

## Fodrin:

# Axonally Transported Polypeptides Associated with the Internal Periphery of Many Cells

JOEL LEVINE and MARK WILLARD

*Departments of Anatomy-Neurobiology and Biological Chemistry, School of Medicine, Washington University, St. Louis, Missouri 63110. Dr. Levine's present address is the Neurobiology Laboratory, Salk Institute for Biological Studies, San Diego, California 92138.*

**ABSTRACT** Fodrin (formerly designated 26 and 27) comprises two polypeptides (250,000 and 240,000 mol wt) that are axonally transported at a maximum time-averaged velocity of 40 mm/d—slower than the most rapidly moving axonally transported proteins, but faster than at least three additional groups of proteins. In this communication, we report the intracellular distribution of fodrin. Fodrin was purified from guinea pig brain, and a specific antifodrin antibody was produced in rabbit and used to localize fodrin in tissue sections and cultured cells by means of indirect immunofluorescence. Fodrin antigens were highly concentrated in the cortical cytoplasm of neurons and also nonneuronal tissues (e.g., skeletal muscle, uterus, intestinal epithelium). Their disposition resembles a lining of the cell: hence, the designation fodrin (from Greek *fodros*, lining). In cultured fibroblasts, immunofluorescently labeled fodrin antigens were arranged in parallel arrays of bands in the plane of the plasma membrane, possibly reflecting an exclusion of labeled fodrin from some areas occupied by stress fibers. The distribution of fodrin antigens in mouse 3T3 cells transformed with simian virus 40 was more diffuse, indicating that the disposition of fodrin is responsive to altered physiological states of the cell. When mixtures of fodrin and F-actin were centrifuged, fodrin cosedimented with the actin, indicating that these proteins interact *in vitro*.

We conclude that fodrin is a specific component of the cortical cytoplasm of many cells and consider the possibilities: (a) that fodrin may be indirectly attached to the plasma membrane via cortical actin filaments; (b) that fodrin may be mobile within the cortical cytoplasm and that, in axons, a cortical lining may be in constant motion relative to the internal cytoplasm; and (c) that fodrin could serve to link other proteins and organelles to a submembrane force-generating system.

A multitude of proteins synthesized in the cell bodies of neurons are conveyed by the process of axonal transport to the axons and synaptic terminals, which are themselves unable to synthesize most proteins. In the retinal ganglion cells (the centrally projecting neurons of the retina) of mammals and amphibians, the axonally transported proteins can be roughly divided into five groups according to their apparent maximum transport velocities (18, 24, 38, 45, 46): the major proteins of the first group (transport velocity  $\geq 240$  mm/d) are associated with membranous organelles resembling the plasma membrane (26); the second group (velocity = 30–60 mm/d) is heterogeneous and includes mitochondrial proteins and proteins asso-

ciated with at least two unidentified organelles (24, 26). The third and fourth groups (velocity = 2–8 mm/d) include (among many other proteins) actin (48) and two forms of myosinlike proteins (44), presumably involved in mechanical and cytoskeletal functions. The fifth group (velocity = 0.7–1.1 mm/d) includes polypeptides associated with neurofilaments and polypeptides resembling tubulin (16, 46, 47). The vast majority of axonally transported polypeptides have been identified only by their behavior in electrophoretic systems; their functions and their precise destinations within the axon or synaptic terminals are unknown.

Among these uncharacterized axonally transported proteins

is a pair of polypeptides (which we previously designated 26 and 27) possessed of a number of intriguing properties: (a) they coelectrophorese with major polypeptides of nervous tissue, suggesting that they may be structural components of the neuron; (b) their electrophoretic mobilities (indicating molecular weights of ~250,000 and 240,000) are similar to those of erythrocyte spectrin and filamin; (c) although the maximum axonal transport velocity (~40 mm/d) of 26 and 27 is characteristic of the second group of transported polypeptides (23, 24), preliminary evidence has suggested that these proteins may, in addition, be transported at slower velocities in association with materials of the third, fourth, or fifth groups (26).

We purified 26 and 27, prepared a specific antibody against them, and used the antibody to localize 26 and 27 antigens in neurons and other cells by means of indirect immunofluorescence. We report here evidence that the 26 and 27 antigens are concentrated in the cortical cytoplasm of many cells. Because their disposition is reminiscent of a cellular lining we have designated them fodrin (from Greek *fodros*, lining) and refer to them as such henceforth.

## MATERIALS AND METHODS

### Purification of Fodrin

Two fresh guinea pig brains (~8 g total weight) were homogenized in 60 ml of a solution containing 10 mM Tris, pH 8.0, 5 mM EDTA, 1 mM dithiothreitol (DTT), 1 mM *o*-phenanthroline and 1 mM phenylmethylsulfonyl fluoride (PMSF); the homogenate was diluted to a total volume of 120 ml with the same solution and centrifuged at a force of 150,000  $g_{max}$  for 60 min at 4°C. The resulting pellet was homogenized in 120 ml of high-salt (HS) buffer (0.6 M KCl, 1 mM DTT, 1 mM Tris, pH 8.0), stirred for 30 min at 4°C, and centrifuged at a force of 150,000  $g_{max}$  for 1 h at 4°C. Ammonium sulfate (Schwartz-Mann, 0.258 g/ml) was added to the supernate and stirred for 30 min at 4°C; the resulting precipitate was collected by centrifugation at 150,000  $g_{max}$  for 30 min at 4°C. The precipitate was dissolved in 5.0 ml of HS buffer, dialyzed extensively against the same buffer at 4°C, and centrifuged at a force of 100,000  $g_{max}$  for 45 min to remove any remaining insoluble material. The supernate was subjected to gel filtration on a column (1.5 × 90 cm) of Sepharose 4B equilibrated with HS buffer, and the eluant fractions enriched in fodrin (assayed by analytical SDS polyacrylamide gel electrophoresis [PAGE]) were combined and the protein was precipitated by the addition of TCA to a final concentration of 8% wt/vol. The precipitate was sedimented by centrifugation at 3,000 g for 15 min, washed with 20 ml of diethyl ether, and dissolved in denaturing solution (D buffer, 2% SDS, 10 mM Tris pH 8.0, 5 mM EDTA, 4 mM DTT). The solution was heated to 90°C for 5 min and cooled to room temperature, and urea was added to a final concentration of 8 M. The resulting sample (0.8 ml) was subjected to preparative SDS PAGE on a cylindrical gel (1.5 cm in diameter) in an apparatus manufactured by Savant Instruments, Inc., Hicksville, N.Y. The running gel (4 cm high) contained 4% acrylamide, 0.03% bisacrylamide, and the stacking gel (1.7 cm high) contained 3% acrylamide, 0.09% bisacrylamide, both in the buffer systems of Laemmli (20). The sample was electrophoresed at 15 mA, and the eluted samples containing fodrin (assayed by means of analytical SDS PAGE) were combined and dialyzed against a solution of SDS (0.05%) and DTT (0.1 mM) and then lyophilized. The purity of each preparation was assayed by analytical SDS PAGE of the lyophilized sample.

### Antibody Production

Lyophilized, electrophoretically homogeneous fodrin was washed twice with absolute acetone at -20°C, and then air-dried, suspended in 1.5 ml of PBS (0.15 mM NaCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.5), emulsified with an equal volume of complete Freund's adjuvant, and subdermally injected at multiple sites (0.2 ml/site) along the backs of two New Zealand White rabbits. The injections were repeated after 10 d, 40 d, and then at 6-mo intervals, with fodrin emulsified in an equal volume of incomplete Freund's adjuvant. 150–300 µg of fodrin was injected each time. The rabbits were bled 10 d after the third and all subsequent injections; in all cases the serum contained anti-fodrin antibodies. The antibody to be used in immunofluorescent studies was purified by affinity chromatography on a column of Sepharose 4B to which electrophoretically homogeneous fodrin had been covalently coupled by the procedures of Parikh et al. (32). Either whole serum diluted 1:1 in phosphate-buffered saline (PBS) or an IgG fraction prepared

by ammonium sulfate precipitation (0.291 g of ammonium sulfate per ml of serum that had been diluted 1:1 with PBS) was applied to an affinity column, which was then washed with PBS. The specific antibodies were eluted by a solution containing NaCl (0.15 M) and glycine (0.2 M, pH 2.2). The fractions were immediately neutralized by adding one-tenth of their volume of Tris (2 M, pH 8.5), and then they were dialyzed against PBS containing sodium azide (0.02% wt/vol).

### Other Proteins

Spectrin was prepared from guinea pig erythrocyte ghosts as described by Marchesi (27). Filamin was partially purified from frozen guinea pig vas deferens (obtained from Pel-Freez Biologicals Inc., Rogers, Ark.) according to the procedure of Wallach et al. (41).

### Preparation of Tissues and Cells for Immunofluorescence

The relevant tissues were rapidly removed from guinea pigs that had been anesthetized with sodium pentobarbital and decapitated. The tissues were frozen in isopentane, cooled on crushed dry ice, and sections (10 µm) were cut inside a cryostat. The sections were mounted on clean glass slides, air dried at -18°C for 20 min, fixed in ethanol (95%) at 4°C for 30 min, and immersed in absolute acetone at room temperature for 1 min. The fixed tissue sections were stored at -70°C before immunofluorescent staining.

Rat superior cervical ganglion cell cultures and retinal explant cultures were prepared as described (8) and were generously provided by Drs. C. Cornbrooks and P. Wood, respectively. Mouse 3T3 cells, simian virus 40(SV40)-transformed 3T3 cells (generously provided by D. Rabin) and C-6 glioma cells (a gift of Dr. L. Glaser) were grown in Dulbecco's modified Eagle's medium (DME; Grand Island Biological Co. [GIBCO], Grand Island, N. Y.) supplemented with 10% heat-inactivated calf serum, 1% penicillin-streptomycin, and glutamine (2 mM). Primary guinea pig fibroblasts (a generous gift from Dr. R. Bischoff) were grown in DME supplemented with 10% horse serum, 10% fetal calf serum, 1% penicillin-streptomycin, and glutamine (2 mM). Additional guinea pig fibroblasts were obtained by enzymatic (0.25% crude trypsin) dissociation of neonatal guinea pig kidneys and were grown in DME supplemented with 10% fetal calf serum, antibiotics, and glutamine. For immunofluorescent staining, cells grown on glass cover slips were washed three times with calcium-magnesium free Hanks' balanced salts (CMF, GIBCO), fixed with 3.7% formaldehyde in CMF for 15–30 min at room temperature, washed three times with CMF, and made permeable to the antibody by acetone treatment as described by Hynes and Destree (17) or by treatment with CMF containing 0.1% Triton X-100 for 5 min at room temperature.

