BIOCHEMISTRY – IMPACT ON MEAT TENDERNESS

Postmortem Changes in the Myofibrillar and Other Cytoskeletal Proteins in Muscle

RICHARD M. ROBSON*, ELISABETH HUFF-LONERGAN', FREDERICK C. PARRISH, JR.,
CHIUNG-YING HO, MARVIN H. STROMER, TED W. HUIATT, ROBERT M. BELLIN
and SUZANNE W. SERNETT

Introduction

The cytoskeleton of “typical” vertebrate cells contains three protein filament systems, namely the ~7-nm diameter actin-containing microfilaments, the ~10-nm diameter intermediate filaments (IFs), and the ~23-nm diameter tubulin-containing microtubules (Robson, 1989, 1995; Robson et al., 1991). The contractile myofibrils, which are by far the major components of developed skeletal muscle cells and are responsible for most of the desirable qualities of muscle foods (Robson et al., 1981, 1984, 1991), can be considered the highly expanded corollary of the microfilament system of non-muscle cells. The myofibrils, IFs, cell membrane skeleton (complex protein-lattice subjacent to the sarcolemma), and attachment sites connecting these elements will be considered as comprising the muscle cell cytoskeleton in this review.

Selected Myofibrillar and Other Cytoskeletal Proteins in Developed Mammalian Skeletal Muscle Cells

The entire cytoskeleton of a muscle cell probably consists of hundreds of different kinds of proteins, including many present in very small amounts, and a large number yet-to-be discovered. A list of some selected cytoskeletal proteins is shown in Table 1. Proteins of the myofibrillar thick filaments (myosin, C-protein, and H-protein), M-line region (myomesin, M-protein, creatine kinase, and skelemin), thin filaments (actin, troponymosin, troponin, tropomodulin, and nebulin), titin filaments (titin), and integral Z-line region (a-actinin, Cap Z), as well as proteins of the intermediate filaments (desmin, paranemin, and synemin), Z-line periphery (filamin) and costameres underlying the cell membrane (filamin, dystrophin, talin, and vinculin) are listed along with an estimate of their abundance, approximate molecular weights, and number of subunits per molecule. Because the myofibrils are the overwhelming components of the skeletal muscle cell cytoskeleton, the approximate percentages of the cytoskeleton listed for the myofibrillar proteins (e.g., myosin, actin, tropomyosin, a-actinin, etc.) also would represent their approximate percentages of total myofibrillar protein.

Some Important Characteristics, Possible Roles, and Postmortem Changes of Key Cytoskeletal Proteins

A subset of the proteins listed in Table 1 are known to undergo postmortem proteolysis and/or to be highly sensitive to proteolysis. These include titin, nebulin, desmin, paranemin, synemin, filamin, and the troponin-T (TN-T) subunit of troponin. Nearly all, if not all, of these proteins are likely to have important roles in organizing and maintaining the integrity and strength of the contractile myofibrils and of the overall cytoskeleton of the skeletal muscle cell. A summary of important characteristics, possible roles, and postmortem changes of these seven proteins are described in a “table format,” which hopefully will enable readers of this review to get a concise overview of the respective proteins. Although space limitations unfortunately do not permit inclusion of many of the original citations for specific characteristics and postmortem changes of proteins described herein, most can be found within the citations that are included. Many of the earlier studies reported on titin, nebulin, desmin, and synemin also can be found in the reviews by Robson and Huiatt (1983), Robson et al. (1984, 1991), and Robson (1995).

Titin (Tables 2 and 3) and nebulin (Tables 4 and 5) are two very large, long proteins that run in parallel with the

