

AXONAL TRANSPORT OF ACTIN IN RABBIT RETINAL GANGLION CELLS

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ABSTRACT

We labeled proteins in the cell bodies of rabbit retinal ganglion cells with [³⁵S]methionine and subsequently observed the appearance of radioactive actin in tissues containing the axons and synaptic terminals of these neurons, i.e., the optic nerve (ON), optic tract (OT), lateral geniculate nucleus (LGN) and the superior colliculus (SC). The temporal sequence of appearance of labeled actin (which was identified by its specific binding to DNase I, its electrophoretic mobility, and its peptide map) in these tissues indicated that actin is an axonally transported protein with a maximum transport velocity of 3.4–4.3 mm/d.

The kinetics of labeling actin were similar to the kinetics of labeling two proteins (M1 and M2) which resemble myosin; these myosin-like proteins were previously found to be included in the groups of proteins (groups III and IV) transported with the third and fourth most rapid maximum velocities. The similarity in transport between actin and myosin-like proteins supports the idea that a number of proteins in the third and fourth transport groups may be functionally related by virtue of their involvement in a force-generating mechanism and suggests the possibility that these proteins may be axonally transported as a preformed force-generating unit.

KEY WORDS axonal transport · retinal ganglion cells · actin · myosin · gel electrophoresis

Since the axons and synaptic terminals of neurons are severely limited in their capacity for protein synthesis (1, 11), many proteins must be supplied from the neuronal cell body. Axonal transport, the process which carries out this function, is complex. In the retinal ganglion cells of the rabbit, newly synthesized proteins undergoing axonal transport can be divided into at least five groups¹

¹ A group has been operationally defined as comprising those polypeptides which appear to become radioactively labeled simultaneously in a particular segment of axons after the cell bodies are labeled.

(9, 28, 29). Each group is composed of a characteristic set of polypeptides, and moves with a characteristic maximum transport velocity (the estimated transport velocities range from ~240 mm/d (group I) to 1 mm/d (group V)). While the identities of the transported proteins are known in only a few cases, certain shared characteristics of the proteins of each group suggest that the organization of the transported proteins is a reflection of the movement of different organelles down the axons at different velocities (17). For example, the group I proteins are associated with an organelle (or organelles) with a density similar to that of the plasma membrane; several (but not all) group II polypeptides appear to be associated with

mitochondria; groups III and IV contain two proteins which resemble muscle myosin (27); and group V includes polypeptides similar to those suggested to be subunits of neurofilaments (8). The identification of additional axonally transported proteins should serve to clarify further the nature of each group.

Transport groups III and IV are intriguing since each includes a protein (M1 and M2, respectively) which, like muscle-myosin, binds to actin in an ATP-reversible manner (27). Analogous to the role of myosin in muscle, it is likely that M1 and M2 are involved in some form of force-generating mechanism and that this mechanism involves interaction between M1, M2, and actin. This consideration suggests the possibility that groups III and IV may represent, at least in part, the movement of such a force-generating unit or organelle down the axon. If so, it would be expected that these transport groups would also include actin and the proteins involved in the regulation of actin-myosin interactions. There are additional reasons to anticipate that actin is an axonally transported protein. First, actin is a major component of neurons (7) (as well as other eukaryotic cells [21]) and is present in the neurites of cultured neuroblastoma cells (4). Second, certain axonal force-requiring processes (e.g., axonal transport itself) which could involve actin are independent of the cell body (19); it would be expected that the proteins involved in such processes would be supplied by axonal transport. In the following experiments, we provide evidence that actin is indeed axonally transported, and that its maximum transport velocity is similar to that of M1 and M2 and other proteins of transport group IV.²

MATERIALS AND METHODS

Labeling of Transported Proteins

[³⁵S]methionine (700 Ci/mmol, obtained from Amersham Searle) was lyophilized, suspended at a concentration of 11 mCi/ml in a solution of dithiothreitol (0.1 mM), and 0.44 mCi (0.04 ml) was injected into the vitreous of the locally anesthetized eyes of adult albino rabbits (28). After appropriate times, the rabbits were killed by intravenous injections of sodium pentobarbital, and the optic nerve (ON), optic tract (OT), lateral geniculate nucleus (LGN), and superior colliculus (SC) were removed and frozen on dry ice.

² Black and Lasek (2) have recently reported similar results from studies of actin transport in the guinea pig visual system.

Local Labeling of Visual System Proteins

A rabbit was anesthetized with sodium pentobarbital, and 100 μ Ci of [³⁵S]methionine (20 μ l) was injected near the OT, LGN, and SC through holes drilled in the skull. The ON was labeled via injection of isotope through the orbit. After 50 min, the animal was killed with sodium pentobarbital and the ON, OT, LGN, and SC were removed and frozen on dry ice.

Analysis of Actin

The visual system tissues containing labeled, transported proteins were homogenized in 5 ml of a solution containing CaCl₂ (1 mM) and Tris (10 mM), pH 7.5, in a glass-on-glass (ON) or teflon-on-glass (OT, LGN, and SC) homogenizer. The samples were subjected to sonic oscillation for 0.5 min at 30 W, centrifuged for 1 h at 100,000 g, and the supernates were subjected to DNase-Sepharose affinity chromatography according to the procedures of Lazarides and Lindberg (15) as follows: The supernates were applied to columns of DNase-Sepharose (0.7 ml, either obtained from Worthington Biochemical Corp., Freehold, N. J., or prepared according to the buffer method of Parikh et al. [20]) in 5-ml disposable pipettes at room temperature. The samples were applied to the columns twice, and then the column was washed with 7 ml of a solution containing CaCl₂ (5 mM) and Tris (10 mM) pH 7.5, followed by 5.5 ml of guanidine·HCl (0.75 M) in Buffer A (0.5 M Na-acetate, 1 mM CaCl₂, 30% glycerol, pH 6.5). Actin was eluted from the column with 5.5 ml of guanidine·HCl (4 M) in Buffer A. The guanidine·HCl washes (0.75 and 4 M) were dialyzed overnight against 10 liters of H₂O (4°C) and precipitated with 9% trichloroacetic acid. The precipitates were washed with diethyl ether and resuspended in 0.15 ml of denaturing solution (1.5% sodium dodecyl sulfate [SDS], 10 mM Tris, 5 mM EDTA, 30% glycerol, 2 mM dithiothreitol, pH 8) with bromphenol blue added as tracking dye, and samples (0.05 ml) were electrophoresed.

