Identification and Localization of Two Triad Junctional Foot Protein Isoforms in Mature Avian Fast Twitch Skeletal Muscle*

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We report evidence for two foot protein isoforms in chicken pectoral muscle. (i) Two polypeptides with molecular masses of ~500 kDa copurify with [3H]ryanodine binding. (ii) Both polypeptides are associated with oligomeric proteins similar in size to the mammalian skeletal muscle foot protein. (iii) The polypeptides are shown to be unique by limited proteolysis. (iv) By using isoform-specific antibodies, the polypeptides are shown to be subunits of different [3H]ryanodine-binding proteins.

Using immunolabeling techniques, we have localized these proteins in chicken breast muscle by both light and electron microscopy. (v) From immunofluorescent light microscopy of longitudinal sections, it was determined that both ryanodine-binding protein isoforms exhibit identical repetitive punctate distributions near the Z-lines. (vi) In serial cross-sections both proteins have similar distributions in the same fibers. (vii) Both proteins were found to be associated with the terminal cisternae of the sarcomplasmic reticulum by immunoelectron microscopy.

Based on their localization to the triadic junction, their large size and their ability to bind [3H]ryanodine, these proteins are identified as foot proteins. In conclusion, two distinct homo-oligomeric foot proteins coexist in avian fast twitch skeletal muscle. We have termed these proteins, α and β foot proteins.

The triad junction, between the sarcoplasmic reticulum and transverse tubule membranes in skeletal muscle cells, is the site where surface membrane depolarization is transduced to release calcium from the sarcoplasmic reticulum and cause muscle contraction (1). The foot protein, a key triad junctional component, is an integral sarcoplasmic reticulum membrane protein that interacts with the transverse tubule membrane in an as yet undefined manner (2, 3). The foot protein is generally believed to participate in transducing the depolarization signal (1) and embodies an ion channel that may provide the pathway for sarcoplasmic reticulum calcium release (4–6). In mammalian fast twitch skeletal muscle, a single foot protein has been identified and purified based on its ability to bind [3H]ryanodine (4, 7, 8). This protein is a homotetramer comprised of a 565-kDa subunit. Recently, cDNA encoding the rabbit skeletal muscle protein monomer has been cloned, sequenced, and functionally expressed (9–11).

The contributions made by the foot protein to coupling electrical excitation to contraction, and to the formation and structural integrity of the triad junctional structure, are unknown. To gain a different perspective on the role of the foot protein in muscle structure and function, we have initiated investigations of the expression of the foot protein and of the identity and nature of the processes involved in the formation of the triad junction during embryonic skeletal muscle development. We have characterized the foot proteins in mature avian fast twitch skeletal muscle to acquire the basic information necessary for these investigations. In this report, we describe biochemical and immunocytochemical evidence for the existence of two foot protein isoforms in mature chicken fast twitch skeletal muscle that co-localize to triad junctions in the same muscle fibers. These results have been presented in abstract (12).

EXPERIMENTAL PROCEDURES

Materials—T-61 euthanasia solution was purchased from American Hoechst Corp. (Somerville, NJ); leupeptin, PMSF, aprotinin, benzamidine, iodoacetamide, pepstatin A, diisopropyl fluorophosphate, CHAPS, L-α-phosphatidylcholine, DEAE-Sepharose, polyethylene glycol, agarose-linked goat anti-mouse IgG, CHES, 2-[N-cyclohexylamino]-1-propanesulfonate; D111, dihydrotestosterone; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CHES, 2-[N-cyclohexylamino]-1-propanesulfonic acid.

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The abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; D111, dihydrotestosterone; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CHES, 2-[N-cyclohexylamino]-1-propanesulfonic acid.

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microsomal membranes were isolated essentially as described by Saito et al. (13). Pectoral muscle was homogenized in 5 ml/gm wet tissue weight of solution A (0.3 M sucrose, 10 mM imidazole, pH 7.4, 1.1 µM leupeptin, and 230 µM PMSF) three times for 1 min at high speed in a Waring Blender. The pH was adjusted to 7.4 between each step using 1 N NaOH (14). The homogenate was centrifuged at 8,000 × g for 14 min, the supernatant discarded, and the pellet rehomogenized and centrifuged as above. Microsomal membrane pellets were prepared from the second supernatant by centrifugation at 130,000 × g for 90 min, resuspended in solution A at a protein concentration of 15–25 mg/ml, rapidly frozen in liquid N₂, and stored at −90 °C. Terminal cisternae membranes comparable with the R₁ fraction described by Saito et al. (13) prepared by sedimentation through a discontinuous sucrose gradient as described by these authors were used for planar lipid bilayer experiments. Membrane protein was measured by the method of Lowry (15) using bovine serum albumin as a standard.

