Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp. 4344–4348, December 1976
Biochemistry

Immunological characterization of the subunit of the 100 Å filaments from muscle cells

(actin/antibodies/immunofluorescence/sodium dodecyl sulfate-gel electrophoresis/tonofilaments)

ELIAS LAZARIDES* AND BRUCE D. HUBBARD

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colo. 80309

Communicated by Keith R. Porter, September 2, 1976

ABSTRACT **We report the immunological characterization of the subunit of the intermediate sized (100 Å) filaments from muscle cells. The protein as isolated from smooth muscle (chicken gizzard) has an apparent molecular weight of 50,000. It is insoluble in buffers that solubilize myosin and the majority of actin, but becomes soluble in the presence of urea. Under a variety of experimental conditions, that include the presence of 8 M urea, this new protein comigrates with actin during purification studies. The two proteins can be separated from each other by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and antibodies have been elicited against the 50,000 dalton protein purified by using this technique. These antibodies crossreact with the partially purified protein in urea, but show no detectable cross reaction with actin or myosin. Indirect immunofluorescence reveals that in skeletal muscle this protein is found in close association with the Z lines of the sarcomeres and extends between the Z lines of adjacent myofibrils; it is also associated with filamentous structures that run along the length of a muscle fiber both in close association with the plasma membrane and between myofibrils. These filaments appear to connect myofibrils to each other or to the plasma membrane at the level of their Z lines. In heart muscle, the protein shows the same distribution as in skeletal muscle. In addition, it is found intimately associated with intercalated disks and areas of membrane interaction between laterally associated heart muscle cells. The immunofluorescent localization to the subunit of the 100 Å filaments suggests that in muscle cells this molecule may serve to link actin filaments at the level of the Z line (or intercalated disk) with the muscle plasma membrane. We believe that it functions in muscle primarily as a three dimensional matrix which interconnects individual myofibrils to one another and to the plasma membrane at the level of their Z lines. In this manner, this molecule may provide a framework that mechanically integrates all the contractile myofilaments during the contraction and relaxation of muscle. As a means of indicating its linking role in muscle, we have termed the protein desmin (from the Greek δεσμός = link, bond).**

Higher eukaryotic cells contain in their cytoplasm three major filamentous structures: microtubules, actin filaments, and a class of intermediate sized filaments known as tonofilaments or 100 Å filaments due to their characteristic diameter as seen in the electron microscope. Intermediate sized filaments are present in such diverse cell types as fibroblasts, epithelial cells, skeletal muscle, smooth muscle, nerve cells, and glial cells (1–10). However, little is known about the cellular function of these filaments, and even less is known about their biochemical composition. A presumptive proteinaceous subunit of these filaments has recently been isolated and characterized biochemically and immunologically from neurons and glial cells (7–10), but the relationship of the protein found in nervous tissues to that in other cell types is presently unknown.

One of the most characteristic morphological features of the intermediate sized filaments is their intimate association in a variety of cell types with desmosomes, those distinctive structures that provide for attachment between adjacent cells within a tissue (for a review see ref. 11). The cytoplasm of both developing and adult smooth muscle cells contains a great number of these filaments which comprise a class distinct from the actin and myosin filaments that are involved in cellular contraction (12, 13). These filaments are known to insert characteristically into desmosome-like structures associated with membrane which are somehow involved in maintaining a strong cell to cell interaction between smooth muscle cells (14). We used smooth muscle as a representative differentiated cell system for the isolation and characterization of the subunit of the 100 Å filaments. In this paper, we report its immunological characterization from this type of muscle, and use indirect immunofluorescence to probe its localization in smooth, skeletal, and cardiac muscle cells.