Analysis of M1 and M2

The ATP-sensitive actin-binding proteins M1 and M2 were recovered from the pellets of the actin extractions by homogenization with 5 ml of a buffer of high ionic strength (20 mM Na₃P₂O₇, 1 mM MgCl₂, 0.6 M NaCl, 1% Triton X-100, 1 mM dithiothreitol, pH 8.2). The supernate of a 1-h, 100,000 g centrifugation was made to 60% of saturation at 0°C with (NH₄)₂SO₄, and the precipitate was sedimented at 100,000 g for 20 min. The precipitate was resuspended in 3 ml of a buffer containing MgCl₂ (5 mM), imidazole (25 mM), NaCl (0.2 M), Triton X-100 (0.1%), pH 7, and centrifuged for 1 h at 100,000 g. The supernates were divided into two aliquots (1.5 ml each); F-actin (100 μ g) was added to each aliquot, and ATP (0.34 mM) was added to one of the two. The actin was sedimented by centrifugation for 40 min at 100,000 g, and the gelatinous pellet was resus-

pended in denaturing buffer (0.1 ml). A sample (0.05 ml) was electrophoresed as described below.

Electrophoresis

Before electrophoresis, samples were heated for 5 min at 90°C in denaturing buffer and applied to SDS gradient polyacrylamide slab gels. The running gels were usually 13 cm high, and contained a gradient of 4–12% acrylamide (acrylamide:bis-acrylamide = 33.3:1) and 5–25% glycerol. The glycerol served to stabilize the acrylamide gradient during polymerization. The stacking gel contained 3% acrylamide, 0.09% bis-acrylamide. The buffer system was that of Laemmli (10). After electrophoresis, the gels were stained and destained according to the procedure of Fairbanks et al. (6). The gels containing M1 and M2 were impregnated with 2, 5-diphenyloxazole (PPO) according to the procedure of Bonner and Laskey (3). The gels were dried on a Bio-Rad gel drier (Bio-Rad Laboratories, Richmond, Calif.), and exposed to x-ray film (Kodak XR-5). In the case of the PPO-impregnated gels, the film was preflashed to an optical density of 0.13 with a shielded electronic flash unit before autoradiography at -70°C. The response of the preexposed film is linear with respect to radioactivity (13). Autoradiographs were developed under standard conditions in an automated film processor.

Quantitative Analysis of Actin, M1, and M2

The amount of radioactivity associated with actin was determined by two methods. First, the autoradiographic band associated with actin was scanned with a microdensitometer (Joyce-Loebl) and the area under the actin peak was determined by computerized planimetry. Second, the actin band was excised from the gel, dissolved in 0.5 ml of 30% H₂O₂, and the radioactivity was determined in a scintillation counter after adding 2.5 ml of H₂O and 10 ml of scintillation medium (3A70, Research Products International, Elk Grove, Ill.). The background radioactivity on the gel was determined as the average of counts in the two pieces of the gel adjacent to the actin band and of the same size. The activities obtained by both methods were corrected for radioactive decay from the time of isotope injection, and the total activity in the sample was calculated. The agreement of the two methods is shown by the similarity of the two actin curves in Fig. 7. In addition to the total radioactivity, the relative specific radioactivity of actin was calculated by dividing the actin-associated radioactivity by the relative protein contents of the actin bands determined by densitometry of the Coomassie blue-stained gels. However, no major differences were apparent between plots of specific activity and total activity; therefore, only total activity is shown in Fig. 7.

The radioactivity associated with M1 and M2 was calculated from densitometry of the fluorographs as follows: The area associated with M1 and M2 was corrected for ATP-insensitive radioactivity with the same

electrophoretic mobility. The resulting activity was corrected for radioactive decay from the time of isotope injection, and the numbers were converted to counts per minute (the conversion factor was obtained by directly counting M2-containing slices of the gel, as described for actin above). The activities were normalized so that the peak activity in each tissue segment corresponded to 1. The labeling pattern obtained in this way did not differ significantly from that obtained when the data were expressed as specific activities and corrected for differences in recovery between the +ATP and -ATP samples.

Peptide Maps

Transported actin was obtained from the ON and OT of a rabbit 8 d after its retina was labeled with 1 mC of [³⁵S]methionine. Actin was extracted and purified by DNase chromatography as described above, except that a 3-ml column of DNase-Sepharose was used. The column eluate was dialyzed against H₂O, concentrated to a volume of 1 ml by ultrafiltration on an Amicon PM 10 filter (Amicon Corp., Scientific Systems Div., Lexington, Mass.), and 530 μg of skeletal muscle actin was added. Reagents were added to achieve the following concentrations: SDS (1.5%), glycerol (15%), dithiothreitol (5 mM), Tris (10 mM), pH 8, and the sample was electrophoresed on a preparative polyacrylamide (8%) running gel (4 cm high, 15 mm inside diameter) with a 3% acrylamide stacking gel (1.2 cm high) using the buffer system of Laemmli (10) and the apparatus from Savant Instruments. The protein was eluted from the bottom of the gel with the same buffer in which it was applied, and the fractions containing actin and radioactivity (the two peaks coincided) were pooled and dialyzed against H₂O and lyophilized. The protein was denatured in guanidine·HCl (8 M), reduced with dithiothreitol, carboxymethylated, digested with trypsin and subjected to two-dimensional thin-layer chromatography-electrophoresis as described by Stephens (23). The thin-layer plates were autoradiographed for 10 d with Kodak XR-5 x-ray film and then stained with fluorescamine as described by Stephens (23). The skeletal muscle actin standard was purified from rabbit muscle by the method of Spudich and Watt (22), followed by preparative electrophoresis and digestion as described above.