### Immunofluorescent Staining

Tissue sections and cells were immunofluorescently stained by a modification of the method of Hartman (11). The specimens were incubated with antifodrin (either whole serum or affinity-purified antibody, diluted in a solution [K-PBS-NGS] of 0.15 M NaCl, 10 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.5, containing 2% goat serum) for 30 min at room temperature in a humidified chamber. The specimens were washed twice for 5 min in K-PBS-NGS containing 0.1% Triton X-100. The rabbit antifodrin antibody was then labeled by incubating the specimens with fluorescein-conjugated goat anti-rabbit IgG (Gateway Serum Co., St. Louis, Mo.); the secondary antibody was used at a concentration of 50 µg/ml in K-PBS-NGS containing 0.1% Triton X-100. The specimens were washed twice in K-PBS-NGS and once in distilled H<sub>2</sub>O, mounted on glass slides with buffered glycerol (60% glycerol, 40% 0.4M KHCO<sub>3</sub>, pH 8.6), and examined with a Letiz orthoplan fluorescence microscope equipped with a Ploem illuminator, a KP 500 activation filter, K530 barrier filter, and a 650-W xenon light source. Photographs were taken on Kodak Tri-X film and exposure times were between 10–30 s.

### Electrophoresis

Protein samples to be analyzed by SDS PAGE were dissolved in D buffer and electrophoresed on 13-cm SDS slab gels containing a gradient of polyacrylamide (4–12%; acrylamide/bisacrylamide = 33.3) in the buffer system of Laemmli (20) as described previously (24) except that in most cases, urea was omitted from the gel solutions and denaturing solution. The gels were stained and destained according to the procedure of Fairbanks et al. (9) and dried under reduced pressure. Radioactive polypeptides were detected by either radioautography or fluorography, using XR5 X-ray film (Kodak); in the case of fluorography, the film was preexposed to an optical density of 0.1 (21). Apparent molecular weights

were estimated by the method of Weber and Osborn (43) as described earlier (24).

### Immunological Methods

Ouchterlony double diffusion was carried out on plates containing 0.85% Difco agar (Difco Laboratories, Detroit, Mich.) in a buffer of 0.15 M NaCl, 1.0 M glycine, 40 mM sodium barbital, 0.5% Triton X-100, and 0.1% SDS. After they were incubated for ~48 h at room temperature in a humid chamber, the plates were washed extensively in PBS and stained with Coomassie Brilliant blue dye according to the procedure of Fairbanks et al. (9).

Labeled axonally transported polypeptides were immunoprecipitated by an adaptation of the methods of Lingappa et al. (25). Tissues containing axons of retinal ganglion cells were homogenized in a solution containing Tris (10 mM, pH 8.0), EDTA (5 mM), DTT (1 mM), and PMSF (1 mM), and separated into particulate and soluble fractions by centrifugation at 100,000  $g_{avg}$  for 1 h at 4°C. The particulate fraction was homogenized in the same solution containing 1% SDS, heated to 90°C for 5 min, and centrifuged at 100,000  $g$  for 1 h at 20°C to remove any insoluble material. An aliquot (0.1 ml) of this extract was added to 0.9 ml of a solution containing NaCl (0.15 mM), Tris (10 mM, pH 8.0), EDTA (5 mM), and Triton X-100 (1%). The samples were mixed with 15  $\mu$ l of either immune or preimmune serum from the same rabbit and incubated at 4°C for 12–15 h; the antibody-antigen complexes were adsorbed to *Staphylococcus aureus* Cowan strain I (SACI, a generous gift from Dr. S. Cullen) according to Kessler (19). The SACI were washed in PBS and the immune complexes were eluted into D buffer containing 30% (vol/vol) glycerol; the eluate was electrophoresed as described above.

### Labeling of Axonally Transported Proteins in Retinal Ganglion Cells

[<sup>35</sup>S]methionine (400 Ci/mmol; Amersham Searle, Chicago, Ill.) was injected into the vitreous humor of adult female guinea pigs (400–600 g) as described previously (24). At appropriate times, animals were killed with sodium pentobarbital, and the optic nerves, optic tracts, lateral geniculate nuclei, and superior colliculi were removed and frozen on dry ice.

### Cosedimentation of Fodrin and Actin

HS buffer extracts of guinea pig brain and gel filtration-purified fodrin (purified as described above) were dialyzed against a solution containing 0.15 M KCl, 1 mM Tris, pH 8.0, 1 mM DTT at 4°C. The dialyzate was centrifuged at 100,000  $g$  for 1 h at 4°C and rabbit skeletal muscle F-actin was added to aliquots of the supernate. After a 5-min incubation on ice, the solutions were centrifuged at 100,000  $g$  for 40 min at 4°C. The supernates were then removed and precipitated by the addition of TCA (8% wt/vol). The pellets, containing F-actin and any proteins bound to the actin, were resuspended by homogenization in cold distilled water; the proteins were subsequently precipitated with TCA, collected by centrifugation at 3,000  $g$  for 15 min, washed in diethyl ether and air-dried. The pellets were dissolved in D buffer, heated to 90°C for 5 min, and electrophoresed as described above.

## RESULTS

### Abundance and Purification of Brain Fodrin

Fig. 1A shows both the Coomassie Blue-stained and axonally transported polypeptides of particulate and soluble fractions (pellet and supernate respectively, of a 1-h centrifugation at 100,000  $g$ ) prepared from a guinea pig optic nerve. It is clear that the stained polypeptides that coelectrophorese with fodrin are enriched in the particulate fraction; they are major polypeptides of this fraction, composing 1–3% of the Coomassie Blue-stained proteins, as judged by densitometric scanning of the gels.

We purified fodrin from a particulate fraction of guinea pig brain by solubilizing it at high ionic strength and subjecting it to ammonium sulfate precipitation, gel filtration chromatography, and preparative SDS PAGE (see Materials and Methods for details). Fig. 1B shows the progressive purification of fodrin. A typical preparation began with 8 g (wet weight) of tissue and yielded 500–700  $\mu$ g of fodrin-enriched protein.

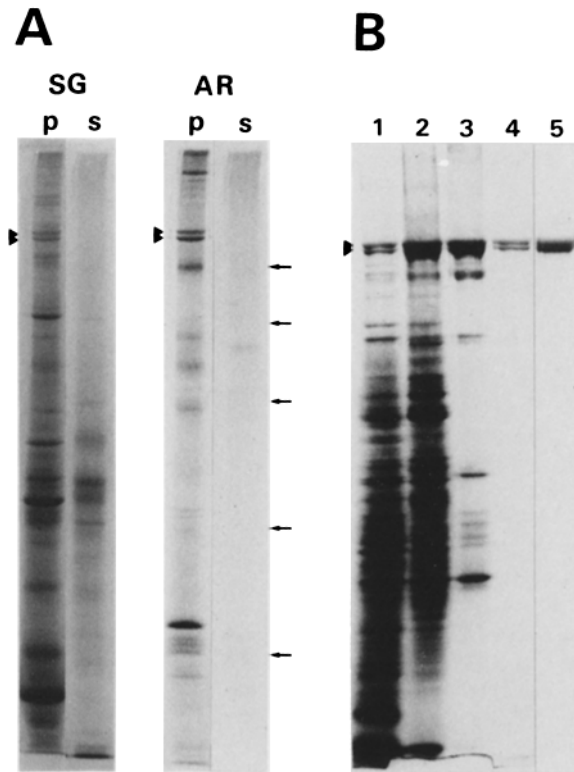
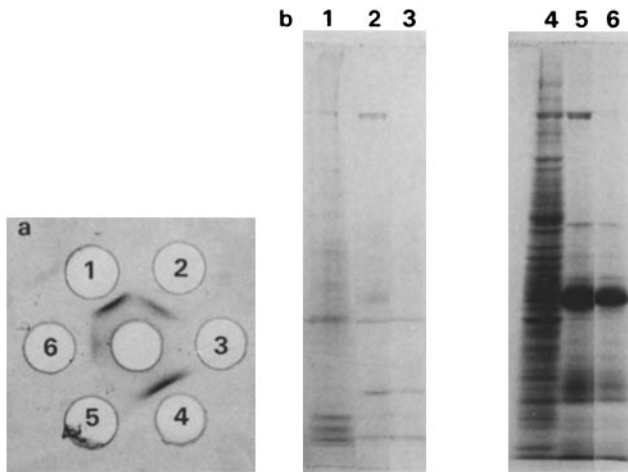


FIGURE 1 (A) A stained SDS gel (SG) and the resulting autoradiograph (AR) showing the polypeptides of particulate (p) and soluble (s) fractions of guinea pig optic nerve prepared 12 h after labeling of the retina with [<sup>35</sup>S]methionine. The double arrowheads to the left denote the fodrin polypeptides and the arrows to the right indicate approximate molecular weights (from top to bottom: 200,000, 150,000, 100,000, 50,000, and 25,000). Autoradiographic exposure time was 10 d. (B) A stained SDS gel showing the progressive purification of fodrin. Column 1, the HS extract of whole guinea pig brain. Column 2, the ammonium sulfate fraction before gel filtration. Column 3, the void volume of the Sepharose 4B column. Column 4, the fodrin-enriched peak eluting behind the void volume. Column 5, fodrin purified by preparative SDS gel electrophoresis from the material shown in Column 4.

### Characterization of Antifodrin

We produced an antibody to guinea pig fodrin in rabbits, as described in Materials and Methods. The resulting antiserum was specific for fodrin by three criteria: first, a single precipitin band was produced when the antibody was subjected to double immunodiffusion against either a crude high-ionic strength extract of guinea pig brain or the most purified fraction of fodrin; the merging of the two precipitin bands indicates that the antigens in the two samples were identical (Fig. 2a). No precipitin band appeared when the antibody was diffused against guinea pig erythrocyte spectrin, or a partially fractionated extract of vas deferens containing filamin (Fig. 2a). Second, Fig. 2b shows that the antibody specifically precipitated only fodrin from either high-ionic-strength or non-ionic-detergent extracts of guinea pig brain. Third, we used the antibody to immunoprecipitate radiolabeled, axonally transported proteins from the axons of guinea pig retinal ganglion cells at various times after labeling the proteins synthesized in the cell bodies of these neurons. Fig. 3 shows that among the labeled, axonally transported polypeptides, only the two that coelectrophorese with fodrin were specifically precipitated, and the time-course



**FIGURE 2** (a) Ouchterlony immunodiffusion illustrating the specificity of the antifodrin serum. The center well contained 20  $\mu$ l of undiluted antifodrin serum and the peripheral wells contained 20  $\mu$ l of the following: (1 and 4) crude HS extract of guinea pig brain (3.5 mg/ml); (2 and 6) purified fodrin; (3) guinea pig erythrocyte spectrin (0.6 mg/ml); (5) partially purified guinea pig vas deferens filamin (3 mg/ml). (b) Stained SDS gels showing the immunoprecipitation of fodrin from crude extracts of guinea pig brain. Column 1, the polypeptides of the crude HS extract (28  $\mu$ g of total protein). Column 2, the polypeptides of the direct immunoprecipitate obtained by incubation of 50  $\mu$ l of immune serum with 70  $\mu$ g of the crude HS extraction in a total volume of 0.5 ml. Column 3, the polypeptides nonspecifically precipitated by 50  $\mu$ l of preimmune serum. Column 4, the polypeptides of a Triton X-100 extract of guinea pig brain (50  $\mu$ g of total protein). Column 5, polypeptides of an immunoprecipitate obtained by mixing 10  $\mu$ l of antifodrin serum with 200  $\mu$ g of the Triton X-100 extract. The immune complexes were collected with SACI as described in Materials and Methods. Column 6, polypeptides nonspecifically precipitated by 10  $\mu$ l of normal rabbit serum under the conditions of column 5.

