Biochemistry and Molecular Architecture of Muscle Cell 10-NM Filaments and Z-Line: Roles of Desmin and α-Actinin

Richard M. Robson*
M. Yamaguchi
T. W. Huiatt
F. L. Richardson
J. M. O'Shea
M. K. Hartzer
W. E. Rathbun
P. J. Schreiner
L. E. Kasang
M. H. Stromer
Y.-Y. S. Pang
R. R. Evans
J. F. Ridpath

Introduction

It is well known (for reviews see Goll et al., 1977b, 1982) that skeletal muscle cells are packed with myofibrils, the protein threads that run the entire length of the cell and that are composed of about ten-twelve proteins. These proteins are assembled into a highly ordered, structural complex composed of a double array of interdigitating thick (~ 14-16 nm in diameter) and thin (~ 6 nm in diameter) filaments (Huxley, 1957, 1963, 1969). The thin filaments are attached at one end to the transverse Z-line and extend between the thick filaments at their other end. Thick filaments are composed primarily of myosin, plus a small amount of C-protein, which is localized in seven bands, 43 nm apart, on each side of the bipolar thick filament (Craig and Offer, 1976; Craig, 1977). C-protein may act to help hold the thick filament in its proper shape during tension development, possibly influence movement of cross-bridges in muscle contraction (Starr and Offer, 1978) and/or affect actin-myosin interaction (Moos et al., 1978; Moos and Feng, 1980). An M-line structure containing two major protein components, creatine kinase (Walliman et al., 1978) and myomesin (Trinick and Lowey, 1977; Eppenberger et al., 1981) cross-links adjacent thick filaments into the proper three-dimensional structure. Thin filaments are composed of tropomyosin and troponin bound to an actin backbone. The Z-line as viewed in cross section at electron microscope magnifications is made up of filaments that form a square lattice arrangement and give it the appearance of a well-ordered structure (Yamaguchi et al., 1980; 1982a). The entire composition of the Z-line is not known with absolute certainty, but it is generally thought that the major protein component of the Z-line is α-actinin (Suzuki et al., 1976). Depending upon how one defines the edges of the Z-line and upon the particular muscle being examined (i.e., one with narrow Z-lines such as fast-twitch skeletal muscle or one with wide Z-lines such as slow-twitch skeletal muscle or cardiac muscle), actin (and perhaps tropomyosin) may be present because the thin filaments insert well into the region of the Z-line proper in muscles having wide Z-lines (Yamaguchi et al., 1980, 1981, 1982a). Very small amounts of additional proteins such as filamin (Bechtel, 1979; Zeece et al., 1979) and desmin (O'Shea et al., 1979, 1981) also are present at the Z-lines.

Studies in several laboratories, including our own (reviewed in Goll et al., 1972, 1974, 1977a; Parrish, 1978; Robson et al., 1980; Goll and Otsuka, 1981), have shown that, aside from the variable degree of shortening and overlap of actin and myosin filaments (depends, in turn, upon such factors as antemortem handling, degree of muscle restraint, muscle temperature, species, and fiber type) and development of the rigor state, the major structural alteration that occurs in muscle postmortem involves the Z-line and Z-line-associated structures. The Z-line gradually disintegrates with postmortem storage. There is also a weakening of intermyofibrillar links or structures at the Z-line (Davey and Gilbert, 1969; Davey et al., 1976; Young et al., 1980-81). The causative factors for loss of Z-line integrity postmortem may, in turn, be due to proteases such as the Ca^{2+}-activated, endogenous muscle protease (termed CAF) that our laboratory has characterized previously (Dayton et al., 1975, 1976) or perhaps to a combination of muscle proteases, including CAF, working together (Bird et al., 1980; Okitani et al., 1980; Goll and Otsuka, 1981). Because 1) so many of the more striking changes that occur in muscle postmortem occur in the region of the Z-line and 2) of the importance of the Z-line region in maintaining overall integrity of the myofibril and muscle cell, we have examined the structure and chemistry of Z-line and Z-line related structures (Robson et al., 1970; Robson and Zeece, 1973; Dayton et al., 1975, 1976; Schollmeyer et al., 1976; Stromer et al., 1976; Suzuki et al., 1976; Singh et al., 1977,

*R. M. Robson, Muscle Biology Group, Iowa State University, Ames, IA 50011

Reciprocal Meat Conference Proceedings, Volume 34, 1981

Journal Paper No. J-10472 of the Iowa Agriculture and Home Economics Experiment Station, Project Nos. 2361 and 2127. Departments of Animal Science, Biochemistry and Biophysics, and Food Technology, cooperating, Iowa State University, Ames, IA 50011.

1981a, 1981b; Davies et al., 1978; Yamaguchi et al., 1978, 1980, 1981, 1982a, 1982b; Zeece et al., 1979; Craig-Schmidt et al., 1981; O'Shea et al., 1979, 1981; Huiatt et al., 1979, 1980, 1982).