RESULTS

We radioactively labeled newly synthesized proteins in the retinal ganglion cells of rabbits by injecting [³⁵S]methionine into the posterior chamber of the eye. The axons of the retinal ganglion cells exit from the eye through the ON (~17 mm long), pass through the contralateral OT³ (~10 mm long), and form synapses in the LGN (~27

³ In albino rabbits, the great majority of retinal ganglion cell axons cross at the optic chiasm.

mm from the eye) and the SC (~35 mm from the eye). We isolated actin by a combination of DNase-Sepharose affinity chromatography and polyacrylamide gel electrophoresis from the ON, OT, LGN, and SC at various time intervals (3 h, 1, 4, 8, 12, and 23 d) after labeling the retina, and determined the actin-associated radioactivity. Fig. 1 shows a stained gel illustrating the purification of actin from visual system structures by DNase I affinity chromatography. Figs. 2, 3, and 4 show autoradiographs of similar gels at 4, 8, and 23 d after the retina was labeled with [³⁵S]methionine. Actin (i.e., a protein which bound to DNase I and had the same electrophoretic mobility as actin) was first labeled in the ON 1 d after the retina was labeled (data not shown). By 4 d the protein was more heavily labeled in the ON (Fig. 2), and by 8 d it was labeled in the OT and LGN as well as in the ON. At 12 and 23 d (Fig. 4) actin was labeled in all four of the visual system tissues analyzed. Assuming that the sequential appearance of labeled actin in visual system structures progressively more distal from the eye reflects its transport down the axon, these experiments suggest that actin moves at a maximum velocity⁴ of 3.4–4.3 mm/d. (This estimate is based on the observation that at 4 d no labeled actin was detected farther than 17 mm from the eye [i.e., in the OT] while by 8 d some labeled actin was detected 27 mm from the eye [i.e., in the LGN]). The maximum transport velocity of actin is therefore most similar to the maximum velocity previously found (28) for group IV proteins, i.e., 2–4 mm/d. Groups I (240 mm/d) and II (34–68 mm/d) contain less actin than can be detected by our methods, since we found no labeled actin in the ON and OT at times when other proteins of these groups were labeled, i.e., 6 h in the ON and OT, and 1 and 4 d in the OT.

We previously observed that two polypeptides (M1 and M2) which resemble skeletal muscle myosin in their ATP-sensitive cosedimentation with actin are axonally transported in rabbit retinal ganglion cells with maximum velocities of 2–8 mm/d (27). Since actin also appears to be transported at a velocity in this range, we attempted to

⁴ By "maximum velocity" we mean the velocity (averaged over time) at which the most rapidly transported actin moves. Operationally, the maximum velocity was estimated as the distance between the retina and a point in the axons, divided by the time of the first appearance of labeled actin at that same point in the axon.

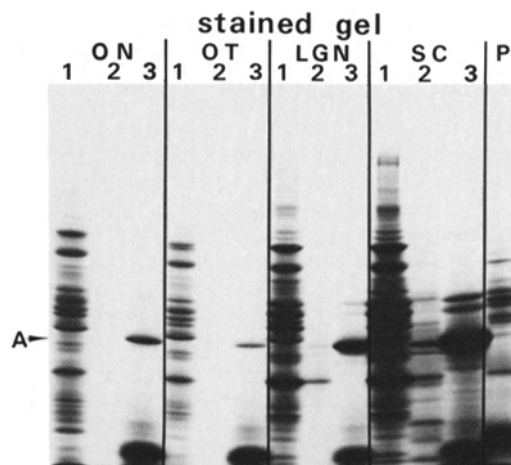
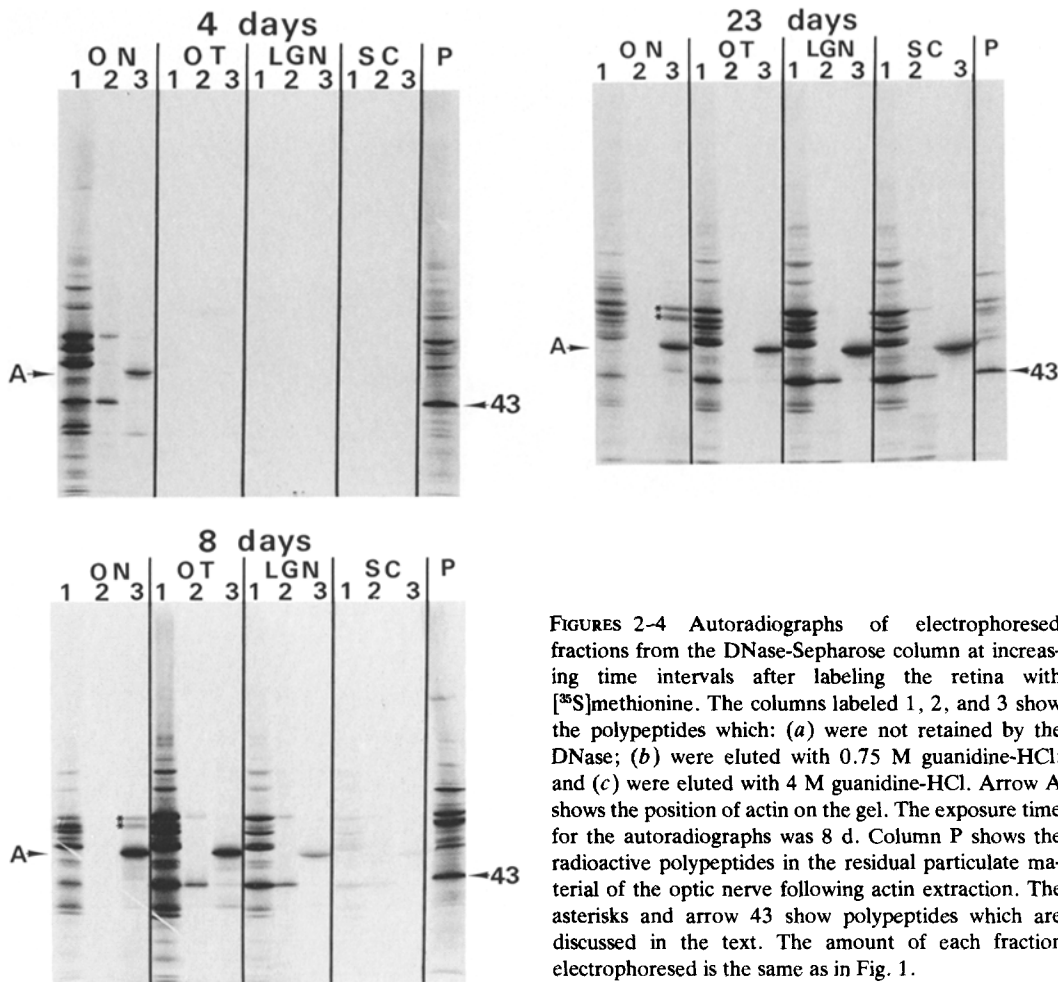