Solubilization and Partial Purification of Ryanodine-binding Proteins—Microsomal membrane proteins were solubilized using the differential solubilization approach described initially by Caswell et al. (16) and by Costello et al. (17) and Beeler. Prior to solubilization, microsomal membranes were diluted 1:1 in solution B (0.3 M sucrose, 10 mM imidazole, pH 7.4, 5 mM DTT, 1.1 µM leupeptin, 230 µM PMSF) and centrifuged at 130,000 × g for 60 min. The pellet was resuspended in solution B containing 10 mM CaCl₂ at a protein concentration of 10 mg/ml and incubated for 15 min at 0 °C. This suspension was diluted 1:1 with solution B containing 10 mM CaCl₂ and 1% CHAPS. The mixture was incubated at 0 °C, centrifuged at 10,000 × g for 30 min. The resulting pellet was resuspended in solution C (0.5 M KCl, 20 mM Tris, pH 7.4, 5 mM DTT, 1.1 µM leupeptin, 230 µM PMSF) at a concentration of 5 mg membrane protein/ml (assuming the recovery of 25% of the starting membrane protein). This suspension was diluted with 10% CHAPS and centrifuged at 130,000 × g for 60 min. The supernatant by centrifugation at 130,000 × g for 90 min, resuspended in solution C (0.5 M KCl, 20 mM Tris, pH 7.4, 5 mM DTT, 1.1 µM leupeptin, 230 µM PMSF) and centrifuged at 130,000 × g for 60 min. The pellet was resuspended in solution C containing 10 mM CaCl₂ and 5% phosphatidylcholine in solution C to yield final protein, CHAPS and phospholipid concentrations of 3 mg/ml, 4 and 2%, respectively (7), and incubated at 0–4 °C for 30 min with periodic mixing. Solubilized proteins were separated from nonsolubilized material by centrifugation at 100,000 × g for 30 min. The solubilize was layered onto continuous 10–30% sucrose gradients (cf. Ref. 4) and centrifuged at 135,000 × g for 14-16 h. Gradient fractions containing the foot proteins were identified using [³H]ryanodine binding and SDS-PAGE analyses as described below. Solubilized proteins were quantitated using the method of Kaplan and Pedersen (18) using bovine serum albumin as a standard. The gradient fractions containing the foot proteins were either used immediately or rapidly frozen in liquid N₂, and stored at −90 °C until use.

For the experiments shown in Fig. 9, the α and β ryanodine-binding protein isoforms were separated from one another by deleting one of the isoforms by immunoprecipitation with an antibody specific for that isoform. The immunoprecipitation protocol is described below. 

DEAE-Sepharose Chromatography—A DEAE-Sepharose column (1 × 4 cm) was equilibrated with 0.1 M NaCl and 20 mM Tris, pH 7.4, 5 mM DTT, 1.1 µM leupeptin, and 230 µM PMSF. Sucrose gradient fractions containing [³H]ryanodine binding activity were diluted 1:1 with distilled water and loaded onto the column. The column was then washed with 10 column volumes of the sample buffer and eluted with a linear 0.5–1.0 M gradient of a KCl solution, which also contained 1% CHAPS, 0.5% phosphatidylcholine, 230 µM PMSF, and 1.1 µM leupeptin, at 0.5 ml/min. Fractions (7.5 ml) were collected and analyzed for the presence of foot proteins by SDS-PAGE.

Polyacrylamide Gel Electrophoresis and Western Blot Analyses—Samples from the sucrose gradient fractions were prepared for SDS-PAGE (19) in a 4–20% linear polyacrylamide gradient SDS resolving minigels and a constant voltage of 130 V applied at 0–4 °C. The separated polypeptides were visualized by staining with 0.2% Comassie Brilliant Blue.

The electrophoretic transfer of the resolved proteins onto nitrocellulose was achieved using a Hoeffer Transphor TE 50 at 200 V for 18 h in 100 ml of 0.25 M Tris, pH 8.3, 10% methanol, and 10% acetic acid. The gel slices used in this study were visualized using either goat anti-rabbit or goat anti-mouse secondary antibodies conjugated to alkaline phosphatase followed by 5-bromo-4-chloro-3-indolyl phosphate/NBT. The positive signals were visualized using a Polaroid camera.