In the remainder of this paper, I will briefly review first what we think the ultrastructure of the myofibril Z-line looks like and the properties of the major Z-line protein, α-actinin, and secondly, acquaint you with a fairly new muscle protein, called desmin, that we have recently purified from both avian smooth (Huiatt et al., 1980) and mammalian skeletal muscles (O'Shea et al., 1981). It is very likely that desmin is the major component of a new class of muscle filaments that are about 10 nm in diameter (thus, are often referred to as "intermediate" filaments because they have diameters between the ~ 6-nm thin and ~ 14-16-nm thick filaments of muscle structure). We (Schollmeyer et al., 1976; O'Shea et al., 1979, 1981; Huiatt et al., 1980, 1982; Richardson et al., 1981) and others (Lazarides and Hubbard, 1976; Granger and Lazarides, 1978; Campbell et al., 1979; Bennett et al., 1978, 1979) think that these filaments have an important cytoskeletal role and that they somehow tie or link adjacent myofibrils together at the level of the Z-lines.

Materials and Methods

Detailed procedures for preparation of highly purified α-actinin can be found in Suzuki et al. (1976), Singh et al. (1977), and Craig-Schmidt et al. (1981). Those for preparation of desmin can be found in O'Shea et al. (1979, 1981) and Huiatt et al. (1980, 1982). Most of the remaining biochemical and structural techniques described in this paper also can be found in those papers. The antibody localization techniques are described in Schollmeyer et al. (1976) and in Richardson et al. (1981).

Results and Discussion

Z-line Structure and Properties of α-Actinin

It is well known that each thin filament entering a Z-line is centrally positioned (when viewed in cross section) among four thin filaments entering the same Z-line from the adjoining sarcomere (Knappeis and Carlsen, 1962). There is considerable disagreement, however, as to the exact structure and composition of filaments (often referred to as Z-filaments) that anchor thin filaments to the Z-line and that may tie thin filaments entering one side of a Z-line to the thin filaments entering the Z-line from the adjoining sarcomere (Knappeis and Carlsen, 1962; Landon, 1970; MacDonald and Engel, 1971; Rowe, 1971; Kelly and Cahill, 1972; Franzini-Armstrong, 1973; Goldstein et al., 1977; Ullrick et al., 1977). A simple model of the Z-line recently worked out in our laboratory by Dr. Mamoru Yamaguchi is shown in Fig. 1. The model depicts how Z-filaments within the Z-line proper are arranged so that actin filaments from apposing sarcomeres are tied together. In cross section, one would see patterns of Z-filaments in what sometimes are referred to as small (11-nm sides) square and large (22-nm) square nets. The model (Yamaguchi

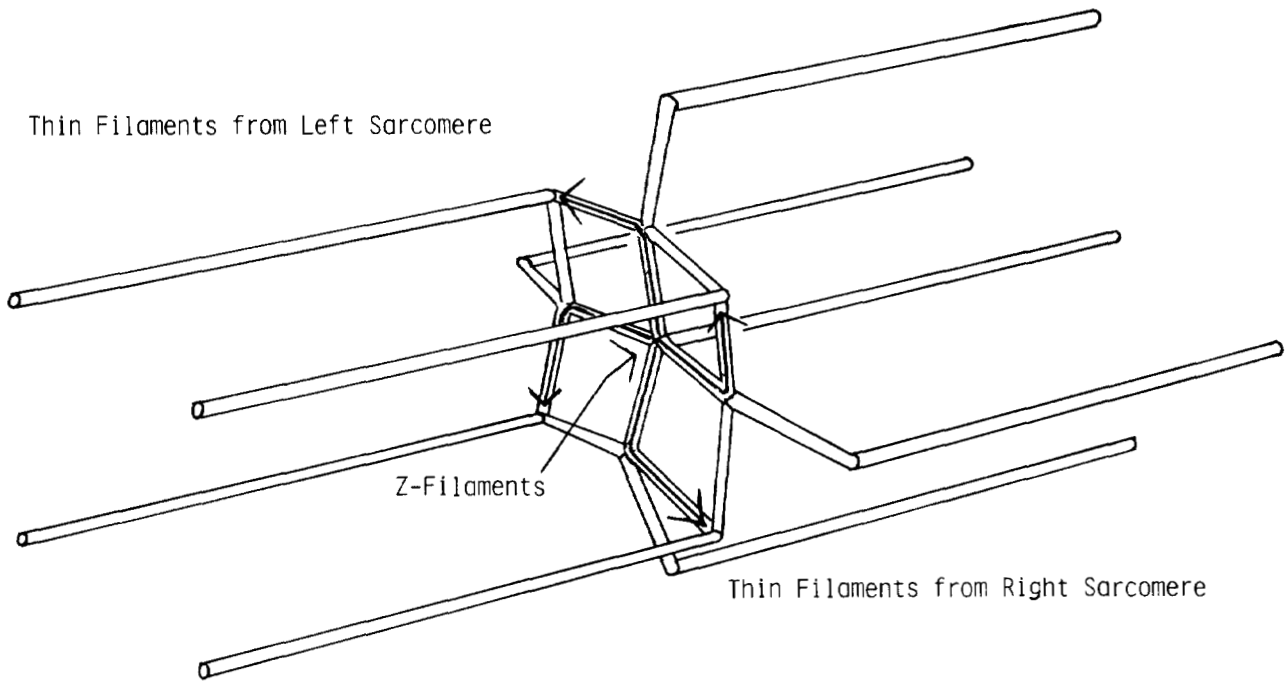

Figure 1. Schematic of simple Z-line model showing how Z-filaments within the Z-line proper are arranged so that an actin filament on one side of a Z-line is attached to four actin filaments on the other side of the same Z-line. For purposes of simplicity, the Z-filaments from the central actin filament shown on the right have been traced with arrows. In the actual model (Yamaguchi et al., 1980; M. Yamaguchi, R. Robson, and M. Stromer, manuscript in preparation), each actin filament would receive two Z-filaments and send two Z-filaments (i.e., two pairs of Z-filaments with reversed polarity).

