TROPOMYOSIN ANTIBODY: THE SPECIFIC LOCALIZATION

OF TROPOMYOSIN IN NONMUSCLE CELLS

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ABSTRACT

An antibody against purified chicken skeletal muscle tropomyosin is used in indirect immunofluorescence to visualize the localization of tropomyosin in a variety of nonmuscle cells. The antibody produces a fluorescent pattern which is very similar to that obtained with an actin-specific antibody. This pattern is composed of fluorescent fibers which are shown to be coincident with the fibers seen with phase-contrast optics. High resolution epifluorescent microscopy reveals that fibers stained with the actin antibody show a continuous fluorescence, while fibers reacted with the tropomyosin antibody show a periodic fluorescence. Measurements indicate that the lengths of the fluorescent segments are variable with an average of 1.2 μm while the spacing between segments is approximately 0.4 μm.

The demonstration that actin and myosin, the two major structural proteins of muscle, are also basic components of nonmuscle cells (see review by Pollard and Weihing, reference 24) has prompted a large amount of research designed to elucidate the cellular role of these proteins. So far, they have been implicated in a variety of cellular functions including motility, endocytosis, exocytosis, cytokinesis, membrane ruffling, and maintenance of cell shape (4, 12, 21, 26, 27, 32).

Much if not most of the actin in nonmuscle cells is localized in the 60-Å thick filaments, called microfilaments, which are one of the three major types of filaments found in eukaryotic cells. This conclusion was first based on the observation that heavy meromyosin, a specific proteolytic fragment of myosin, forms characteristic arrowhead complexes with microfilaments *in situ* (16); these arrowhead structures are similar to those seen with isolated actin filaments from skeletal muscle (14). Until recently, the cellular localization of myosin was not completely resolved, although myosin-like

thick filaments have been observed in a variety of nonmuscle cells (1, 3, 11, 25).

More precise localization of these structural proteins within the cell has been approached recently using the technique of indirect immunofluorescence with antibodies specific for these proteins. An actin-specific antibody has been obtained using as an antigen-purified actin denatured with sodium dodecyl sulfate (18, 19). When this antibody is used in indirect immunofluorescence to visualize the intracellular distribution of actin, the fluorescence is found primarily associated with long fibers which frequently span the entire length of the cell. These fibers often converge to "focal points" and are intimately associated with the plasma membrane. Further work has shown that the fibers visualized with the actin antibody are identical to the fibers observed in cells by phase-contrast light microscopy and correspond to the microfilament bundles seen with electron microscopy (13). Subsequent application of this immunofluorescent technique with an antibody against

native chicken gizzard myosin has shown that a myosin-like protein is also associated with the microfilament bundles in nonmuscle cells giving rise to fluorescent interruptions or "striations" (31).

In addition to the structural proteins, actin and myosin, muscle cells contain tropomyosin, a protein which helps to regulate the Ca^{2+} ion-dependent interaction of actin and myosin (8). A protein with properties similar to those of skeletal muscle tropomyosin has also been isolated from human platelets (5) and chick brain (10). However, up until now, the localization of tropomyosin in nonmuscle cells has not been established. In this paper an antibody against chicken skeletal muscle tropomyosin is used in indirect immunofluorescence to localize the intracellular distribution of this protein in nonmuscle cells.

MATERIALS AND METHODS

Cell Cultures

Human skin fibroblasts, designated CRL 1139 Enson, from patients with cystic fibrosis, were obtained from the American Type Culture Collection, Rockville, Md. These cells were used because they are somewhat easier to maintain in culture than normal human skin fibroblasts. The fluorescent patterns observed with these cells are indistinguishable from those observed with the normal fibroblasts. The cells were grown in Dulbecco's modification of Eagle's medium (DME) containing 10% fetal calf serum, 1 mg/ml gentamycin, 100 Units/ml penicillin, and 100 µg/ml streptomycin. The established mouse epithelioid cell line 3T3 was grown in DME medium containing 10% calf serum and 50 µg/ml gentamycin.

Tropomyosin Purification

Purified chicken skeletal muscle tropomyosin used as the antigen was a generous gift from Dr. Susan Lowey, Brandeis University, Waltham, Mass. Rabbit skeletal muscle tropomyosin was purified from a skeletal muscle acetone powder following the procedure of Spudich and Watt (28).

Mouse fibroblast tropomyosin was partially purified from the cell line SV101, a clone of mouse fibroblast 3T3 cells transformed by Simian virus 40 (kindly provided by Dr. R. Pollack, Cold Spring Harbor Laboratory). The cells were grown in roller bottles in DME containing 10% calf serum and 50 µg/ml gentamycin. At confluency, the medium was removed and the cells were washed extensively with phosphate-buffered saline (PBS). The cells were then scraped off the bottles, collected by low speed

centrifugation, and stored at −70°C. An ethanol-ether powder of the SV101 cells was prepared by homogenizing them in 95% ethanol, collecting the residue by centrifugation, rapidly washing it with ether and air drying the residue. 1 g of ethanol-ether powder was stirred for 3 h at 4°C in 7.5 ml 0.01 M sodium phosphate buffer pH 6.8, 5 mM $MgCl_2$, and 1 mM diethiothreitol (buffer A). The supernate was passed, without dialysis, through a DEAE-cellulose Sephadex A-50 column equilibrated with buffer A (5 ml of equilibrated resin per 1 g of ethanol-ether powder packed in a plastic disposable syringe). The flow through of the column was collected and concentrated by lyophilization. Sodium dodecyl sulfate (SDS) gel electrophoretic analysis of the proteins retained by the column indicated that more than 95% of the tropomyosin was recovered in the flow through of the column. Under the chromatographic conditions employed, actin was retained by the column and eluted at salt concentrations above 0.25 M. 1 g of ethanol-ether powder yielded between 1.5 and 2.0 mg of tropomyosin in the DEAE-cellulose Sephadex A-50 flow through. The protein at this stage was approximately 70% pure and could be purified further by precipitation with 50 mM $MgCl_2$.

