Cap Z(36/32), a Barbed End Actin-capping Protein, Is a Component of the Z-Line of Skeletal Muscle

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Abstract. Various biological activities have been attributed to actin-capping proteins based on their in vitro effects on actin filaments. However, there is little direct evidence for their in vivo activities. In this paper, we show that Cap Z(36/32), a barbed end, actin-capping protein isolated from muscle (Casella, J. F., D. J. Maack, and S. Lin, 1986, J. Biol. Chem., 261:10915-10921) is localized to the barbed ends of actin filaments by electron microscopy and to the Z-line of chicken skeletal muscle by indirect immunofluorescence and electron microscopy. Since actin filaments associate with the Z-line at their barbed ends, these findings suggest that Cap Z(36/32) may play a role in regulating length, orienting, or attaching actin filaments to Z-discs.

Materials and Methods

Production and Affinity Purification of the Anti-Cap Z(36/32) Antibodies

Antisera against the M, 32,000 (beta) subunit of Cap Z(36/32), was prepared as previously described (3). Affinity purification of the antibody was carried out essentially as described by Davis and Bennett (6). Pooled immune sera was diluted with an equal volume of 0.15 M NaCl, 10 mM sodium phosphate, 1 mM NaEDTA, 1 mM NaN3, 0.2% (vol/vol) Triton X-100 and heated to 60°C for 20 min in the presence of 200 µg/ml phenylmethylsulfonyl fluoride (PMSF) to minimize protease activity. All subsequent procedures were carried out at 4°C unless otherwise specified. 15 ml of the diluted antisera was then passed over a 1.5-ml Cap Z(36/32)-agarose column (purified by a previously described procedure [3], and dialyzed into 500 mM KCl, 10 mM potassium phosphate, pH 8 before coupling). The column was then washed serially with 20 ml of 0.5 M NaCl, 10 mM sodium phosphate, 1 mM NaN3, 0.2% Triton X-100, pH 7.5, 10 ml of 2 M urea, 0.1 M glycine, 1% Triton X-100, and then 20 mM Na acetate, pH 5, until the absorbance at 280 nm was 0. The bound antibody was then eluted using 1 M acetic acid.
Acid and collected in 1-ml fractions containing one-tenth volume of 1 M Tris base. The antibody was then extensively dialyzed against 0.15 M NaCl, 10 mM sodium phosphate, and 1 mM NaN₃, albumin was added to 1 mg/ml, and the solution was stored at −20°C. Approximately 0.075 mg of affinity-purified antibody was recovered using this procedure. The affinity-purified antibody was later thawed and absorbed for 1 h using 1 ml of Sepharose 4B which an M-line extract had previously been coupled. The M-line extract consisted of an "M-protein" preparation, prepared as described by Trinick and Lowey (32) and modified by Eppenberger and Strehler (8), but with omission of the adenosine 5'-monophosphate-Sepharose 4B affinity-chromatography step. M-protein prepared in this way is heavily contaminated with phosphorylase b. Approximately 5 mg of the M-line extract was used per milliliter of Sepharose 4B in the coupling procedure.

Immunoblots were performed as previously described (3). Samples of muscle for electrophoresis were prepared from small pieces of pectoralis muscle, which were frozen in liquid nitrogen immediately after slaughter. The muscle strips were then homogenized in a solution containing 30% sucrose, 2 mM EGTA, 0.4 mM diisopropyl fluorophosphate, 0.5 mM PMSF, 5 lag/ml leupeptin, 5 µg/ml pepstatin A, and 5 µg/ml aprotinin at 0°C for 1 rain using a Polytron homogenizer (Brinkmann Instruments, Inc., Palo Alto, CA) at 100,000 rpm for 5 min. The pellets were then recovered and subjected to electrophoresis on 12% SDS polyacrylamide separating gels with 5% stacking gels (19). The gels were then acrylamide and collected in 1-ml fractions containing one-tenth volume of 1 M Tris base. The antibody was then extensively dialyzed against 0.15 M NaCl, 10 mM sodium phosphate, and 1 mM NaN₃, albumin was added to 1 mg/ml, and the solution was stored at −20°C. Approximately 0.075 mg of affinity-purified antibody was recovered using this procedure. The affinity-purified antibody was later thawed and absorbed for 1 h using 1 ml of Sepharose 4B which an M-line extract had previously been coupled. The M-line extract consisted of an "M-protein" preparation, prepared as described by Trinick and Lowey (32) and modified by Eppenberger and Strehler (8), but with omission of the adenosine 5'-monophosphate-Sepharose 4B affinity-chromatography step. M-protein prepared in this way is heavily contaminated with phosphorylase b. Approximately 5 mg of the M-line extract was used per milliliter of Sepharose 4B in the coupling procedure.