MATERIALS AND METHODS

Antibody Preparation. Analytical and preparative slab gel electrophoresis with 12.5% gels was performed according to the discontinuous Tris-glycine system of Laemmli (15). The 50,000 molecular weight protein (desmin), for immunization, was purified from the 8 M urea extract of smooth muscle (see legend to Fig. 1f), by introducing as a final purification step preparative sodium dodecyl sulfate ($NaDodSO_4$)/slab gel electrophoresis (refs. 16 and 17; see *Results*). After preparative electrophoresis of the 8 M urea extract (Fig. 1f), the gels were stained for 15 min in 0.25% Coomassie brilliant blue, made up in 50% methanol (vol/vol) and 7.5% acetic acid (vol/vol), and then destained for 3 hr in 20% methanol (vol/vol) 7.5% acetic acid (vol/vol). The bands corresponding to a molecular weight of 50,000 were removed with a razor blade, and neutralized in the $NaDodSO_4$ running buffer (per liter: 14.4 g of glycine, 3 g of Tris base, and 1 g of $NaDodSO_4$ at pH 8.3). Several such bands were pooled and the 50,000 dalton protein was eluted electrophoretically as previously described (16, 17). Some of the polyacrylamide bands were homogenized directly in phosphate-buffered saline at pH 7.2 with the use of a Dounce homogenizer. The eluted protein and the protein containing gel homogenate were separately emulsified by using Freund's complete adjuvant. Because the eluted protein still contained appreciable amounts of $NaDodSO_4$, it was difficult to emulsify it directly with the adjuvant. Complete emulsification was achieved by inducing the protein/$NaDodSO_4$ solution to form a precipitate with $AlCl_3$. This was done by making the eluted protein solution 1% in $AlCl_3$ (wt/vol), and slowly neutralizing the mixture to pH 7.4 with 1 M NaOH. The Al/$NaDodSO_4$/protein precipitate that formed could be now emulsified completely with the adjuvant. Two white rabbits (New Zealand females) were injected subcutaneously each with approximately 500 μg of the eluted antigen and approximately 200 μg of the

Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; DTT, dithiothreitol.

* To whom correspondence should be addressed at Division of Biology, California Institute of Technology, Pasadena, Calif. 91125.

homogenized gel at multiple separate sites on their backs. This procedure was repeated with the same quantities of antigen on the day 8, and the rabbits were bled by ear puncture on the day 15. Precipitating antibodies could be already detected by this time (Fig. 3B). The rabbits were reinjected subcutaneously on the day 21 with 500 μg of the eluted protein only, emulsified in Freund's incomplete adjuvant. The rabbits were bled by ear puncture a week later, and the last procedure of injection and bleeding was repeated once more. Serum was prepared by allowing the blood to clot, and the globulins were partially purified by precipitation with 48% ammonium sulfate at 4°. The precipitated globulins were dialyzed against 0.15 M NaCl, 0.02 M Tris·HCl at pH 7.8, 1 mM NaN_3, 5 mM ϵ-amino-*n*-caproic acid and stored at −20°, at an approximate concentration of 25 mg/ml. Both animals responded efficiently to the injected antigen. The results obtained with the globulin preparations from either of the two animals have been indistinguishable.

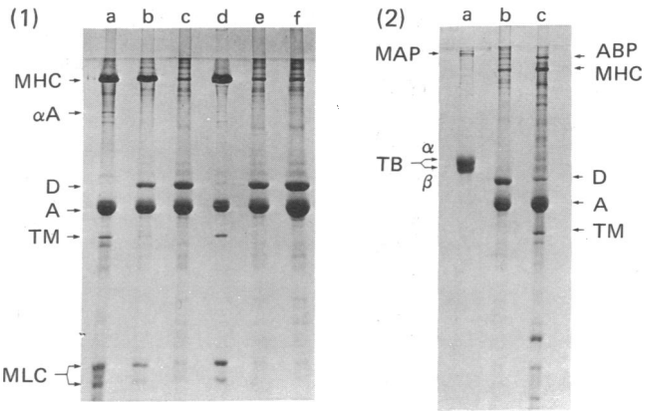
Indirect Immunofluorescence. For the preparation of skeletal myofibrils, skeletal muscle was allowed to stand for 48 hr at 4° in a muscle-relaxing solution containing 50% glycerol, 2.5 mM KCl, 0.014 M NaCl, 1 mM sodium phosphate buffer at pH 7.2, 0.2 mM $MgCl_2$, and 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid before blending. Heart muscle cells were incubated for 24 hr at 4° in the above relaxing solution in the presence of 25% glycerol (vol/vol). Exposure of heart muscle cells to low concentrations of Ca^{2+} ions causes the separation of individual heart muscle cells at their intercalated disks (18). The cardiac tissue was gently disrupted with a Dounce homogenizer, thus causing the dispersal of individual myocardial cells which retained one or both of their terminal intercalated disks as well as their lateral cellular connections. Frozen sections of skeletal muscle and intestine were obtained by using conventional techniques after embedding the material in OCT compound (Tissue-Tek II; Lab-Tek Products), and immersing them in liquid nitrogen. The sections, approximately 4 μm thick, were placed on slides, immersed into 95% ethanol at room temperature, and air dried before the application of the antibodies. Skeletal myofibrils and heart muscle cells were concentrated by centrifugation, and suspended directly in approximately 0.5 mg/ml of the globulin fraction containing antibody against desmin. An equivalent concentration of globulin from control serum showed no detectable reaction with any of the sarcomere structures. Indirect immunofluorescence was performed as previously described (19). The specimens were viewed under a Leitz microscope equipped with epifluorescent optics. Pictures were taken with TriX film using a 100X immersion lens, and developed in Diafine (Acufine, Inc.).