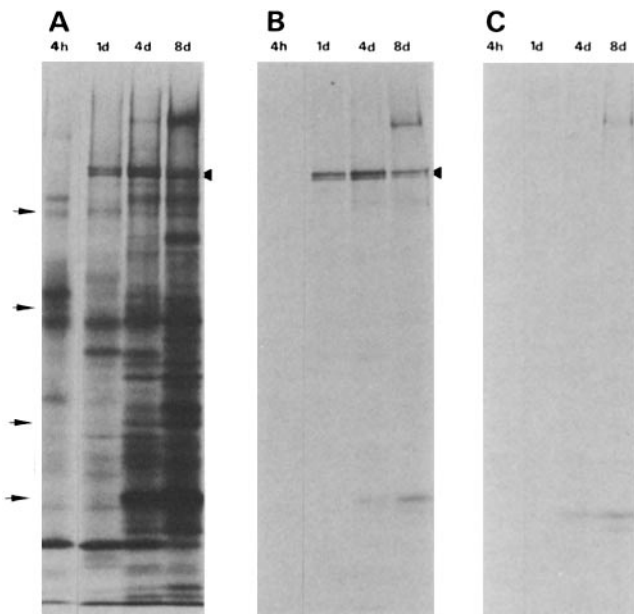
of their labeling was the same as that previously determined for the axonally transported polypeptides 26 and 27 (24). These observations establish that: (a) The antibody is specific for fodrin among the antigens we have tested. In particular, we have detected no immunological relationship between fodrin and two other high molecular weight proteins (spectrin and filamin) nor to any other axonally transported polypeptide. (b) The protein we have purified contains the antigens of the axonally transported proteins that we formerly designated 26 and 27. We can, therefore, be confident that the following immunofluorescence localization of these antigens reflects the steady-state distribution of those axonally transported polypeptides.

### Immunofluorescent Localization of Fodrin Antigens in Nervous Tissue

Figs. 4-6 show the indirect immunofluorescent staining of fodrin antigens in sections of guinea pig nervous tissue. Intense rings of fluorescence proximal to the plasma membranes of axons cut in cross section were the major fluorescent profiles in coronal sections of the spinocerebellar tract (Fig. 4a), optic tract (Fig. 4b), and medial longitudinal fasciculus (Fig. 4c). Fig. 5 shows that the fodrin antigens are also concentrated near the perikaryal plasma membrane of dorsal root ganglion neurons (Fig. 5a) as well as the plasma membrane of the large dendrites and the axon initial segments of spinal motor neurons

(Figs. 5b and c). Cross sections of the sciatic nerve also showed striking rings of fluorescence corresponding to the periphery of the axons (Fig. 6a); higher magnification (*inset* to Fig. 6a) revealed an unstained annulus, 2-3  $\mu$ m thick, surrounding the intensely stained axolemmal region, and beyond this annulus a faintly stained ring (arrow). The unstained annulus corresponds to the region of the myelin sheath surrounding the axon, whereas the stained outer ring is close to the plasma membrane of the Schwann cell. This same bilaminar staining pattern can also be appreciated in longitudinal sections of the sciatic nerve (Fig. 6b and c). In such sections, the axolemma frequently displays longitudinal variation in the intensity of the fluorescent staining (arrowhead), and the Schwann cell specializations at the nodes of Ranvier (arrow) are clearly resolved.

Fig. 7 shows the immunofluorescent staining of neurons grown in tissue culture. When dissociated rat superior cervical ganglion cells were grown under conditions that suppress the growth of nonneuronal cells, antifodrin specifically stained the region of the perikaryal plasma membrane (Fig. 7b) and unmyelinated neurites (Fig. 7c). The specific staining of these cells did not occur if they were not made permeable to the antibody by treatment with acetone, indicating that fodrin antigens are not exposed on the cell surface. When neuritic processes produced by explanted cultures of embryonic rat retina were stained with antifodrin, the fluorescence was most intense at the tips of the elongating processes (Fig. 7d); the fodrin antigens thus appear to be components of the growth cones of these neurons.



**FIGURE 3** Fluorographs illustrating the specificity of the antifodrin serum when tested against extracts containing radiolabeled axonally transported proteins. At the designated intervals after labeling the retina with [<sup>35</sup>S]methionine, 1% SDS extracts of the optic tract contralateral to the injected eye were immunoprecipitated as described in Materials and Methods. Panel A, polypeptides of the SDS extracts. Panel B, polypeptides of the immune precipitates. Panel C, polypeptides precipitated by preimmune serum. The double arrowheads to the right indicate the electrophoretic mobilities of the fodrin polypeptides and the arrows to the left indicate approximate molecular weights of 200,000, 100,000, 50,000 and 25,000, from top to bottom.

The following controls indicate that the fluorescent profiles we have described here reflect the distribution of fodrin antigens. They were not observed when the specific antibody was omitted or replaced either with preimmune serum (Fig. 4 *a'*) or with IgG pooled from unimmunized rabbits and used at twice

the concentration of the immune IgG (not shown), or with IgG (from the immunized rabbit) that had been passed over a fodrin-Sepharose affinity column to specifically remove anti-fodrin antibodies (not shown). In addition, the staining of fodrin antigens was not altered when tissue sections were fixed by immersion in a solution of chloroform-methanol (3:1) at  $-20^{\circ}\text{C}$  for 3 h, or when the sections were obtained from animals that had been perfused with 3.7% formaldehyde in PBS.

We conclude that antigens of the axonally transported protein fodrin are highly concentrated at the internal periphery of the entire neuron. They occupy a similar location in Schwann cells. They are absent (or present at much lower concentrations) in myelin.

#### *Fodrin Antigens in Nonneuronal Tissues*

To determine whether fodrin is a component of nonneuronal tissues, we attempted to precipitate fodrin antigens from extracts of other tissues with antifodrin. Fig. 8 shows that extracts of kidney, liver, and cardiac muscle contained immunoprecipitable polypeptides that were electrophoretically indistinguishable from fodrin. In the case of smooth muscle (vas deferens, Fig. 8, panel 3), a major polypeptide of the molecular weight (260,000) of the protein filamin was not specifically precipitated by the antibody; small amounts of fodrin, not easily visible in Fig. 8, were specifically immunoprecipitated from this same tissue.

We examined the intracellular distribution of fodrin antigens in sections of intestinal epithelium, skeletal muscle, and uterus by means of indirect immunofluorescence. As was the case in neurons, the fodrin specific fluorescence was most intense at the periphery (Fig. 9).

#### *Fodrin Organization in Cultured Fibroblasts*

Antifodrin specifically precipitated fodrin from extracts of [ $^{35}\text{S}$ ]methionine-labeled cultures of guinea pig fibroblasts (Fig. 8, panel 5). To visualize the organization of fodrin in the plane of the plasma membrane, we immunofluorescently stained these fibroblasts. As with cultured neurons, fibroblasts (either living or formaldehyde-fixed) were only stained if they were first made permeable with acetone or Triton X-100; thus the fodrin antigens are not accessible on the cell surface. Fig. 10 shows that fodrin-specific fluorescence is arranged in arrays of parallel bands, often 2–3  $\mu\text{m}$  wide, but of variable width. In some cells, two parallel arrays at different orientations formed a criss-cross pattern (Fig. 10 *c*). The linear organization was evident regardless of whether the cells were viewed as usual