FIGURE 1 Stained SDS polyacrylamide gels showing the following: polypeptides which were not retained by DNase-Sepharose (columns labeled 1); polypeptides which were retained and eluted by 0.75 M guanidinium-HCl (columns labeled 2); polypeptides which were retained and eluted by 4 M guanidinium-HCl (columns labeled 3). Column P shows the polypeptides in the residual particulate fraction of the optic nerve after the extraction of soluble proteins. Arrow A indicates the electrophoretic mobility of skeletal muscle actin. In columns 1, 2, 3, and P, 0.16, 0.33, 0.33 and 0.10, respectively, of the entire fraction was electrophoresed.

characterize more precisely the relationship between the transport of actin and that of the two myosin-like proteins. We therefore repeated the above experiment, analyzing radioactively labeled M1 and M2 as well as actin in rabbits whose retinas were labeled with [³⁵S]methionine at intervals of 6 h, 1, 2, 4, 6, 8, 12, 23, and 36 d before the analysis. Actin was isolated from a soluble fraction of each tissue segment while M1 and M2 were extracted at high ionic strength from the particulate fractions of the same tissues. M1 and M2 were identified by their ATP-sensitive cosedimentation with F-actin. Figs. 5 and 6 show examples of autoradiographs of electrophoresed samples of actin-cosedimented M1 and M2, and DNase-purified actin at increasing time intervals after labeling the retina, while Fig. 7 summarizes the relative amounts of label associated with these proteins at each time. The temporal sequences of labeling of actin, M1, and M2 in the visual system tissues were similar; in the ON, OT, LGN, and SC, respectively, actin was first labeled at 2, 6, 8, and 23 d, M2 at 2, 6, 6, and 23 d, and M1 at 1, 6, 6, and 8 d. The data summarized in Fig. 7 suggest

