An Antiactin Antibody that Distinguishes between Cytoplasmic and Skeletal Muscle Actins

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ABSTRACT We elicited antibodies in rabbits to actin purified from body wall muscle of the marine mollusc, *Aplysia californica*. We found that this antiactin has an unusual specificity: in addition to reacting with the immunogen, it recognizes cytoplasmic vertebrate actins but not myofibrillar actin. Radioimmunoassay showed little or no cross-reaction with actin purified from either chicken gizzard or rabbit skeletal muscle. Immunocytochemical studies with human fibroblasts and L6 myoblasts revealed intense staining of typical cytoplasmic cables. Myofibrils were not stained after treatment of human and frog skeletal muscle with the antibody, although the distribution of immunofluorescence suggested that cytoplasmic actin is associated with membrane systems in the muscle fiber. The antibody may therefore be especially suited for studying the localization of cytoplasmic actin in skeletal muscle cells even in the presence of a great excess of the myofibrillar form.

In addition to its role in muscle contraction, actin is thought to participate in other processes that involve intracellular motility (including fast axonal transport) and to serve as a universal cytoskeletal element in eukaryotic cells (see references 14, 35, 43). Although greatly conserved during phylogeny, the actin molecule occurs in a variety of forms (24). In vertebrate muscle, three types have been separated because of differences in charge: α-actin is present in sarcomeres of the skeletal muscle fibril; β- and γ-actins are found in embryonic and smooth muscle (13, 38, 45). Actins have also been isolated from brain and other nonmuscle eukaryotic tissues; these have been called cytoplasmic, and are more closely related to the actins of embryonic muscle than to myofibrillar actin (24).

Immunocytochemical localization has been one of the main tools for obtaining evidence that actin is involved in intracellular motility. The antiactin antibodies previously used, whether raised against actins of smooth muscle or against α-actin, have generally reacted with both cytoplasmic and myofibrillar actins (see below). To determine how actin might be involved in the rapid movement of organelles along axons of identified neurons of the marine mollusc, *Aplysia californica*, we have raised antibodies in rabbits using *Aplysia* body wall muscle actin as immunogen. We found that the antibody obtained can be used to localize actin within neurons (reference 29 and Lubit et al., manuscript in preparation). We also found that the specificity of the antibody is unlike all previous antiactins because it reacted only with certain forms of cytoplasmic actin and not with myofibrillar actin. This unique specificity was determined by competitive binding studies and by immunohistochemical examination of vertebrate cells in which the distribution of actin is known. Reactivity of the antibody with cytoplasmic actin was assessed by determining its localization in myoblasts and in fibroblasts that have been intensively studied with other antiactin antibodies. Lack of reactivity with α-actin was tested, using adult skeletal muscle.

MATERIALS AND METHODS

Muscle Preparations

Cultures of L6 muscle cells were provided by Dr. Halina Den, and primary human fibroblasts by Dr. Armand Miranda, both in the Department of Neurology, College of Physicians and Surgeons, Columbia University. 10- to 12-μm cryostat sections of normal adult human quadriceps muscle were generously supplied by Dr. Arthur Hays in the Department of Neuropathology. Single muscle fibers were dissected from the m. cutaneous pectoris of the frog (8).

Aplysia actin was prepared by differential extraction of thin filaments by a modification (Sherbany and Schwartz, unpublished observations) of Szent-Györgyi et al. (40). Briefly, washed strips of body wall muscle from animals weighing 100–250 g (Pacific Bio-Marine Supply Co., Venice, Calif.) were homogenized in 40 mM NaCl, 0.1 mM EDTA, 5 mM ATP, 5 mM sodium phosphate (pH 6.0) in a Sorvall Omnimix (DuPont Instruments Co., Sorvall Biomedical Div., Wilmington, Del.) for 2–3 s. The extract was clarified by centrifugation at 80,000 *g* for 20 min, and actin was purified by chromatography on DEAE-cellulose (15) followed by two cycles of polymerization with 2 mM $MgCl_2$.

Immunological Methods

The actin used for immunization was subjected to polyacrylamide gel electrophoresis in SDS (44). The region of the gel containing actin appeared opalescent

when chilled and was readily differentiated from the rest of the gel, presumably because actin, by far the predominant protein on the gel, binds SDS, which is insoluble below 15°C. The opalescent zone was cut out and freeze-dried. The lyophilized gel was then homogenized in 0.85% NaCl (two parts) and emulsified in complete Freund's adjuvant (one part) (Grand Island Biological Co., Grand Island, N. Y.) (41). The rest of the gel was stained with Coomassie Brilliant Blue to verify that actin had been cut out of the gel accurately. New Zealand white rabbits were immunized intramuscularly with 30 μg of actin each week for 4 wk and boosted monthly. Globulin (39) was purified from weekly bleeds pooled at monthly intervals. The appearance of precipitating antibody was monitored by Ouchterlony immunodiffusion performed at 4°C in 1% agarose (Sigma Chemical Co., St. Louis, Mo.) containing borate buffer (pH 8.2). A single precipitin line was observed when *Aplysia* thin filaments were used as the test antigen (Lubit et al., manuscript in preparation).