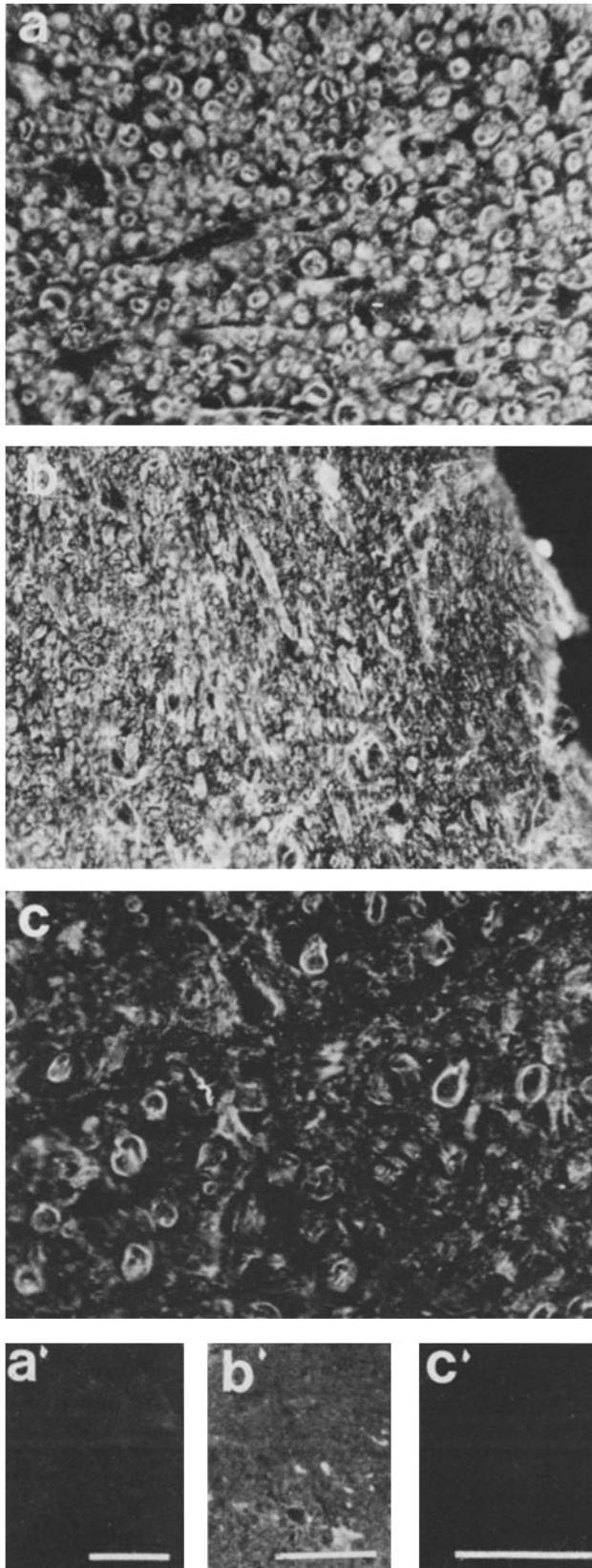


FIGURE 4 Immunofluorescent photomicrographs illustrating the disposition of fodrin antigens in frozen sections of the guinea pig central nervous system. *a* is a coronal section of the cervical spinal cord containing axons of the spinocerebellar tract stained with antifodrin serum diluted 1:150.  $\times 1,100$ . *a'* is a similar section stained with preimmune serum at the same dilution.  $\times 1,100$ . *b* is a section of the optic tract stained with antifodrin serum diluted 1:100.  $\times 1,370$ . *b'* is a control section of optic tract stained with normal rabbit serum diluted 1/100.  $\times 1,370$ . *c* is a section containing axons of the medial longitudinal fasciculus stained with antifodrin serum diluted 1:150.  $\times 1,800$ . *c'* is a similar section stained with serum that had been absorbed on columns of fodrin-Sepharose as described in Materials and Methods.  $\times 1,800$ . Bars, 10  $\mu\text{m}$ .

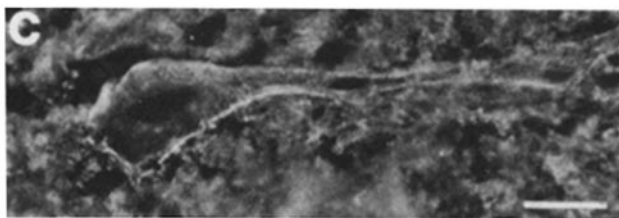
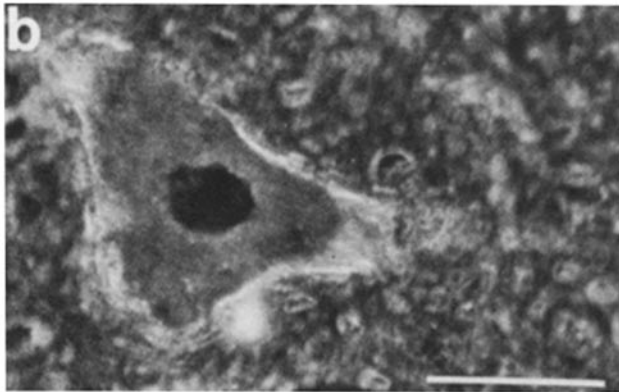
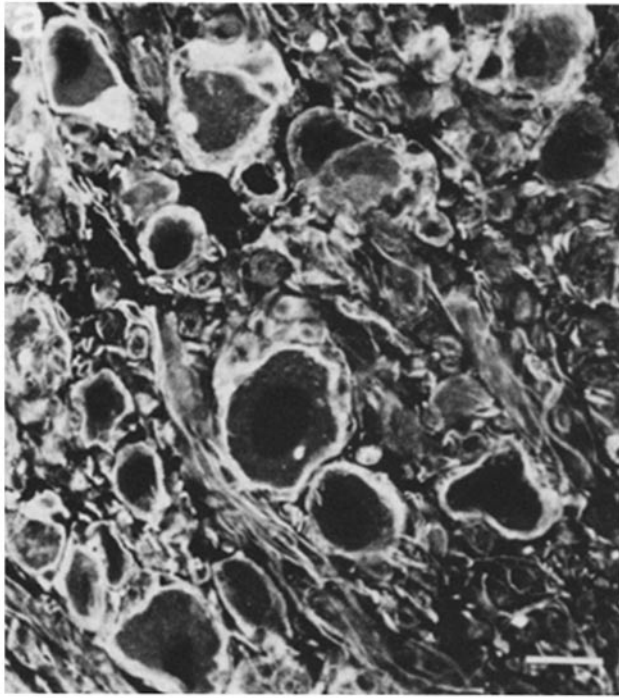


FIGURE 5 Fodrin-specific immunofluorescence of neuronal cell bodies. (a) Dorsal root ganglion neurons stained with affinity-purified antifodrin IgG at 10  $\mu\text{g}/\text{ml}$ .  $\times 940$ . Antifodrin that had been adsorbed with fodrin did not stain these cells. (b and c) Spinal motor neurons stained with antifodrin serum diluted 1:150.  $\times 2,340$  and  $\times 1,150$ , respectively. Bars, 10  $\mu\text{m}$ .

from their under (attached) surface or their upper (unattached) surface (Fig. 10c).

Stress fibers, which appear as dark bands in phase-contrast micrographs of fibroblasts, are also arranged in parallel linear arrays. To compare the organization of fodrin and stress fibers, we superimposed fluorescence (Fig. 10a) and phase-contrast (Fig. 10b) micrographs of the same cell; the stress fibers often corresponded to the dark areas on the fluorescence micrographs, indicating that fodrin is not a major component of

stress fibers, and further suggesting that the striated organization of fodrin fluorescence may be a consequence of its exclusion (or prevention from interaction with the antibodies) by stress fibers in some region of the cell. Fibroblast cytoskeletons (prepared by extraction of the membranes and cytoplasm with nonionic detergents [6]), which contain stress fibers, showed very little reaction with antifodrin (as judged by indirect immunofluorescence) and none at all when 0.6M KCl was included in the extraction medium.

Certain conditions that alter the organization of stress fibers also altered the organization of fodrin. When guinea pig fibroblasts were treated with cytocholasin B (10  $\mu\text{g}/\text{ml}$  in dimethyl sulfoxide [DMSO]), an agent that disrupts stress fibers and other actin-containing structures, the organization of fodrin became more random (Fig. 10e). DMSO alone had no apparent effect; nor did colchicine ( $10^{-6}$  M, or  $10^{-4}$  M; Fig. 10f), an agent that disrupts microtubules. Fodrin antigens that were normally organized in linear arrays in mouse 3T3 cells (Fig. 11a) became diffusely distributed when the cells were transformed with SV40 (Fig. 11c); transformation of these cells also results in the disappearance of stress fibers (33). A similar diffuse pattern of antifodrin fluorescence was apparent in C<sub>6</sub> glioma cells (Fig. 11c). The organization of fodrin is thus sensitive to the physiological state of the cell.

### Distribution of Fodrin among Species

The antibody against guinea pig fodrin cross-reacted with antigens in rat and cow brain (assayed by immunofluorescence, immunoprecipitation, and immunodiffusion) and in toad (*Bufo marinus*) brain and embryonic chick neural retina (assayed by immunoprecipitation). Thus, fodrin is widely distributed in the animal kingdom, and at least some aspects of its structure have been conserved among animals as evolutionary divergent as amphibians and mammals.

### Interaction with F-actin In Vitro

When F-actin was sedimented through extracts containing fodrin, fodrin was concentrated in the resulting pellet (Fig. 12A) indicating that it interacts either directly or indirectly with actin in vitro. A direct interaction is suggested by the observation that fodrin appeared to cosediment with F-actin to a greater extent than did other proteins in the extract (Fig. 12A) and retained its ability to cosediment with actin in more purified fractions (Fig. 12B).

### DISCUSSION

The region underlying the plasma membrane forms an interface that must mediate the transfer of information and material between a cell's environment and the internal cytoplasm, and also be compatible with such processes as endocytosis (39), cytokinesis (35), the regulation of cell shape (37), the regulation of the disposition of integral membrane proteins (30), and cell motility (34). The architecture of the inner aspect of the plasma membrane appears to comprise a complex meshwork of filamentous material (14). The contractile proteins actin (organized as microfilaments attached to the membrane [7, 28, 29], or in the case of the erythrocyte membrane, polymerized to only a limited extent [5, 40]) and, in most cases, myosin, are components of this region, although both of these proteins are also located elsewhere within the cell (34). In the case of