**Immunoprecipitation of ¹²⁵I-Cap Z₃₆/₃₂ Using Antibodies against the Beta-Subunit of Cap Z₃₆/₃₂**

Approximately 40 µg of Cap Z₃₆/₃₂ in 10% sucrose, 50 mM KCl, 0.01% NaN₃, 10 mM K₂HPO₄, pH 8.0 in a volume of 0.315 ml was exposed to dry Bolton-Hunter reagent containing 1 nCi of ¹²⁵I for 90-100 min at 0°C. The reaction was then quenched with 10 µl of 0.1 M glycine and diluted 1:3 (vol/vol) with a solution containing 1.33 mg/ml gelatin, 20% sucrose, 100 mM KCl, 0.01% NaN₃, 10 mM Na₂HPO₄, pH 8.0, and dialyzed against the same solution without gelatin overnight with two exchanges of buffer. Approximately 41% of the radioactivity of the labeling reagent was incorporated into protein as judged by TCA precipitation of the labeled material, yielding an average labeling of 0.3 mol of ¹²⁵I per mole of Cap Z₃₆/₃₂.

Either 0.5 µg of affinity-purified anti-Cap Z₃₆/₃₂ IgG or the same amount of preimmune IgG was then incubated with 0.1 µg of ¹²⁵I-Cap Z₃₆/₃₂ in the presence of 0.75 mg/ml albumin in PBS for 2 h at 24°C. Pansorbin (Pharmacia Fine Chemicals, Piscataway, NJ), previously equilibrated with 110 µg/ml unlabeled Cap Z₃₆/₃₂, was then added to the solution (IgG binding capacity 2 µg). This mixture (total vol 50 µl) was then incubated for 10 min, layered over 125 µl of 20% sucrose, and centrifuged in a Beckman Airfuge (Beckman Instruments, Inc., Palo Alto, CA) at 100,000 rpm for 5 min. The pellets were then recovered and subjected to electrophoresis on 12% SDS polyacrylamide separating gels with 5% stacking gels (19). The gels were then dried, and an autoradiogram was prepared (see Fig. 1B).

**Localization of Cap Z₃₆/₃₂ on Actin Filaments**

Glutaraldehyde-fixed, subfragment-1 of myosin (S₁)–decorated actin nuclei were prepared as previously described (3, 33) with the modification that 0.6 µM Cap Z₃₆/₃₂ was added to the actin filaments (2.3 µM, in a 0.5-ml vol) before shearing with a 25-gauge needle (40 passes). After a 15-min rest period, the filaments were decorated with S₁ and fixed with 0.1% glutaraldehyde for 4 min. The fixation was then quenched with 500 mM ethanolamine as described. The nuclei/Cap Z₃₆/₃₂ mixture was then gel-filtered on a 0.9 × 6 cm Sepharose 2B column equilibrated with 2 mM MgCl₂, 100 mM KCl, 0.2 mM CaCl₂, 0.5 mM 2-mercaptoethanol, 0.2% NaN₃, 5 mM Tris, pH 8.0 (nuclei suspension buffer). Peak fractions containing the nuclei and depleted of unbound cap were collected and used for the following experiments.