RESULTS

For the isolation and characterization of the putative subunit of the 100 Å filaments, we made use of the observation that in smooth muscle (chicken gizzard) this class of filaments is insoluble at salt concentrations that render the myosin and actin filaments soluble (14, 20). Fig. 1 shows that after prolonged extraction of actin and myosin with 0.6 M KCl, the insoluble residue (Fig. 1b) is enriched in a new protein with an apparent molecular weight of 50,000 (hereafter called desmin, see *Discussion*). The majority of the salt insoluble myosin and a substantial amount of the insoluble actin can now be extracted by moderate concentrations of potassium iodide (0.6 M KI; Fig. 1d), an agent that is known to depolymerize actin filaments and to dissociate actin from myosin (21). The KI insoluble material is highly enriched in desmin, but still contains a considerable amount of actin which does not seem to be soluble under conditions that solubilize the majority of muscle actin (Fig. 1c and e). Because such salt insoluble extracts do not contain any actin or myosin filaments, but are highly enriched in 100 Å filaments (20), we believe that the protein which is enriched in these salt insoluble extracts (desmin) is probably the major subunit of these filaments. The solubility properties of the actin that remains insoluble with desmin are unusual for conventional muscle actin. It has been tentatively identified as actin on the basis of its molecular weight (42,000), its reaction with antibodies raised against smooth muscle (gizzard) actin, and its isoelectric point. Actin and desmin can now be extracted in the presence of urea (Fig. 1f).

Assuming that the molecular weights of the α and β subunits of tubulin are 56,000 and 54,000, respectively (22), and that of actin is 42,000 (23), Fig. 2 indicates that the apparent molecular weight of desmin is 50,000.

Because desmin comigrates with the residual salt insoluble actin, we used preparative $NaDodSO_4$/gel electrophoresis as a final step in the purification of this protein prior to its use as



FIGS. 1 AND 2. *Fig. 1.* Partial purification of desmin from smooth muscle. Frozen chicken gizzards (500 g), were thawed and freed of the surrounding cartilagenous matrix. The muscle was thoroughly washed at 4° with 2 liters of distilled H_2O containing 1 mM dithiothreitol (DTT), 1 mM *p*-tosyl-L-arginine methyl ester, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 25 units/ml of Trasylol (Aprotinin, Sigma). The muscle was subsequently blended in the above solution in a Sorvall omnimixer for approximately 5 min, and the blended muscle was recovered at 8000 × *g*. The pellet was then successively extracted for 3 hr, 6 hr, 12 hr, 12 hr, and finally 24 hr in 500 ml of fresh buffer containing 0.6 M KCl, 20 mM Tris·HCl at pH 8.7, 1 mM DTT, 1 mM *p*-tosyl-L-arginine methyl ester, 0.5 mM phenylmethylsulfonyl fluoride, and 25 units/ml of Trasylol and the insoluble material was recovered at 8000 × *g*. (a) 0.6 M KCl final soluble proteins. (b) 0.6 M KCl final residue. This residue was freshly extracted three times for 12 hr each with 500 ml of 0.6 M KI, 20 mM sodium thiosulfate, 1 mM DTT, 20 mM Tris·HCl, at pH 8.7, and 1 mM ATP. Practically all of the residual actomyosin is solubilized within the first 2 hr. The insoluble material was recovered by centrifugation at 9000 × *g*. (c) Initial KI residue. (d) KI soluble material. (e) Final KI residue. This KI insoluble material (Fig. 1e) was extracted with 500 ml of a buffer containing 8 M urea (Ultra Pure, Schwarz/Mann), 2 mM DTT, 20 mM Tris·HCl at pH 8.7, for 12 hr, and the insoluble material was removed at 20,000 × *g* for 20 min and subsequently at 100,000 × *g* for 2 hr. (f) Final soluble proteins. *Fig. 2.* Comparative electrophoresis of porcine brain tubulin and desmin. Porcine brain tubulin was a generous gift of J. A. Snyder and J. R. McIntosh. (a) Approximately 8 μm of tubulin. (b) Ten micrograms of 8 M urea-soluble material of gizzard. (c) Approximately 10 μg of gizzard from a day-old chicken which was denatured and reduced directly in $NaDodSO_4$ sample buffer by placing in a boiling H_2O bath for 6 min. A, actin; D, desmin; MHC, myosin heavy chain; MLC, myosin light chains; ABP, actin binding protein (24, 25); α-A, α-actinin; TM, tropomyosin; TB, tubulin; MAP, microtubule associated proteins.