The results were consistent with the finding of epiryanodine binding 50%, indicating that epiryanodine has an approximately 4-fold lower binding affinity than ryanodine.

* T. J. Beeler, personal communication.

Fig. 1. Competitive binding between [³H]ryanodine (100 nM) and the indicated concentrations of ryanodine. A ryanodine concentration of 22 nM reduced [³H]ryanodine binding by 50%, indicating that epiryanodine has an approximately 4-fold lower binding affinity than ryanodine.
part of the total protein present in the precipitate and could not be measured directly in an accurate manner.

When \(^{[\text{H}]}\)ryanodine or \(^{[\text{H}]}\)pipyranodine binding was measured after immunoprecipitation, the contents of individual binding assay tubes were added to antibody beads that had been pre-equilibrated with the appropriate primary antibody and incubated for 3 h at room temperature. The supernatants were saved and the beads were washed three times in solution D. The precipitated material was resuspended in 0.3 ml of solution D, added directly to scintillation vials, and counted. Protein bound radioactivity remaining in the supernatants was determined by filtration as described above. The immunoprecipitated proteins were visualized by SDS-PAGE in parallel experiments, in which an equivalent amount of unlabelled ryanodine was substituted for the radioligand.

Limited Proteolysis of Avian Muscle Foot Proteins—Limited proteolysis of the foot proteins was achieved using two different protocols. First, the foot proteins were solubilized, separated by precipitation with polypeptide-specific antibodies, and then proteolyzed at room temperature. Second, solubilized foot protein isoforms, either as mixtures or individually, were proteolyzed at room temperature and the resulting proteolytic fragments immunoprecipitated as described above. The peptide fragments resulting from both protocols were separated by SDS-PAGE and visualized by staining the gel with Coomassie Brilliant Blue and as densitometric scans of the stained gels using a Model GS 300 Scanning Densitometer (Hoeffer Scientific Instruments, San Francisco, CA).

Light and Electron Immunomicroscopy—Three-day-old chicks were fixed by vascular perfusion with 4% paraformaldehyde in 0.1 M phosphate-buffered saline, and breast muscle was removed and placed in the same solution for 2 h at 4°C. Tissue was cryoprotected in 2.3 M sucrose (30) and rapidly frozen in liquid propane cooled to −185°C. For immunofluorescence, 1-μm-thick sections were cut on a Reichert-Jung PC-45 ultracryomicrotome (Cambridge Instruments) and immunostained as described previously (31). Photomicrographs were obtained on a Zeiss Universal microscope using a Nikon UFX camera and Kodak T-Max film. For immunoelectron microscopy, identically prepared tissue was cut into 100-nm-thick sections on the Reichert-Jung FC-45 ultracryomicrotome at −100°C and prepared as described previously (32). Electron micrographs were obtained with a JEOL 100CX electron microscope at 100 Kev.

RESULTS

Avian and Mammalian Fast Twitch Skeletal Muscles Contain Distinct High Molecular Mass Proteins—Three initial observations indicated that ryanodine-binding proteins in avian and mammalian skeletal muscle were different. First, the application of solubilization (4, 5) and purification (4) protocols designed to purify skeletal muscle foot proteins resulted in the co-purification of two high molecular mass polypeptides and [\(^{[\text{H}]}\)ryanodine binding activity from chicken pectoral muscle (Figs. 2 and 3A). Under similar conditions, only a single high molecular mass polypeptide is obtained from mammalian muscle (Refs. 4 and 5 and Fig. 3A). As shown in Fig. 2B, the differential detergent extraction/sucrose gradient sedimentation procedure resulted in a significant purification of avian skeletal muscle high molecular mass foot proteins. From comparison with solubilized rabbit skeletal muscle foot protein separated in parallel on identical gradients, the chicken muscle proteins appear to have native and denatured subunit molecular masses of ~2000 and ~500 kDa, respectively. Overloading a SDS gel with sample revealed minor contaminating polypeptides of 200, ~160, ~100, and 45 kDa. The 200- and 45-kDa polypeptides are likely to be myosin and actin. The material apparent between 60 and 80 kDa is due to the CHAPS/phospholipid and is observed in the absence of any added muscle protein. Using this procedure, the solubilized [\(^{[\text{H}]}\)ryanodine binding activity in the sucrose gradient fraction shown in Fig. 2, lane 6, was enriched by 78-82-fold (270–285 pmol/mg protein) over that present in the sarcoplasmic reticulum membranes (3.45 pmol/mg protein).