The specificity of antiactin was determined by a double antibody radioimmunoassay method (18), using [^{3}H]actin as the radiolabeled probe. Assays were carried out at 4°C in 0.01 M Tris-HCl (pH 7.4) containing 0.15 M NaCl and 0.25% gelatin (TBS). All sera (including preimmune) were heated at 56°C for 30 min before use (9).

Antiactin globulin was purified by adsorption to an immunoadsorbent consisting of 0.9 mg *Aplysia* body wall muscle actin coupled to 1 g of cyanogen bromide-activated Sephacryl S200 (11) by batch procedure (1). The amount of *Aplysia* actin coupled was determined by the difference in the protein concentration of the actin solution before and after coupling. Protein concentration was measured colorimetrically using Coomassie Blue (5). Antiactin globulin was similarly coupled to cyanogen bromide-activated Sephacryl (11). The concentration of globulin was determined spectrophotometrically at A_{280nm} before and after coupling to determine the amount of antibody protein bound to Sephacryl.

Immunohistochemical Methods

Antiactin globulin was directly coupled to fluorescein isothiocyanate (21). For testing specificity, some samples were absorbed for 16 h at 4°C with purified rabbit skeletal muscle actin (36) or chicken gizzard actin (17). The antiactin used for immunohistochemistry was purified by adsorption as described in the legend to Table I.

Cells and tissues were fixed either in paraformaldehyde or in 95% ethanol at −20°C for 1 min and air-dried. Paraformaldehyde powder (1% wt/vol) was dissolved in PBS by adjusting the pH of the suspension to 11 with 1 N NaOH. The resulting solution was then stirred for 10 min and the pH brought to 7.3 with 1 N HCl (46). Cells and tissue sections were fixed at 4°C for 30 min, whereas frog muscle was fixed for 1 h, and all were washed briefly with PBS. To prevent nonspecific staining, they were treated with a 1:5 dilution of normal sheep serum in a moist chamber for 10 min at room temperature before application of the fluoresceinated antiactin for 30 min. 0.5% Nonidet P-40 (NP-40) (Gallard Schlesinger Chemical Mfg. Corp., Carle Place, N. Y.) was included in all antibody solutions to make the cells permeable to antibody. In some experiments, paraformaldehyde-fixed frog muscles were also treated with 0.2% Triton X-100 and acetone (19, 20). After the slides were rinsed with PBS and wet mounted under coverslips in PBS-glycerol (4:1), they were examined by illumination from above with a Leitz Ortholux fluorescence microscope fitted with a TK510 dichroic mirror, a K515 suppression filter, a 1.5-mm BG12 excitation filter, and a × 2 interference blue filter (KP490). Either a × 25 (0.75 numerical aperture) or × 63 (1.3 numerical aperture) oil immersion objective was used. Photographs were taken with Kodak high-speed Ektachrome color film (ASA 400) and duplicated using Ilford Pan F (ASA 50) black and white film.

Cryostat sections of adult human muscle, fixed for 30 min at 4°C in 1% buffered paraformaldehyde and treated with normal sheep serum, were also examined by the peroxidase-antiperoxidase (Miles Laboratories, Inc., Elkhart, Ind.) procedure (37): 0.5% NP-40 was added to all solutions except the final substrate mixture, which contained 3,3'-diaminobenzidine (Sigma Chemical Co.).

TABLE I

Purification of Antiactin

Antibody	Antiactin activity	Protein	Specific activity
	pg/ml	*mg/ml*	*pg antiactin/ mg protein*
Starting material			
Serum	6,927	109	64
Antibody recovered			
Excluded	1,930	89	22
Bound	2,915	1.3	2,208

Purification of antiactin by affinity chromatography on an *Aplysia* actin-Sephacryl immunoadsorbent. The actin, isolated by DEAE-cellulose (Materials and Methods), was further purified by chromatography on a DNase I-agarose column as previously described (26). The actin fraction to be coupled to Sephacryl was eluted from the DNase column in 3 M guanidine-HCl. 3.7 ml of antiactin serum was applied to a slurry of the Sephacryl immunoadsorbent containing 0.54 mg of *Aplysia* actin. 72% of the antiactin activity applied was adsorbed after three washes each with 3 ml of 0.15 M NaCl, 0.01 M sodium phosphate (pH 7.3) (PBS). The rest was recovered in the excluded fraction. To elute bound antiactin, we added 1 ml of 3 M ammonium thiocyanate. After repeating this elution twice, we dialyzed the protein eluted against PBS, and sterile filtered. Antiactin titers were determined using the protein A immunoassay described in Fig. 2. We recovered 42% of the antiactin activity present in the serum initially applied, which was 58% of the antiactin adsorbed to the immunoadsorbent.