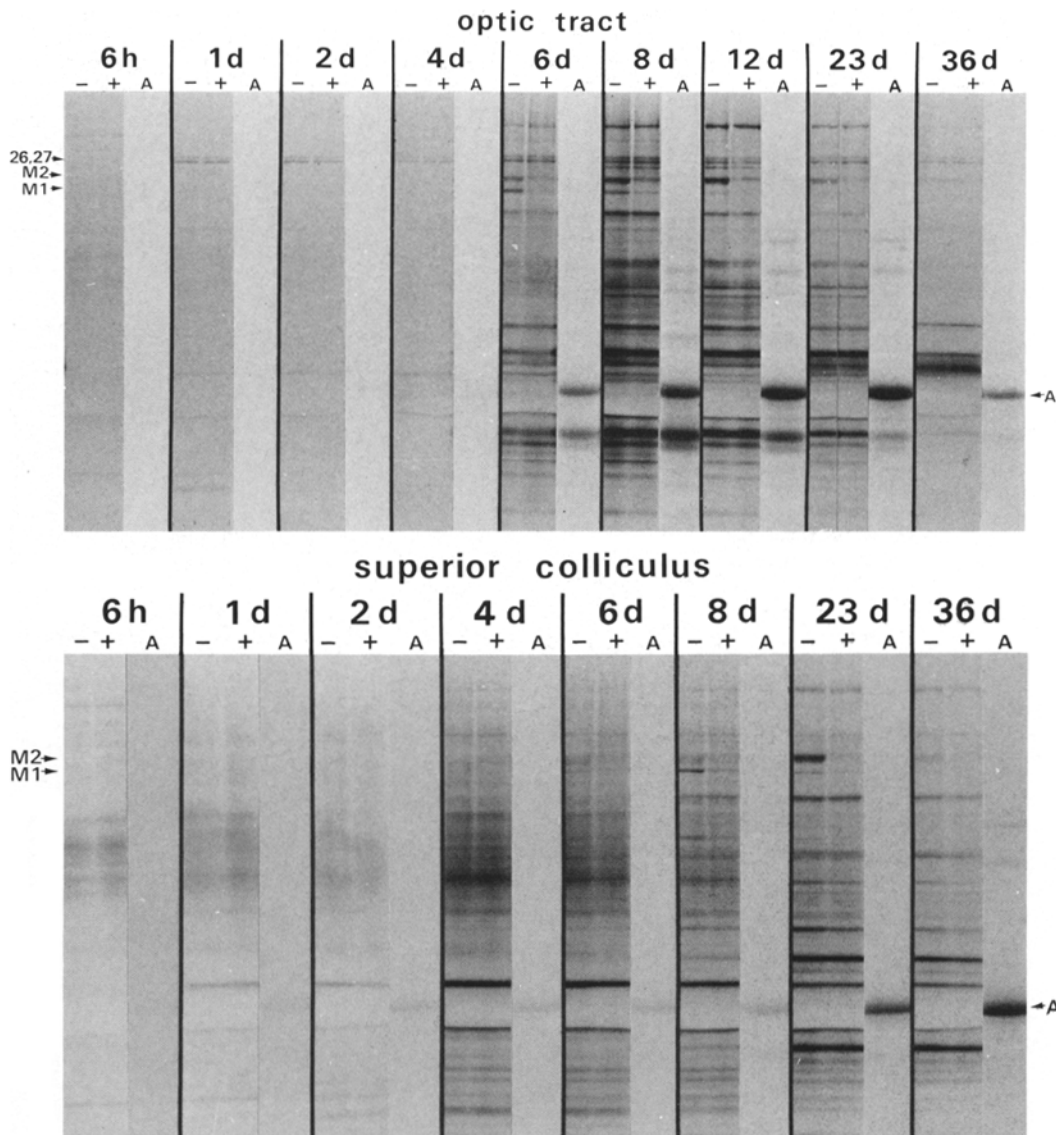


FIGURES 2-4 Autoradiographs of electrophoresed fractions from the DNase-Sepharose column at increasing time intervals after labeling the retina with [³⁵S]methionine. The columns labeled 1, 2, and 3 show the polypeptides which: (a) were not retained by the DNase; (b) were eluted with 0.75 M guanidine-HCl; and (c) were eluted with 4 M guanidine-HCl. Arrow A shows the position of actin on the gel. The exposure time for the autoradiographs was 8 d. Column P shows the radioactive polypeptides in the residual particulate material of the optic nerve following actin extraction. The asterisks and arrow 43 show polypeptides which are discussed in the text. The amount of each fraction electrophoresed is the same as in Fig. 1.

three additional conclusions. First, much more actin-associated label was recovered than label associated with M1 or M2 (see the legend of Fig. 7). At the time of maximum labeling of each protein in the optic nerve, the ratios of actin: M2: M1 were 1.0:0.014:0.0025. This difference indicates that much more newly synthesized actin is transported than M1 or M2, although the exact proportions cannot be determined without knowing the relative amounts of methionine in these three proteins and the relative efficiencies of their recovery. (However, we estimate that at least 30% of the total M1 and M2 was recovered; therefore, the excess of labeled actin cannot be entirely explained by differences in the efficiencies of recovery.) The second conclusion drawn from Fig. 7 is that the maximum labeling of M1 precedes the maximum labeling of actin and M2 in all

structures more distal from the eye than the ON. The times of maximum labeling of M2 and actin were similar, although M2 became maximally labeled more rapidly than actin in the OT and SC. Finally, Figs. 2-7 show that the label associated with actin disappeared from the visual system tissues more slowly than the label associated with M1, M2, and certain other group IV polypeptides, e.g., 43.

It is worth noting the behavior of several additional labeled proteins which were retarded by the DNase-Sepharose column. While most of the labeled polypeptides other than actin passed unretarded through the DNase-Sepharose (columns 1 of Figs. 2-4), several were retained. One such protein (No. 43 in Figs. 2-4, column 2) was eluted from the column by 0.75 M guanidine-HCl and co-electrophoresed with a previously described



FIGURES 5 and 6 Fluorographs of electrophoresed actin, M1, and M2 extracted from the same tissue segments at increasing time intervals after labeling the retina with [³⁵S]methionine. Actin was purified by DNase-Sepharose chromatography. The columns labeled A show the radioactive polypeptides which were eluted from the DNase with 4 M guanidine-HCl. The columns labeled (-) and (+) show the labeled proteins which cosedimented with actin in the absence (-) and presence (+) of ATP. "A" indicates the electrophoretic mobility of actin. One-third of each actin sample and one-half of each M1 and M2 sample were electrophoresed. Autoradiographic exposure times were: Fig. 5, actin = 10 d, M1 and M2 = 5 d; Fig. 6, actin = 10 d, M1 and M2 = 5 d.

transported polypeptide (No. 43 in reference 28). This polypeptide became labeled in a temporal sequence similar to that of actin, but (like M1 and M2) the label associated with it disappeared from the ON and OT more rapidly than was the case

for actin (Fig. 4). Polypeptide 43 migrates on SDS polyacrylamide gels the same way as skeletal muscle β tropomyosin (data not shown); if 43 and tropomyosin were related, it would seem possible that 43 was retained on the column by virtue of

an interaction with the actin bound to the column. Two labeled polypeptides (mol wt \approx 61,000 and 58,000) were, like actin, eluted from the DNase-Sepharose column with 4 M guanidine·HCl (asterisks in Figs. 3 and 4). The labeling pattern of these polypeptides was peculiar; while they were radioactively labeled in the ON from 8 d after the retina was labeled until the end of the experiment (23 d), they never became labeled in the OT, LGN, or SC. These labeled polypeptides co-electrophoresed on polyacrylamide gels with two stained polypeptides, one of which we have routinely observed in the ON but not in the OT (data not shown); these polypeptides may therefore be associated with the nerve sheath. The simplest explanation for their pattern of labeling is that the two polypeptides are not axonally transported from the cell bodies but, instead, are locally synthesized by optic nerve satellite cells,⁵ presumably from the labeled amino acids supplied by the breakdown of transported polypeptides.