Second, the chicken muscle high molecular mass protein polypeptides have lower molecular masses than the rabbit muscle protein subunit (Fig. 3A), which has been identified as ~565 kDa based on cDNA sequence analysis (9). We have termed the higher and lower molecular mass polypeptides from chicken muscle, α and β, respectively. Third, the polypeptides from avian and mammalian skeletal muscle exhibit limited immunologic cross-reactivity. Affinity-purified polyclonal sera against both chicken muscle polypeptides reacts weakly with rabbit muscle protein (Fig. 3B), and polyclonal sera against rat muscle foot protein reacts with only the α polypeptide from avian muscle (Fig. 3C). In both cases Western blots had to be developed beyond when the primary antigen was clearly visible to demonstrate that the rabbit protein was recognized by anti-chicken foot protein sera (B) or that the chicken muscle α polypeptide was identified by the anti-rat foot protein sera (C).

**Fig. 3.** Two avian muscle high molecular mass polypeptides copurify with [\(^{[\text{H}]}\)ryanodine binding and differ from the mammalian muscle protein in molecular mass and immunological cross-reactivity. A, partially purified chicken and rabbit skeletal muscle [\(^{[\text{H}]}\)ryanodine-binding proteins were separated on 4–20% continuous gradient SDS-PAGE gels and stained for protein with Coomassie Brilliant Blue. B, Western blot probed with rabbit anti-chicken foot protein sera affinity-purified against both avian skeletal muscle polypeptides. C, Western blot probed with rabbit anti-rat skeletal muscle foot protein sera. In both B and C, the blots had to be developed beyond when the primary antigen was clearly visible to demonstrate that the rabbit protein was recognized by anti-chicken foot protein sera (B) or that the chicken muscle α polypeptide was identified by the anti-rat foot protein sera (C).
2, yielded fractions containing different amounts of the α and β polypeptides (Figs. 4B). This differential enrichment of these polypeptides could be due to either the purification of individual polypeptide subunits or a selective enrichment of large oligomeric proteins differing in subunit composition. To decide whether individual polypeptides or large oligomeric proteins were present, aliquots from selected DEAE column fractions were centrifuged in individual sucrose gradients. In each case the two polypeptides were found to be associated with large proteins that sedimented to the same gradient fractions as the freshly solubilized ryanodine-binding protein (compare Fig. 4, A and C). These results suggested that large proteins comprised of different ratios of the two polypeptide subunits were present in the DEAE column fractions.

Avian Pectoral Muscle Contains Two Distinct Homo-oligomeric High Molecular Mass Proteins—The preceding results could indicate the existence of either hetero-oligomeric high molecular mass proteins composed of variable ratios of the two subunits or of two distinct homo-oligomeric proteins containing different subunits. To decide between these possibilities, monoclonal antibodies specific for each polypeptide were used to precipitate proteins containing each polypeptide. The antigen specificity of the monoclonal antibodies used in these studies was determined for both denatured and native antigen by Western blotting (Fig. 5) and immunoprecipitation (Fig. 6A), respectively. Western blots of pectoral muscle microsomal membranes probed with anti-α polypeptide and anti-β polypeptide antibodies are shown in Fig. 5, B and C, respectively.

If hetero-oligomeric isoforms are present, then antibodies specific for either polypeptide should precipitate proteins containing both polypeptides. On the other hand, if two homo-oligomeric isoforms exist, then the antibodies should precipitate proteins containing only the polypeptide recognized by that antibody. As shown in Fig. 6A, polypeptide-specific antibodies precipitated proteins containing only a single polypeptide, indicating the existence of two homo-oligomeric foot protein isoforms.

Both Avian Muscle High Molecular Mass Proteins Bind [3H]Ryanodine—Two variations of the immunoprecipitation protocol were used to determine whether both high molecular mass proteins bound [3H]ryanodine. The proteins were solubilized and either labeled with [3H]ryanodine and precipitated with polypeptide-specific antibodies or immunoprecipitated and then allowed to bind [3H]ryanodine. As described under “Experimental Procedures” and shown in Fig. 6A, the conditions used for immunoprecipitation resulted in precipitation of equivalent amounts of both isoform. The proteins precipitated by antibodies specific for either polypeptide bound [3H]ryanodine in a specific manner (Fig. 6B) to such an extent that the observed binding could not be attributed to contamination of the precipitate by the alternative isoform (Fig. 6A, lanes 3 and 4). These data indicate the existence of two distinct high molecular mass ryanodine-binding proteins, comprised of either α or β polypeptide subunits.