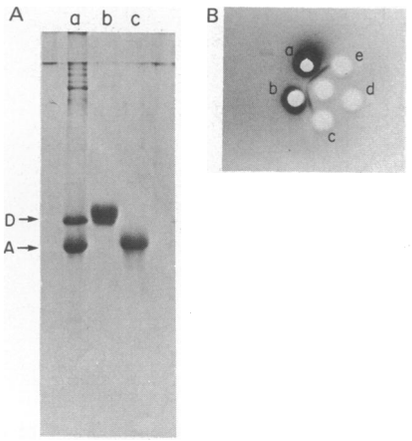


FIG. 3. (A) Analytical electrophoresis of desmin and actin after purification by preparative $NaDodSO_4$/polyacrylamide slab gel electrophoresis. (a) The 8 M urea extract (Fig. 1f) that was used for preparative purification of the two proteins. (b) Re-electrophoresed desmin and (c) re-electrophoresed actin. Preparations similar in purity to those depicted in this picture were used as antigens for the production of antibodies to desmin and actin (19). When only small amounts of desmin (approximately 1 μg) are re-electrophoresed, the protein separates into a closely spaced doublet that is poorly resolved under the reducing and nonalkylating conditions of electrophoresis employed here. (B) Double immunodiffusion of the partially purified desmin and the globulin preparation obtained against the electrophoretically purified antigen. The immunodiffusion plates were 1% agar equilibrated in 0.6 M KCl, 0.02 M Tris·HCl at pH 8.7. (a) Ten micrograms of the 8 M urea soluble extract (Fig. 1f). The buffer of this protein solution was 8 M urea, 0.02 M Tris·HCl at pH 8.7, 2 mM DTT. (b) Approximately a 10-fold dilution of the protein solution in (a). (c) Ten micrograms of smooth muscle actomyosin (Fig. 1a) in 0.6 M KCl, 0.2 M Tris·HCl at pH 8.7, 1 mM DTT, and the proteolytic inhibitors described in the legend of Fig. 1. (d) Eight microliters of the urea buffer of the samples in (a) and (b). (e) Five micrograms of skeletal muscle α-actinin in 0.6 M KCl, 0.02 M Tris·HCl at pH 8.7 (19). Immunodiffusion was for 72 hr at 4°. The agar plates were subsequently washed for 76 hr in 0.6 M KCl, 0.02 M Tris·HCl at pH 8.7, stained for 30 min with 0.25% Coomassie brilliant blue made up in 50% methanol and 7.5% acetic acid, and destained with 20% methanol and 7.5% acetic acid. For abbreviations, see legend to Figs. 1 and 2.