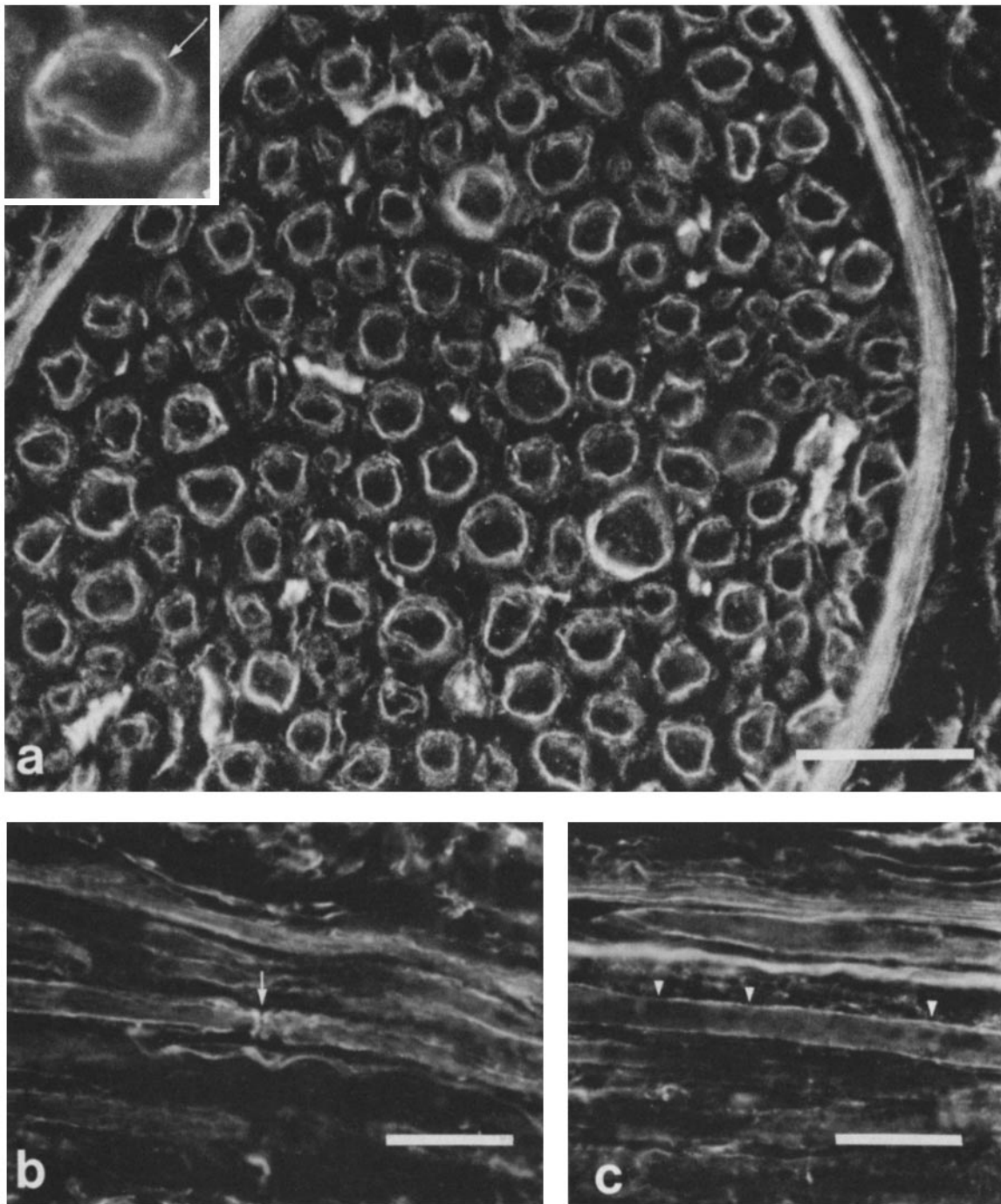


FIGURE 6 (a) Coronal section of sciatic nerve stained with antifodrin serum diluted 1:200.  $\times 2,750$ . *Inset* shows staining of regions near both the axolemma and the Schwann cell plasma membrane (arrow)  $\times 5,400$ . (b and c) Longitudinal sections of sciatic nerve stained with affinity-purified antifodrin IgG at  $20 \mu\text{g}/\text{ml}$ . The arrow in *b* points to a node of Ranvier and the arrowheads in *c* mark variations in the longitudinal distribution of the fodrin antigens.  $\times 1,900$ . Bars,  $10 \mu\text{m}$ .

erythrocytes, a protein designated spectrin, that is antigenically related to the myosin of smooth muscle (36), is exclusively localized to the inner surface of the membrane (31). Spectrin has not been demonstrated in any other type of cell (15). The cortical cytoplasm is apparently a dynamic region; for example, in lymphocytes, a redistribution of actin and myosin in this region accompanies the capping of cell surface molecules (3, 4), and in fibroblasts, this region participates in clathrin-mediated endocytosis (1). The experiments reported here indicate

that a pair of polypeptides, initially recognized for their participation in axonal transport, are specific components of this region of the cell. The evidence is as follows: Indirect immunofluorescence showed that fodrin-associated antigens are concentrated in the periphery of cells. However, the antigens were not exposed on the external surface, because cultured cells were not reactive unless their membranes were made permeable to the antibody. The polypeptides were not integrally associated with the plasma membrane, because they were easily extracted

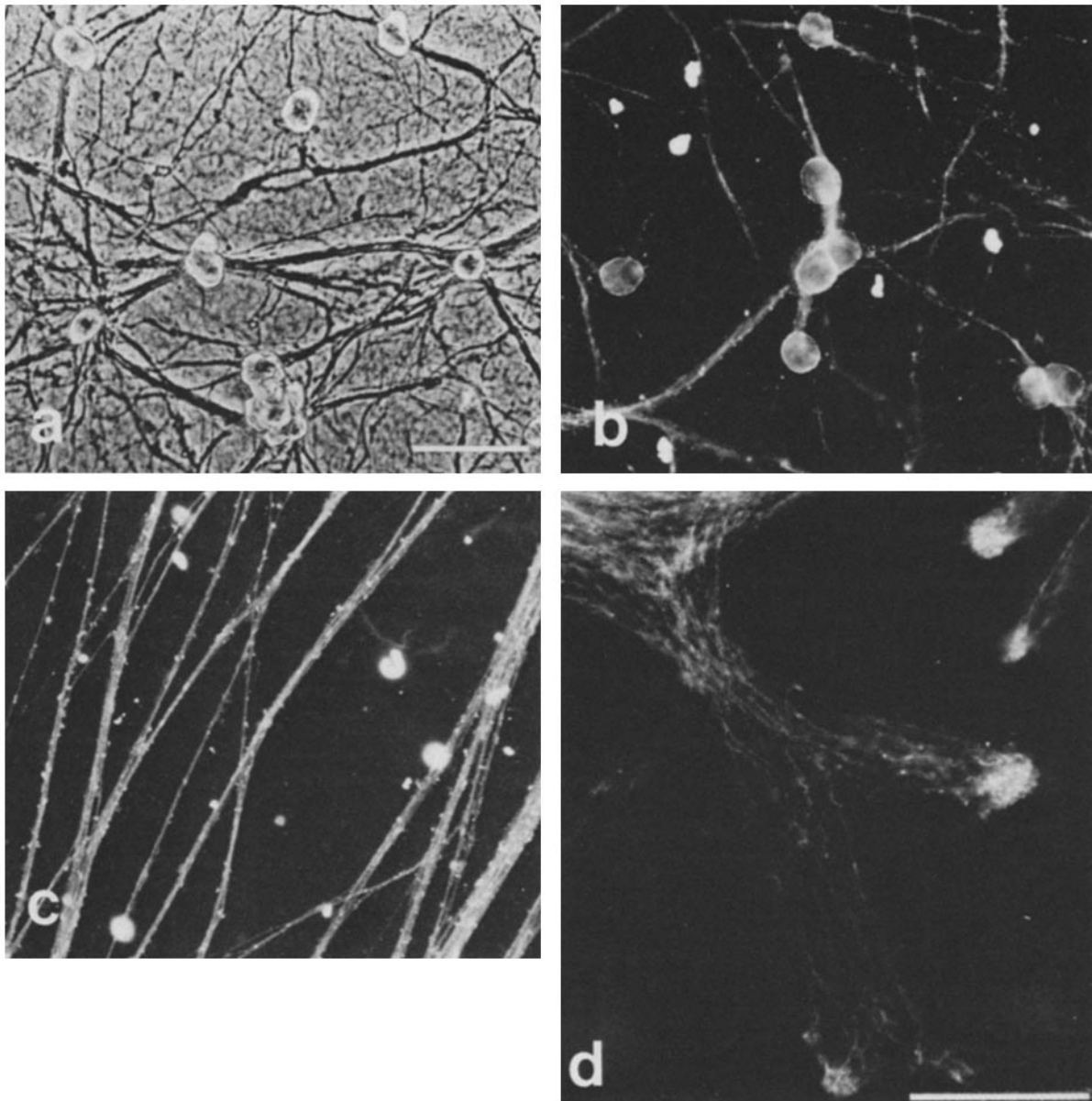


FIGURE 7 Neurons in culture stained with the antifodrin antibodies. (a) Phase-contrast photomicrograph of the dissociated superior cervical ganglia (SCG) cultures. (b) A similar culture stained with antifodrin IgG at 20  $\mu\text{g}/\text{ml}$ . (c) Neurites produced by SCG explant cultures stained with antifodrin serum diluted 1:100. Preimmune serum did not stain the cultures at this concentration. (a-c)  $\times 840$ . d, Neuritic outgrowth of explant cultures of embryonic rat retina stained with affinity-purified antifodrin IgG at 12  $\mu\text{g}/\text{ml}$ . Normal rabbit serum (25  $\mu\text{g}/\text{ml}$ ) did not stain these cultures.  $\times 1,300$ . Bars, 10  $\mu\text{m}$ .

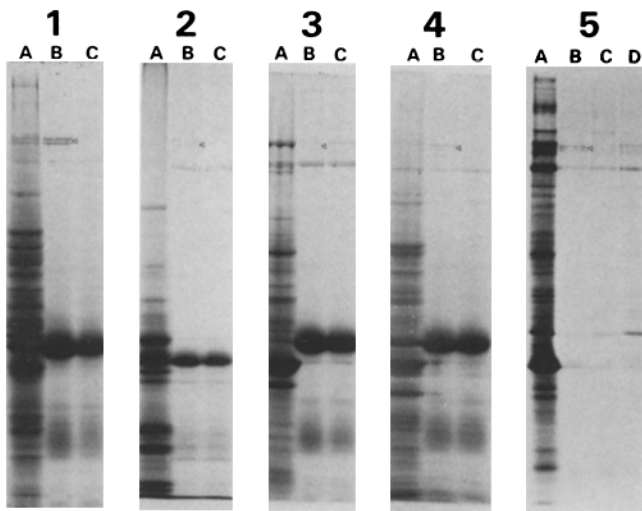
into aqueous solutions of high ionic strength. They are thus apparently specific components of the peripheral cytoplasm.<sup>1</sup>

The location of fodrin raises the possibility that it may interact with the interior side of the plasma membrane. In previous subcellular fractionation experiments (26), fodrin did

<sup>1</sup> It should be noted that there is no direct evidence that the two components of fodrin are subunits of the same protein, although this interpretation is suggested by their coordinate movement down the axons of a number of different neuronal systems (24, 45). In any case, it seems reasonable to conclude that both components are located in the cortical cytoplasm because the ability of our antibody to precipitate both components from solution implies that it either reacts with both components or, alternatively, that if it only reacts with one component, the two components are in the same complex.

not cofractionate with a specific plasma membrane enzyme, although both were most enriched in the same fraction. Thus, if fodrin interacts with the membrane, the interaction is probably indirect, reversible, or subject to partial disruption by subcellular fractionation procedures. It is interesting to consider that, if fodrin were attached to cortical actin filaments that were in turn associated with the plasma membrane, it might be easily sheared from the membrane during the homogenization of the tissue. The observed interaction between fodrin and F-actin *in vitro* would be consistent with this possibility. If fodrin interacts with actin within the cell, it probably does so selectively; antifodrin did not appear to stain fibroblast stress fibers that contain major concentrations of actin (10) (but also other proteins [e.g., tropomyosin] that might render the actin unavailable for interaction with fodrin [22]). On the contrary,