*Richard M. Robson, Muscle Biology Group, 3110 Molecular Biology Building, Iowa State University, Ames, IA 50011-3260.
'Elisabeth Huff-Lonergan, Department of Animal and Diary Sciences, Auburn University, Auburn, AL 36849.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Primary Myofibrillar/Cytoskeletal Location</th>
<th>Approximate % of Cytoskeleton</th>
<th>Approx. MW (# of Subunits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin</td>
<td>Thick filament</td>
<td>45</td>
<td>520,000 (6)</td>
</tr>
<tr>
<td>C-Protein (MyBP-C)</td>
<td>Thick filament</td>
<td>2</td>
<td>130,000 (1)</td>
</tr>
<tr>
<td>H-Protein (MyBP-H)</td>
<td>Thick filament</td>
<td>1</td>
<td>74,000 (1)</td>
</tr>
<tr>
<td>Myomesin</td>
<td>M-line</td>
<td>2</td>
<td>185,000 (1)</td>
</tr>
<tr>
<td>M-Protein</td>
<td>M-line</td>
<td>1</td>
<td>165,000 (1)</td>
</tr>
<tr>
<td>Creatine Kinase</td>
<td>M-line (peripheral)</td>
<td>&lt;1</td>
<td>80,000 (2)</td>
</tr>
<tr>
<td>Skelemin</td>
<td>M-line (peripheral)</td>
<td>&lt;1</td>
<td>195,000 (1)</td>
</tr>
<tr>
<td>Actin</td>
<td>Thin filament</td>
<td>20</td>
<td>42,000 (1)</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>Thin filament</td>
<td>5</td>
<td>66,000 (2)</td>
</tr>
<tr>
<td>Troponin1</td>
<td>Thin filament</td>
<td>5</td>
<td>69,000 (3)</td>
</tr>
<tr>
<td>Tropomodulin</td>
<td>Thin filament-free end</td>
<td>&lt;1</td>
<td>41,000 (1)</td>
</tr>
<tr>
<td>Titin1</td>
<td>Longitudinal sarcomeric filaments (Z- to M-line)</td>
<td>10</td>
<td>3,700,000 (1)</td>
</tr>
<tr>
<td>Nebulin2</td>
<td>Parallels (part of) thin filaments to the Z-line</td>
<td>3</td>
<td>773,000 (1)</td>
</tr>
<tr>
<td>α-Actinin</td>
<td>Z-line (integral)</td>
<td>2</td>
<td>204,000 (2)</td>
</tr>
<tr>
<td>Cap Z</td>
<td>Z-line (integral)</td>
<td>&lt;1</td>
<td>66,000 (2)</td>
</tr>
<tr>
<td>Desmin1</td>
<td>Intermediate filaments at Z-line (peripheral)</td>
<td>&lt;1</td>
<td>212,000 (4)</td>
</tr>
<tr>
<td>Paranemin1</td>
<td>Intermediate filaments at Z-line (peripheral)</td>
<td>&lt;1</td>
<td>356,000 (2)</td>
</tr>
<tr>
<td>Synemin1</td>
<td>Intermediate filaments at Z-line (peripheral)</td>
<td>&lt;1</td>
<td>372,000 (2)</td>
</tr>
<tr>
<td>Filamin2</td>
<td>Z-line (peripheral)</td>
<td>&lt;1</td>
<td>560,000 (2)</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>Costamere-Membrane</td>
<td>&lt;1</td>
<td>854,000 (2)</td>
</tr>
<tr>
<td>Talin</td>
<td>Costamere-Membrane</td>
<td>&lt;1</td>
<td>536,000 (2)</td>
</tr>
<tr>
<td>Vinculin</td>
<td>Costamere-Membrane</td>
<td>&lt;1</td>
<td>116,000 (1)</td>
</tr>
</tbody>
</table>

1Adapted from Robson (1995).
2Proteins emphasized herein.

long axis of the sarcomeres, myofibrils, and muscle cell. These two proteins, therefore, should contribute significantly to maintaining the longitudinal continuity and integrity of muscle cells. Both proteins undergo proteolysis early postmortem and are excellent substrates for the calpain propeptolytic system.

Filamin is generally considered as playing an important cytoskeletal cross-linking role in cells, but much more information is needed to understand its role in postmortem muscle (Table 6). Two recent reports indicate that filamin undergoes postmortem proteolysis (Uytterhaegen et al., 1994; Huff-Lonergan et al., 1996a), but not as early postmortem as do nebulin and titin (Huff-Lonergan et al., 1996a).