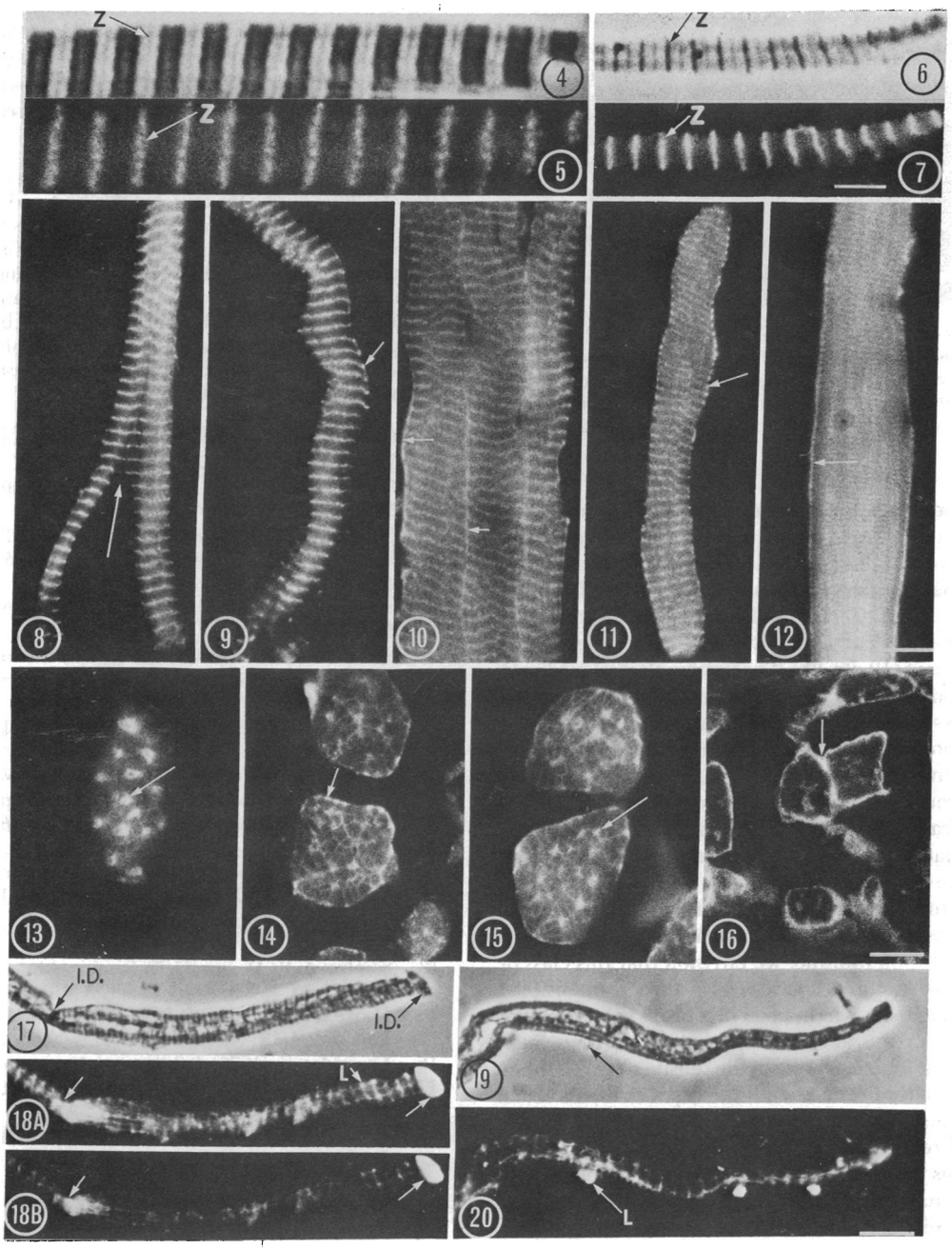
an antigen. Fig. 3A indicates that upon re-electrophoresis on an analytical $NaDodSO_4$/gel, desmin migrates with the same molecular weight as the original protein and it shows no detectable contamination by actin or any other protein of different molecular weight. In double immunodiffusion, the antiserum against desmin reveals only one detectable precipitin line with the protein partially purified in the presence of urea, but no detectable crossreaction with actin, myosin, α-actinin, or the 34,000 subunit of smooth muscle tropomyosin (Fig. 3B). These results, in conjunction with the immunofluorescent results reported below, indicate that the antiserum against desmin is specific for the 50,000 molecular weight desmin, and carries no detectable antibodies to any of the other known major contractile proteins of smooth muscle.