et al., 1980, 1982a) was derived after careful examination of 1) longitudinal and cross-sectional views of simple Z-line structures, such as those found in many fish or amphibian muscles, 2) the seemingly more complex, wider Z-line structures, such as those found in "red" skeletal muscle and cardiac muscle, and 3) expanded Z-line-type structures, such as the rod body structures that are present in several muscle abnormalities (Yamaguchi et al., 1978, 1980, 1981, 1982a, 1982b). In the model, the width of the Z-line proper is determined by the degree of insertion of thin filaments (i.e., in wide Z-lines, the thin filaments insert into the Z-line further and overlap with the ends of the thin filaments coming into the Z-line from the adjacent sarcomere). Wider Z-lines can be explained as having additional sets or repeating units (approx. 40 nm apart) of Z-filaments.

The major protein of the myofibril that is limited in location to the Z-line is α-actinin (see Suzuki et al., 1976, and references therein). The protein first was prepared in a homogeneous state in our laboratory over ten years ago (Robson et al., 1970). This permitted considerable clarification of the molecule's properties. Some of these physical properties of skeletal muscle α-actinin are summarized in Table 1 (see Suzuki et al., 1976). The molecule has a molecular weight of about 200,000 and is composed of two subunits, each of 100,000 molecular weight. α-Actinin contains a large amount (~ 75%) of α-helical secondary structure. The size of the α-actinin molecule is approximately 40 × 400-500 Å, which is long enough for α-actinin to be the Z-filaments of the Z-line. Possible roles of α-actinin in skeletal muscle are listed in Table 2. It is generally assumed that α-actinin has the structural role in the Z-line of anchoring thin filaments from apposing sarcomeres. Because thin filaments on each side of a Z-line have opposite polarity, it is also possible that α-actinin helps to determine actin polarity. Because F-actin filaments prefer to grow *in vitro* at their barbed (equivalent to Z-line end) ends (Woodrum and Pollard, 1975), it also is possible that α-actinin has a role in regulation of thin filament growth.

Table 1. Some physical properties of skeletal muscle α-actinin[a]

Parameter	*Experimental Value*
$E^{1\%}_{278}$	9.71
A278/A260	1.79
$S^{\circ}_{20,w}$	6.21
Molecular weight in nondenaturing solvents	206,000
Molecular weight in denaturing solvents (*subunit size*)	100,000
$[\eta]$	20.6 ml/g
α-helix content	74%
Approximate size	
From physical-chemical measurements	~40 × 500Å
From EM of negatively stained molecules	~44 × 400Å

[a]Most of the data are taken from Suzuki et al. (1976).

Table 2. Possible roles of α-actinin in skeletal muscle

A. Comprise Z-filaments that help anchor thin filaments from apposing sarcomeres.
B. Help determine directionality and regulate growth of thin filaments.
C. Modify structures of actin in thin filaments and thereby enhance contractile-promoting activity of actin.

The role proposed in item 3 in Table 2 (i.e., that α-actinin can modify actin structure in thin filaments and thereby enhance contractile-promoting activity of actin) is based primarily upon *in vitro* experiments in which it has been established (Robson et al., 1970; Robson and Zeece, 1973) that α-actinin increases the Mg^{2+}-modified ATPase activity and rate of turbidity development of reconstituted actomyosin suspensions (i.e., α-actinin accelerates *in vitro* measures of muscle contraction). Implicit in the latter proposed role (enhancement of contraction) for α-actinin is that α-actinin exerts, at least in part, its effect(s) on the contractile apparatus via its interaction with actin and that α-actinin located at the Z-line has the ability to cause long-range changes in structure of the F-actin filaments. Therefore, we recently have studied the effects of α-actinin on F-actin ATPase activity (Singh et al., 1981a) and on rate of exchange of bound nucleotide of F-actin (Craig-Schmidt et al., 1981). Our results demonstrated that α-actinin has substantial and specific effects on F-actin ATPase activity and that α-actinin markedly increases the rate of release of F-actin bound nucleotide. Thus, our results are consistent with the possibility that each α-actinin can affect the structure of multiple actin monomers present in an actin filament.