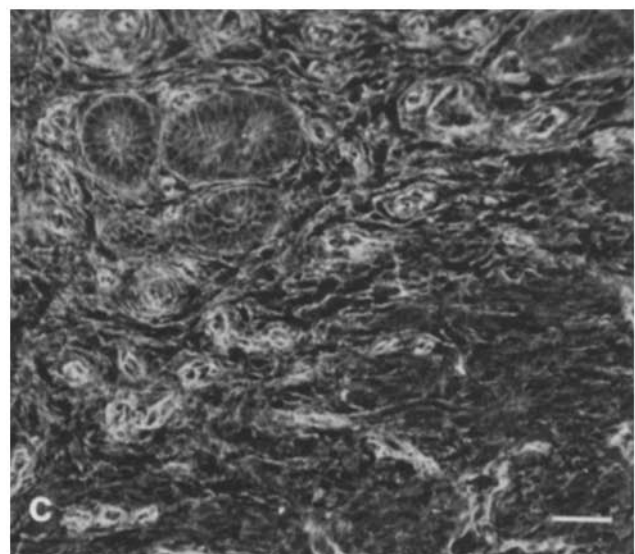
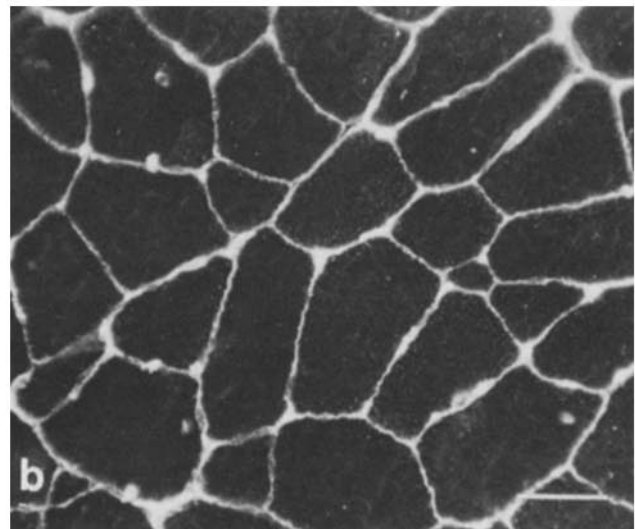
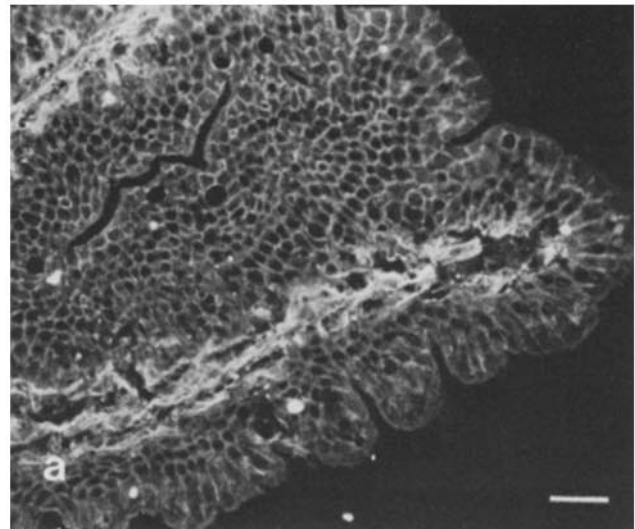


**FIGURE 8** The immunoprecipitation of fodrin antigens from extracts of nonneuronal tissues and cells. Panels 1–4: Triton X-100 extracts of particulate (pellet of a 100,000-*g*, 1-h centrifugation) fractions of tissues (200  $\mu$ g total protein) were incubated with 10  $\mu$ l of either antifodrin serum or preimmune serum, and the immune complexes were collected onto SACI as described in Materials and Methods. In each panel, *A* indicates polypeptides of the total extract (50  $\mu$ g of protein), *B* the polypeptides precipitated by the antifodrin serum, and *C* the polypeptides nonspecifically precipitated by the preimmune serum. The arrowheads indicate fodrin. The tissues were as follows: 1, kidney; 2, liver; 3, vas deferens, and 4, heart. Panel 5 is a fluorograph of polypeptides precipitated from extracts of exponentially growing guinea pig fibroblasts labeled with [<sup>35</sup>S]methionine (5  $\mu$ Ci/ml of media). (*D*) Polypeptides bound by SACI alone. Fluorograph exposure time was 30 h.

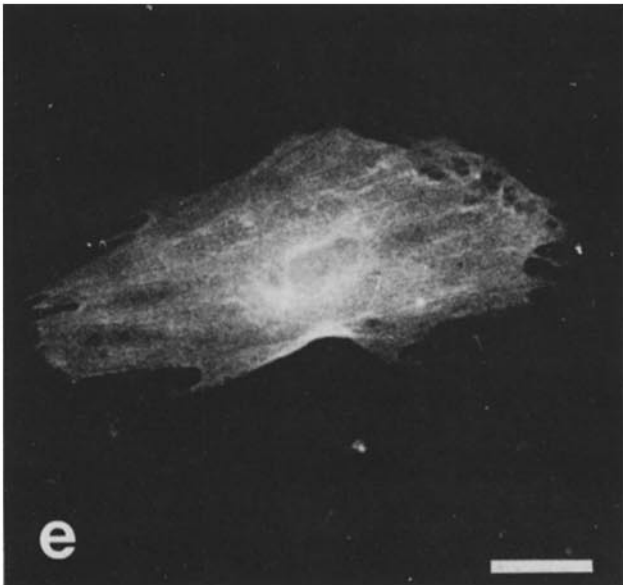
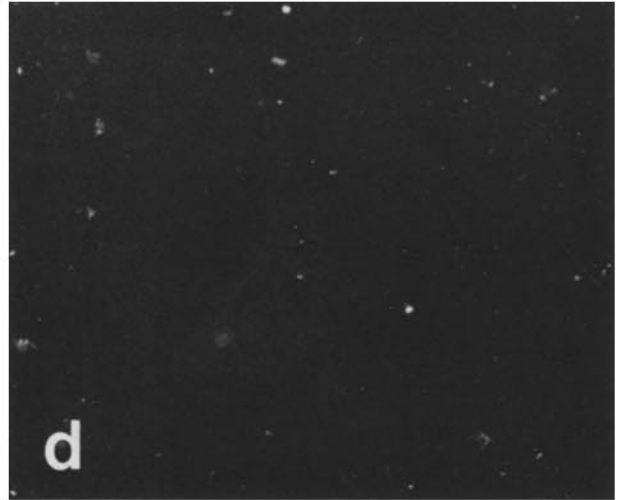
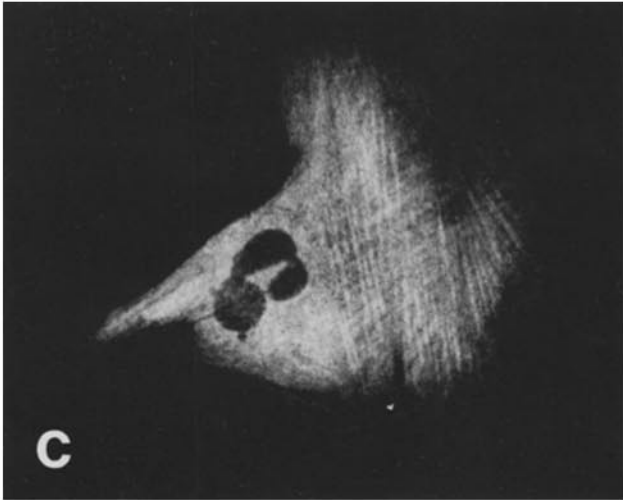
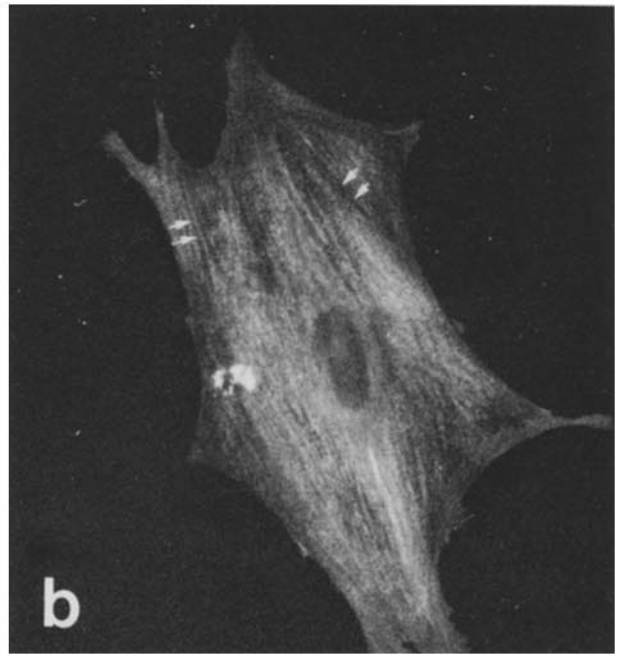
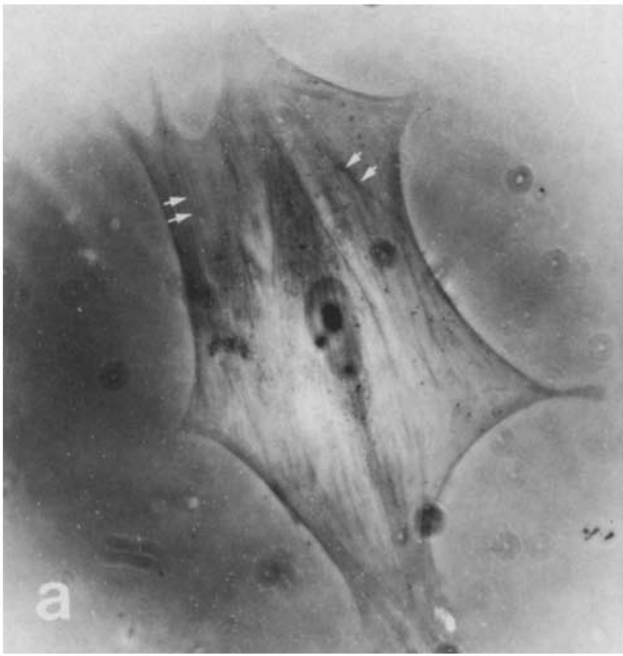
labeled fodrin appeared to be depleted from some regions of the cell occupied by stress fibers, suggesting that these fibers, which are often close to the plasma membrane (10), may exclude fodrin from the cortical cytoplasm. Alternatively, we cannot rule out the possibility that stress fibers simply limit the access of the antibody to fodrin. A similar pattern of apparent stress fiber avoidance is shown by certain proteins accessible from the external surface of fibroblasts (2); whether these similar distributions are independent or reflect some form of transmembrane linkage between fodrin and proteins of the external surface remains to be established. The reorganization of fodrin in SV40-transformed and cytochalasin B-treated fibroblasts could be simply a reflection of the absence of stress fibers in these cells, or a direct effect of these agents on the organization of fodrin itself.