Indirect immunofluorescence shows desmin localized along the Z lines of isolated, relaxed, or contracted skeletal myofibrils (Figs. 4–7). Mechanical shearing of myofibrils results in the stretching of a slender desmin containing link between two adjacent Z lines (Fig. 8) that indicates this molecule extends between the Z lines of adjacent myofibrils. Myofibrils separated this way can sometimes be seen to contain a "knob"-like fluorescent structure at the ends of their Z lines (Fig. 9, arrow). These are presumably the sites at which desmin snaps back when the two adjacent myofibrils are finally sheared away from each other at their respective Z lines. The split appears to take place on one or the other side of each "knob"-like structure with the result that desmin snaps back only on one or the other Z line (compare Figs. 8 and 9). Within a muscle fiber, desmin is found associated with fluorescent filamentous structures that run along the long axis of the fiber both in close association with the plasma membrane and within the fiber between the Z lines. These structures appear to interconnect myofibrils to each other and to the plasma membrane at the level of their Z lines (Figs. 10 and 12, arrows). When a bundle of myofibrils is sheared away from the rest of the muscle fiber, the shearing takes place along the filaments found inside along the long axis of the fiber (Fig. 10 upper). The resulting myofibril bundles have the same fluorescent "knob"-like structures at the ends of their Z lines (Fig. 11), as they do in individual myofibrils. These "knob"-like structures appear to be the sites of attachment of the myofibril bundle's Z lines to the filaments that run along the length of the fiber either within the fiber or in close association with the fiber's plasma membrane. In a whole muscle fiber, desmin is localized in a diffuse form as well as at the Z lines and in close association with the plasma membrane (Fig. 12). The close association of desmin with filamentous structures that are in turn associated with the fiber's plasma membrane, is clearly demonstrated in cross sections of the fibers (Figs. 13–16). Desmin is also found in areas where two muscle fibers make contact (Fig. 16), as well as in a fine transverse filamentous matrix that appears to interconnect the filaments that run along the long axis of the fiber, within the fiber and parallel to the plasma membrane (Figs. 14–15).

In isolated heart muscle cells, desmin is found intimately associated with intercalated disks (Figs. 17–18), and with areas where adjacent heart muscle cells make contact (Figs. 19–20). In addition, desmin shows the same basic distribution as it does in skeletal myofibrils; it is found associated with cardiac myofibril Z lines and in filamentous structures that extend between the two intercalated disks of the myocardial cell and onto which a great number of Z lines appear to connect (Figs. 17–20). In frozen sections of intestine, the desmin antibody reacts with filamentous structures in the cytoplasm of the intestinal smooth muscle cells that run along the whole length of these cells (Fig. 21). The antibody does not seem to react with the intestinal brush border which can be shown to react intensely with antibodies to actin (unpublished observations). Furthermore, the desmin antibody also reacts with filamentous structures distinct from actin filaments in gizzard smooth muscle and in blood vessels. Desmin also shows a perinuclear localization in differentiating muscle cells growing in tissue culture and treated with colcemid, which is a characteristic distribution of the 100 Å filaments in cells exposed to this drug (1–3) (E. Lazarides, in preparation).

DISCUSSION

In smooth muscle, 100 Å filaments and actin filaments appear to terminate in electron dense material that is found associated all along the cell's plasma membrane, especially in areas where two smooth muscle cells share an intercellular junction (12, 14). This electron-dense material associated with membranes is morphologically indistinguishable from the electron-dense material of the fasciae adherentes which are the sites within a cardiac intercalated disk where the actin filaments of the terminal sarcomere terminate and insert into the plasma membrane. Quite frequently, the electron-dense material of the fasciae adherentes is continuous with intervening desmosomes and 100 Å filaments were observed in association with these two structures in both developing and adult cardiac muscle cells



