion of the axon (c), but does not extend to the site of the original growth cone (a and c, arrow). There is faint 13H9 staining (b) associated with the growth cone of a short axonal branch that is stained along its length with tubulin, but no 13H9 staining in the long process. (d–f) and (g–i) Cells 1.5 h after removal of nocodazole. In some cells with apparently complete microtubule assembly (f) there is no detectable 13H9 staining (e) at the axonal growth cone. In cells with processes of quite different lengths, the tubulin staining may reach the ends of minor processes and a shorter axonal branch (i, arrows) but not the longer axonal branch (i, arrowhead). The appearance of 13H9 staining (i) in this cell occurs only in the growth cones of the shorter axonal branch and minor processes. (j–l) Cell 2 h after removal of nocodazole. Microtubule repolymerization is complete in this cell (l), and 13H9 staining (k) is present at each growth cone. The pattern of 13H9 staining in these cells is indistinguishable from that in cells never exposed to nocodazole.
A Growth Cone Component Interacts with Both Microtubules and Microfilaments

The experiments above suggest that a growth cone component, the antigen recognized by the mAb 13H9, interacts directly or indirectly with two different cytoskeletal elements, microfilaments and microtubules. The interaction with microfilaments is suggested strongly by colocalization: 13H9 immunoreactivity aligns closely with assembled actin in the growth cone, as visualized by phalloidin. Although the patterns are not identical, there are regions of overlap in the staining, including striking correlations at the radial elements of lamellipodia. In contrast, 13H9 staining is clearly distinguishable from that of assembled tubulin in neurons. Indeed, the two have nearly complementary distributions. Instead, the interaction between 13H9 antigen and microtubules is indicated by the concomitant effects of nocodazole on them both. Depolymerization of microtubules leads to the rapid disruption of the 13H9 staining pattern. The growth cone-associated 13H9 antigen spreads proximally along the axon, always remaining distal to the receding edge of assembled tubulin. This result suggests that the 13H9 antigen relies upon intact microtubules for normal localization in the cell. Both effects of the microtubule depolymerizing drug are reversible. Upon removal of the nocodazole, the microtubules in processes reassemble in the proximal-to-distal direction. Only after they again reach the end of the process does 13H9 return to its original position. Although the 13H9 antigen is associated with assembled microtubules in other systems (Birgbauer and Solomon, 1989), only in the growth cone is its localization obviously dependent upon intact microtubules.

Taken together, these observations raise the possibility that the 13H9 antigen may participate in interactions between axonal microtubules and elements of the growth cone cytoskeleton. There are many results suggesting that such an interaction might occur and be necessary for process extension. Of course, intact microtubules are necessary but not sufficient for axonal outgrowth (Seeds et al., 1970; Daniels, 1973). Viewing the central regions of growth cones with EM, Letourneau and Ressler (1983) observed close associations between the ends of microtubules and bundles of actin filaments that extend to the growth cone periphery. In another system, disruption of the growth cone microfilaments with cytochalasin affected the microtubules: instead of terminating in the central region of the growth cone, they rapidly extend to its periphery (Forscher and Smith, 1988). Two sorts of drug interference experiments indicate a functional connection between microtubules and growth cone microfilaments. Bray and colleagues (1978) showed that colchicine-mediated depolymerization of microtubules induced the elaboration of ectopic growth cone structures all along the axon, suggesting that intact microtubules may be responsible for restricting motility to the axon terminus. Microfilaments also may be required for the loss of cellular asymmetry that can accompany microtubule disassembly. Neuroblastoma (Solomon and Magendantz, 1981) and PC12 cells (Joshi et al., 1985) incubated with nocodazole typically retract their processes rapidly, but cells incubated simultaneously with nocodazole and cytochalasin D retain their neurites but no longer have intact microtubules. These observations raise provocative questions about coordinated interactions among cytoskeletal elements. To date, however, they have not produced candidate molecules that might be involved in that coordination.

The Identity of the 13H9 Antigen

Could the 13H9 antigen be such a molecule? The 13H9 antibody was raised against a protein that coassembles with chick brain tubulin in vitro. In chicken erythrocytes, it binds to a protein that is associated with the marginal band (Solomon and Birgbauer, 1989), and indeed it was identified from a library of monoclonal antibodies because of that staining pattern. A significant portion of phalloidin staining in the chicken erythrocyte aligns with the marginal band (Kim et al., 1987). In these cells, then, 13H9 recognizes a protein that colocalizes in vivo with microtubules, and with some but not all of the assembled actin. Also, unlike other microtubule-associated proteins, the 13H9 antigen remains associated with detergent extracted preparations of chicken erythrocytes even when the microtubules themselves are completely removed. Because of these results, the 13H9 antigen appears to have properties of both a microtubule- and a microfilament-associated protein (Birgbauer and Solomon, 1989).

We have not determined the identity of the 13H9 antigen responsible for the singular staining pattern observed in hippocampal neurons. Several properties of the 13H9 antigen from erythrocytes, described in the accompanying manuscript (Birgbauer and Solomon, 1989), suggest that it may be ezrin. Ezrin was first isolated from brush border, and has been found in other cell types localized to motile projections (Bretscher, 1983, 1989; Gould et al., 1986). These are sites rich in polymerized actin structures. Despite this cellular localization, it does not appear to associate with actin in vitro (Bretscher, 1983). The identification of the antigen responsible for the immunofluorescent staining in hippocampal neurons is more problematic because 13H9 binds to several bands in immunoblots. Among them is a doublet of 80-kD that comigrates with the chicken erythrocyte protein uniquely stained by 13H9 (data not shown). Similar results are obtained with other cell lines (E. Birgbauer, unpublished observations). It is possible that, because the epitope recognized by 13H9 is less abundant in these cells, the higher concentrations of cellular protein and the longer exposures necessary incur binding of nonspecific bands as well. Defini-
tive demonstration of these relationships will require both eliciting new antibodies and direct structural tests.

Summary

Although conspicuously dissimilar in many ways, marginal bands and axons do have properties in common. Arguments for studying the former to understand the latter have been presented in detail (Solomon, 1988). The identification of an antigen common to both structures invites further analysis of potential common functions. Clear from results in both chicken erythrocytes and hippocampal neurons is that the 13H9 antigen is not a typical cytoskeleton-associated protein. In both systems, its properties include some but not all of the properties of both microtubule- and microfilament-associated proteins. Perhaps this complex nature reflects a dual role for the 13H9 antigen, mediating specific interactions between subsets or domains of cellular cytoskeletal elements.

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