Gel Electrophoresis

SDS gel electrophoresis was performed according to the Tris-glycine system of Laemmli (17), as described for slab gels by Anderson et al. (2). The gels contained 12.5% acrylamide and 0.1% bisacrylamide. The samples were prepared for electrophoresis by dissolving them in sample buffer (0.05 M Tris-HCl pH 6.8, 2% SDS, 15% glycerol, 0.001% bromphenol blue, and 0.1 M dithiothreitol) and by incubation in a boiling water bath for 3–4 min. The SDS electrophoresis buffer contained per 4 liters: 12.2 g of Tris base, 57.7 g of glycine, and 4 g of SDS, final pH 8.3.

Preparation of Tropomyosin Antibody

For the preparation of antibodies native chicken skeletal muscle tropomyosin was dissolved in 0.15 M NaCl, 0.01 M Tris-HCl pH 7.4. Approximately 1 mg of protein in 0.5 ml of buffer was emulsified with complete Freund's adjuvant and injected subcutaneously into a rabbit. 3 wk later 0.3 mg of protein was injected intravenously in the form of an aluminum sodium sulfate (alum) precipitate. This injection was repeated at weekly intervals two additional times. At the end of the third wk the rabbit was bled by cardiac puncture. The immunization and bleeding was kindly performed by Dr. F. Miller and Ms. E. Villamarzo at the State University of New York at Stony Brook.

After the clot had formed, the serum was collected by centrifugation and the gamma globulins were partially purified by precipitation with ammonium sulfate at 50% saturation. They were subsequently dialyzed against 0.15 M NaCl, 0.01 M Tris-HCl pH 7.8, and stored at −20°C.

Preparation of Actin Antibody

Antibodies to actin were obtained against calf thymus actin which was purified by column chromatography, denatured in SDS, and reduced with dithiothreitol before immunization (18), or against mouse fibroblast actin purified through preparative SDS slab gel electrophoresis (19).

In the latter case high speed supernates of SV101 cells were prepared by homogenizing cells in 10 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, centrifuging at 10,000 rpm for 10 min to remove nuclei and mitochondria, and centrifuging further for 2.5 h at 50,000 rpm in the SW 50.1 rotor. The soluble protein was lyophilized and dissolved in electrophoresis sample buffer at a concentration of 2 mg/ml. Preparative SDS slab gel electrophoresis was performed on slabs 2 mm thick, 135 mm long, and 215 mm wide as described by Anderson et al. (2). Approximately 1 mg of protein (200 μg of actin) was electrophoresed for 2 h at 35 mA, until the bromphenol blue dye marker reached the front of the gel. The gels were stained with 0.25% Coomassie Brilliant Blue (CBB) in 50% methanol, 7.5% acetic acid for 1 h, and destained with 7.5% methanol, 7.5% acetic acid. The band at 42,000 mol wt corresponding to actin was removed from the remainder of the gel with a sharp razor blade. The protein was then eluted from the gel electrophoretically as described by Anderson et al. (2): the sliced gel was packed between two "Kimwipe" tissue plugs fitted in the lower 5 ml of a 10-ml glass pipette, which was capped at the anode end with a small piece of no. 8 dialysis tubing. Elution of the protein was achieved at 4 mA per cylinder overnight in SDS electrophoresis buffer. At the end of the run, the eluted protein and dye were dialyzed against 0.1 M NH_4HCO_3, 0.05% SDS and lyophilized. The residue was washed once with $-20°C$ acetone to remove excess SDS and CBB, collected by centrifugation, and relyophilized. Under these conditions the recovery of the eluted proteins is approximately 60%. The purified antigen was resuspended in a minimum amount of water and dissolved in SDS sample buffer as described above. Reelectrophoresis of a portion of the eluted actin on SDS slab gels indicated that the protein had an electrophoretic mobility indistinguishable from that of chicken skeletal muscle actin, with no visible contamination by proteins with different mobilities.

The antibody was prepared in rabbits as described previously (19). In the initial injection, 400-500 μg of protein in SDS sample buffer was emulsified with complete Freund's adjuvant and injected subcutaneously; in subsequent injections 100 μg of protein was injected intravenously.

Indirect Immunofluorescence

The cells grown on coverslips were drained of excess medium, immersed for 30 min in phosphate-buffered saline (PBS) containing 3.5% formaldehyde, at room temperature, and treated with absolute acetone for 5 min at $-10°C$. They were then incubated for 1 h at 37°C with the actin or the tropomyosin-specific antibody, washed with PBS, and incubated for 1 h at 37°C with fluorescein-labeled goat antirabbit IgG serum (Miles Laboratories Inc., Miles Research Div., Elkhart, Ind.) (19). The tropomyosin and the actin antibodies were diluted in PBS and used at an approximate final concentration of 2.5 mg/ml. The goat antirabbit serum was used at a final concentration of 0.8 mg/ml in PBS and a final absorbance ratio at 280/495 nm of approximately 0.13. The specimens were viewed under a Zeiss microscope equipped with fluorescent and epifluorescent optics. Pictures were taken on Plus X film (Eastman Kodak Co., Rochester, N. Y.); under oil immersion (100×) the exposure time was approximately 30-45 s.

RESULTS

Characterization of a Tropomyosin Specific Antibody

The validity of this technique is dependent upon the specificity of the antibody for the desired antigen. For this purpose the antigen preparation used to elicit the antibody must be highly pure. Fig. 1 A shows an SDS polyacrylamide gel electrophorogram of the purified chicken skeletal muscle tropomyosin preparation used as the antigen and, for comparison, purified rabbit skeletal muscle tropomyosin. The rabbit muscle tropomyosin gives two major polypeptide chains, α and β (6, 7), with electrophoretic mobilities corresponding to mol wt of 34,000 and 36,000, respectively. The chicken tropomyosin shows one polypeptide chain with an electrophoretic mobility intermediate to that of the α and β subunits of rabbit tropomyosin and corresponding to a mol wt of 35,000. The gel in Fig. 1 A, which is purposely overloaded to reveal any possible contaminants, shows that the chicken tropomyosin used as an antigen is approximately 90% pure with no detectable contamination with actin or myosin.