FIGS. 4–20. *Figs. 4–7.* The localization of desmin in the Z lines of chicken skeletal myofibrils. Myofibrils were reacted with the desmin antibody in indirect immunofluorescence, and viewed with phase contrast (Figs. 4 and 6) and epifluorescent (Figs. 5 and 7) optics. The arrows termed Z refer to the localization of the Z-line within a sarcomere. Figs. 4 and 5 are those of a relaxed myofibril (distance between two Z-lines approximately 2.6 μm), while Figs. 6 and 7 are those of a contracted myofibril (distance between two Z-lines approximately 1.6 μm; bar = 3.2 μm). Within a sarcomere, a I band seems to be the weakest part of the myofibril structure, and mechanical shearing of muscle breaks myofibrils through the I bands on one or the other side of the Z line (18). Such myofibrils have a Z line as their terminal structure. Note in Figs. 4–7 (right) that this is indeed the case in these myofibrils and that the terminal Z lines react with the desmin antibody. Occasional longitudinal fluorescent connections between Z lines are evident (sixth fluorescent Z line from the right in Fig. 7; see also below). *Figs. 8–12.* Sheared products of muscle revealing the localization of desmin in a muscle fiber. See *text* for figure explanation. Bar = 7 μm for Figs. 8–12. *Figs. 13–16.* The localization of desmin in frozen cross sections of skeletal muscle. For details see the *text*. Bar = 11 μm for Figs. 13–15. Fig. 16, bar = 8 μm. Pictures were taken using an oil immersion 63× lens. *Figs. 17–20.* The localization of desmin in isolated heart muscle cells. Figs. 17 (phase optics) and 18 (epifluorescent optics) show a heart muscle cell which has retained its intercalated disc (I.D.) on the right side and is still connected to another heart cell with its intercalated disc on the left side. Fig. 18B is a repeat of Fig. 18A, but which has been printed darker to reveal more clearly the presence of desmin at the I.D.s. Figs. 19 (phase optics) and 20 (epifluorescent optics) show the presence of desmin in areas where two heart cells make lateral (L; see also Fig. 18A) connections. Note the apparent attachment of these lateral fluorescent connections to fluorescent "knob"-like material that is associated with the heart myofibril Z lines (bar = 7 μm in Figs. 17–20).

(26–28). Morphologically, the area of the fascia adherens was considered as the terminal Z line and it was assumed that these two structures are biochemically related (26–28). In developing skeletal or in heart muscle cells, Z lines are quite frequently seen to originate from intercellular desmosomes or from electron dense membrane continuations of such areas which resemble morphologically the electron-dense material of the fascia adherens (26, 27, 29). Similarly in adult cardiac or skeletal muscle,

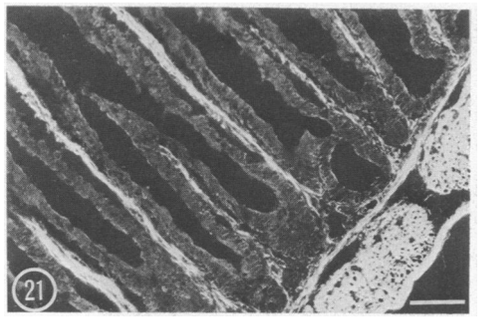


FIG. 21. The localization of desmin in intestinal smooth muscle. Similar results have been obtained with gizzard smooth muscle. Note that the epithelial cells also show a small amount of reaction which may indicate an antigenic crossreaction of the epithelial and muscle desmin (100 Å filaments; bar = 70 μm). Pictures were taken with a 25× lens.

Z lines are often seen in association with intercellular junctions that are located along the longitudinal surfaces of these cells (30). On the basis of this morphological evidence, the assumption was made that the electron-dense material found in association with the muscle Z lines and the muscle intercellular junctions are biochemically related and share at least one structural component (12, 26–28). The immunological evidence presented here indicates that one of the structural proteins they may share is the subunit of the 100 Å filaments.

By the immunofluorescent localization of this molecule, we believe that in muscle cells the 100 Å filaments and/or their protein subunit may mediate the attachment of actin filaments to specialized areas of the plasma membrane (Z lines to plasma membrane associated intercellular junctions terminal sarcomere actin filaments to fasciae adherentes in intercalated disks, and actin filaments to dense bodies in smooth muscle). Because muscle cells strongly interact at areas where 100 Å filaments insert to the plasma membrane, we envision that this class of filaments or their subunit functions as a three dimensional matrix which interconnects individual myofibrils to one another and to the plasma membrane at the level of their Z lines. Such an interconnecting matrix may provide tensile strength to the muscle and ensure that each and all the myofibrils, and each and all the muscle cells, are mechanically integrated during the contraction and relaxation of muscle. To indicate the linking function that this molecule might have in muscle cells and as a means of summarizing its fluorescent localization, we have termed the protein desmin (from the Greek δεσμός = link, bond).

The name desmin denotes that this protein, in addition to being the subunit of the 100 Å filaments, is also a component of muscle desmosome-like structures. It should be realized that we do not know yet whether the same protein is also a component of the more conventional types of desmosomes and 100 Å filaments that are seen in epithelial cells. Similarly, it should be emphasized that the conclusive identification of desmin as the subunit of the 100 Å filaments requires further purification of this molecule and the demonstration *in vitro* that it can assemble into filaments with an approximate diameter of 100 Å. The role of the actin that copurifies with desmin in the structure of 100 Å filaments is presently unknown. Its comigration with desmin is intriguing, especially because it has solubility properties which are characteristically distinct from those of conventional muscle actin.