RESULTS

The antibody raised against *Aplysia* actin displayed an unusual specificity for certain molecular forms of actin. Specificity was determined by conventional double antibody radioimmunoassay, using [^{3}H]actin from *Aplysia* body wall as the radiolabeled probe (Fig. 1). As expected, the immunogen itself competed effectively: 50% inhibition of binding occurred in the presence of 15 ng of body wall actin. In preliminary experiments we have found that bovine brain actin (generously supplied by Dr. Soll Berl, Mount Sinai School of Medicine, New York) also inhibited binding: 50% inhibition occurred at a 29× greater protein concentration with the brain actin than with the im-

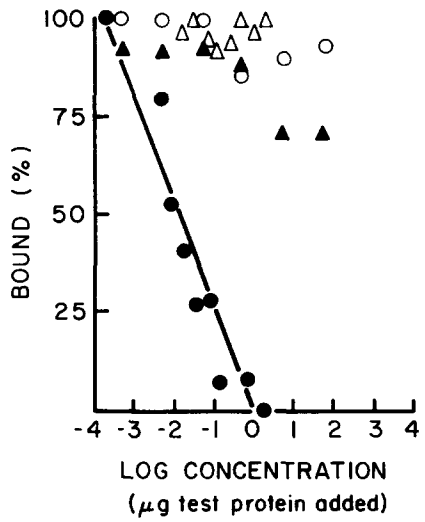

FIGURE 1 Specificity of antiactin antiserum determined by conventional radioimmunoassay. 0.5 mg of purified *Aplysia* actin was tritiated by acetylation with 8 μmol [^{3}H]acetic anhydride (3.5 Ci/mmol, Amersham Corp., Arlington Heights, Ill.) first at room temperature for 15 min and then at 4°C for an additional 135 min (26). The reaction mixture was maintained at pH 9.5 by the addition of sodium bicarbonate. [^{3}H]Actin (specific activity, 0.25 μCi/μg) was purified by gel filtration on Sephadex G-25 and used as the radiolabeled probe in a double antibody radioimmunoassay. 25 μl of immune serum was added to a 50-μl vol containing the competing test proteins (●, *Aplysia* body wall muscle actin; ▲, rabbit skeletal muscle actin; △, chicken gizzard actin; ○, *Aplysia* tropomyosin) in a series of dilutions and the mixtures kept for 1 wk. This long incubation period has been reported to increase the sensitivity of the assay by giving preference to the unlabeled test protein for the antibody combining sites (33). At this time, 50 μl of the [^{3}H] *Aplysia* actin probe (10,000 cpm) was added; 16 h later, sheep antirabbit globulin (prepared as described in reference 39) was added. After another 16 h at 4°C, the tubes were centrifuged at 6,500 *g* for 5 min. The immune precipitates were washed twice with TBS and dissolved in 0.2 ml of 0.5 N acetic acid. Radioactivity was measured by scintillation counting. Preimmune serum was used as the control.

munogen. Cross-reaction between brain actin and *Aplysia* actin is likely to be greater than these results would indicate, however, because the preparation from brain contained <20% actin, as estimated by inspection of gel electropherograms stained with Coomassie Blue. In contrast, actin purified from rabbit skeletal muscle inhibited only at much higher concentrations, and chicken gizzard actin was without effect. We did not obtain 50% inhibition with either form of vertebrate muscle actin even when as much as 7 μg were added. *Aplysia* tropomyosin and myosin also did not inhibit.

We purified the antiactin by affinity chromatography, using *Aplysia* actin as a ligand. Chromatography of the immune globulin on a Sephacryl-*Aplysia* actin immunoadsorbent resulted in a 35-fold purification of antiactin IgG (Table I) when tested by the new assay method using ^{125}I-labeled protein A described in Fig. 2.

Immunohistochemical studies revealed that the antibody reacted with *Aplysia* body wall muscle and cross-reacted with the cytoplasmic actin in individual *Aplysia* neurons (Lubit et al., manuscript in preparation). These studies will be reported in detail elsewhere. To characterize the specificity of the antibody further, we first tested its reactivity in vertebrate cells. We found that the antiactin raised against the invertebrate immunogen reacted strongly with vertebrate cytoplasmic actins. When primary human fibroblasts were treated with fluoresceinated antiactin purified by affinity chromatography, we found intense staining of cytoplasmic cables as described by others (17, 25, 27) (Fig. 3 *A* and *B*). Stress fibers were oriented parallel to the longitudinal axis of the cell, traversing the nucleus, and appeared to insert in the plasma membrane. Intensely granular fluorescence was also present around the cell nucleus; the nucleus itself, however, was not usually fluorescent. Intensity of staining appeared to be proportional to the concentration of the antiactin serum applied in the range of dilutions used (1:2–1:10). Staining was not obtained when fibroblasts were treated with the serum fraction that was excluded from the actin immunoadsorbent (Fig. 3 *C*). This fraction contained only 1% of the antiactin activity of the purified antiactin fraction when applied at the same protein concentration (see Table I).