The major protein composing the IFs of adult skeletal muscle cells is desmin (Table 7). The IFs in skeletal muscle cells primarily run perpendicular to the long axis of the myofibrils and skeletal muscle cell (Robson et al., 1981; Robson, 1995). As a result, the IFs are believed to play a major role in maintaining overall muscle cell integrity (Table 8). In contrast to the titin and nebulin components, postmortem degradation of the IFs would be expected to result in loss of integrity perpendicular to the long axis of muscle cells (Table 8). Two proteins, paranemin and synemin, which heretofore were considered to be IF-associated proteins (for review, see Bellin et al., 1997), have recently been shown in our lab to actually be IF proteins (Table 9). Whereas both proteins are present in all types (skeletal, cardiac, and smooth) of developing and mature muscle cells, paranemin is down-regulated and present in only very small amounts in mature skeletal muscle cells, and synemin is down-regulated and present in only very small amounts in mature cardiac muscle cells. It is likely that these two proteins form heteropolymeric IFs with desmin in mature skeletal muscle cells, with paranemin present in a very low molar ratio, and synemin present in a low molar ratio, to desmin. Both paranemin and synemin may play an important cytoskeletal role in linking desmin IFs to other structures in muscle cells (Table 10).

One of the proteins that has been studied extensively, both with regard to its role in regulation of contraction/relaxation of muscle (for review of its characteristics and role in muscle, see Farah and Reinach, 1995) and in postmortem proteolysis (Ho et al., 1994; Huff-Lonergan et al., 1995,
4. That part of titin located within the sarcomeric A-band is evidently bound firmly to the outside of the thick filament, and is relatively inelastic.

5. The cDNA sequence of titin is known (Labeit and Kolmerer, 1995a). Much of the part of titin located between the end of a thick filament and the Z-line is associated with the thin filaments (Trombitas et al., 1997), but, overall, titin is not as firmly attached to thin filaments as it is to the thick filaments.

6. Purified titin is degraded to T2 (b-connectin) by calpain (Kimura et al., 1992). Titin in purified bovine myofibrils also is degraded by calpain (Zeece et al., 1986; Huff-Lonergan et al., 1996a; Suzuki et al., 1996). Interestingly, muscle-specific calpain (p94) binds to at least two sites (N-terminal region and near C-terminus) on the titin molecule (Kinbara et al., 1997).

7. Several titin isoforms varying in size have been identified (Wang et al., 1991; Labeit and Kolmerer, 1995a), and have been correlated of titin near the Z-line is associated with the thin filaments (Trombitas et al., 1997), but, overall, titin is not as firmly attached to thin filaments as it is to the thick filaments.

8. Titin interacts in vitro with myosin (rod part, not the S1 heads), C-protein, AMP deaminase, M-line proteins (myomesin and M-protein), actin, and the integral Z-line protein, a-actinin (Trinick, 1994; Keller, 1995; Labeit et al., 1997; Maruyama, 1997; Ohtsuka et al., 1997).

Adapted from Robson et al. (1991) and Robson (1995).

TABLE 3. Possible Roles and Postmortem Changes of Muscle Titin.

Possible Roles of Titin

1. In developing muscle, titin is one of the first sarcomeric proteins detected during myogenesis, and may play a role as part of a morphogenetic scaffolding during sarcomeric organization (Handel et al., 1989; Fulton and Isaacs, 1991; van der Loop et al., 1996; Maruyama, 1997).

2. In mature muscle, titin forms a third filament system within the myofibrils that provides sarcomeric alignment (e.g., keeps myosin thick filaments in register, regulates the length of the myosin thick filaments; Bennett and Gautel, 1996), and helps maintain overall structural integrity of the sarcomeres, myofibrils and muscle cells (for reviews, see Labeit et al., 1997; Maruyama, 1997). Titin appears to act as a molecular blueprint or ruler for the layout of much of the sarcomere, even including width and structure of the Z-lines (Gautel et al., 1996).

3. A part of the titin molecule/filament in the I-band and, thus, not associated with the A-band (thick filaments), appears to provide an elastic element of the sarcomeres, accounts for the “gap filaments” observed in over-stretched bovine skeletal muscle many years ago by Dr. Ron Locker and associates (1975), and appears to be involved in the generation of resting (passive) tension (Wang et al., 1993; Labeit and Kolmerer, 1995a; Labeit et al., 1997; Maruyama, 1997).