A major cellular protein with an electrophoretic mobility identical to that of chicken muscle tropomyosin has been observed in a variety of primary and established cell types grown in culture (e.g., mouse, hamster, human, and chicken fibroblasts, KB and Hela cells [18, 34]). SDS gel electrophoretic analysis of extracts of these cells reveals that this protein is soluble in low salt buffer and constitutes approximately 3-5% of the cell's soluble protein. It has been partially purified by extracting an ethanol powder of these cells with low salt buffer and passing the extract through a column of DEAE-cellulose Sephadex A-50. Fig. 1 B shows that this protein comprises 60-70% of the

protein which is not retained by the column. In both purified and unpurified form this protein displays properties characteristic of rabbit and chicken skeletal muscle tropomyosin, i.e., it is precipitable by vinblastine and by high concentrations of Mg^{2+} or Ca^{2+} ions (50 mM and 20 mM, respectively). Fig. 1 B shows that this protein purified from mouse fibroblasts coelectrophoreses with chicken skeletal muscle tropomyosin with a mol wt of 35,000, a value slightly higher than the 30,000 value obtained for tropomyosin purified from human platelets (5) and chick brain (10).

The specificity of the rabbit antiserum obtained against chicken skeletal muscle tropomyosin has been tested by double immunodiffusion. The antibody cross reacts with the preparations of chicken muscle tropomyosin, rabbit muscle tropomyosin, and mouse fibroblast tropomyosin shown in Fig. 1

A and B, as well as with purified gizzard smooth muscle tropomyosin, but shows no detectable cross reaction with purified chicken skeletal muscle actin or purified chicken breast muscle myosin (data not shown). Therefore, the antibody shows a preferred specificity for tropomyosin from both muscle and nonmuscle cells and can be used as a probe to localize tropomyosin in these cells.

Indirect Immunofluorescence with an Actin-Specific Antibody

When antibody made against purified actin (18, 19; see also Materials and Methods) is reacted with the large flat cells in a culture of primary human skin fibroblasts, the fluorescence is associated with a highly developed fiber network shown in Fig. 2 a. These typically straight fibers span portions of the cell and frequently converge
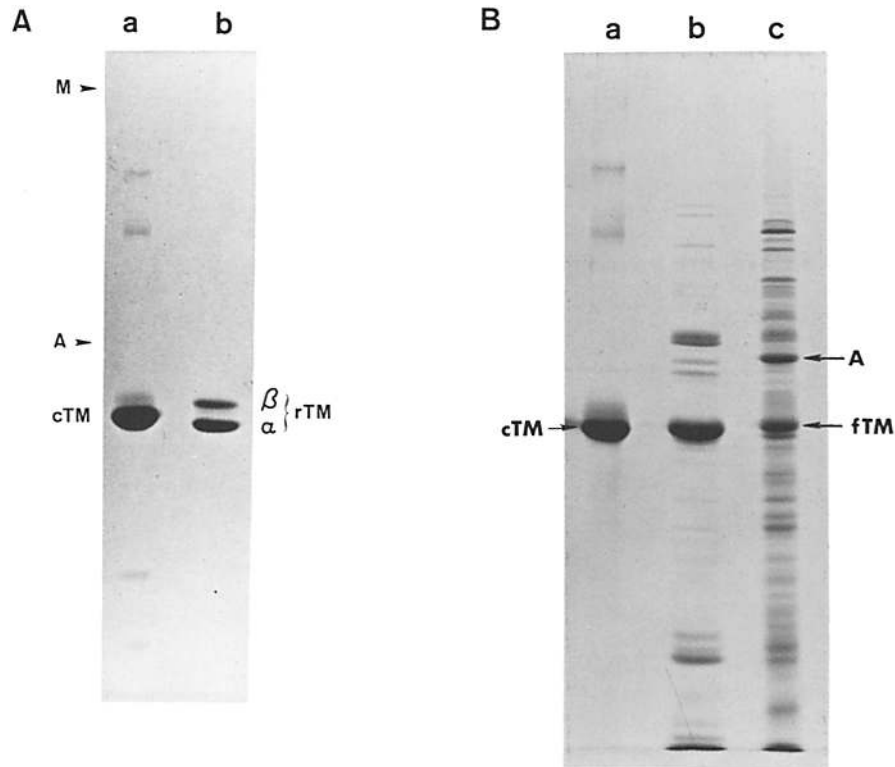