α-Actin normally appears during development of skeletal muscle only after myoblasts fuse (13, 45). We therefore examined the distribution of immunofluorescence in myoblasts of the rat myogenic cell line, L6. An intracellular network of fibers appeared intensely fluorescent (Fig. 4 *A*). A similar distribution of immunofluorescence was seen with cells fixed in 95% ethanol. No immunofluorescence was observed when the myoblasts were treated with serum excluded from the Sephacryl-actin immunoadsorbent (Fig. 4 *B*). This distribution of immunofluorescence indicates that the antibody cross-reacts with embryonic muscle actin.

Immunohistochemical studies with fully differentiated skeletal muscle provided further support for the idea that the antibody does not react with α-actin, because it failed to stain myofibrils. When a frog muscle fiber was treated with antiactin in the presence of 0.5% NP-40, only diffuse immunofluorescence was observed (Fig. 5). (No immunofluorescence was obtained in the absence of detergent.) A similar distribution of immunofluorescence was seen after fixation with 95% ethanol or after treatment with Triton X-100 and acetone, a procedure shown to permit penetration of antibody to myofibrils in muscle fibers (19, 20). Furthermore, when we treated cross sections of human skeletal muscle with the antiactin, myofibrils

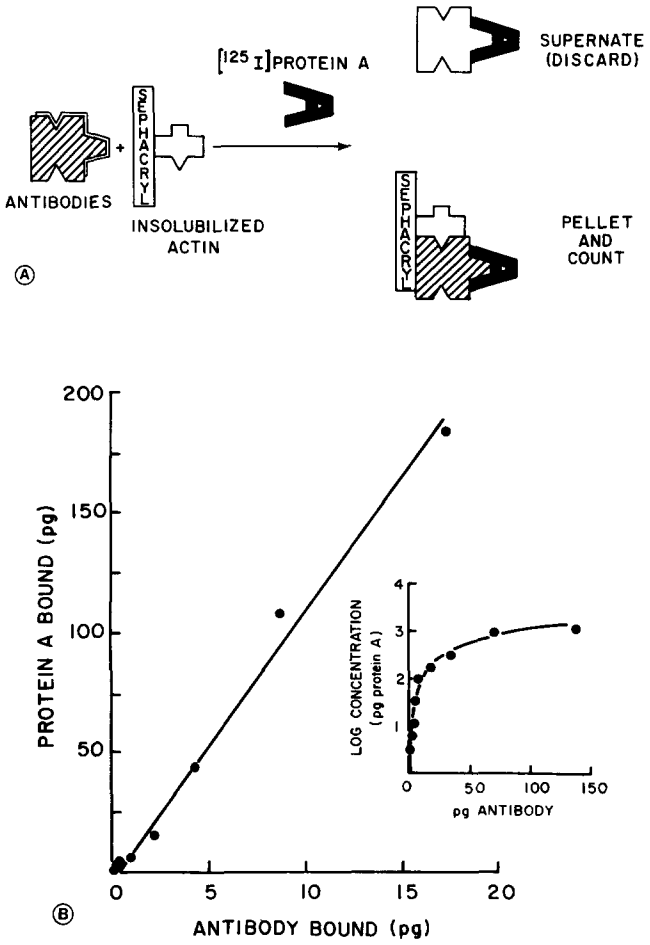

FIGURE 2 Titration of antiactin antibody by use of [^{125}I]protein A. (*A*) Diagram of assay procedure. Tubes each contained 0.5 μg of insolubilized *Aplysia* actin as a suspension of the protein coupled to 0.54 mg of Sephacryl (see Materials and Methods) and a dilution of the serum to be tested both in a total volume of 125 μl of TBS. They were shaken at 4°C for 18 h; we then added an amount of [^{125}I]protein A in 0.1 ml TBS containing 1% Triton X-100 determined in preliminary assays to be in a 10- to 20-fold molar excess of the lowest dilution of antiactin globulin used, and shook the tubes at room temperature for 1.5 h. [^{125}I]Protein A bound to the insolubilized antigen-antibody complexes was separated by centrifugation at 6,500 *g* for 5 min from free protein A and from protein A bound to serum IgGs other than antiactin. Before centrifugation we added 4 mg of untreated Sephacryl to each tube as carrier to help in the handling of the pellets. The pellets were then washed twice and counted in a gamma counter. A similar procedure was developed independently (28). (*B*) Standard curve used for measuring the amount of antiactin bound. Antiactin protein was calibrated by measuring the amount of [^{125}I]protein A sedimented by centrifugation after incubation with known amounts of insolubilized serum globulin under the assay conditions described above. A serum globulin standard was prepared by precipitating antiactin serum in one-third saturated ammonium sulfate solution at room temperature; the globulin was then coupled to Sephacryl (see Materials and Methods); the immobilized standard contained 22 pg globulin bound per μg Sephacryl. The amount of protein A sedimented was determined from the radioactivity in the pellets, using the nominal specific activity of 30 cpm/pg corrected for decay. In these experiments, we added ~3 ng of protein A. Under these conditions, amounts of insolubilized globulin <20 pg can be conveniently assayed, because the relationship between globulin added and protein A sedimented becomes exponential when more than ~5% of the protein A is sedimented (see *inset*).