Several observations that may pertain to the function of fodrin are worth noting. First, judging from its axonal transport in neurons, fodrin is capable of quite rapid directional intracellular movements; when newly synthesized fodrin is labeled in the cell bodies of mammalian retinal ganglion cells, it

subsequently appears to move down the axon at a maximum time-averaged velocity on the order of 500 nm/s (24, 45). Taken together with the immunofluorescence localization of fodrin in the periphery of axons, this observation suggests the



**FIGURE 9** The immunofluorescent localization of fodrin antigens in nonneuronal tissues. (*a*) Intestinal epithelium stained with affinity-purified antifodrin IgG at 12  $\mu$ g/ml. When the IgG was adsorbed on a column of sepharose, it no longer stained this tissue.  $\times$  750. (*b*) Skeletal muscle stained with antifodrin serum diluted 1:100. Preimmune serum at the same dilution minimally stained this tissue.  $\times$  1,200. (*c*) Uterus stained with affinity-purified antifodrin IgG at 12  $\mu$ g/ml. Normal rabbit serum (25  $\mu$ g/ml) did not stain this tissue.  $\times$  7,500. Bars, 10  $\mu$ m.



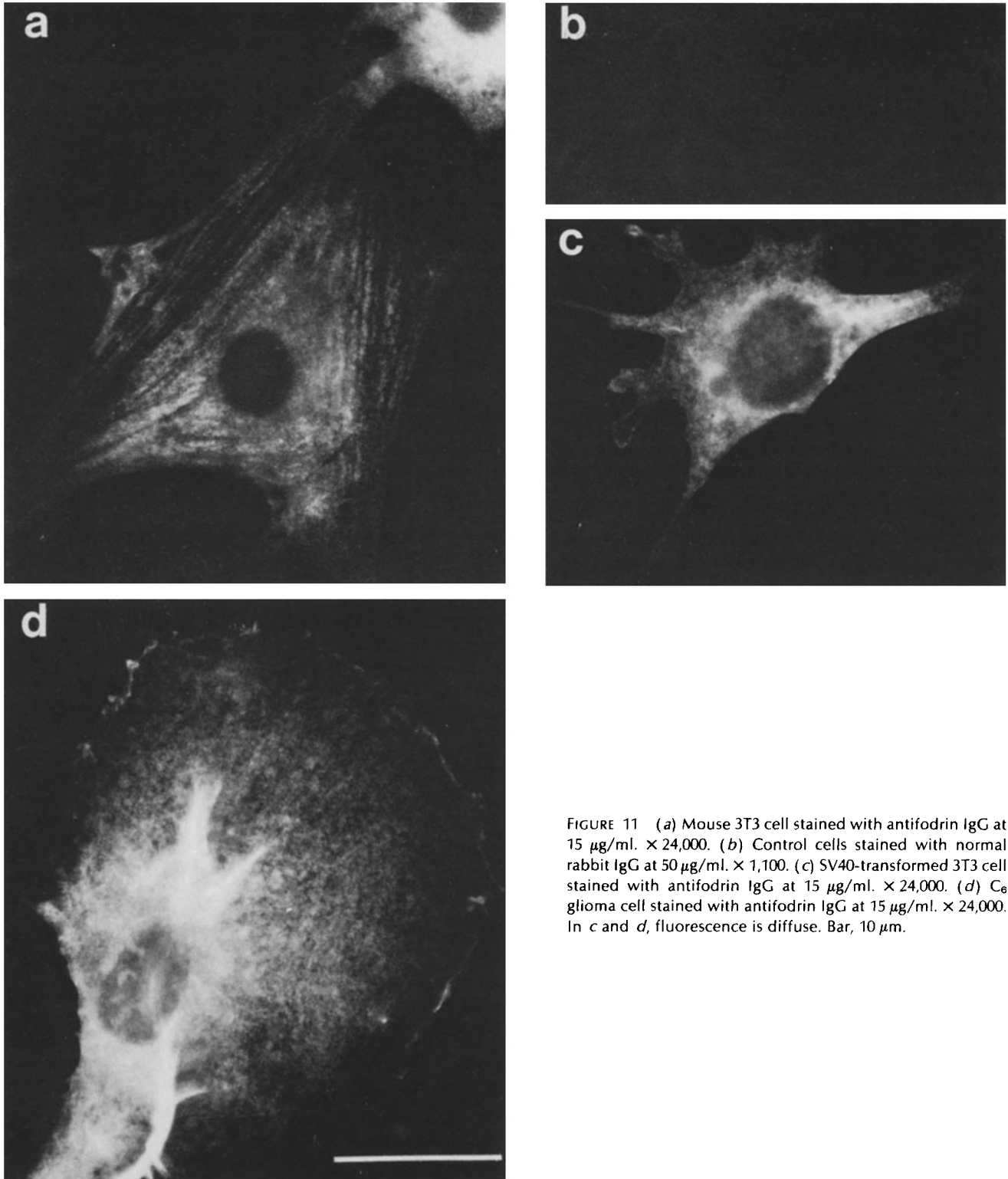


FIGURE 11 (a) Mouse 3T3 cell stained with antifodrin IgG at 15  $\mu\text{g}/\text{ml}$ .  $\times 24,000$ . (b) Control cells stained with normal rabbit IgG at 50  $\mu\text{g}/\text{ml}$ .  $\times 1,100$ . (c) SV40-transformed 3T3 cell stained with antifodrin IgG at 15  $\mu\text{g}/\text{ml}$ .  $\times 24,000$ . (d)  $\text{C}_6$  glioma cell stained with antifodrin IgG at 15  $\mu\text{g}/\text{ml}$ .  $\times 24,000$ . In c and d, fluorescence is diffuse. Bar, 10  $\mu\text{m}$ .

FIGURE 10 Cultured guinea pig fibroblasts stained with antifodrin. (a and b) The same fibroblast viewed in phase-contrast and epifluorescent optics, respectively. In b, the cell was stained with antifodrin IgG at 20  $\mu\text{g}/\text{ml}$ . The arrows point to stress fibers visible in a and the corresponding areas of the cell in b that have reduced fodrin-specific fluorescence. (c) A cell viewed from its dorsal surface; note the crossed linear arrays of antifodrin fluorescence. (d) Control cell stained with normal rabbit IgG at 50  $\mu\text{g}/\text{ml}$ . (e) A cell treated with cytochalasin B (10  $\mu\text{g}/\text{ml}$ ) in media for 30 min at 37°C before fixing and staining with antifodrin IgG at 15  $\mu\text{g}/\text{ml}$ . (f) A cell incubated with colchicine ( $10^{-4}$  M) for 30 min at 37°C and then stained with antifodrin IgG at 20  $\mu\text{g}/\text{ml}$ .  $\times 1,350$ . Bar, 10  $\mu\text{m}$ .

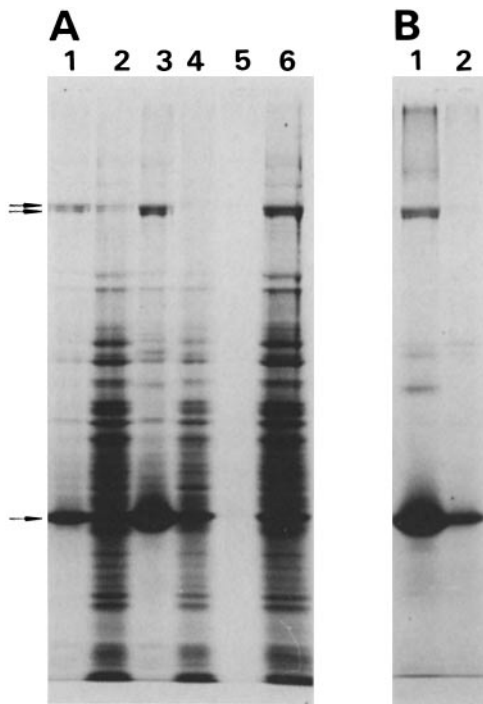


FIGURE 12 Cosedimentation of fodrin and F-actin. (A) Crude HS extracts of brain (500  $\mu$ g of protein) were mixed with F-actin and centrifuged, and the pellets and supernates were analyzed by SDS PAGE. Lanes 1 and 2: pellet and supernate, respectively, with 30  $\mu$ g of actin in the mixture. Lanes 3 and 4: pellet and supernate, respectively, with 100  $\mu$ g of actin in the mixture. Lanes 5 and 6: pellet and supernate, respectively, with no added actin. (B) Fodrin purified by gel filtration, and treated as in A. Lanes 1 and 2: pellet and supernate, respectively, with 100  $\mu$ g of actin in the mixture. The double arrow indicates fodrin. The single arrow indicates actin.

possibility that fodrin may have the potential to move quite rapidly in the cortical cytoplasm of cells, and that in neurons, the lining of the axolemma may be in constant motion away from the cell body. However, we cannot yet exclude the alternative possibility that only a small fraction of the axonal fodrin is actually moving at any one time in some other subcellular compartment that is not detected by immunofluorescence, and that this moving fodrin serves to replenish a stationary axonal lining of fodrin.<sup>2</sup> A second observation that may prove to bear upon the function of fodrin is that, although labeled newly synthesized fodrin first enters the retinal ganglion cell axons with the second most rapidly transported group of polypeptides, labeled fodrin persists in the axon through the passage of the fourth and fifth groups (24). Furthermore, preliminary subcellular fractionation experiments have indicated that the labeled fodrin is associated with denser material when the fifth group of transported proteins is in the axons than at earlier times (26). These observations suggest that newly synthesized fodrin becomes associated with a number of different organelles, which are subsequently transported down the

<sup>2</sup> When we ligated a sciatic nerve 48 h before determining the intracellular distribution of fodrin proximal to the ligation, we found only the usual bilaminar profile of fodrin-specific immunofluorescence. It might be expected that if fodrin were transported in a different subcellular compartment, that the moving fodrin would have accumulated in this compartment at the ligation; however, without knowing the fraction of fodrin that moves, we cannot consider this negative result as conclusive evidence against a separate transport compartment.

axons at different velocities. It seems reasonable to imagine that transported organelles and proteins must be linked in some fashion to the structures that provide the mechanical force for axonal transport; these properties of fodrin might be expected of a component of such a linkage system. More specifically, fodrin might serve to link organelles and proteins of any or all of groups II, III, IV, and V to a subaxolemmal force-generating system, possibly involving the cortical actin filaments (48). Analogously, fodrin could serve to link proteins (including membrane proteins) and organelles to submembranous force-generating systems in other cells, and participate in a system governing their distribution.