Postmortem Changes in Titin

1. It is reasonable to expect that titin plays a very significant role in determining degree of overall integrity, or strength, of myofibrils, muscle cells and muscle tissue. Titin comprises longitudinal structures in the myofibril. It is the only protein that is present throughout the entire length of the sarcomere, and, for that matter, throughout the entire length of the very long myofibrils.

2. Titin is one of the most proteolytically-susceptible myofibrillar proteins, and undergoes significant degradation during postmortem aging of bovine muscle (Lusby et al., 1983; Bandman and Zdania, 1988; Fritz and Greaser, 1991; Huff-Lonergan et al., 1995, 1996a,b; Taylor et al., 1995). The rate of degradation of T1 (intact titin) is related to tenderness measurements, with significant degradation occurring in tender beef samples by one day postmortem, and nearly complete degradation occurring by three days postmortem. In tougher beef samples, rate of postmortem degradation of T1 is slower, but significant degradation occurs between one and three days postmortem (Huff-Lonergan et al., 1996a).

3. The rate of postmortem degradation of titin (T1) in muscle from Bos indicus crossbred cattle (less tender meat) is slower (Ho et al., 1997) than the rate in muscle (relatively tender meat) from Bos taurus cattle (Ho et al., 1996).

4. The rate of postmortem degradation of T1 in muscle from Callipyge lambs, which produce tougher meat, is slower than occurs in muscle samples from normal lambs (Koohmaraie et al., 1995).

5. The titin in purified bovine myofibrils is an excellent substrate for the calpains using postmortem-like conditions (Huff-Lonergan et al., 1996a). The resulting major proteolytic fragments of titin appear similar to those observed during postmortem aging of bovine muscle.
4. Complete primary structure studies (Labeit and Kolmerer, 1995b) of human nebulin revealed 185 repeating copies of ~35-residue modules, which
3. There is a positive correlation between the size of nebulin isoforms and the length of thin filaments from different species and from different
6. Electron microscope studies of postmortem bovine muscle (e.g., Taylor et al., 1995; Ho et al., 1996, 1997) suggest that one of the more
7. Overall, the degree of titin degradation appears to parallel measurements of meat tenderness.
5. Results of electron microscope studies of postmortem bovine muscle (e.g., Taylor et al., 1995; Ho et al., 1996, 1997) suggest that one of the more
1. Nebulin is an insoluble, very high molecular weight myofibrillar protein (M, = 6 to 9 x 10^5) present in skeletal (not cardiac or smooth) muscle cells
2. Nebdin may help link or anchor the thin filament
4. Nebulin may participate in active contraction of skeletal muscle, and help
3. Nebdin in purified bovine myofibrils is an excellent substrate for the calpains using postmortem-like conditions (Huff-Lonergan et al., 1996a).
1. Nebulin is one of the most proteolytically-susceptible myofibrillar proteins, and undergoes significant degradation during postmortem aging of
2. Nebulin is one of the more pronounced ultrastructural changes is the occurrence of breaks near the myofibrillar Z-lines, a region occupied by nebulin.
6. Overall, the degree of nebulin degradation appears to parallel measurements of meat tenderness.

Important Characteristics of Filamin
1. Filamin (also called ABP = actin-binding protein) is an ~500,000 molecular weight homodimer (Wang et al., 1975; Hartwig and Stossel, 1975). Several filamin isoforms exist, but adult skeletal and cardiac muscle filamins are extremely similar, if not identical (Price et al., 1994).
2. The C-terminal ends of the two polypeptides are linked to each other to form ~V-shaped homodimers, whereas the two N-termini form the tips of the V-shaped molecule, and contain actin-binding domains (Gorlin et al., 1990).
3. Calpain cleaves the ~250 kDa filamin subunits into ~240 and ~10 kDa fragments (Davies et al., 1978). The small cleavage fragment is from the C-termini of each of the two subunits in the homodimer. As a result, the 240 kDa fragment can bind to, but no longer crosslink, actin filaments.
4. Filamin is located at the periphery of myofibrillar Z-lines (see Price et al., 1994, and refs. therein), and probably at the costameres located along the sarcolemma that have a periodicity that matches the Z-lines of the nearby peripheral layer of myofibrils. The costameric location of filamin has not been clearly documented.