FIGURE 1   SDS slab gel electrophoresis of tropomyosin. Panel A: (a) cTM = 12 μg of chicken skeletal muscle tropomyosin. (b) rTM = 12 μg of rabbit skeletal muscle tropomyosin. α and β refer to the two subunits of tropomyosin, following the nomenclature of Cummins and Perry (6, 7). The arrows M and A indicate the positions where myosin and actin would migrate. Panel B: (a) cTM = 12 μg of chicken skeletal muscle tropomyosin. (b) Approximately 20 μg of mouse fibroblast tropomyosin purified through DEAE-cellulose Sephadex A-50. The protein not retained by the column was concentrated by lyophilization. A portion of the lyophilized protein was dissolved in SDS electrophoresis sample buffer by incubation in a boiling water bath for 3 min. (c) The proteins extracted from the SV101 ethanol-ether powder into buffer A. fTM and A indicate the locations of fibroblast tropomyosin and actin, respectively.
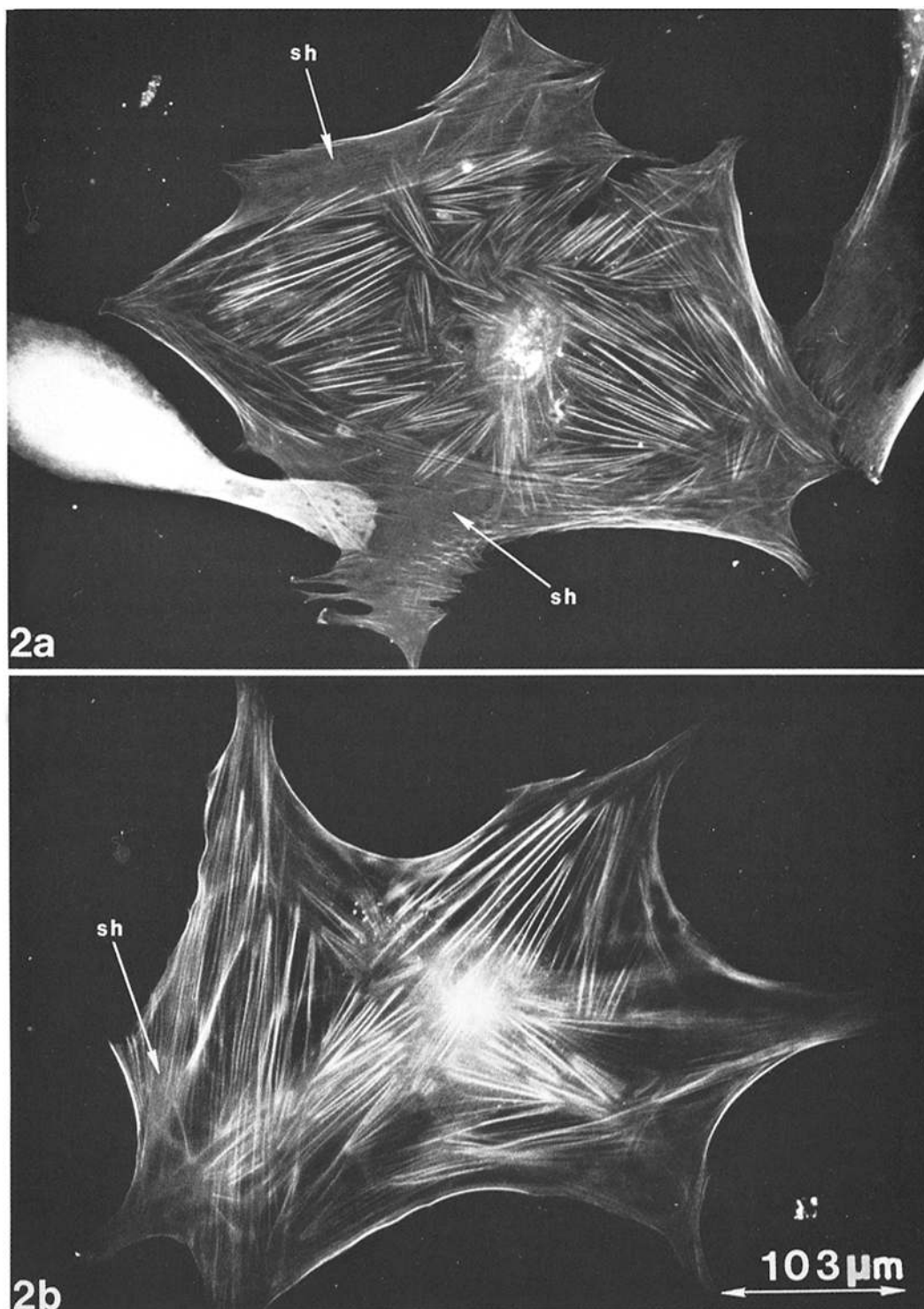
FIGURES 2 and 3   Indirect immunofluorescence with actin and tropomyosin antibodies. Photographs show immunofluorescent staining of human skin fibroblasts with the actin-specific antibody (Figs. 2 *a* and 3 *d*) and with the tropomyosin antibody (Figs. 2 *b*, 3 *a*, *b*, and *c*). Pictures were taken with fluorescent optics using the 16× objective for Fig. 2 and the epifluorescent optics under oil immersion using a 100× objective for Fig. 3. *sh* indicates the sheath-like structures observed with the actin and tropomyosin antibodies (Fig. 2 *a* and *b*).
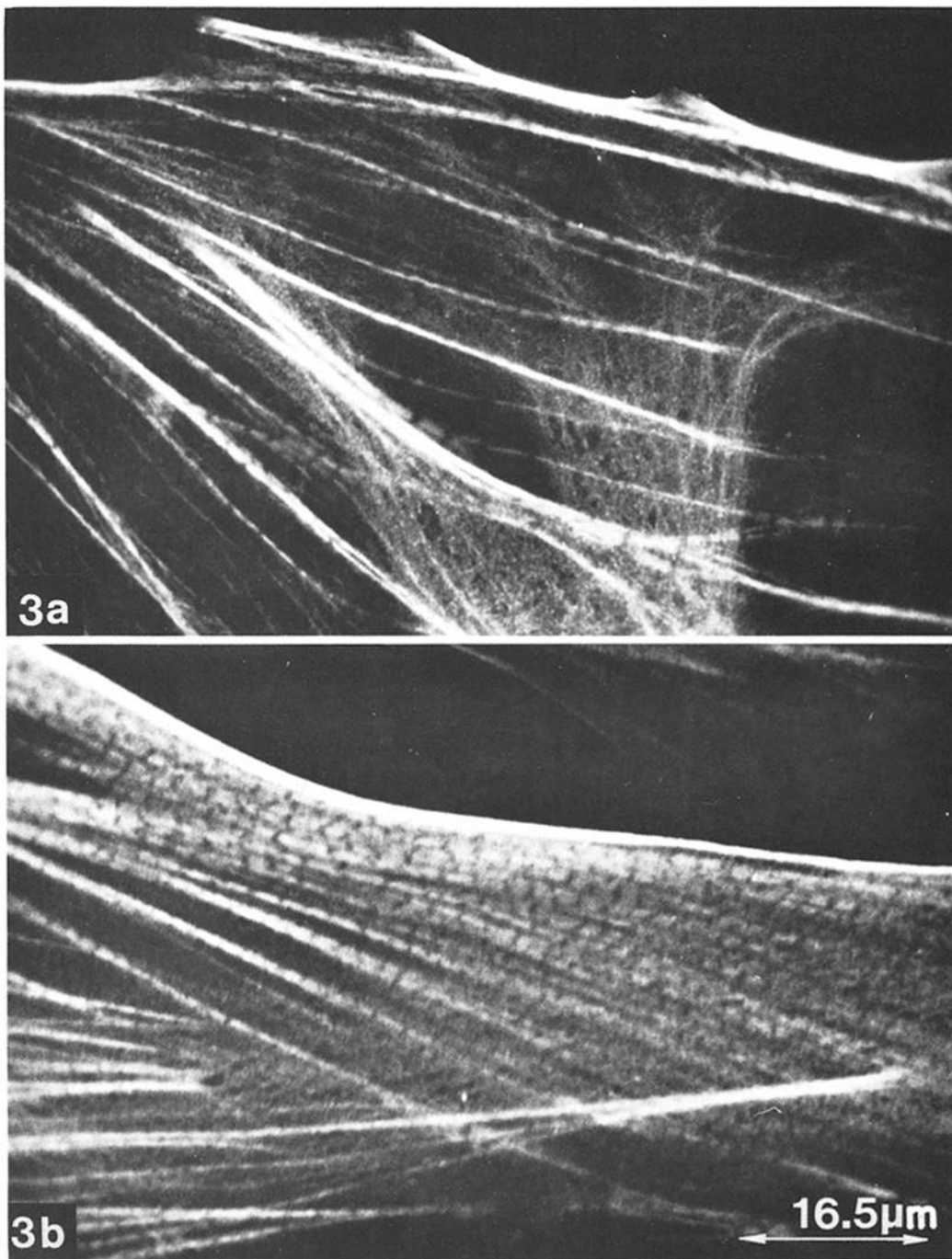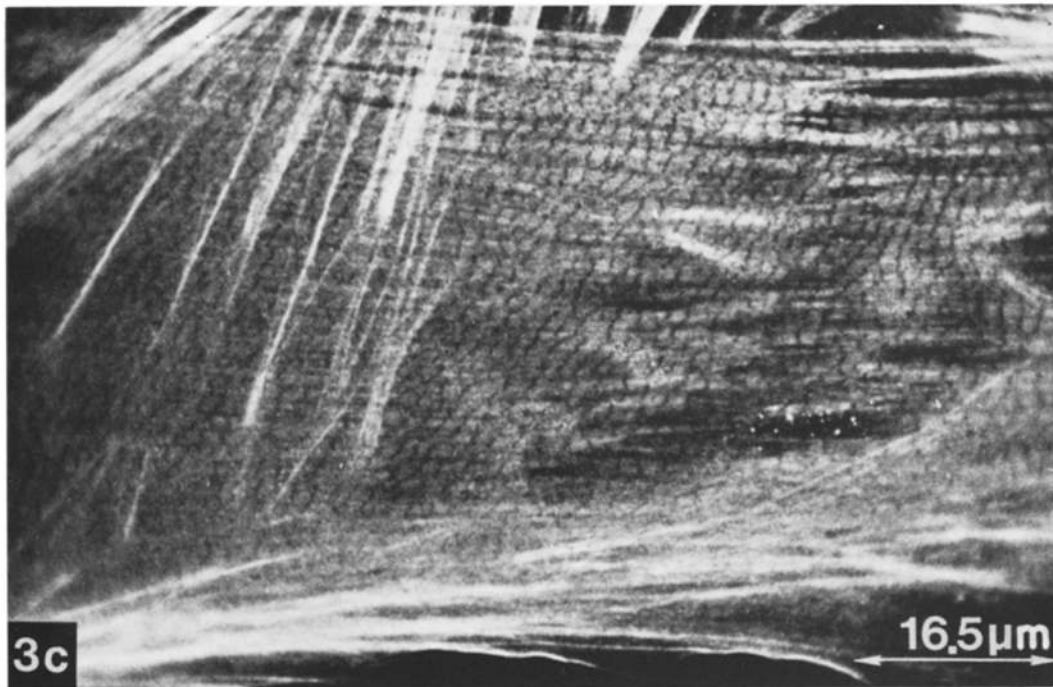
FIGURE 3 a–d   Legend on preceding page.