Desmin and Muscle Cell 10-nm Filaments

One of the remarkable features of skeletal muscle cells is the precise degree of alignment of adjacent myofibrils. It seemed reasonable to us that there may be transverse structural elements, possibly at the level of the Z-lines, that link or tie adjacent myofibrils together and that may be responsible for alignment of myofibrils and overall muscle cell integrity. Essentially four observations (Table 3) led us to our detailed studies on 10-nm filaments and desmin in muscle cells; namely: 1) In experiments in which we were preparing skeletal muscle fractions enriched in Z-line material, a small

Table 3. Observations that led us to 10-nm filaments and desmin in muscle cells

A. Skeletal muscle fractions enriched in Z-line material had a small amount of a new 55,000 dalton component.
B. We saw significant amounts of a 55,000 MW component in smooth muscle crude α-actinin extracts.
C. Peter Cooke and others had observed a large number of 10-nm filaments that inserted into or encircled the "dense bodies" in smooth muscle cells.
D. We had shown that "dense bodies" were Z-line analogs by use of anti-α-actinin antibodies.

amount of a new 55,000 dalton component was identified by electrophoretic separation; 2) In comparative experiments that we were conducting on smooth muscle α-actinin, we observed significant amounts of a similar-sized 55,000 molecular weight component in the initial crude α-actinin extracts; 3) Peter Cooke (Cooke and Chase, 1971; Cooke, 1976) and others (Somlyo et al., 1973) had observed a large number of 10-nm diameter filaments that inserted into or encircled the "dense bodies" in smooth muscle cells; 4) We had shown that these "dense bodies" were Z-line analogs in that they were labeled by anti-α-actinin antibodies (Schollmeyer et al., 1976). As a result of those observations, we thought it possible that the 55,000-dalton protein, later termed desmin by Lazarides and Hubbard (1976) and "skeletin" by Small and Sobieszek (1977), may be one of the components of the 10-nm filaments.

Because desmin makes up about 5% of the total protein in homogenized gizzard muscle but only about 0.18% of the total protein in homogenized skeletal muscle, we first developed methods for isolation and purification of desmin from smooth muscle (Huiatt et al., 1980). This information was then used as the basis for working out procedures for isolating the very small amount of desmin present in mature mammalian skeletal muscle. Because it was known (Cooke and Chase, 1971; Cooke, 1976; Schollmeyer et al., 1976) that 10-nm filaments in smooth muscle remained insoluble after extraction of actomyosin at high ionic strength from myofibril fractions, a similar method was used in our experiments on avian smooth and porcine skeletal muscle desmin to initially obtain a fraction enriched in desmin before solubilization and further purification by chromatography in urea. For both (smooth and skeletal) preparations, well-washed (included washes with Triton X-100) myofibrils were first prepared (see Table 4 for summary of major steps involved in isolation and purification of porcine skeletal muscle desmin). The myofibrils were then exhaustively extracted with high-ionic-strength solutions (KCl for smooth samples and both KCl and KI for skeletal samples) to remove actomyosin. Examination of these residues with the electron microscope demonstrated that the smooth muscle samples contained large numbers of native 10-nm filaments, together with dense bodies (Z-line analogs), insoluble collagen fibrils, and some residual membranes (Huiatt et al., 1980). To date, we have been unable to unambiguously identify 10-nm filaments in the skeletal muscle residues.

The smooth muscle desmin can be quantitatively solubilized by overnight extraction of these crude 10-nm filament preparations with 6 M urea-containing solutions (Huiatt et al., 1980). For preparation of crude desmin from skeletal muscle (Table 4), the actomyosin-extracted residues are subjected to one cycle of desmin-solubilization in acetic acid followed by precipitation of desmin by pH neutralization in order to additionally enrich the fractions in desmin before solubilization in urea (O'Shea et al., 1981). Desmin comprises about 25% and 20% of the total protein in the urea-solubilized, crude desmin fractions of the smooth (Huiatt et al., 1980) and skeletal (O'Shea et al., 1981) muscle preparations, respectively. After trying many chromatographic methods for purification of desmin from the crude desmin extracts, we settled upon successive chromatography of the crude desmin on hydroxylapatite and DEAE-Sepharose CL-6B in the presence of urea. Routine preparations of purified desmins contained no actin contamination. Both the smooth and skeletal desmins contained a small amount (less than 3% of protein) of a 48,000- to 50,000-dalton proteolytic breakdown product of desmin in the purified samples. Routine yields of purified desmin averaged 175 mg from preparations initiated with 100 g minced gizzard (Huiatt et al., 1980) and 6 mg from 100 g minced porcine skeletal muscle (O'Shea et al., 1981), reflecting the higher concentration of desmin in smooth muscle (desmin comprises 8% of avian gizzard myofibrils, but only 0.35% of mature mammalian skeletal muscle myofibrils).

Avian gizzard muscle desmin is composed of two major isoelectric variants, α-(acidic) and β-(more basic) desmins, with the β-desmin constituting approximately 75% of the total protein. In contrast, the purified porcine skeletal muscle desmin consisted overwhelmingly (>90%) of one variant. Analysis of the purified smooth and skeletal muscle desmins by one- and two-dimensional gel electrophoresis demonstrated that no significant amount of the fibroblastic 10-nm filament protein (termed vimentin by Franke et al., 1978) was present in the purified desmins.

The purified desmins (see Table 5 for summary of some physical and chemical properties of purified turkey gizzard desmin) remain soluble after removal of urea by dialysis against 10 mM. Tris-acetate, pH 8.5, and centrifugation at 183,000 × g for 1 hr. Analytical ultracentrifugation demonstrated that the purified desmins in the 10-mM Tris-acetate, pH 8.5, had no large aggregates present. The ultraviolet absorption spectrum of desmins showed a maximum at 278 nm,

Table 4. Summary of major steps used in isolation and purification of porcine skeletal muscle desmin[a]

A. Prepare well-washed myofibrils from ground muscle.
B. Extract myofibrils with high-ionic-strength solutions to remove much of the actomyosin.
C. Solubilize desmin in 1 M acetic acid to remove additional actin.
D. Purify desmin by chromatography in the presence of urea.