Possible Role(s) of Filamin
1. Filamin, with its homodimeric structure, can crosslink actin filaments in parallel and in orthogonal arrays (see Price et al., 1994). There is suggestive (circumstantial) evidence that filamin also may associate with intermediate filaments (see discussion in Price et al., 1994). Taken in toto, filamin appears to be a cytoskeletal cross-linking protein.
2. It seems we don’t know much about the precise role(s) of filamin in skeletal muscle cells!

Postmortem Changes in Filamin
1. Filamin in skeletal muscle samples, which have been injected with CaCl₂ to stimulate proteolysis and tenderization, exhibits increased degradation (Uytterhaegen et al., 1994).
2. Filamin is degraded at different rates in myofibrils isolated from naturally-aged beef muscles having different shear force values, with some filamin degraded by three days postmortem in tender beef samples, but not until about seven to fourteen days in tougher beef samples. Thus, filamin is degraded more slowly postmortem than are nebulin and titin (Huff-Lonergan et al., 1996a). Filamin degradation may be involved in tenderization, via disruption of key cytoskeletal linkages, and/or is at least an indicator of postmortem proteolysis.
3. Filamin in purified bovine myofibrils is a substrate for the calpains using postmortem-like conditions (Huff-Lonergan et al., 1996a). The resulting major proteolytic fragment of filamin is similar to that observed during postmortem aging of bovine muscle.
4. Much more must be learned regarding filamin’s properties (e.g., location, interacting proteins, etc.) and function in skeletal muscle in order for us to address in detail the significance of postmortem changes in filamin.

TABLE 7. Some Important Characteristics of Muscle Desmin.

1. Desmin is one of the major types/isomers of protein comprising 10-nm diameter intermediate filaments (IFs) that, in turn, are part of the cytoskeleton of nearly all animal cells (Robson, 1989, 1995).
2. Desmin is a fairly insoluble cytoskeletal protein (Mr of subunit = 53,000) present in skeletal, cardiac and most smooth muscle cells of vertebrates. A tetramer (Mr = 212,000) of desmin comprises the protofilament building block of the IFs (Ip et al., 1985). Desmin is easily degraded by several proteases, including calpain (O’Shea et al., 1979).
3. Desmin was successfully purified from mammalian skeletal muscle in our lab (O’Shea et al., 1979, 1981). The purified protein has the ability, via several structural intermediates, starting with the tetrameric protofilament building block, to self-assemble into synthetic, 10-nm diameter, very long (>1-2 mm) filaments (Ip et al., 1985). IFs are much more dynamic structures than was originally believed (e.g., their assembly/disassembly is mediated by covalent modifications; Robson, 1989; Huang et al., 1993; Zhou et al., 1996, Graves et al., 1997; Inagaki et al., 1997), but our understanding of the control of their assembly/disassembly properties is still very incomplete.
4. Immunoelectron microscope localization studies in our lab indicated that desmin IFs encircle the Z-line and radiate out perpendicular to the myofibril axis to ensnare and connect adjacent myofibrils (Richardson et al., 1981). The desmin IFs also link myofibrillar Z-lines to subcellular organelles, such as nuclei and mitochondria, and the Z-lines of the peripheral layer of cellular myofibrils to the cell membrane skeleton (Robson, 1995).

*Adapted from Robson et al. (1991) and Robson (1995).*
TABLE 8. Possible Roles and Postmortem Changes of Muscle Desmin.