to "focal points." Fig. 3 d shows that at higher magnification (100× oil immersion) the fibers retain the basic characteristics observed at low magnification, but in addition a multitude of smaller fibers is resolved. The fibers are nonuniform in diameter and in orientation, and, within the resolution of the microscope, they show a continuous fluorescence. These fluorescent fibers are coinci-

dent with fibers seen with phase-contrast optics and correspond to the microfilament bundles observed with electron microscopy[1], as shown previously for the fibers in the established mouse epithelioid cell line 3T3 (13). Fig. 2 *a* shows that ac-

tin can also be organized in closely spaced fibers which give the appearance of a filamentous "sheath." At higher magnification the fibers in the sheaths also show a continuous fluorescence; characteristically they display a high degree of organization and uniformity of diameter, and frequently run parallel to the plasma membrane (data not shown).

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[1] Lazarides, E., and R. D. Goldman. Manuscript in preparation.

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Indirect Immunofluorescence with the Tropomyosin-Specific Antibody

In Fig. 2 b the low magnification picture of a cell from a culture of primary human skin fibroblasts reacted with the tropomyosin antibody shows that the fluorescent pattern observed with this antibody is very similar to that seen in Fig. 2 a with the actin-specific antibody. The antibody reacts with straight fibers which are well organized, which span sections of the cell, and which converge to "focal points." Fig. 3 c shows that it also reacts with the closely spaced parallel fibers which give the appearance of "sheaths." Even at high magnification the fiber pattern observed with the tropomyosin antibody in Fig. 3 a is similar to that observed with the actin antibody in Fig. 3 d. Fig. 4 shows a portion of a human skin fibroblast which has been reacted with the tropomyosin antibody and observed with phase-contrast and epifluorescent optics. The pattern of fibers visible with phase-contrast optics in Fig. 4 a is coincident with the fluorescent pattern observed with epifluorescent optics in Fig. 4 b.

Cells from several primary cultures and established cell lines (e.g., bovine kidney epithelial cells, mouse, hamster, chicken, and guinea pig fibroblasts) give fluorescent patterns similar to those described above when reacted with the tropomyosin antibody. The fluorescent pattern observed for the established epithelioid cell line 3T3 seen in Fig. 5 a is also very similar to that previously reported for 3T3 cells with the actin antibody (13, 19).

Analysis of the "Periodicity" Observed with the Tropomyosin Antibody

Although the fluorescent patterns seen with the actin and tropomyosin antibodies are similar, a characteristic difference in the reactivity of these two antibodies is that the tropomyosin antibody produces a periodic fluorescence which is well resolved at high magnification. As mentioned above (see Fig. 3 d), such a periodicity is not apparent when the cells are reacted with the actin antibody. Fig. 3 a-c shows three general patterns of the fluorescent segments in human skin fibroblasts. In some fibers the fluorescence shows a well resolved periodicity while in other fibers the periodicity is barely detectable; in the fibers in sheaths the periodicity is usually well resolved. In the sheaths the alignment of the fluorescent segments in adjacent fibers may be either "in register" as shown in Fig. 3 c or "out of register" as shown in

Fig. 3 b. Measurements made on pictures taken at high magnification indicate that the length of the fluorescent segments is variable but is sometimes uniform within a given fiber. Fig. 6 gives a diagrammatic representation of 123 measurements of the fluorescent segments made on pictures taken from ten different cells. Approximately 80% of the segments are between 0.8 and 1.7 μm long with an average length of 1.2 μm. The small number of fluorescent segments (less than 10%) which have much larger lengths, up to 5 μm or more, may be multiples of the shorter fluorescent segments. An equal number of measurements of the lengths of the spacings observed between the fluorescent segments indicates that they vary between 0.3 and 0.5 μm with an average of 0.4 μm.

The periodic fluorescence described for human skin fibroblasts has also been observed in a number of other cell types grown in culture; e.g., Fig. 5 a shows the pattern observed for 3T3 cells reacted with the tropomyosin antibody. As shown in Fig. 5 b, cells reacted with a preimmune serum, purified and adjusted to the same protein concentration as the antitropomyosin serum, show no cytoplasmic fluorescence and only a weak perinuclear fluorescence. When either human skin fibroblasts or 3T3 cells are reacted with a mixture of the actin and the tropomyosin-specific antibodies, the fluorescent segments are no longer evident; the fibers show a continuous fluorescence similar to that shown in Figs. 2 a and 3 d for the actin antibody (data not shown).

The results reported above have also been obtained using a different preparation of antibodies to chicken skeletal muscle tropomyosin (kindly provided by Dr. F. Pepe at the University of Pennsylvania School of Medicine, Philadelphia. These antibodies have been previously shown to specifically react with tropomyosin in the I bands of chicken skeletal muscle myofibrils (23, see Discussion). Both antibody preparations give indistinguishable immunofluorescent patterns in nonmuscle cells.