[a]See O'Shea et al. (1981) for additional details.

Table 5. Summary of some physical and chemical properties of purified turkey gizzard desmin[a]

Parameter	*Experimental Value*
M_r (from SDS-gel electrophoresis)	55,000
$S^{\circ}_{20^{\circ}}$ (10 mM Tris-acetate, pH 8.5)	5.20
$E^{1\%}_{278}$	5.57
A278/A260	1.64
% carbohydrate (by weight)	<0.3%
α-helix content	45%
Isoelectric points α	5.43
in urea β	5.48

[a]Most of the data are taken from Huiatt et al. (1980).

a measured $A_{278}^{1\%}$ of 5.6, and a ratio of absorbance at 278 nm to that at 260 nm of 1.64. The ultraviolet circular dichroism spectra of soluble smooth and skeletal desmins (in 5 mM sodium phosphate, pH 8.5) are typical of α-helical proteins (Huiatt et al., 1980; W. E. Rathbun and R. M. Robson, unpublished observations), with two negative extrema at 208 and 222 nm. The significant amount of α-helix present (Table 5) suggests that the purified desmins adopt a native structure after removal of urea (Huiatt et al., 1980, 1982).

Amino acid compositions of the avian smooth and mammalian skeletal muscle desmins are quite similar (O'Shea et al., 1979). Comparative peptide maps of the two desmins obtained after limited cleavage by CAF or trypsin (O'Shea et al., 1979) also indicate some homology in primary structure between the avian and mammalian desmins, but some distinct differences as well. One of the most characteristic properties of desmin is its high degree of susceptibility to proteolysis.

One of the more intriguing and important properties of highly purified desmin is its ability to form "synthetic" filaments *in vitro* (Huiatt et al., 1980, 1982; O'Shea et al., 1981). Both smooth and skeletal muscle desmins assembled into filaments that appeared as long, flexible strands with a diameter of approximately 10 nm after dialysis against 100 mM NaCl, 1 mM MgCl, 10 mM imidazole-HCl, pH 7.0. The morphology of these synthetic filaments was quite similar to that of native 10-nm filaments present in crude intermediate filament fractions before urea extraction (Huiatt et al., 1980).

One of our more exciting results in the past year resulted from our attempts to ascertain the location of desmin in muscle cells. We prepared antibodies to DEAE-purified desmins that had been further purified by preparative electrophoresis. Indirect immunofluorescence localization studies (Richardson et al., 1981; F. Richardson, J. O'Shea, R. Robson, M. Stromer, and P. Bechtel, unpublished work) demonstrated localization of desmin near the Z-lines of striated muscle cells. A connecting link between Z-lines of adjacent myofibrils often could be seen. The immunofluorescence results suggested that desmin was located around the periphery of the Z-line as opposed to being an integral protein of the Z-line proper as is α-actinin. Although the immunofluorescence results were useful, they did not demonstrate that the desmin near the Z-line was actually present in skeletal muscle cells in the aggregated form of 10-nm filaments. Thus, the localization experiments also were done by immunoelectron microscope localization using the immunoperoxidase technique (Richardson et al., 1981). The electron-dense reaction product followed an approximately linear course between Z-lines of adjacent myofibrils and also indicated that the desmin antibodies had decorated a small number of ~10-nm (9-12 nm) filaments spanning this region. To our knowledge, these studies are the first to demonstrate that desmin is present in a filamentous form in normal mature mammalian striated muscle cells and, thus, that these filaments can actually serve to connect adjacent myofibrils at the level of the Z-line. A schematic depicting the location of desmin filaments and their role in tying the myofibrils into the muscle cell cytoskeleton is shown in Figure 2.

We have examined the effects of postmortem storage on bovine skeletal muscle desmin (Robson et al., 1980; J. O'Shea, R. Robson, and M. Stromer, unpublished; L. Kasang, M. Stromer, and R. Robson, unpublished). In general, desmin is degraded at about the same rate as the highly proteolytically susceptible troponin-T. Similar results have recently