The α and β Polypeptides Are Unique—Several pieces of circumstantial evidence argued that the α and β polypeptides are unique and that β does not result from either in vitro or in vivo proteolysis of α. First, the relative abundance of the two polypeptides did not vary between microsomal membrane preparations. Similar ratios of α and β proteins were consistently observed when sarcoplasmic reticulum membranes were isolated in the absence or presence of the following combinations of protease inhibitors: leupeptin + PMSF (7), leupeptin +...
The differences observed in the respective maps are clearest in the region of the gels above 100 kDa as can be appreciated from comparison of the densitometric scans of lanes 4 and 5. The two high molecular mass polypeptides do not result from proteolysis. The α and β ryanodine-binding proteins were solubilized, precipitated with polypeptide-specific antibodies under conditions that allowed precipitation of equivalent amounts of each isoform (cf. lanes 2 and 3), and then proteolyzed with trypsin (1.0 μg/ml) at room temperature for the times indicated. The proteolytic products were separated in a 4-20% polyacrylamide gradient gel and visualized by Coomassie Brilliant Blue staining. Densitometric comparisons of the polypeptides resolved in lanes 2-5 are presented in the lower part of this figure. The arrows indicate the portion of the scanned. The molecular mass standards are as identified in Fig. 2. Mab, monoclonal antibody.

Two different experimental protocols utilizing limited proteolysis were employed to demonstrate further that the two polypeptides are unique. α or β polypeptides were either immunoprecipitated and then proteolyzed, or solubilized proteins were proteolyzed and the resulting fragments precipitated with polypeptide-specific antibodies. Both approaches yielded different peptide maps for each polypeptide. The results obtained with the first protocol are shown in Fig. 7. The differences observed in the respective maps are clearest in the region of the gels above 100 kDa as can be appreciated from comparison of the densitometric scans of lanes 4 and 5 shown in the lower portion of Fig. 7. Polypeptides at 25, 55, 80 and 105 kDa are immunoglobulin light and heavy chains and were observed in the absence of antigen.

In the second approach, the α and β polypeptides were proteolyzed both as a mixture (Fig. 9A) and individually (Fig. 9, B-D). The resulting peptides were immunoprecipitated in the former case with isoform-specific antibodies (cf. Fig. 5) and in the latter with an antibody that recognized an epitope common to both isoforms (Fig. 8). Again, comparison of the resulting immuno-maps shows a lack of similarity between the proteolytic fragments resulting from the trypsination of the two polypeptides. Comparable differences were obtained using S. aureus V8 protease and the calcium-activated neutral protease (data not shown).

The α and β polypeptides can also be differentiated by the greater post-solubilization lability of the α polypeptide (cf. Fig. 7, compare lanes 2 and 3, and Fig. 9A, compare lanes 9–12) and by the greater susceptibility of this polypeptide to proteolysis by trypsin (Fig. 7B, compare lanes 1–4). The Avian Muscle Ryanodine-binding Proteins Are Present in the Same Muscle Cells—The biochemical identification of two ryanodine-binding protein isoforms in avian skeletal muscle raised questions concerning whether they are phenotype-specific isoforms expressed in different muscle fiber types or if both isoforms co-exist in the same fiber.

The muscle fiber distribution of the α and β ryanodine-binding proteins was compared using immunofluorescent detection of isoform-specific monoclonal antibodies on 1-μm thick serial cryosections of chick breast muscle. The immunofluorescent localization of the α and β isoforms in longitudinal sections appeared as discrete points equidistant along the length of the fibers in close proximity to the Z-lines (Fig. 10, A and B). This was confirmed by co-labeling muscle sections with an antibody against the Z-line protein, α-actinin (data not shown). The staining pattern of the α and β isoforms in serial sections appear identical (Fig. 10). In serial cross-sections both the α (Fig. 10C) and β (Fig. 10D) isoforms have

**Fig. 7.** The two high molecular mass polypeptides do not result from proteolysis. α and β ryanodine-binding proteins were solubilized, precipitated with polypeptide-specific antibodies under conditions that allowed precipitation of equivalent amounts of each isoform (cf. lanes 2 and 3), and then proteolyzed with trypsin (1.0 μg/ml) at room temperature for the times indicated. The proteolytic products were separated in a 4-20% polyacrylamide gradient gel and visualized by Coomassie Brilliant Blue staining. Densitometric comparisons of the polypeptides resolved in lanes 2-5 are presented in the lower part of this figure. The arrows indicate the portion of the scanned. The molecular mass standards are as identified in Fig. 2. Mab, monoclonal antibody.
the same interfiber distribution, indicating that both proteins are present in the same cells.