DISCUSSION

The results presented above show that the actin and tropomyosin antibodies each react with cellular fibers which are coincident with the fibers seen with phase-contrast optics. The difference in the reactivity of the two antibodies is that the actin antibody gives a continuous fluorescence while the tropomyosin antibody gives a periodic fluores-
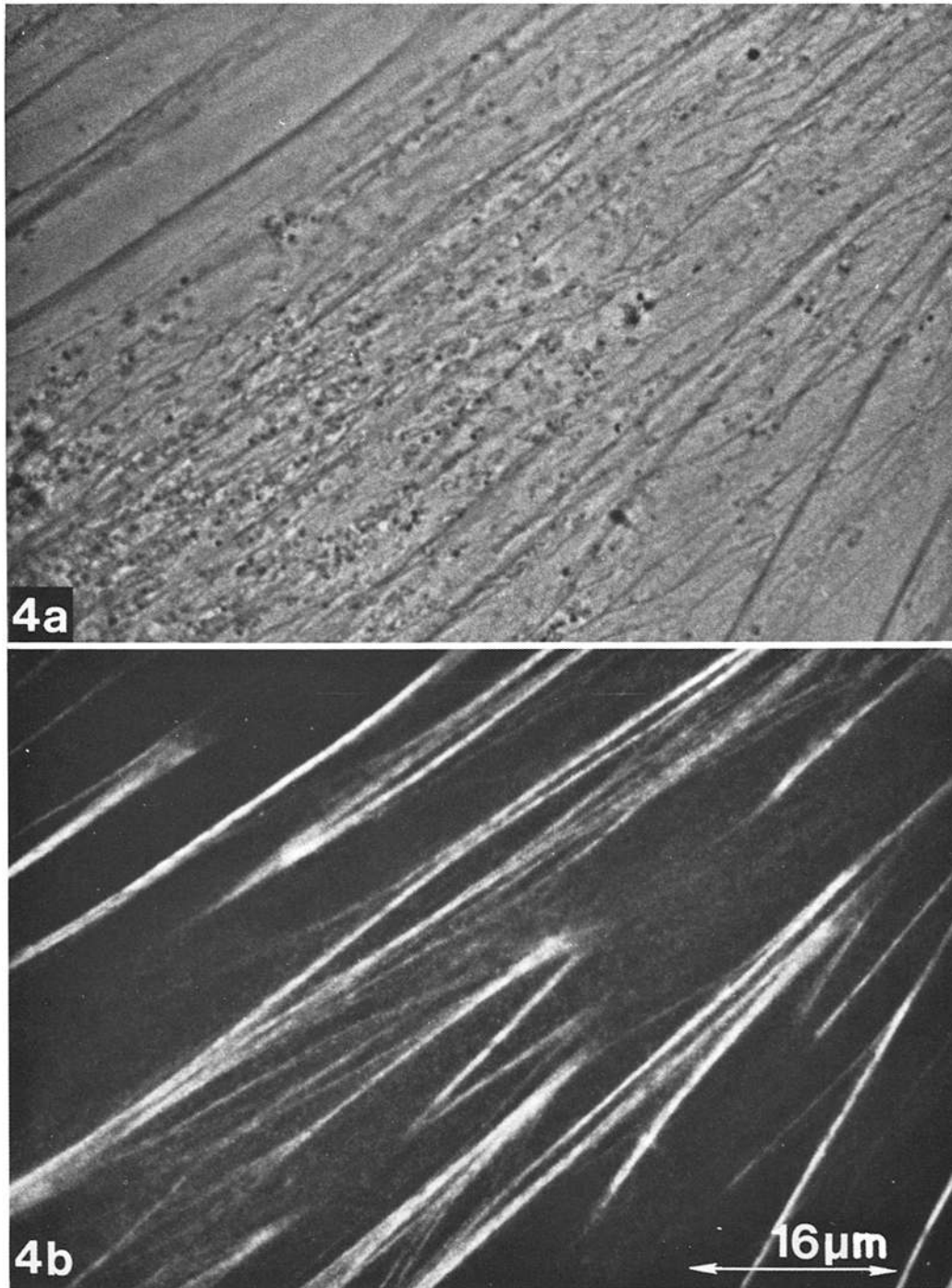
FIGURE 4 Comparison of phase-contrast and tropomyosin antibody immunofluorescent patterns. Human skin fibroblasts were prepared for immunofluorescence with the tropomyosin antibody as described in Materials and Methods. Fig. 4 a shows a portion of a cell photographed with phase-contrast optics. Figure 4 b shows the same section of the cell photographed with epifluorescent optics. The pictures were taken under oil immersion with a 100× objective.
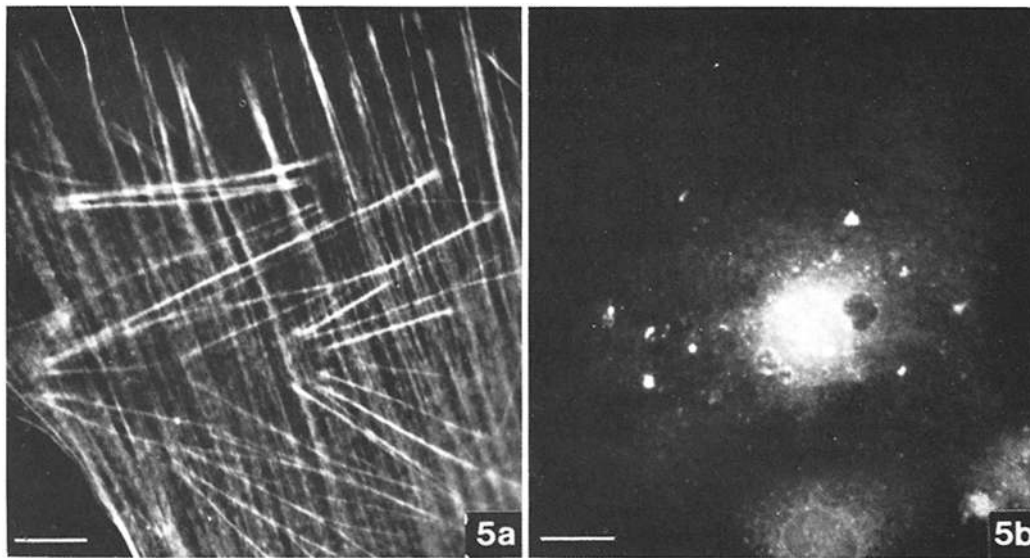
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FIGURE 5 (a) Tropomyosin antibody staining of mouse epithelioid cell line 3T3. The picture was taken with epifluorescent optics under oil immersion with a 100× objective. The bar represents a length of 8 μm. (b) Preimmune serum staining of mouse epithelioid cell line 3T3. Serum taken from the rabbit before immunization with tropomyosin was partially purified with ammonium sulfate and adjusted to the same protein concentration used for the tropomyosin antibody. The picture was taken with epifluorescent optics with a 40× objective. The exposure time was 90 s, approximately twice that of the tropomyosin antibody. The bar represents a length of 21 μm. The outline of the cytoplasm of the cell is barely visible.
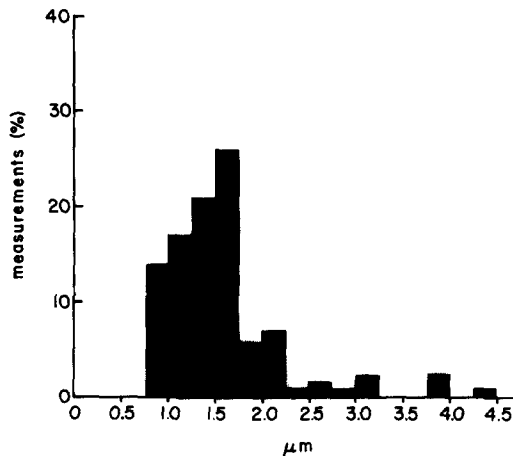

FIGURE 6 Distribution of the lengths of the fluorescent segments observed with the tropomyosin antibody. The histogram is derived from 123 measurements made at random on 10 photographs taken under oil immersion with a 100× objective from a population of human skin fibroblasts. Each mm on the photograph was approximately equal to 0.5 μm. Approximately 80% of the values are distributed between 0.8 and 1.7 μm and give an average of 1.2 μm. The average of all the values is 1.5 μm.

cence. Since the fiber pattern seen with both antibodies is the same and since the addition of the actin antibody masks the periodicity of the fluorescence observed with the tropomyosin antibody alone, it seems likely that the two antigens are present in the same fiber.