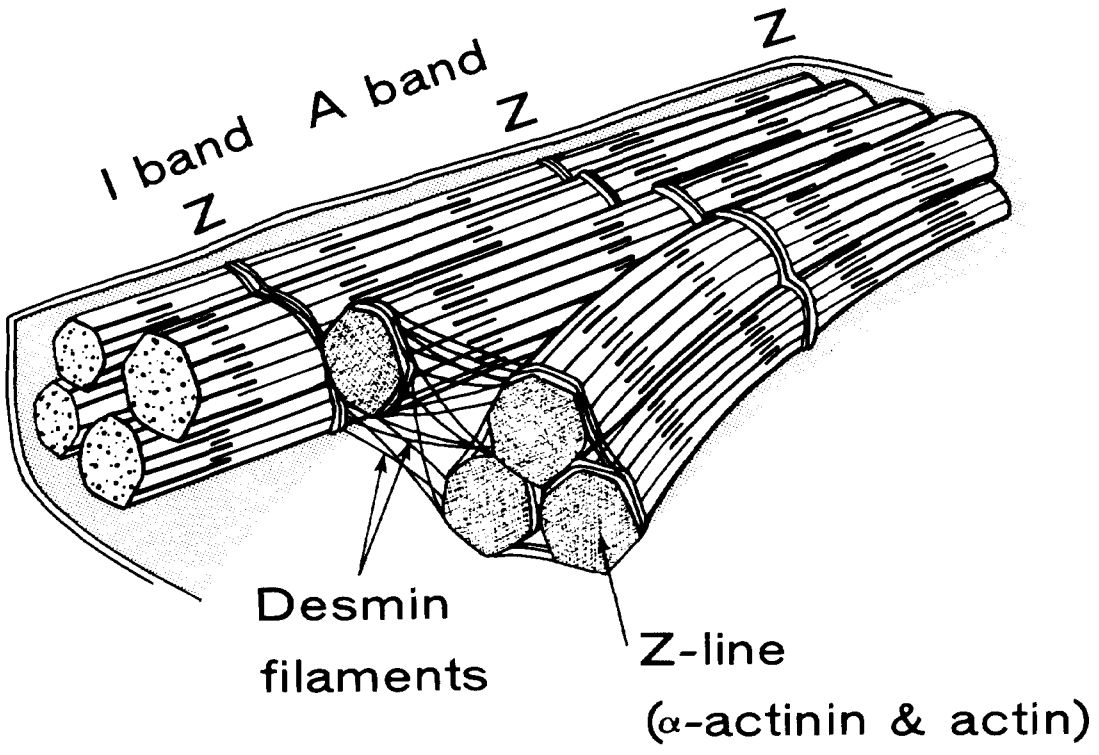

Figure 2. Schematic showing desmin filament location in mature vertebrate skeletal muscle.

been reported by Young et al. (1980-81). It seems quite plausible that the degradation of desmin and the cytoskeletal 10-nm filaments that they comprise is responsible for many of the postmortem physical changes in muscle and for the beneficial increase in tenderness that occurs in fresh meat during postmortem storage. In a very recent study, Honikel et al. (1981) have suggested that longitudinal changes in bovine muscle that occur during contraction postmortem have much less influence on water-holding capacity than transversal alterations (particularly in the cross-linking between myofilaments). Thus, alterations in desmin postmortem also may have important implications to the processing characteristics of muscle tissue.

Acknowledgments

We are especially grateful to Mary Bremner for expert technical assistance and to Joan Andersen for typing the manuscript. This work was supported in part by grants from the National Institutes of Health (HL-15679), the Muscular Dystrophy Association, and by a grant-in-aid from the American Heart Association (79-948) with funds contributed in part by the Iowa Affiliate. This is Journal Paper No. J-*10472* of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Projects No. 2361 and 2127.

Discussion

Janet Williams, University of Wyoming: In your slide on skeletal muscle, it looked like there was more gamma actinin, which is from the smooth muscles, and I wondered why you had more gamma than alpha actinin from skeletal muscles.

Robson: There is, essentially, no gamma in skeletal muscle. I added that. In other words, I made purified alpha and purified gamma and I put those in as internal standards. That is why it showed up.

R. G. Kauffman, University of Wisconsin: How can you tie this information to an application for meat scientists? Just exactly, down the road aways, do you see the information on desmin and, as far as that goes, alpha actinin, indicating roles in improving meat tenderness, or making better sausage emulsions, or have you even thought about that yet?

Robson: When you work at the Ag. Experiment Station, you have to think about it. The answer is, we have thought about it quite a bit, and I do think it's very directly applicable to meat science. If you remember some of the work that came out in the early 70's, the major changes that occur in muscle postmortem are centered around the Z-line structure. That's actually what originally led us into looking at "what is a protein" and "what is the structure in the Z-line." It also branched out and turned into a rather intensive investigation trying to find out the factors involved—which Drs. Dayton, Goll, and so on, worked on with the calcium activated protease. At the same time, we noted (it was actually noticed by some workers in New Zealand many years ago) that one of the first things that happens in postmortem muscle is a breakdown in the lateral register. There is something that ties these things togehter. Now, when you get into meat emulsions, one has to start wondering about the importance there. I kind of worried about that until I gave the paper last fall, and Dr. Reiner Hamm jumped to his feet and said that, finally, we had discovered what he had been trying to tell people about for years. It turns out that the emulsification properties of meat are more directly related to the lateral spacing of the myofibrils in a meat product than they are to the longitudinal tieing together of the myofibrils. I kind of have to take Dr. Hamm's word for that, but that appeared in a paper that came out in the *Journal of Food Science* in January. If you'll refer to that paper, and then you recall all of this, why, I sure hope that they go together pretty well.

A. M. Mullins, Louisiana State University: Would you care to venture a comment as to what effect electrical stimulation is having on desmin?

Robson: Well, I certainly don't know for sure. We do have a graduate student in the Food Science Department who is looking at that right now. My guess—I don't think I'll guess, because I'm really not sure what we'll find. I think it's going to be related to whether or not we really feel that the electrical stimulation increases the activity of some of the muscle proteases. We've actually been looking at that, and, quite frankly, as you assay some of these proteases, the activity often times is lower in the electrically stimulated. We initially thought, of course, that it would be up. It is very difficult to interpret those experiments because of the things that occur in a muscle cell during electrical stimulation. A lot of times, they happen very quickly, and, it might be that, by the time we're able to assay for proteolytic enzymes, they have actually already gone through and 'done their thing', and they are now self-destructing—they are essentially going through autolysis. So I guess I'd better just wait and hope that Len Kasang will have the answer to that, then he can tell you.