**1. Abbreviation used in this paper:** S₁, subfragment-1 of myosin.

**Figure 1.** Characterization of the affinity-purified anti-Cap Z₃₆/₃₂ antibodies. (A) Specificity of the affinity-purified antibodies was demonstrated by examining immunoblots of whole muscle (lane 1), myofibrils (lane 2), and purified Cap Z₃₆/₃₂ (lane 3). Care was taken to prevent proteolysis of proteins in whole muscle and myofibrillar samples as described in Materials and Methods. Immune replicas of the Coomassie Blue–stained gel (left) were obtained after overnight incubation of the nitrocellulose-bound proteins in 0.3 µg/ml anti-Cap Z₃₆/₃₂ (middle) or preimmune IgG (right) at 4°C, followed by a 2-h incubation with ¹²⁵I-protein A (2 × 10⁶ cpm/ml). Autoradiograms were developed for 2 h at −70°C using Cronex (Dupont) intensifier screens. (B) ¹²⁵I-labeled Cap Z₃₆/₃₂ was immunoprecipitated using affinity-purified anti–Cap Z₃₆/₃₂ as described in Materials and Methods. Autoradiograms of gels of supernatants (S) and immunoprecipitates (P) are shown for immune and preimmune samples.
Figure 2. Immunolocalization of Cap Z_{36(32)} on S1-decorated actin filaments. Examples of protein A–colloidal gold labeling of the barbed ends of actin filaments exposed to Cap Z_{36(32)} and affinity-purified anti–Cap Z_{36(32)} antibodies as described in Materials and Methods are shown at representative low (top) and high (bottom) power views. Bar, 0.2 μm.

bound Cap Z_{36(32)} were then diluted to ~65 μg protein/ml, and 10 μl of this solution was incubated with 6 μl of protein A–coated 10-nm colloidal gold particles (a kind gift of Dr. Douglas Murphy) and 10 μl of 15 μg/ml affinity-purified anti–Cap Z_{36(32)} or nonimmune IgG at the same concentration. 5 μl of this solution was then injected into 2 μl of the nuclei suspension buffer described above on a glow-discharged, carbon-coated, 400-mesh copper electron microscopy grid. After 1 min, the grid was washed and stained by touching the grid to three successive drops of the nuclei suspension buffer, three drops of distilled water, and three drops of 0.1% uranyl acetate. Excess stain was removed by blotting the edge of the grid on filter paper and gentle suctioning of the edge of the grid using a finely drawn pasteur pipet. The grids were then photographed at 5000×, 60 kV in a Zeiss EM 10 A microscope. Negatives were then examined in a microfiche reader (at 22×; Atlantic Microfilm Corp., Spring Valley, NY) to determine the direction of S1 labeling and the percentage of filaments labeled. The photographs of actin filaments shown in Fig. 2 were taken at 20,000×, 60 kV.

**Immunofluorescence Microscopy of Myofibrils**

Glycerinated chicken muscle was prepared as described by Knight and Trinick (18). Myofibrils were prepared from this muscle in the presence of protease inhibitors (0.4 mM diisopropyl fluorophosphate, 0.5 mM PMSF, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 μg/ml pepstatin A, and 5 mM EDTA) as previously described (3). The rest of the procedure was carried out at 24°C. 10-μl vol of a fresh concentrated myofibrillar suspension was allowed to settle on glass slides for 2 min. The slides were then rinsed with PBS
and fixed for 5 min in 0.1% glutaraldehyde in PBS. The fixation reaction was then quenched with two 5-min washes with 0.1% sodium borohydride in PBS. The slides were then subjected to two 10-min washes with PBS and exposed to the first antibody (10 μl of 15 μg/ml affinity-purified anti-Cap Z(36/32) antibody, nonimmune IgG, rabbit anti-keyhole limpet hemocyanin antibody, or affinity-purified anti-Cap Z(36/32) plus excess anti-Cap Z(36/32) for 30 min. After repeat PBS washes, the slides were incubated with the FITC-conjugated anti-rabbit goat IgG (Cappel Laboratories, Cochranville, PA) at a 1:100 dilution for 30 min, followed by washing with PBS. The specimens were then mounted in 0.1 M NaCl, 50 mM Tris-HCl, 0.2% NaN₃, containing 10% glycerol and 1 mg/ml paraphenyldiamine (final pH 9.0) to reduce quenching of the fluorescence.

**Tissue Preparation for Immunolabeling**

Tissue was prepared for both immunofluorescence and immunoelectron microscopy by the following method. 9-wk-old chickens were decapitated and a thin (~5-mm wide by 1-mm thick) strip of pectoralis muscle was removed from the surface of the muscle with a razor blade, making sure that the origin and insertion remained attached. Working quickly, the strip of muscle was tied firmly to a flat wooden splint with dental floss. The muscle was removed and submerged in 100 ml of 0.5% glutaraldehyde in PBS plus 10 mM EGTA, pH 7.4 on ice. After 20 min, the splint was removed, and the muscle was cut into 5-mm squares and left in fixative on ice for 40 min. Afterwards, the pieces were trimmed to 1-mm square and set in a fresh 100 ml of 0.5% glutaraldehyde in PBS at 0°C for 2 h. The muscle pieces were then infiltrated with 2.3 M sucrose in deionized water by rotating them at 4°C for 1 h in the sucrose solution. After infiltration, the muscle was oriented transversely or longitudinally, placed on copper pegs, and immersed in liquid nitrogen for storage and later ultracryomicrotomy.