**Both Avian Muscle Ryanodine-binding Protein Isoforms Localize to the Triad Junction**—The Z-line localized immunofluorescent staining observed at the light microscopic level was consistent with localization of both isoforms to the triad junction. This question was examined further by more precisely localizing the isoforms with immunogold labeled secondary antibodies and α and β protein-specific primary antibodies in ultrathin cryosections of chick breast muscle at the electron microscopic level. These studies yielded two major observations. First, both the α (Fig. 11A) and β (Fig. 11B) isoforms are localized to the terminal cisternae membrane of the sarcoplasmic reticulum at the triad junction. Second, both isoforms were detected in virtually every triad junction observed in serial sections of the same fiber suggesting that both proteins co-exist in the same junctions. A similar intracellular localization has been observed for the mammalian fast twitch skeletal muscle foot protein (33).

**DISCUSSION**

The results of the present studies suggest that avian and mammalian fast twitch skeletal muscle fibers differ in that the former contain two ryanodine-binding protein isoforms. The large size of these proteins, their ability to bind [3H]ryanodine, and their localization to the triad junction support the conclusion that the avian muscle proteins are foot protein isoforms. The molecular similarities between these proteins have not yet been determined. It is not known whether the isoforms are the products of separate genes or represent either post-transcriptional or post-translational modifications of a single gene product. The differences in subunit molecular mass and peptide maps and the identification of isoform-specific antibodies suggest that the avian muscle α and β foot proteins differ in both their primary and secondary structures.

The existence of multiple foot protein isoforms in avian muscle raises several questions concerning the phylogenetic pattern of expression of single or multiple foot proteins and the functional contributions made by the different protein isotypes. Both birds and mammals arose from reptilian ancestors (34). Therefore, whether the expression of multiple foot protein isoforms is unique to avian fast twitch skeletal muscle or a trait shared with muscles from other nonmammalian vertebrates is relevant to considerations of the functional significance of the multiple foot protein isoforms. In preliminary studies we have observed multiple high molecular polypeptides that are immunologically related to the avian skeletal muscle foot protein subunits in microsomes prepared from both frog and fish muscles.3 We are currently determining the

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3E. Olivares, S. J. Tanksley, J. A. Airey, C. F. Beck, T. Deerinck, M. H. Ellisman, and J. L. Sutko, submitted for publication.
tissue and cellular distributions of the frog and fish muscle proteins. The identification of multiple foot protein isoforms in fish muscle could provide a biochemical correlate to the morphological evidence for two foot protein types reported by Block et al. (35). In these studies a tetrameric transverse tubule membrane protein was observed to associate with alternating foot proteins suggesting the existence of structurally distinct foot protein types. The present results suggest that mammalian fast twitch skeletal muscle may be distinguished from similar muscles in other vertebrates by the expression of a single foot protein type. If this is the case, then comparisons of the different foot protein isoforms may provide insights into whether the functional characteristics of these proteins are such that expression of a single foot protein isotype provided a selective advantage during the evolution of mammalian striated muscle.

Whether the presence of single or multiple foot proteins confers advantages for specific types of muscle function can only be determined once the molecular and functional characteristics of the various foot protein isotypes have been established. We are currently investigating the \(^{3}H\)ryanodine binding abilities and calcium channel characteristics of the avian muscle proteins. In other studies, we have established that the avian muscle foot protein isoforms are expressed at different times during the embryonic development of the pectoral muscle. The differential expression of these proteins during muscle development and their continued expression in mature muscle strongly suggests that the two isoforms make different functional contributions to the muscle cell.

In conclusion, we have presented evidence for the existence of two foot protein isoforms in chicken pectoral muscle. Both isoforms are found in the same muscle cell and both are localized to the triad junction. The functional significance of these proteins is currently being investigated.

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