<table>
<thead>
<tr>
<th>Possible Roles of Desmin</th>
<th>Postmortem Changes in Desmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Desmin IFs may help align and tie together adjacent myofibrils in developing muscle cells, but this remains unclear. Studies involving isometric force measurements of single myotubes, with truncated desmin expression, indicate a critical role for desmin IFs in providing mechanical stability for force development (Feng et al., 1994).</td>
<td>1. Desmin is degraded in postmortem muscle (Robson et al., 1980, 1981; Koohmaraie et al., 1984a,b; Hwan and Bandman, 1989; Taylor et al., 1995; Ho et al., 1996, 1997; Huff-Lonergan et al., 1996a). The rate of degradation of desmin, however, is slower than that of nebulin and titin (Huff-Lonergan et al., 1996a; Ho et al., 1996).</td>
</tr>
<tr>
<td>2. Desmin IFs contribute to resting tension in adult muscle cells, but only at unusually long sarcomere lengths (&gt;4.5mm) (Wang et al., 1993).</td>
<td>2. The rate of postmortem degradation of desmin in bovine muscle (Robson et al., 1980, 1981; Koohmaraie et al., 1984a,b; Hwan and Bandman, 1989; Taylor et al., 1995; Ho et al., 1996, 1997; Huff-Lonergan et al., 1996a). The rate of degradation of desmin, however, is slower than that of nebulin and titin (Huff-Lonergan et al., 1996a; Ho et al., 1996).</td>
</tr>
<tr>
<td>3. In the developed muscle cell, desmin IFs are believed to play an important cytoskeletal role in connecting the myofibrils and, in turn, tie or anchor the myofibrils to subcellular organelles and the cell membrane, i.e., desmin IFs may play a significant role in maintaining overall integrity and organization of the skeletal muscle cell (Robson, 1995). In some muscles (especially those heavily used such as the diaphragm), desmin &quot;knockout&quot; mice exhibit severe disruption of muscle cellular organization (Milner et al., 1996), which also indicates a major role for desmin in maintaining integrity of the muscle cell cytoskeleton.</td>
<td>3. The rate of postmortem degradation of desmin in bovine muscle is related to tenderness measurements, with some degradation occurring in tender samples by three days postmortem, but not until about seven to fourteen days in tougher beef samples (Huff-Lonergan et al., 1996a).</td>
</tr>
<tr>
<td>4. Both purified porcine skeletal muscle desmin (O'Shea et al., 1979) and the desmin in purified bovine myofibrils (Huff-Lonergan et al., 1996a) are good substrates for the calpains.</td>
<td>4. Both purified porcine skeletal muscle desmin (O'Shea et al., 1979) and the desmin in purified bovine myofibrils (Huff-Lonergan et al., 1996a) are good substrates for the calpains.</td>
</tr>
<tr>
<td>5. Overall, postmortem desmin degradation is consistent with increased meat tenderness.</td>
<td>5. Results of electron microscope studies of postmortem bovine muscle (e.g., Taylor et al., 1995) suggest that filamentous structures, presumably desmin IFs (a) linking adjacent myofibrils laterally at their Z-lines are degraded, resulting in gaps between myofibrils, and (b) linking the peripheral layer of myofibrils to costameres, are degraded.</td>
</tr>
<tr>
<td>6. Overall, postmortem desmin degradation is consistent with increased meat tenderness.</td>
<td>6. Overall, postmortem desmin degradation is consistent with increased meat tenderness.</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Important Characteristics of Paranemin</th>
<th>Important Characteristics of Synemin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Until recently, paranemin has generally been considered as one of the types of intermediate filament-associated proteins (IFAPs). It is generally found together with desmin and/or vimentin, a desmin homologue (Price and Lazarides, 1983).</td>
<td>1. Until recently, synemin has generally been considered as one of the types of intermediate filament-associated proteins (IFAPs). It is generally found together with desmin and/or the desmin homologue, vimentin (Price and Lazarides, 1983).</td>
</tr>
<tr>
<td>2. Paranemin has recently been cloned and completely sequenced in our lab (Hemken et al., 1996, 1997). Although the M_\text{r} \text{ of the paranemin polypeptide subunit} = 280,000 \text{ by SDS-PAGE}, the predicted M_\text{r} \text{ of the paranemin polypeptide subunit} = 178,000 \text{ from the sequence. The sequencing studies also have shown that paranemin is a novel IF protein rather than an IFAP. Paranemin likely forms heteropolymeric IFs with desmin in mature skeletal muscle cells, with paranemin present in a very low molar ratio to desmin.}</td>
<td>2. Synemin is a rather insoluble cytoskeletal protein (M_\text{r} \text{ of polypeptide subunit} = 230,000; forms a dimer of M_\text{r} = 460,000) present in skeletal, cardiac and smooth muscle cells of vertebrates. Partial primary structure studies in our lab (Becker et al., 1995) first revealed that synemin is a novel IF protein rather than an IFAP. We (Bellin et al., 1996, 1996) have now obtained synemin's complete sequence (M_\text{r} \text{ of polypeptide subunit} = 186,000 \text{ from sequence}). Synemin, by itself, will not form IFs in vitro. It more likely forms heteropolymeric IFs with desmin in which synemin is present in a low molar ratio to desmin.</td>
</tr>
<tr>
<td>3. Localization studies show that paranemin is localized with desmin/vimentin IFs in developing muscle cells, and with the desmin IFs at the Z-line (periphery) of mature striated muscle myofibrils (Hemken et al., 1997).</td>
<td>3. Immunofluorescence localization studies show that synemin is co-localized with desmin at the Z-line (periphery) of striated muscle myofibrils. Immunoelectron microscope studies in our lab indicate that synemin is attached to, or more likely is part of, the desmin IFs that link myofibrils together, and myofibrils to other cellular structures.</td>
</tr>
<tr>
<td>4. Synemin interacts in vitro with desmin, altering IF assembly and dynamics, and, under some conditions, also with the integral Z-line protein α-actinin and with the costameric protein vinculin (Bellin et al., 1996; Sernett et al., 1996, 1997a,b).</td>
<td>4. Synemin interacts in vitro with desmin, altering IF assembly and dynamics, and, under some conditions, also with the integral Z-line protein α-actinin and with the costameric protein vinculin (Bellin et al., 1996; Sernett et al., 1996, 1997a,b).</td>
</tr>
</tbody>
</table>
TABLE 10. Some Possible Roles and Postmortem Changes of Muscle Paranemin and Synemin.