Cryostat sections, 0.8-0.5-μm thick, were made on an LKB ultratome U with an attached cryo kit. Glass knives were broken with a LKB knife-breaker using the Griffth setting, and then tungsten-coated (26). Sections for light microscopy were cut at ~60°C and were >0.5-μm thick. Sections were mounted on slides that were previously coated with a solution of 0.5% gelatin and 0.05% chromium alum and allowed to dry. 0.5-μm sections were picked up with a drop of 2.3 M sucrose suspended on a copper wire loop. The frozen sucrose drop with section was allowed to thaw and then was touched to the prepared slides. The slides were placed in PBS for 5 min, and then quenched in 0.05% sodium borohydride in PBS three times, for 5 min each wash. The slides were then washed in PBS for 2 min and incubated in 5% BSA for 5 min. Immunostaining of the tissue sections for light microscopy was carried out as described above for myofibrils.

For electron microscopy, sections were cut to 80 nm at ~90°C. The sections were picked up and placed on paratobrian- and carbon-coated 200-mesh copper grids. Grids with sections were floated on the solutions as described by Tokuyasu et al. (31). The grids were first floated on 5% BSA for 5 rain. Staining of Sections with Heavy Metals

1 ml of 0.25 M sucrose was saturated with uranyl formate (93%). The sections were stained for 5 min on a drop of uranyl formate (pH 7.0) for 5 min, and then on uranyl formate (pH 6.0) for 5 min. Grids were then embedded in methylcellulose as described by Tokuyasu (31).

**Results**

**Specificity of the Anti-Cap Z(36/32) Antibodies**

Affinity-purified antibodies prepared as described in Materials and Methods recognized only the beta (M, 32,000) subunit of Cap Z(36/32) in immunoblots of myofibrils and whole muscle (Fig. 1 A). As in previous studies in which antibodies were affinity-purified on immunoblots (3), no crossreactivity between the alpha- and beta-subunits of Cap Z(36/32) was seen. Absorption of the antibodies against crude M-line extracts was necessary, however, since even antibodies prepared using Cap Z(36/32) affinity columns showed weak reactivity with M-line components in immunoblots and immunofluorescence assays. Whether this M-line reactivity is a result of true crossreactivity between Cap Z(36/32) and other M-line proteins or the presence of contaminating antibodies has not been determined. We were unable to obtain antibodies against the alpha-band of Cap Z(36/32) suitable for immunofluorescence studies. The blot purified antibodies against only the beta-subunit of Cap Z(36/32) used in previous studies immunoprecipitated equal amounts of the alpha- and beta-subunits of radiolabeled native protein (Fig. 1 B), confirming previous physical data that indicate that they are associated in solution (3).

**Localization of Cap Z(36/32) to the Barbed End of Actin Filaments**

Cap Z(36/32) was localized to the barbed end of cross-linked, SI-decorated actin—Cap Z(36/32) complexes using the affinity-purified antibodies and protein A—coated colloidal gold particles (Fig. 2). 17% of the filaments were labeled solely at the barbed end. The ratio of labeled barbed ends to labeled pointed ends was greater than 100:1. This finding corroborates previous biochemical data indicating that Cap Z(36/32) binds selectively to the barbed end of actin filaments and has the advantage of allowing evaluation of the association of the protein with the sides of actin filaments as well. The quantitative analysis of the labeling in this experiment (Table I) shows that slightly more gold particles were seen in association with the sides of actin filaments in samples containing anti-Cap Z(36/32) than in those containing preimmune antibodies. Although this difference is statistically significant (P < 0.01), the number of side-labeled filaments is small; the actual significance of this finding is unclear.