The Local Synthesis of Actin and Myosin-like Proteins in the ON, OT, LGN, and SC

The possibility that proteins can become labeled in the visual system via the local incorporation of radioactive precursors supplied by the breakdown of axonally transported proteins led us to investigate the ability of the ON, OT, LGN, and SC to synthesize actin, M1, and M2 from locally supplied amino acids. We injected [³⁵S]methionine near these visual system tissues in an anesthetized rabbit, and after 50 min we assayed labeled actin, M1, and M2 by the same methods used in the transport experiments. Approx. 0.2% of the locally incorporated label was recovered in actin (Fig. 8). An ATP-sensitive actin-binding protein with an electrophoretic mobility similar to M2 was also locally labeled, but the major fraction of the local ATP-sensitive actin-binding label appeared to electrophorese slightly farther than M2 (Fig. 8). M1 was not labeled by the local application of [³⁵S]methionine.

Peptide Mapping of Transported Actin

As further confirmation of the relationship between the labeled DNase I binding protein and skeletal muscle actin, we compared the methio-

⁵ Satellite cells, as used here, means all nonneuronal cells (e.g., glia and mesodermal and meningeal elements) in the ON and OT.

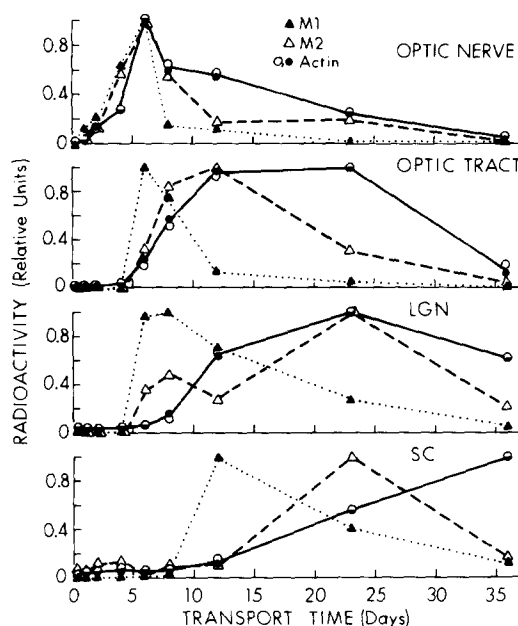


FIGURE 7 Radioactivity associated with actin, M1, and M2 after labeling the retina with [³⁵S]methionine. The symbols are: (○) actin-associated radioactivity determined by densitometry of autoradiographs or (●) by directly counting excised gel slices; (▲) M1, and (△) M2, determined by densitometry of fluorographs. The curves are normalized so that in each case the highest value is 1.0. This peak value corresponds to the following values (cpm) of total recovered radioactivity in each tissue for M1, M2, and actin, respectively: ON: 152, 889, 61,326; OT: 130, 274, 11,867; LGN: 43, 132, 6,775; SC: 31, 318, 9,187.

nine-containing tryptic peptides derived from these two proteins. We used DNase-Sepharose chromatography to purify the presumptive transported actin from the ON and OT of a rabbit 8 d after labeling its retina with 1 mCi of [³⁵S]methionine. The presumptive transported actin was mixed with an 18-fold excess of rabbit skeletal muscle actin and further purified by preparative gel electrophoresis. The resulting preparation gave rise to a single stained band on analytical polyacrylamide gels and one major radioactive band which comigrated with actin (Fig. 9a). 16 of the 29 radioactive methionine-containing tryptic peptides (arising from the transported protein) coincided on the peptide map with fluorescamine-stained peptides arising predominantly from the excess muscle actin (Fig. 9c). Most of these same labeled peptides also corresponded to stained peptides on a simultaneously

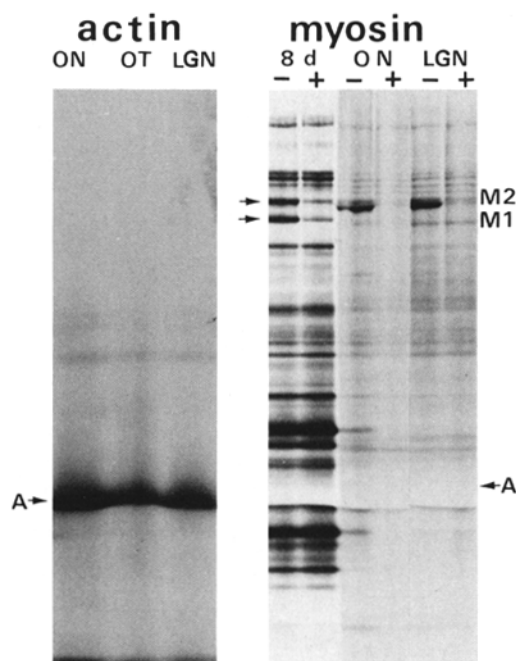


FIGURE 8 Synthesis of actin and ATP-sensitive actin-binding proteins after local application of [³⁵S]methionine to the ON, OT, and LGN. Columns labeled "actin" show autoradiographs of gels upon which actin purified from locally labeled ON, OT, and LGN was electrophoresed. Columns labeled myosin show fluorographs of gels containing polypeptides which cosedimented with actin in the absence (-) or presence (+) of ATP. The starting material in columns ON and LGN was locally labeled tissue, while the column labeled 8 d shows authentic M1 and M2 extracts from an ON + OT 8 d after labeling the retina with [³⁵S]methionine.

prepared map of muscle actin alone, but, due to the large number of spots and the variability in peptide separations, their coincidence in many cases was not a convincing proof of their identity. However, two of the labeled peptides corresponded to actin peptides with reproducibly distinctive positions on the peptide map, and could, therefore, be confidently identified as actin peptides. Since only methionine-containing peptides were radioactive, the presence of stained but nonradioactive peptides was expected. Labeled polypeptides which did not comigrate with muscle actin peptides were also expected since there are at least 25 amino acid differences between skeletal muscle actin and cytoplasmic actins (24). The similarities between the peptide maps of the transported protein and muscle actin provide additional evidence that the two are related.