References

Bechtel, P. J. 1979. J. Biol. Chem. 254:1755-1758.
Bennett, G. S., Fellini, S. A., and Holtzer, H. 1978. Differentiation 12:71-82.
Bennett, G. S., Fellini, S. A., Toyama, Y., and Holtzer, H. 1979. J. Cell Biol. 82:577-584.
Bird, J. W. C., Carter, J. H., Triemer, R. E., Brooks, R. M. and Spanier, A. M. 1980. Fed. Proc. 39:20-25.
Campbell, G. R., Chamley-Campbell, J., Gröschel-Stewart, U., Small, J. V., and Anderson, P. 1979. J. Cell Sci. 37:303-322.
Cooke, P. 1976. J. Cell Biol. 68:539-556.
Cooke, P. H. and Chase, R. H. 1971. Exp. Cell Res. 66:417-425.
Craig, R. 1977. J. Mol. Biol. 109:69-81.
Craig, R. and Offer, G. 1976. Proc. R. Soc. Lond. B. 192:451-461.
Craig-Schmidt, M. C., Robson, R. M., Goll, D. E., and Stromer, M. H. 1981. Biochem. Biophys. Acta 670:9-16.
Davey, C. L. and Gilbert, K. V. 1969. J. Food Sci. 34:69-76.
Davey, C. L., Niederer, A. F., and Graafhuis, A. E. 1976. J. Sci. Food Agric. 27:251-256.
Davies, P. J., Wallach, D., Willingham, M. C., Pastan, I., Yamaguchi, M. and Robson, R. M. 1978. J. Biol. Chem. 253:4036-4042.
Dayton, W. R., Goll, D. E., Stromer, M. H., Reville, W. J., Zeece, M. G., and Robson, R. M. 1975. In: Vol. II. Proteases and Biological Control. (Reich, E., Rifkin, D. B., and Shaw, E., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 551-577.
Dayton, W. R., Goll, D. E., Zeece, M. G., Robson, R. M., and Reville, W. J. 1976. Biochemistry 15:2150-2158.
Eppenberger, H. M., Perriard, J.-C., Rosenberg, U. B., and Strehler, E. E. 1981. J. Cell Biol. 89:185-193.
Franke, W. W., Schmid, E., Osborn, M., and Weber, K. 1978. Proc. Natl. Acad. Sci. USA 75:5034-5038.
Franzini-Armstrong, C. 1973. J. Cell Biol. 58:630-642.