Although fodrin shares certain properties with other partially characterized proteins, it differs from each. The most striking similarity is with erythrocyte spectrin, which occupies a similar position in the submembranous region in erythrocytes, forms complexes that interact with actin *in vitro* (5), and is a heterodimer composed of polypeptides with electrophoretic mobilities similar to those of fodrin. However, antisera against spectrin have not been observed to react with proteins from cells other than erythrocytes (15), and we were unable to demonstrate a reaction between antifodrin and spectrin (Fig. 2). In addition, the electrophoretic mobilities of fodrin and spectrin, although similar, are distinguishable. It is, therefore, clear that spectrin and fodrin are not identical proteins, although it remains possible that they perform related functions in different cells. Fodrin is also electrophoretically and functionally distinct from two forms of neuronal myosin, whose interaction with actin is, unlike fodrin's, sensitive to ATP (44). A high molecular weight, actin-binding protein designated filamin has been identified in a number of cells (41, 42), and a similar polypeptide has been described in macrophages (12). However, antifodrin antibodies did not precipitate filamin from smooth muscle tissue. In addition, unlike fodrin, filamin has been reported to be a component of the stress fibers of fibroblasts (13).

Finally, it is worth noting that these experiments illustrate that immunofluorescence localization techniques, which have proven effective in pursuing many other problems, provide a means of determining the steady-state distribution (the "destination") of axonally transported polypeptides, most of which are known only as bands or spots on polyacrylamide gels.

We thank Drs. R. Bischoff, C. Cornbrooks, P. Wood, R. Munson, and D. Rabin for generous gifts of cultured cells; Dr. B. Hartman and S. Kalmbach for sharing immunofluorescence advice and equipment; Dr. L. Glaser, Dr. P. Skene, Dr. C. Baitinger, and C. Simon for cogent comments; and Dr. P. R. Vagelos and D. Vagelos for Greek translation.

This work was supported by grant EYO-2682, a Jerry Lewis Neuromuscular Research Center grant, and Research Career Development Award NS00170. J. Levine was supported in part by Vision Research Training Grant 1 EY 00092.

Received for publication 15 September 1980, and in revised form 13 April 1981.

#### REFERENCES

- Anderson, R. G. W., M. S. Brown, and J. L. Goldstein. 1977. Role of the coated endocytic vesicle in the uptake of receptor-bound low density lipoprotein in human fibroblasts. *Cell* 10:351-364.
- Ash, J. F., D. Louvard, and S. J. Singer. 1977. Antibody-induced linkages of plasma membrane proteins to intracellular actomyosin-containing filaments in cultured fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 74:5584-5588.
- Bourguignon, L. Y. W., and S. J. Singer. 1977. Transmembrane interactions and the mechanism of capping of surface receptors by their specific ligands. *Proc. Natl. Acad. Sci. U. S. A.* 74:5031-5035.
- Braun, J., K. Fujiwara, T. D. Pollard, and E. R. Unanue. 1978. Two distinct mechanisms for redistribution of lymphocyte surface macromolecules. I. Relationship to cytoplasmic myosin. *J. Cell Biol.* 79:409-418.
- Brenner, S. L., and E. D. Korn. 1980. Spectrin/actin complex isolated from sheep

- erythrocytes accelerates actin polymerization by simple nucleation. *J. Biol. Chem.* 255: 1670-1676.
6. Brown, S., W. Levinson, and J. A. Spudich. 1976. Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. *J. Supramol. Struct.* 5:119-130.
  7. Chang, C. M., and R. D. Goldman. 1973. The localization of actin-like fibers in cultured neuroblastoma cells as revealed by heavy meromyosin binding. *J. Cell Biol.* 57:867-874.
  8. Estridge, M., and R. Bunge. 1978. Compositional analysis of growing axons from rat sympathetic neurons. *J. Cell Biol.* 79:138-155.
  9. Fairbanks, G., T. L. Steck, and D. F. H. Wallach. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry.* 10:2606-2617.
  10. Goldman, R. D., E. Lazarides, R. Pollack, and K. Weber. 1975. The distribution of actin in non-muscle cells. *Exp. Cell Res.* 90:333-344.
  11. Hartman, B. K. 1973. Immunofluorescence of dopamine- $\beta$ -hydroxylase. Application of improved methodology to the localization of the peripheral and central noradrenergic nervous system. *J. Histochem. Cytochem.* 21:312-332.
  12. Hartwig, J. H., and T. P. Stossel. 1975. Isolation and properties of actin, myosin and a new actin binding protein in rabbit alveolar macrophages. *J. Biol. Chem.* 250:5696-5705.
  13. Heggeness, M., K. Wang, and S. J. Singer. 1977. Intracellular distributions of mechanochemical proteins in cultured fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* 74:3883-3887.
  14. Heuser, J. E., and M. W. Kirschner. 1980. Filament organization revealed in platinum replicas of freeze-dried cytoskeleton. *J. Cell Biol.* 86:212-234.
  15. Hiller, C., and K. Weber. 1977. Spectrin is absent in various tissue culture cells. *Nature (Lond.)* 266:181-183.
  16. Hoffman, P., and R. Lasek. 1975. The slow components of axonal transport. *J. Cell Biol.* 66:351-366.
  17. Hynes, R. O., and A. T. Destree. 1978. 10 nm filaments in normal and transformed cells. *Cell.* 13:151-163.
  18. Karlsson, J., and J. Sjöstrand. 1971. Synthesis, migration and turnover of protein in retinal ganglion cells. *J. Neurochem.* 18:749-767.
  19. Kessler, S. W. 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* 115:1617-1624.
  20. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* 227:680-685.
  21. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of  $^3\text{H}$  and  $^{14}\text{C}$  in polyacrylamide gels by fluorography. *Eur. J. Biochem.* 56:335-341.
  22. Lazarides, E. 1975. Immunofluorescence studies on the structure of actin in tissue culture cells. *J. Histochem. Cytochem.* 23:507-528.
  23. Levine, J. 1980. Fodrin: two axonally transported polypeptides associated with the submembrane space of many cells. PhD Thesis. Washington University, St. Louis, Mo.
  24. Levine, J., and M. Willard. 1980. The composition and organization of axonally transported proteins in the retinal ganglion cells of the guinea pig. *Brain Res.* 194:137-154.
  25. Lingappa, V., J. Lingappa, R. Prasad, K. Ebner, and G. Blobel. 1978. Coupled cell-free synthesis, segregation and core glycosylation of a secretory protein. *Proc. Natl. Acad. Sci. U. S. A.* 75:2338-2342.
  26. Lorenz, T., and M. Willard. 1978. Subcellular fractionation of intra-axonally transported polypeptides in the rabbit visual system. *Proc. Natl. Acad. Sci. U. S. A.* 75:505-509.
  27. Marchesi, V. T. 1974. Isolation of spectrin from erythrocyte membranes. *Methods Enzymol.* 32:275-277.
  28. Metzuzals, J., and I. Tasaki. 1978. Subaxolemmal filamentous network in the giant nerve fiber of the squid (*Loligo pealei* L.) and its possible role in excitability. *J. Cell Biol.* 78: 597-621.
  29. Mooseker, M. S., and L. G. Tilney. 1975. Organization of an actin filament-membrane complex: filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. *J. Cell Biol.* 67:725-743.
  30. Nicolson, G. L. 1976. Transmembrane control of the receptors on normal and tumor cells. I. Cytoplasmic influence over cell surface components. *Biochim. Biophys. Acta.* 457:57-108.
  31. Nicolson, G. L., V. T. Marchesi, and S. J. Singer. 1971. The localization of spectrin on the inner surface of human red blood cell membranes by ferritin-conjugated antibodies. *J. Cell Biol.* 51:265-272.
  32. Parikh, I., S. March, and P. Cuatrecasas. 1974. Topics in the methodology of substitution reactions with agarose. *Methods Enzymol.* 34:77-102.
  33. Pollack, R., M. Osborn, and K. Weber. 1975. Patterns of organization of actin and myosin in normal and transformed cultured cells. *Proc. Natl. Acad. Sci. U. S. A.* 72:994-998.
  34. Pollard, T. D., and R. R. Werhling. 1974. Actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* 2:1-65.
  35. Schroeder, T. E. 1973. Actin in dividing eggs: contractile ring filaments bind heavy meromyosin. *Proc. Natl. Acad. Sci. U. S. A.* 70:1688-1692.
  36. Sheetz, M. P., R. G. Painter, and S. J. Singer. 1976. Relationships of the spectrin complex of human erythrocyte membranes to the actomyosins of muscle cells. *Biochemistry.* 15: 4486-4492.
  37. Sheetz, M. P., and S. J. Singer. 1977. On the mechanism of ATP-induced shape changes in human erythrocyte membranes. I. The role of the spectrin complex. *J. Cell Biol.* 73: 638-646.
  38. Skene, J. H. P., and M. Willard. 1981. Electrophoretic analysis of axonally transported proteins in toad retinal ganglion cells. *J. Neurochem.* In press.
  39. Stossel, T. P., and J. M. Hartwig. 1976. Interactions of actin, myosin and a new actin-binding protein of rabbit pulmonary macrophages. II. Role in cytoplasmic movement and phagocytosis. *J. Cell Biol.* 68:602-619.
  40. Tilney, L. G., and P. Detmers. 1975. Actin in erythrocyte ghosts and its association with spectrin. *J. Cell Biol.* 66:508-520.
  41. Wallach, D., P. J. Davies, and I. Pastan. 1978. Purification of mammalian filamin. *J. Biol. Chem.* 253:3328-3335.
  42. Wang, K., J. F. Ash, and S. J. Singer. 1975. Filamin, a new high molecular weight protein found in smooth muscle and non-muscle cells. *Proc. Natl. Acad. Sci. U. S. A.* 72:4483-4486.
  43. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl-sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.
  44. Willard, M. 1977. The identification of two intra-axonally transported polypeptides resembling myosin in some respects in the rabbit visual system. *J. Cell Biol.* 75:1-11.
  45. Willard, M., W. M. Cowan, and P. R. Vagelos. 1974. The polypeptide composition of intra-axonally transported proteins: evidence for four transport velocities. *Proc. Natl. Acad. Sci. U. S. A.* 64:191-195.
  46. Willard, M., and K. Hulebak. 1977. The intra-axonal transport of polypeptide H: evidence for a fifth (very slow) group of transported proteins in the rabbit visual system. *Brain Res.* 1361:289-306.
  47. Willard, M., C. Simon, C. Baitinger, J. Levine, and P. Skene. 1980. Association of an axonally transported polypeptide (H) with 100-Å filaments: use of immunoaffinity electron microscope grids. *J. Cell Biol.* 85:587-596.
  48. Willard, M., M. Wiseman, J. Levine, and P. Skene. 1979. Axonal transport of actin in rabbit retinal ganglion cells. *J. Cell Biol.* 81:581-591.