**Immunolocalization of Cap Z(36/32) in Myofibrils and Frozen Sections of Muscle**

Isolated myofibrils stained with the antibodies against the beta-subunit of Cap Z(36/32) showed fluorescence in the Z-line of myofibrils with no other staining above background (Fig. 3, A–D). The specificity of this reaction was established by the absence of Z-line staining when preimmune antibodies or an irrelevant IgG (rabbit anti—keyhole limpet hemocyanin) was substituted for the affinity-purified antibodies, and by obliteration of the immunofluorescent staining by preincubation of the affinity-purified antibodies with purified Cap Z(36/32). This pattern of localization was also observed in unfixed myofibrils and in myofibrils fixed with either 0.1%

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**Table I. Immunolocalization of Cap Z(36/32) to the Barbed End of Actin Filaments**

| Site of label | Barbed | Pointed | Side | None | Total filaments |
|--------------|--------|---------|------|------|----------------|
| Immune       | 116    | 1       | 19   | 533  | 668            |
|              | (17.4%)| (0.15%) | (2.84%)| (79.8%)|
| Preimmune    | 1      | 2       | 10   | 698  | 711            |
|              | (0.14%)| (0.28%) | (1.4%)| (98.2%)|

SI-decorated filament—Cap Z(36/32) complexes were exposed to affinity-purified anti-Cap Z(36/32) antibodies and labeled with protein A—coated colloidal gold as described in Materials and Methods. Results are expressed as the number and percent (in parentheses) of filaments showing gold particles on their sides and on barbed and pointed ends.
Figure 3. Immunolocalization of Cap Z\textsubscript{36/32} in sections of whole muscle and in myofibrils. Fluorescent (A and B) and phase (C and D) images of myofibrils exposed to affinity-purified anti-Cap Z\textsubscript{36/32} (A and C) and preimmune (B and D) antibodies. Fluorescent (E) and phase (G) images of a 0.5-μm frozen section of chicken pectoralis muscle exposed to affinity-purified anti-Cap Z\textsubscript{36/32} antibodies are compared with fluorescent (F) and phase (H) images of a similar section exposed to preimmune antibodies. Bar: (A–D) 3 μm; (E–H) 20 μm.
Figure 5. Immunolocalization of Cap Z(36/32) in frozen sections of muscle by electron microscopy (5-nm gold particles). Sections of skeletal muscle prepared identically to those shown in Fig. 4 were labeled with 5-nm gold particles, as compared with the 15-nm particles shown in the previous figure. Note the higher density of labeling of the Z-line. Bar, 0.3 μm.

gluteraldehyde, 3% paraformaldehyde, or Nakane's fixative, although all of these fixatives reduced the intensity of the Z-line staining.

Longitudinal cryostat sections of whole chicken pectoralis muscle showed a pattern of staining identical to that seen in myofibrils; in addition, there was no visible staining of sarcolemma or intracellular organelles (Fig. 3, E–H). Transverse sections of muscle (data not shown) showed no other staining as well. These findings suggest that Cap Z(36/32) is a constituent of, and possibly limited to, the Z-line of skeletal muscle, the site of insertion of the barbed ends of actin filaments in the contractile apparatus.

Immunolocalization of Cap Z(36/32) in Ultrathin Frozen Sections of Muscle by Electron Microscopy

Cap Z(36/32) was also localized to the Z-line in ultrathin cryostat sections of chicken pectoralis muscle (Figs. 4 and 5). These ultrastructural studies confirm the result obtained at the level of light microscopy. In addition, the electron microscopy results suggest that Cap Z(36/32) is distributed throughout the Z-disk, in contrast to some other Z-line proteins, e.g., desmin, synemin, and spectrin, which are found only at the periphery of the Z-disk (7, 9, 10, 13). As was the case in light microscopy studies, no other staining above background was observed. However, these results do not preclude the presence of Cap Z(36/32) at lower concentrations at other sites or in structures that may be poorly preserved by the techniques used in this study.

Discussion

The studies described in this paper provide a direct demonstration that an actin-capping protein localizes specifically to the barbed ends of actin filaments in vitro and to an anatomical site associated with the barbed ends of actin filaments in situ. Previous conclusions on the site of action of actin-capping proteins have been drawn from kinetic and stoichiometric data; however, attempts to localize capping proteins on purified actin filaments using immunological techniques...
have been unsuccessful (25). The success of the experiments reported herein is most likely due to experimental procedures involving cross-linking of the antigen to its binding site before exposure of the ligand–receptor complex to washes, thereby preventing inadvertent removal of bound protein. The frequency of labeling of actin filaments in these experiments was also markedly enhanced by removal of unbound protein by gel filtration before exposure to antibodies. These techniques supplement and extend available biochemical methods of determining binding of proteins to actin, in which small amounts of protein binding to the sides of actin filaments may be difficult to detect. It will be important to determine whether proteins that appear to sever as well as cap actin filaments (e.g., gelsolin [38], villin [1, 5, 12, 23], fragmin, [14] and severin [31]) show a higher incidence of binding to the sides of actin filaments than those that appear to bind only to the ends of actin filaments (e.g., the Acanthamoeba [4, 16], Dictyostelium [28], brain [17] capping proteins, and Cap Z(36/32)).