were unstained (Fig. 6*A*). Intensely fluorescent foci were observed in the sarcoplasmic spaces lying between myofibrils. Occasionally these focal patches appeared to be interconnected by a fine reticular network of thin fluorescent strands. A similar distribution was observed by use of the immunoperoxidase procedure (Fig. 6*B*). Actin immunofluorescence and immunoperoxidase reaction product were also associated with the sarcolemma and the endomesium. Much of the staining in the endomesium may have resulted from cytoplasmic actin in fibroblasts and macrophages that are normally present in this area. Punctate orange autofluorescence from lipofuscin also contributed to some of the brightness in this connective tissue layer (Fig. 6*C*). Insignificant immunofluorescence was observed in sections of the muscle treated with either serum excluded from the Sephacryl-actin immunoadsorbent (Fig. 6 *D* and *E*) or fluoresceinated normal rabbit globulin (data not shown). Furthermore, the pattern of immunofluorescence did not change after we absorbed samples of the antibody with actin purified from chicken gizzard or rabbit skeletal muscle. The distribution of immunofluorescence and peroxidase reaction product is consistent with the idea that cytoplasmic actin is associated with the membranes of mature striated muscle.

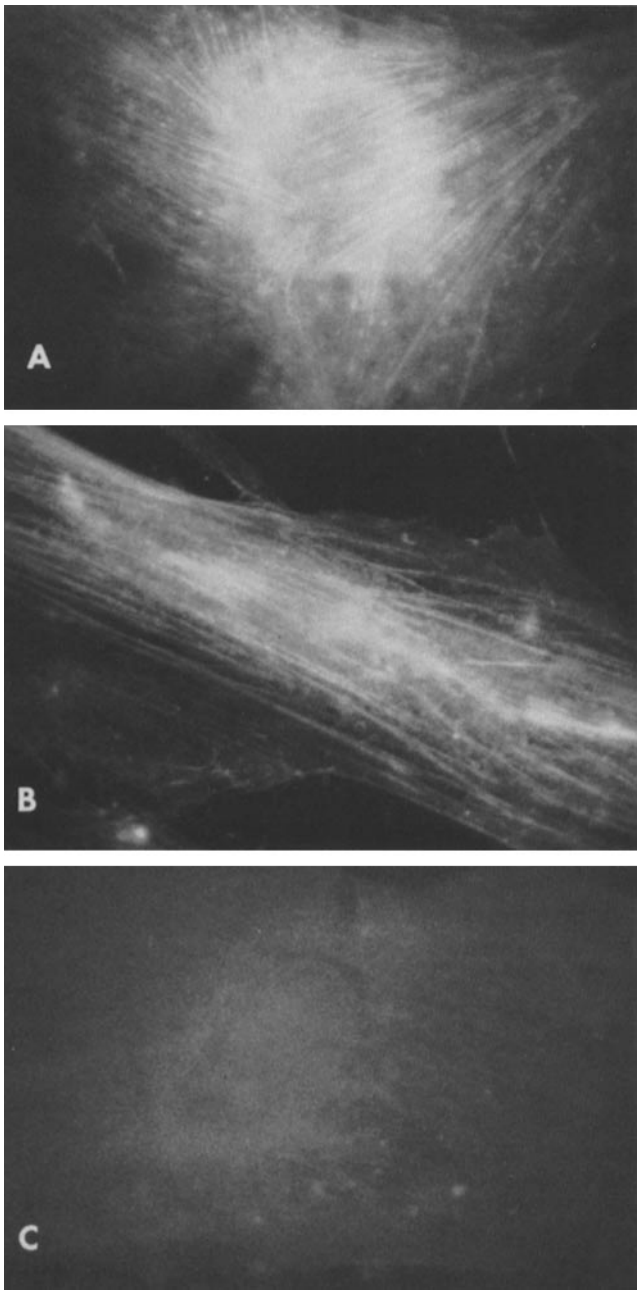

FIGURE 3 Localization of actin immunofluorescence in primary human fibroblasts. (*A*) Treated with 415 μg/ml of purified fluoresceinated antiactin. (*B*) Treated with 125 μg/ml of purified fluoresceinated antiactin. (*C*) Control, treated with 415 μg/ml of the fluoresceinated serum fraction excluded during purification of the antiactin serum on the actin-Sephacryl immunoadsorbent described in Table I. × 550.