DISCUSSION

We conclude that actin (identified by its affinity for DNase I, its electrophoretic mobility, and its peptide map) is a transported protein which moves down the axons of the retinal ganglion cells with a maximum transport velocity of 3.4–4.3 mm/d. This conclusion is significant in three respects. First, it establishes the identity of one of the transported proteins. Second, the transport velocity of actin supports the idea that a number of proteins with maximum transport velocities between 2 and 4 mm/d (group IV proteins) may be related to each other by virtue of their mutual involvement in a force-generating mechanism (17). And third, the similarity in transport of actin and M2 suggests the possibility that the two proteins may be transported as part of a preformed force-generating unit or organelle. These possibilities are considered below.

The transport of proteins at similar velocities (the basis for their division into transport groups) suggests that the proteins may move as an interacting unit, e.g., an assembled organelle, especially when the proteins within the group are functionally related. For example, the transport of multiple mitochondrial proteins in group II can most easily be understood as the movement of pre-assembled mitochondria down the axon (17). The similarity in the transport velocities of actin and M2, as well as their potential functional relationship, suggests that the two proteins (perhaps together with other group IV proteins) are part of a force-generating organelle which is pre-assembled into a functional unit before its transport down the axon. Such a functional unit might then be distributed along the length of the axon, where it would participate in mechanochemical events, such as the movements of materials in other transport groups. The precise destination of such a force-generating unit can only be surmised from what is known about the organization of actin within neurons. Two possibilities are considered here. First, Chang and Goldman (4) found actin filaments (identified by their decoration with heavy meromyosin) along the length of neurites. The density of these filaments was greatest underlying the plasma membrane. An actual attachment of actin to the plasma membrane has been demonstrated in the microvilli of the intestine (18). In cultured fibroblasts, the distribution of actin (16), myosin (25), and tropomyosin (14) can be similar, indicating that these three proteins may be components of the same structure. It therefore seems