In other studies of the localization of capping proteins in cells, gelsolin and the Acanthamoeba capping proteins have been localized to actin-rich areas of cells. Gelsolin has been immunolocalized to the area around actively phagocytic regions of the cytoplasm in leukocytes (37). Gelsolin has also been localized to actin-containing rosette structures in cells transformed by Rous sarcoma virus (34) and to the I-band of skeletal and cardiac muscle (27, 37). However, one recent study failed to localize gelsolin in the I-band of muscle and suggested that the previous results need to be re-evaluated (2). The Acanthamoeba capping protein has been localized to the actin-rich subcortical region of the amoeba (4). However, in none of these studies was the capping protein in question identified at a known site of termination of the barbed ends of actin filaments.

The present study defines Cap Z(36/32) as a new Z-line protein. Although a number of the Z-line proteins, including alpha-actinin (30), zeugmatin (20), Z-proteins (21, 24), and spectrin (22), have been implicated in anchorage of actin filaments at the Z-line by their location, only Cap Z(36/32) has been shown to bind to the barbed end of actin filaments in vitro. Alpha-actinin binds to the side of actin filaments in vitro. In fact, the molecular reason for restriction of alpha-actinin to the Z-line of intact muscle is uncertain, although evidence suggests that at 37°C tropomyosin is an effective competitor for side-binding to actin filaments (30). Nevertheless, no direct evidence exists to suggest that alpha-actinin binds directly to the ends of actin filaments. In contrast, Wilkins et al. (35) have recently reported that polyclonal antibodies to HAI, a heterogeneous group of proteins from avian smooth muscle, localized to the Z-line of striated muscle and to focal adhesive sites of nonmuscle cells. This finding is of interest in that HAI was first identified by its ability to inhibit monomer association at the barbed end of actin filaments. The same study also showed that many of the HAI polypeptides are most likely proteolytic fragments of larger (200 and 150 kD) polypeptides. As the authors indicate, however, it is not clear that the 200- and 150-kD polypeptides are responsible for the actin-capping activity of the HAI preparation, since the 200- and 150-kD polypeptides have not yet been purified; although the ability of HAI to affect the low-shear viscosity of actin was reduced after immunoprecipitation with the anti-HAI antibodies, antibodies affinity-purified against the 200- and 150-kD polypeptides did not have the same effect. Further characterization of the HAI polypeptides and their parent proteins will be required before meaningful comparisons with Cap Z(36/32) can be made. However, the possibility that the HAI polypeptides and Cap Z(36/32) represent proteins from smooth and striated muscle, respectively, with similar activities is an exciting one.

The presence of Cap Z(36/32) in the Z-line of skeletal muscle indicates that this protein is in an appropriate position to regulate the number, length, orientation, and attachment of actin filaments in muscle. Unfortunately, localization of Cap Z(36/32) with respect to the known substructure of the Z-line was not possible because antigenicity was lost in fixatives more stringent than 0.1% glutaraldehyde. Such fine structural localization of Cap Z(36/32) will be necessary to relate the localization of Cap Z(36/32) to existing models of Z-line structure, such as those proposed by Yamaguchi et al. (36), in which actin filaments of opposite polarity are tethered to one another by diagonal Z-filaments consisting primarily of alpha-actinin. One can speculate that Cap Z(36/32) might function in such a model by either (a) mediating Z-filament to actin filament connections, or (b) forming an additional site for attachment of actin filaments at their barbed ends. Further conclusions must await a systematic investigation of the interactions between Cap Z(36/32) and other Z-line constituents.

This study raises several interesting questions. For example, is Cap Z(36/32) a constituent of other sites where actin filaments terminate, such as the plasma membrane, adhesion plaques, or intercalated discs? Are there "Z-line equivalents" containing Cap Z(36/32) or other capping proteins in nonmuscle cells that allow force transduction between opposing actin filaments? Answers to these questions will require further investigation of Cap Z(36/32) at a cellular level.

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