Goldstein, M. A., Schroeter, J. P., and Sass, R. L. 1977. J. Cell Biol. 75:818-836.
Goll, D. E. and Otsuka, Y. 1981. In: Reciprocal Meat Conference Proceedings, Vol. 34 (this issue).
Goll, D. E., Robson, R. M., and Stromer, M. H. 1977a. In: Food Proteins. Proc. Symposium on Fundamental Aspects of Proteins Basic to Foods. (Whitaker, J. R. and Tannenbaum, S., eds.). pp. 121-174, AVI Publishing Company, Westport, Connecticut.
Goll, D. E., Stromer, M. H., Olson, D. G., Dayton, W. R., Suzuki, A., and Robson, R. M. 1974. In: Proc. Meat Ind. Res. Conf., pp. 75-98, Am. Meat Inst. Foundation, Arlington, Virginia.
Goll, D. E., Stromer, M. H., and Robson, R. M. 1977b. In: Duke's Physiology of Domestic Animals, 9th Edition (Swensen, M. H., ed.). Chapter 39, pp. 504-530. Cornell University Press, Ithaca, New York.
Goll, D. E., Stromer, M. H., and Robson, R. M. 1982. In: Duke's Physiology of Domestic Animals, 10th Edition (Swensen, M. J., ed.). Cornell University Press, Ithaca, New York (submitted).
Goll, D. E., Stromer, M. H., Suzuki, A., Busch, W. A., Reddy, M. K., and Robson, R. M. 1972. In: Proc. 18th Eur. Meeting of Meat Res. Workers, pp. 1-13, Guelph, Canada.
Granger, B. L. and Lazarides, E. 1978. Cell 15:1253-1268.
Honikel, K. O., Hamid, A., Fischer, C., and Hamm, R. 1981. J. Food Sci. 46:23-25, 31.
Huiatt, T. W., Robson, R. M., Arakawa, N., and Stromer, M. H. 1980. J. Biol. Chem. 255:6981-6989.
Huiatt, T. W., Robson, R. M., O'Shea, J. M., Hartzer, M. K., and Stromer, M. H. 1979. Abstracts of papers presented at the May 16-20, 1979 meeting on "The cytoskeleton, membranes and movement," Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Huiatt, T. W., Robson, R. M., Stromer, M. H., and Rougvie, M. A. 1982. (submitted).
Huxley, H. E. 1957. J. Biophys. Biochem. Cytol. 3:631-646.
Huxley, H. E. 1963. J. Mol. Biol. 7:281-308.
Huxley, H. E. 1969. Science 164:1356-1366.
Kelly, D. E. and Cahill, M. A. 1972. Anat. Rec. 172:623-642.
Knappeis, G. G. and Carlsen, F. 1962. J. Cell Biol. 13:323-335.
Landon, D. N. 1970. J. Cell Sci. 6:257-276.
Lazarides, E. and Hubbard, B. D. 1976. Proc. Natl. Acad. Sci. USA 73:4344-4348.
MacDonald, R. D. and Engel, A. G. 1971. J. Cell Biol. 48:431-437.
Moos, C. and Feng, I.-N. M. 1980. Biochem. Biophys. Acta 632:141-149.
Moos, C., Mason, C. M., Besterman, J. M., Feng, I.-N. M., and Dubin, J. H. 1978. J. Mol. Biol. 124:571-586.
Okitani, A., Matsukura, U., Kato, H., and Fujimaki, M. 1980. J. Biochem. 87:1133-1143.
O'Shea, J. M., Robson, R. M., Hartzer, M. K., Huiatt, T. W., Rathbun, W. E., and Stromer, M. H. 1981. Biochem. J. 195:345-356.
O'Shea, J. M., Robson, R. M., Huiatt, T. W., Hartzer, M. K., and Stromer, M. H. 1979. Biochem. Biophys. Res. Commun. 89:972-980.
Parrish, Jr., F. C. 1978. In: Proc. 24th Eur. Meeting of Meat Res. Workers, pp. E4:1-14, Kulmbach, West Germany.
Richardson, F. L., Stromer, M. H., Huiatt, T. W., and Robson, R. M. 1981. Eur. J. Cell Biol. (in press).
Robson, R. M., Goll, D. E., Arakawa, N., and Stromer, M. H. 1970. Biochem. Biophys. Acta 200:296-318.
Robson, R. M., Stromer, M. H., Huiatt, T. W., O'Shea, J. M., Hartzer, M. K., Richardson, F. L., and Rathbun, W. E. 1980. Proc. 26th Eur. Meeting of Meat Research Workers, Colorado Springs, Colorado, USA. Vol. 1, pp. 22-25.
Robson, R. M. and Zeece, M. G. 1973. Biochem. Biophys. Acta 295:208-224.
Rowe, R. W. D. 1971. J. Cell Biol. 51:674-685.
Schollmeyer, J. E., Furcht, L. T., Goll, D. E., Robson, R. M., and Stromer, M. H. 1976. In: Cell Motility, Cold Spring Harbor Conferences on Cell Proliferation (Goldman, R. D., Pollard, T. D. and Rosenbaum, J. L., eds.) Vol. 3, Book A, pp. 361-387, Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
Singh, I., Goll, D. E., and Robson, R. M. 1981a. Biochem. Biophys. Acta 670:1-8.
Singh, I., Goll, D. E., Robson, R. M., and Stromer, M. H. 1977. Biochem. Biophys. Acta 491:29-45.
Singh, I., Goll, D. E., Robson, R. M., and Stromer, M. H. 1981b. Biochem. Biophys. Acta 669:1-6.
Small, J. V. and Sobieszek, A. 1977. J. Cell Sci. 23:243-268.
Somlyo, A. P., Devine, C. E., Somlyo, A. V., and Rice, R. V. 1973. Phil. Trans. R. Soc. Lond. B. 265:223-230.
Starr, R. and Offer, G. 1978. Biochem. J. 171:813-816.
Stromer, M. H., Tabatabai, L. B., Robson, R. M., Goll, D. E., and Zeece, M. G. 1976. Exp. Neurol. 50:402-421.
Suzuki, A., Goll, D. E., Singh, I., Allen, R. E., Robson, R. M., and Stromer, M. H. 1976. J. Biol. Chem. 251:6860-6870.
Trinick, J. and Lowey, S. 1977. J. Mol. Biol. 113:343-368.
Ullrick, W. C., Toselli, P. A., Saide, J. D., and Phear, W. P. C. 1977. J. Mol. Biol. 115:61-74.
Wallimann, T., Pelloni, G., Turner, D. C., and Eppenberger, H. M. 1978. Proc. Natl. Acad. Sci. USA 75:4296-4300.
Woodrum, R. and Pollard, T. 1975. J. Cell Biol. 67:231-237.
Yamaguchi, M., Robson, R. M., and Stromer, M. H. 1982a. (submitted).
Yamaguchi, M., Robson, R. M., Stromer, M. H., and Cholvin, N. R. 1981. J. Cell Biol. 91:344a.
Yamaguchi, M., Robson, R. M., Stromer, M. H., Dahl, D. S., and Oda, T. 1978. Nature 271:265-267.
Yamaguchi, M., Robson, R. M., Stromer, M. H., Dahl, D. S., and Oda, T. 1982b. (submitted).
Yamaguchi, M., Stromer, M. H., Robson, R. M., Anderson, B., and Anderson, W. D. 1980. J. Cell Biol. 87:259a.
Young, O. A., Graafhuis, A. E., and Davey, C. L. 1980-81. Meat Science 5:41-55.
Zeece, M. G., Robson, R. M., and Bechtel, P. J. 1979. Biochem. Biophys. Acta 581:365-370.