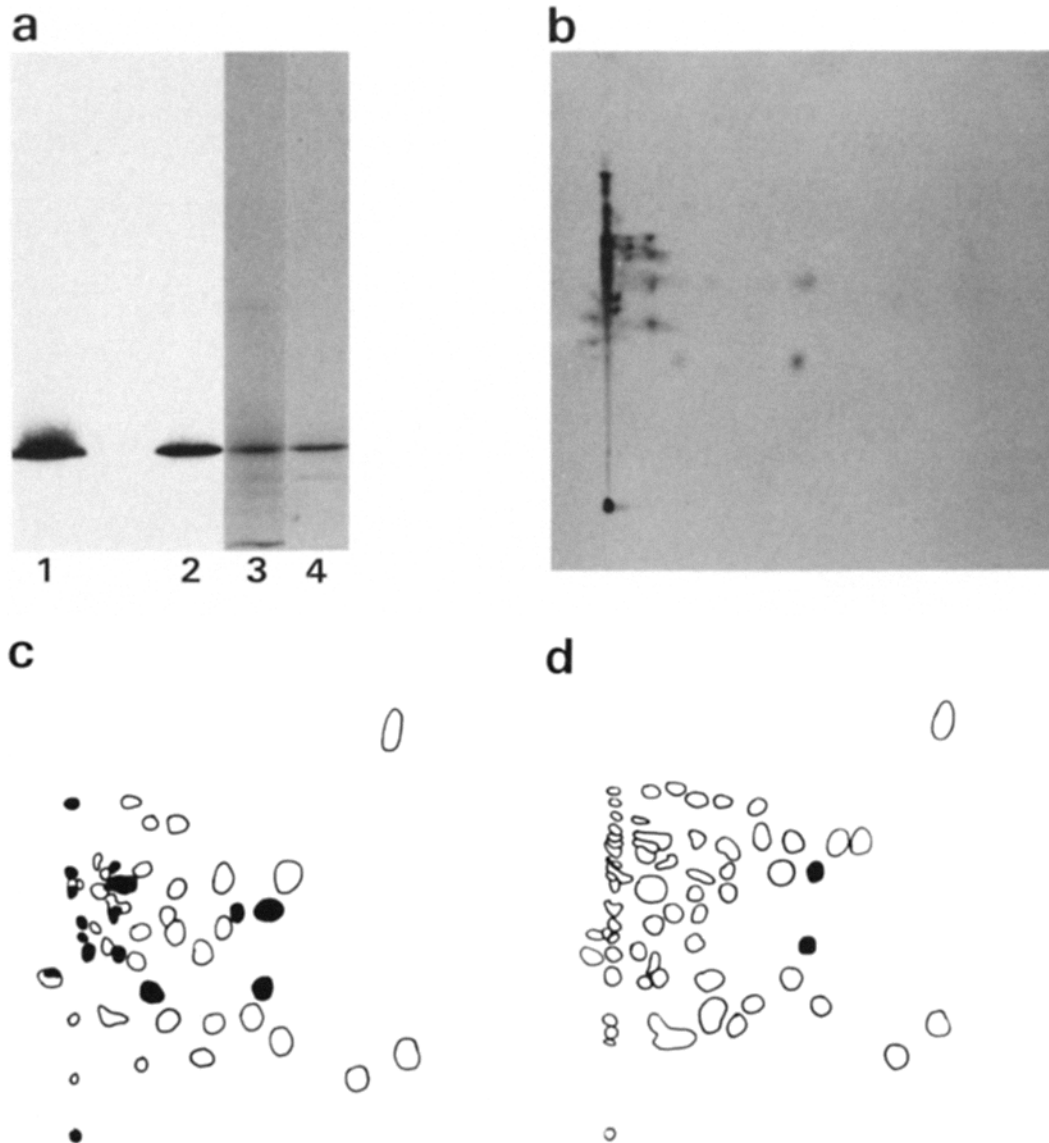


FIGURE 9 Tryptic peptide analysis of transported actin. The labeled transported polypeptide resembling actin was purified from ON + OT 8 d after labeling the retina with [³⁵S]methionine. (a) Column 1: a stained gel showing a skeletal muscle actin standard; Column 2: a stained gel showing material which subsequently served as the substrate for trypsin, i.e., the DNase purified ON + OT extract mixed with an excess of skeletal muscle actin, and further purified by preparative electrophoresis; Column 3: an autoradiograph of a gel upon which the labeled ON + OT material was electrophoresed after purification on DNase-Sepharose; Column 4: an autoradiograph of the gel in Column 2, showing the labeled material which was subsequently used as the substrate for trypsin. (b) Autoradiograph of the tryptic peptide map of the labeled transported protein mixed with an excess of skeletal muscle actin (220 μ g total). Chromatography is in the vertical direction while electrophoresis at pH 3.5 is in the horizontal direction. (c) Fluorescamine-stained peptide map of the labeled transported protein mixed with excess skeletal muscle actin. Fluorescamine-staining polypeptides which were also radioactive are indicated by solid spots. (d) Fluorescamine-stained peptide map of muscle actin (220 μ g) alone. The solid spots indicate the two peptides which are most clearly identical to the methionine-containing peptides arising from the transported protein shown in 9b.

reasonable to imagine that the actin transport observed here serves at least in part to replenish a sub-axolemmal force-generating apparatus, perhaps comprising M2, tropomyosin, and other group IV polypeptides, in addition to actin. It has been suggested by Lasek and Hoffman (12) that an interaction of neurofilaments with sub-axolemmal actin filaments may provide the motile force for the slow movement of neurofilaments down the axon. It seems quite reasonable to suppose that a sub-axolemmal force-generating unit such as described above might be responsible for the transport of more rapidly moving materials, i.e., groups I or II.

Alternatively, the morphological correlate of a group IV force-generating system could be the microtrabecular ground substance of the axoplasm. The microtrabecular system of axons comprises a lattice of interwoven strands which appears to interconnect microtubules, neurofilaments, smooth endoplasmic reticulum, and plasma membrane (5). The proteins that make up this system have not been directly determined, but similar systems in non-neuronal cytoplasm appear to be closely associated with actin-containing fibers (26). The maintenance of the microtrabecular system has not been accounted for in terms of axonally transported proteins. It is therefore worth considering that the axonal transport of actin, M2, and other group IV proteins may represent the replenishment of the elements of the microtrabecular system, either by deposition of its elements along the axon or by a growth of a continuous system down the axon.

While the axonal transport of actin and myosin as part of a preformed functional force-generating unit is an intriguing possibility, other explanations for the similarity in axonal transport of several contractile proteins obviously have not been excluded. For example, noninteracting proteins which shared the same transport mechanism might move at similar velocities, as might proteins whose interactions during transport did not reflect their functional interaction at their destination (e.g., if one protein was responsible for carrying another down the axon). On the surface, the difference in the apparent relative turnover rates of actin and M2 appears to favor such alternative explanations; while the various elements of a preformed functional force-generating unit might be expected to decay in the axon at similar rates, we observed that actin-associated label disappeared from the ON, OT, LGN, and SC more slowly than the

label associated with M2, 43, and other group IV proteins. However, since the apparent turnover rate of actin in the ON, OT, LGN, and SC may be complicated by the synthesis of actin by satellite cells as discussed below, it remains possible that actin, M2, and certain other group IV proteins decay at similar rates within the axons.

Two observations raise the possibility that actin may have become labeled on the ON, OT, LGN, and SC via local synthesis in these tissues as well as by axonal transport. First, satellite cells in these tissues can incorporate locally supplied amino acids into actin (Fig. 8). Second, since an ON protein, which was apparently not transported, eventually became labeled after the retina was labeled (asterisks in Figs. 3 and 4), it appears that some labeled amino acids became available for incorporation by satellite cells during the transport experiments. (This labeled amino acid was probably supplied by the transport process itself [e.g., by the intra-axonal breakdown of transported proteins] rather than by systemic uptake, since the ON contralateral to the labeled eye did not accumulate labeled actin [data not shown] although its access to systemic label was similar to that of the ON originating in the labeled eye.) However, we think it is unlikely that the labeling of actin by local synthesis can account for the pattern of actin labeling observed in the transport experiments, for the following reasons. The nontransported ON protein did not become labeled until 4 d after actin in the ON; if this nontransported protein is typical of locally synthesized proteins, this difference in labeling time argues that at least the initial appearance of labeled actin in the ON and OT is the result of transport rather than local synthesis. Furthermore, we estimate (based upon the fraction of locally synthesized protein which was recovered as actin) that a minimum of 4×10^7 cpm of transported label would have to be locally incorporated to achieve the observed labeling of actin in the ON 6 d after labeling the retina. Since the maximum total label in the ON at any time (also 6 d) was 1.5×10^7 cpm, a substantial breakdown of transported proteins would be required to supply enough label for a significant fraction of the observed labeled actin to have arisen from local incorporation. However, it does seem possible that the slow disappearance of labeled actin from the ON, OT, and LGN could be due in part to the continued local synthesis of actin from radioactive amino acid provided by the breakdown of transported proteins.

The distinction between transport and local synthesis is unambiguous in the case of M1, since it was not labeled by local administration of labeled amino acids and, in addition, its maximum labeling during transport preceded the maximum labeling of total protein in the OT, LGN, and SC. The failure to detect M1 among locally synthesized proteins is interesting since it suggests either that M1 may be involved in neuronal processes not shared by satellite cells or that M1 is enriched by the process of transport. The major ATP-sensitive actin-binding protein synthesized locally in the ON appeared to have a slightly greater electrophoretic mobility than M2; this observation makes it unlikely that M2 is labeled by local synthesis in the transport experiments.

We thank the members of the 1976 Neurosciences Techniques course for their participation in some phases of this work, and Dr. R. Waterston and C. Baitinger for suggestions on the manuscript.

This work was supported by grant EYO-2682, a Jerry Lewis Neuromuscular Research Center Grant, and Research Career Development Award NS00170.

Received for publication 10 August 1978, and in revised form 16 January 1979.

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