DISCUSSION

Molluscan actins can be expected to differ from vertebrate muscle actin. *Aplysia* body wall muscle cannot be characterized as either smooth or skeletal: as in other invertebrates, it is

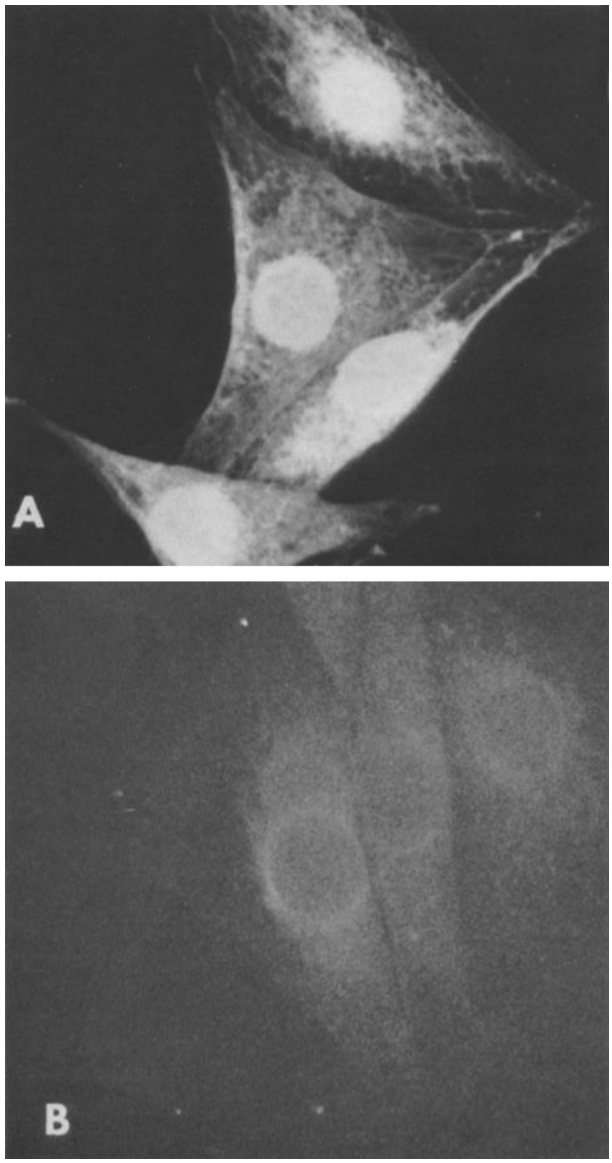

FIGURE 4 Localization of actin immunofluorescence in L6 myoblasts. (*A*) Treated with 125 μg/ml of purified fluoresceinated antiactin. (*B*) Control, treated with 125 μg/ml of the excluded fraction. × 733.

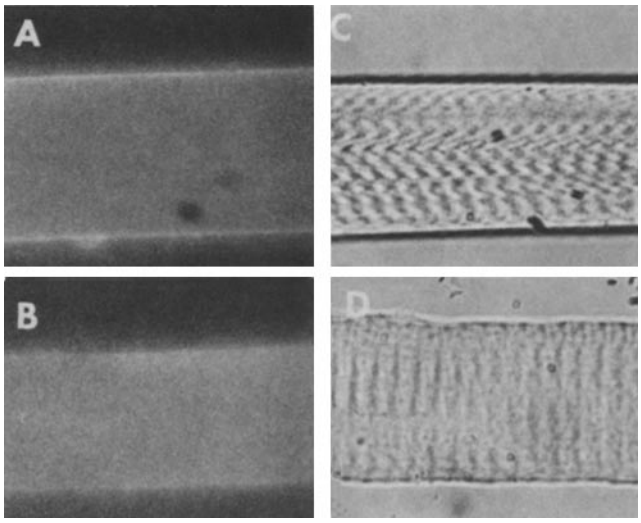

FIGURE 5 Localization of actin immunofluorescence in single frog muscle fibers. (*A*) An m. cutaneus pectoris fiber was fixed in 1% buffered paraformaldehyde (see Materials and Methods) and then treated with 125 μg/ml of the purified fluoresceinated antiactin containing 0.5% NP-40. (*B*) A fiber fixed in 3.7% paraformaldehyde and treated with the antiactin after exposure to Triton X-100 and acetone (19, 20). (*C*) Light micrograph of same fiber as in *A*. (*D*) Light micrograph of same fiber as in *B*. × 233.