In skeletal muscle, actin filaments have a uniform length of approximately 1 μm and are aligned in parallel arrays with opposite polarities on either side of the Z band, as shown in Fig. 7. In relaxed muscle the length between two Z bands is approximately 2.6 μm. Since the actin filaments extend for only 1 μm from the Z band, the spacing between the ends of actin filaments attached to opposite Z bands is approximately 0.5 μm (15, 22). The bipolar myosin filaments are located between the antiparallel actin filaments; muscle contraction is mediated by the sliding of actin and myosin filaments past each other resulting in a reduction of the spacing between the ends of opposite actin filaments. Electron microscopic observations indicate that tropomyosin is coiled along the groove of the actin double helix throughout the entire length of the actin filament (9, 20, 23, 30). Since the actin filaments are organized in a periodic manner, the

reaction of relaxed myofibrils with either an actin or a tropomyosin antibody results in a periodic fluorescence (9, 19, 23). If the myofibrils were completely contracted, the spacing between opposite actin filaments would be eliminated, and reaction with an actin or a tropomyosin antibody would be expected to produce a continuous fluorescence.

In nonmuscle cells treated with the actin antibody the microfilament bundles show a continuous fluorescence. This might result if actin filaments were organized into microfilament bundles in a periodic manner similar to that in skeletal muscle, but are observed in a contracted state. The continuous fluorescence might also reflect an organization of actin filaments in microfilament bundles which is different from their organization in myofibrils; for example, actin filaments may be overlapping. Since little is known about the organization of microfilament bundles and since it is not clear if the terms "relaxed" and "contracted" are even applicable to them, it is difficult to decide between these possibilities at present.

If the association of actin and tropomyosin in nonmuscle cells is the same as in skeletal muscle, the fluorescent pattern produced by the tropomyosin antibody should be identical to that produced by the actin antibody, i.e., continuous. Thus, the observation of a periodic fluorescence produced by this antibody is unexpected. The periodicity might be accounted for by one of the following possibilities: (a) tropomyosin is bound over the entire length of the actin filament bundle, but within the "spacing" region is unable to react with the antibody. This might be due to a localized alteration in the tropomyosin molecule which prevents its recognition by the antibody; alternatively, tropomyosin might be hidden from the antibody by

the presence of an unknown substance, called "S," the spacing substance, which might possibly be myosin. (b) Tropomyosin may actually be missing from the actin filament in the spacing region. This might be due to an alteration in the action which prevents the tropomyosin from binding, or due to the presence of S which itself binds to actin and thereby prevents the tropomyosin from binding. Since the conformation of actin in the filaments is similar to its conformation in muscle (24, 29, 33), it seems unlikely that an alteration in actin itself could prevent the tropomyosin from binding. Similarly, it is extremely unlikely that an alteration in tropomyosin could completely prevent the binding of the antibody. Thus, we favor the hypothesis that the spacing is due to the presence of an S substance which either prevents tropomyosin from binding to actin or prevents the antibody from binding to tropomyosin. It should be noted that in the hypothetical case where the S substance prevents the tropomyosin antibody from binding to tropomyosin, it does not prevent the binding of the actin antibody.

In some of the microfilament bundles treated with the tropomyosin antibody, the periodicity of the fluorescence is poorly resolved or undetectable (see Figs. 3 a and 4 b). It is possible that the proteins in these bundles are organized in a different manner than they are in the bundles showing periodicity; for example, the S substance might be missing. Alternatively, a continuous fluorescence would result if the actin filaments within a microfilament bundle were "out of register" by as little as 0.4 μm.

Although the organization of tropomyosin which results in the periodicity is not known, it is interesting to note that the average lengths of the fluorescent segments, 1.2 μm, and the spacing
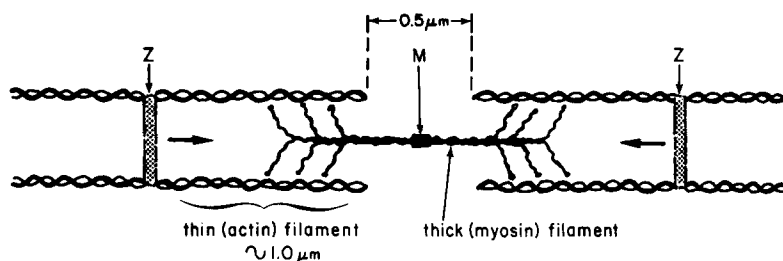
FIGURE 7   A diagrammatic representation of the structure of a skeletal muscle sarcomere. Z indicates the location of the Z bands; M indicates the M line seen in myosin filaments. The thin line in the actin filaments represents actin while the heavy line represents tropomyosin. The arrows within the Z bands indicate the direction of polarity of actin filaments and the direction of movement of the filaments during contraction.

regions, 0.4 μm, in these microfilament bundles closely parallel the lengths of individual actin filaments and the spacing between opposite actin filaments within a sarcomere, 1.0 μm and 0.5 μm, respectively.

The data available at present leave unresolved many questions concerning the organization of microfilament bundles in nonmuscle cells. However, the presence of tropomyosin, myosin, and actin in these microfilament bundles suggests the possibility that the interaction of actin and myosin in these bundles may be regulated by tropomyosin in a manner similar to that in skeletal muscle.

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