We are grateful to Dr. K. R. Porter for the generous use of his laboratory facilities during the course of this work and to Drs. J. R. McIntosh, D. Hirsh, K. R. Porter, and the reviewers for their valuable comments on the manuscript. We are also grateful to Drs. D. Irwin, G. Brownlee, D. Hirsh, and J. P. Revel for advising us to modify the originally proposed name for the protein. We thank Ms. S. Nolan for her technical assistance. This work was supported by a grant from the Muscular Dystrophy Association of America (number 171073) and by a post-doctoral fellowship from the Muscular Dystrophy Association of America to one of us (E.L.).

1. Goldman, R. D. & Knipe, D. M. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **37**, 523–534.
2. Ishikawa, H., Bischoff, R. & Holtzer, H. (1968) *J. Cell Biol.* **38**, 538–555.
3. Brecher, S. (1975) *Exp. Cell Res.* **96**, 303–310.
4. Huneeus, F. C. & Davison, P. F. (1970) *J. Mol. Biol.* **52**, 415–428.
5. Spooner, B. S., Yamada, K. M. & Wessells, N. K. (1971) *J. Cell Biol.* **49**, 595–613.
6. Shelanski, M. L. & Feit, H. (1972) in *Structure and Function of Nervous Tissue*, ed. Bourne, G. (Academic Press, New York), Vol. 6, pp. 47–80.
7. Shu-Hui, Y., Dahl, D., Schachner, M. & Shelanski, M. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 529–533.
8. Bignami, A. (1975) in *Modern Trends in Neurology*. ed. Williams, D. (Butterworths, London), Vol. 6, pp. 1–16.
9. Dahl, D. & Bignami, A. (1975) *Biochim. Biophys. Acta* **386**, 41–51.
10. Dahl, D. & Bignami, A. (1974) *J. Neurol. Sci.* **23**, 551–563.
11. Staehelin, L. A. (1974) *Int. Rev. Cytol.* **39**, 191–283.
12. Uehara, Y., Campbell, G. R. & Burnstock, G. (1971) *J. Cell Biol.* **50**, 484–497.
13. Somlyo, A. P., Devine, C. E., Somlyo, A. V. & Rice, R. V. (1973) *Phil. Trans. R. Soc. London Ser. B.* **265**, 223–229.
14. Cooke, P. (1976) *J. Cell Biol.* **68**, 539–556.
15. Laemmli, U. K. (1970) *Nature* **227**, 680–685.
16. Lazarides, E. (1976) *J. Supramol. Struct.*, in press.
17. Lazarides, E. (1975) *J. Cell Biol.* **67**, 549–561.
18. Muir, A. R. (1967) *J. Anat.* **101**, 239–261.
19. Lazarides, E. (1976) *J. Cell Biol.* **68**, 202–219.
20. Cooke, P. H. & Chase, R. H. (1971) *Exp. Cell Res.* **66**, 417–425.
21. Szent-Gyorgyi, A. G. (1951) *J. Biol. Chem.* **192**, 361–369.
22. Eipper, B. A. (1974) *J. Biol. Chem.* **249**, 1407–1416.
23. Elzinga, M., Collins, J. H., Kuehl, W. N. & Adelstein, R. S. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2687–2691.
24. Stossel, T. P. & Hartwig, J. H. (1975) *J. Biol. Chem.* **250**, 5706–5712.
25. Wang, K., Ash, J. F. & Singer, S. J. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4483–4486.
26. Rash, J. R., Biesele, J. J. & Gey, G. O. (1970) *J. Ultrastruct. Res.* **33**, 408–435.
27. Hagopian, M. & Spiro, D. (1970) *J. Cell Biol.* **44**, 683–687.
28. Fawcett, D. W. & McNutt, N. C. (1969) *J. Cell Biol.* **42**, 1–45.
29. Heuson-Stienon, J. A. (1965) *J. Microsc. (Paris)* **4**, 657–678.
30. Baldwin, K. M. (1970) *J. Cell Biol.* **46**, 455–476.