obliquely striated (34). In preliminary electrofocusing studies, *Aplysia* body wall actin migrated with chicken gizzard actin; it therefore has a higher isoelectric point than that of α-actin (R. Spangler, unpublished observations). Our evidence strongly indicates that the antibody raised against *Aplysia* body wall muscle actin is specific for vertebrate cytoplasmic actins and does not react with vertebrate myofibrillar actins. Reactivity with *Aplysia* actins was shown by radioimmunoassay and by affinity chromatography on an *Aplysia* actin immunoadsorbent. *Aplysia* tropomyosin and myosin, possible contaminants of the highly purified protein used as immunogen, did not cross-react. Actins isolated from vertebrate skeletal and smooth muscle did not inhibit binding of *Aplysia* actin when added at concentrations in the range in which *Aplysia* actin was found to be effective. Slight inhibition was observed with the actin from rabbit skeletal muscle in 100-fold greater concentrations. Although this inhibition, which is only barely significant, may indicate cross-reactivity between myofibrillar actin and *Aplysia* actin, it is also consistent with the possibility that the skeletal muscle preparation used contained a small proportion of cytoplasmic actin. We also have preliminary evidence from competitive binding studies that actin from vertebrate brain cross-reacts.

We do not have sufficient information to explain why the antibody can discriminate between cytoplasmic actin and α-actin. The *Aplysia* actin used as immunogen was an SDS-denatured protein and may contain determinants not shared with α-actin. Alternatively, these determinants may also exist in α-actin but not be exposed to the antibody under the conditions used for testing. If the determinants are cryptic, resistance of different actins to denaturation could be the variable that defines the extent of cross-reactivity. Although differential resistance to denaturation is a possible explanation, it seems unlikely because we found that α-actin failed to react under a variety of conditions. Thus, myofibrils did not become fluorescent when skeletal muscle was fixed in cold ethanol or when treated according to several procedures involving detergent. It may not be important to draw a distinction between these two explanations, because both the existence of different antigenic determinants and differential resistance to denaturation imply that the invertebrate actin differs from α-actin in some aspect of its molecular structure which the antibody can recognize.

Presumably also there must be some similarity in structure between invertebrate muscle actin and vertebrate cytoplasmic actins that would account for the cross-reactivity observed. Owaribe and his collaborators have found that an antiactin raised against a fungal actin did not cross-react with vertebrate skeletal actin although it did react with sea urchin egg actin (30) and vertebrate cytoplasmic actins (31). Invertebrate contractile proteins have been used extensively to characterize the mechanisms controlling contraction of myosin (40), but these abundant sources of actin have not yet been exploited for immunochemical purposes. Use of phylogenetically different but closely related forms of proteins as immunogens is a fruitful approach for examining small structural differences in molecules (3).

The specificity of the anti-*Aplysia* actin antibody differs from that previously reported for antibodies raised against vertebrate actins (Table II). Immunization has most frequently resulted in the production of antibodies that fail to discriminate between muscle and cytoplasmic actins. In most instances the specificities of these antibodies were assessed by immunohistochemical techniques. Groschel-Stewart et al. (16) obtained an antibody that reacted with muscle actin but not with cytoplasmic actins. They also obtained antisera with unusual specificities after immunizing with immobilized chicken actins from either smooth or skeletal muscle coupled to agarose; these antibodies, which were found to react exclusively with chicken tissue, exhibited unique reactivity for the type of muscle from which the immunogen had been purified. They were not tested against cytoplasmic actins, however.

Antibodies without reactivity to myofibrillar actin should be

TABLE II

Specificities of Antiactin Antibodies

Immunogen		Reactivity			
Source	State	CY	SK	SM	Refs.
Smooth	D	+	+	+	26, 42
	D	−	+	+	16
	Immobilized	NT	−	+	16
	N	+	+	+	17, 19
	N	−	+	+	7
Skeletal	D	+	+	+	19, 32
	D	+	+	NT	3, 22
	Immobilized	NT	+	−	16
	N	+	+	NT	9
Cytoplasmic (calf thymus)	D	+	+	+	6, 28
Unknown (hepatitis serum)	—	+	+	+	12

The table summarizes previous reports of antibodies raised against vertebrate actins from a variety of sources. Immunization was carried out either with denatured (*D*) or native (*N*) actin: immobilized refers to actin coupled to agarose (16). Reactivities were usually tested immunohistochemically with nonmuscle cells or tissues (*CY*), vertebrate skeletal muscle (*SK*) or vertebrate smooth muscle (*SM*). *NT*, not tested.

especially useful for examining the distribution of cytoplasmic actin in skeletal muscle. Using both immunofluorescence and the immunoperoxidase method, we found cytoplasmic actin associated with the sarcolemma and membranous structures surrounding individual myofibrils in mature skeletal muscle. Identification of these structures under the light microscope is tentative at best, but the distribution of the intensely stained reticular network surrounding the myofibrils suggests that they represent elements of the sarcoplasmic reticulum or transverse tubules (T-system). Definitive identification awaits immuno-