**Possible Roles of Paranemin and Synemin**
1. In developing muscle cells, paranemin and synemin may play an important cytoskeletal role in linking desmin and/or vimentin IFs to other structures such as the Z-lines of assembling myofibrils.
2. In mature striated muscle cells, paranemin and synemin may play a significant cytoskeletal role in linking desmin IFs to other structures such as the myofibrillar Z-lines and discrete sites, called costameres, along the cell membrane skeleton (i.e., contribute to overall muscle cell integrity and organization) (Bellin et al., 1997; Hemken et al., 1997; Sernett et al., 1997a,b).

**Postmortem Changes in Paranemin and Synemin**
1. Our results to date suggest that paranemin and synemin will form heteropolymeric IFs with desmin and/or vimentin.
2. Both paranemin and synemin are proteolytically labile, and require use of potent protease inhibitor cocktails during their purification (Sernett et al., 1996; Bellin et al., 1997; Hemken et al., 1997). We also have found that purified synemin is an excellent substrate for the calpains. Because both paranemin and synemin are very proteolytically sensitive, it is reasonable to suggest that postmortem degradation of these newly identified IF proteins would hinder the ability of desmin IFs to link adjacent myofibrils, and the peripheral layer of cellular myofibrils to the costameric regions at the sarcolemma. Interestingly, synemin binds in vitro to vinculin (Sernett et al., 1997a,b) and dystrophin (S.W. Sernett and R.M. Robson, unpublished observations), two costameric proteins other investigators (Taylor et al., 1995) have shown are degraded during postmortem aging of bovine muscle.
3. The precise role(s) of paranemin and synemin in postmortem muscle will require further studies.

**Postmortem Changes in Muscle Troponin-T.**
1. Troponin-T (TN-T), first shown in the early 1970’s by Greaser and Gergely (1971) to be one of the three TN subunits, is a well characterized component of the tropomyosin-troponin regulatory complexes located along the length of the thin filaments (for review, see Farah and Reinach, 1995).
2. TN-T (~37 kDa by SDS-PAGE) is extensively degraded postmortem, coincident with appearance of polypeptides at ~30 kDa (MacBride and Parrish, 1977; Olson and Parrish, 1977; Penny and Dransfield, 1979). Ho et al. (1994) have recently shown unambiguously, by use of Western blotting, that the ~30 kDa polypeptides in aged bovine muscle are products of TN-T degradation.
3. The rate of degradation of TN-T is related to tenderness measurements, with significant degradation occurring in more tender beef samples by three days postmortem. In tougher beef samples, rate of TN-T degradation is slower, but significant degradation occurs by seven days postmortem (Huff-Lonergan et al., 1996a).
4. The rate of postmortem degradation of TN-T in muscle from Callipyge lambs, which produce tougher meat, is slower than occurs in muscle samples from normal lambs (Koohmaraie et al., 1995).
5. The TN-T in purified bovine myofibrils is a substrate for the calpains using postmortem-like conditions (Huff-Lonergan et al., 1996a). The resulting major proteolytic fragments of TN-T appear similar to those observed during postmortem aging of bovine muscle.
6. Whether degradation of TN-T may just be an indicator of postmortem proteolysis, and/or specifically contribute to the increase in tenderness postmortem, remains unclear (see discussions in Ho et al., 1994; Huff-Lonergan et al., 1995, 1996a).