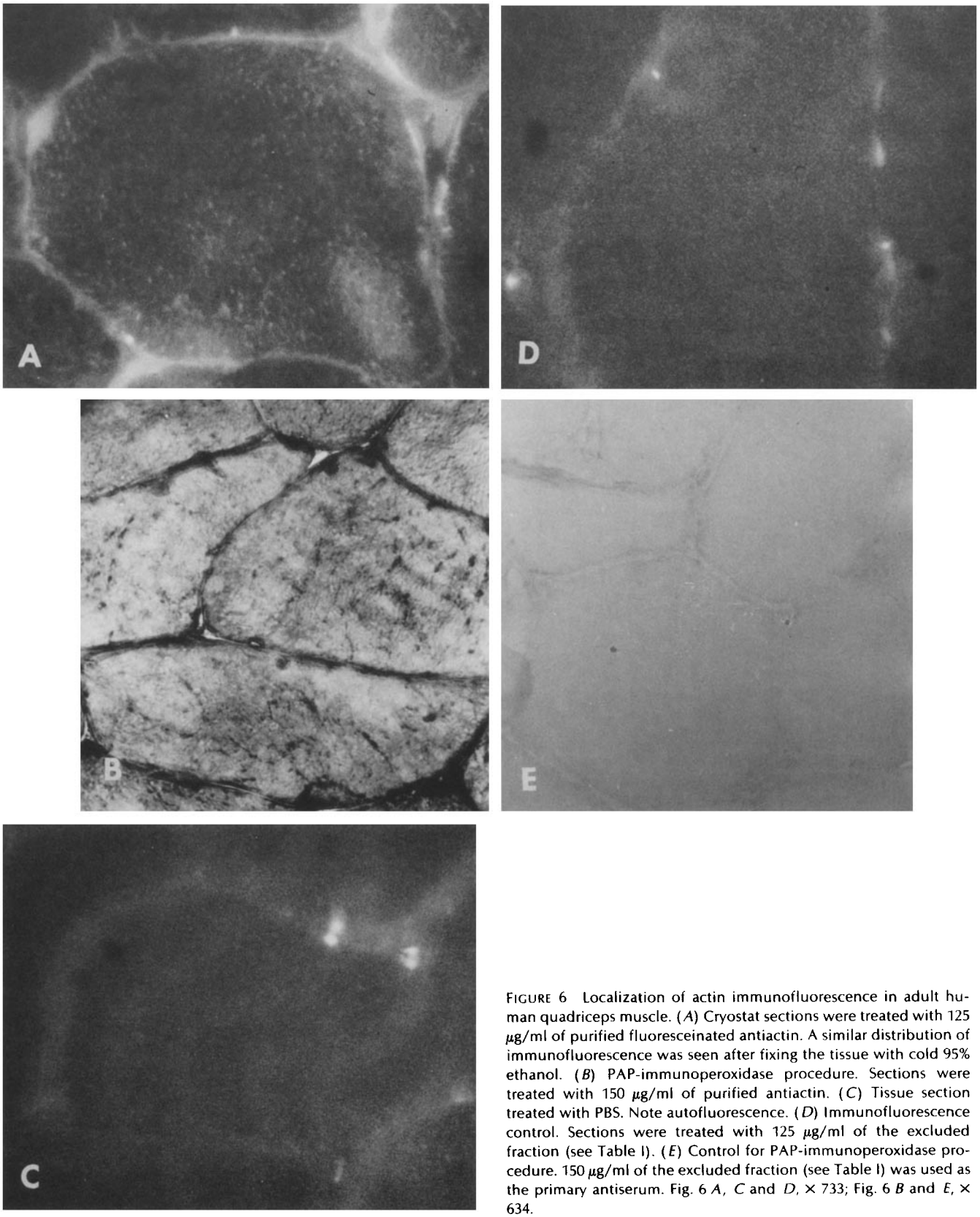

FIGURE 6 Localization of actin immunofluorescence in adult human quadriceps muscle. (*A*) Cryostat sections were treated with 125 μg/ml of purified fluoresceinated antiactin. A similar distribution of immunofluorescence was seen after fixing the tissue with cold 95% ethanol. (*B*) PAP-immunoperoxidase procedure. Sections were treated with 150 μg/ml of purified antiactin. (*C*) Tissue section treated with PBS. Note autofluorescence. (*D*) Immunofluorescence control. Sections were treated with 125 μg/ml of the excluded fraction (see Table I). (*E*) Control for PAP-immunoperoxidase procedure. 150 μg/ml of the excluded fraction (see Table I) was used as the primary antiserum. Fig. 6 *A*, *C* and *D*, × 733; Fig. 6 *B* and *E*, × 634.

histochemical analysis in the electron microscope. It has often been proposed that cytoplasmic actin is involved in the localization and transmembrane movement of integral membrane proteins (4). The acetylcholine receptor is a complex of proteins whose insertion and distribution in the sarcolemma changes during development and after denervation (10). Immunohistochemical studies with muscle cells under a variety of conditions that affect the distribution of the receptor could provide information about whether cytoplasmic actin plays a determining role in receptor localization.

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