**TABLE 12. Summary of Postmortem Changes in the Myofibrillar and Other Cytoskeletal Proteins in Muscle.**
1. The major myofibrillar/structural proteins, myosin and actin, are not proteolytically degraded in conventionally-aged meat.
2. Postmortem proteolysis of key myofibrillar (titin, nebulin, TN-T) and other cytoskeletal (dystrophin, filamin, vinculin, desmin) proteins (and probably ones not yet examined) is a significant factor contributing to meat tenderness.
3. We must have a reasonable understanding of properties and role(s) of a protein in muscle before we can easily address the importance/impact of postmortem changes in that protein to meat tenderness.
4. Exactly which protein(s) being degraded postmortem is most important in tenderness is not known, and it may not be important that we know. It is more likely that it is the overall proteolysis of several cytoskeletal proteins that is important.
5. It is unlikely that all of, or even much of, a given protein undergoing postmortem proteolysis must be degraded to impact tenderness (i.e., a small number of proteolytic cleavages/breaks within the cytoskeleton may result in a large, disproportionate increase in tenderness).
6. The proteins undergoing proteolysis are directly attached to (e.g., titin, nebulin), closely associated with (filamin, desmin, paranemin, synemin, some TN-Ts), or near (dystrophin, vinculin) the myofibrillar Z-lines.
7. One of the more likely and useful results of knowing which proteins are degraded, as well as the identity of their degraded products, is that someone will use this information as an indicator of proteolysis and devise a simple, practical test/ assay for predicting meat tenderness.
8. Postmortem changes, other than proteolysis, have not been addressed in this presentation (e.g., protein denaturation effects due to changes in pH and ionic strength).
9. Rich Robson’s major professor, Darrel E. Goll, was correct when he told him as a young graduate student in 1964 that the biochemical changes in postmortem muscle are complex!
1996a), is the TN-T subunit of troponin (Table 11). However, it remains unclear as to whether degradation of TN-T is simply an indicator of postmortem proteolysis, and/or may play a direct role in the increase in tenderness during postmortem aging. Two costameric proteins listed in Table 1, namely dystrophin and vinculin, also have been shown to undergo postmortem proteolysis (see Taylor et al., 1995, and references therein), but have not been described in detail in this review. The other costameric protein listed in Table 1, talin, has not yet been examined in postmortem muscle, but is proteolytically labile (Zhang et al., 1996).

Conclusions

A summary of the postmortem changes in the myofibrillar and other cytoskeletal proteins in muscle that have been reviewed in this paper is presented in Table 12. Although a great deal of progress has been made in understanding the properties and roles of proteins and structures that undergo postmortem proteolysis, we obviously have a lot of work yet to do. Although there is a growing consensus among many scientists that postmortem proteolysis, and especially that resulting from the calpain system, is the major mechanism underlying postmortem tenderization (Koohmaraie, 1992, 1994, 1996; Uytterhagen et al., 1994), other provocative views do exist (Goll et al., 1997).

Acknowledgments

We are very grateful to Lynn Newbold for typing the manuscript. R. M. Robson also thanks Rob Bellin, Suzy Sernett and Elisabeth Lonergan for lots of assistance in preparation of the oral presentation of this paper. The work described in this review and that came from our lab was supported in part by grants from the USDA-NRICGP (Award 96-35206-3744), American Heart Association, Iowa Affiliate, Muscular Dystrophy Association and Beef Industry Council of the National Live Stock and Meat Board. Journal Paper Number J-17491 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011, Projects 3349, 3444 and 2127, and supported by Hatch Act and State of Iowa Funds.

References


