Elastic Behavior of Connectin Filaments during Thick Filament Movement in Activated Skeletal Muscle

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Abstract. Connectin (also called titin) is a huge, striated muscle protein that binds to thick filaments and links them to the Z-disc. Using an mAb that binds to connectin in the I-band region of the molecule, we studied the behavior of connectin in both relaxed and activated skinned rabbit psoas fibers by immunoelectron microscopy. In relaxed fibers, antibody binding is visualized as two extra striations per sarcomere arranged symmetrically about the M-line. These striations move away from both the nearest Z-disc and the thick filaments when the sarcomere is stretched, confirming the elastic behavior of connectin within the I-band of relaxed sarcomeres as previously observed by several investigators. When the fiber is activated, thick filaments in sarcomeres shorter than 2.8 μm tend to move from the center to the side of the sarcomere. This translocation of thick filaments within the sarcomere is accompanied by movement of the antibody label in the same direction. In that half-sarcomere in which the thick filaments move away from the Z-disc, the spacings between the Z-disc and the antibody and between the antibody and the thick filaments both increase. Conversely, on the side of the sarcomere in which the thick filaments move nearer to the Z-line, these spacings decrease. Regardless of whether I-band spacing is varied by stretch of a relaxed sarcomere or by active sliding of thick filaments within a sarcomere of constant length, the spacings between the Z-line and the antibody and between the antibody and the thick filaments increase with I-band length identically. These results indicate that the connectin filaments remain bound to the thick filaments in active fibers, and that the elastic properties of connectin are unaltered by calcium ions and cross-bridge activity.

POSSESSING a subunit molecular weight of 2.5–3 million D (Maruyama et al., 1984; Kurzban and Wang, 1988) and comprising ~10% of myofibrillar protein (Maruyama et al., 1977; Wang et al., 1979; Trinick et al., 1984), connectin (also called titin) is an extremely large protein component of skeletal muscle. Connectin is found bound to isolated thick filaments (Trinick et al., 1984; Gassner, 1986; Hill and Weber, 1986), and in intact sarcomeres connectin extends from the thick filaments to the Z-lines (Maruyama et al., 1985; Furst et al., 1988). Previous studies using mAbs have demonstrated that the connectin molecule contains many different epitopes located in various parts of the sarcomere and arranged symmetrically about the M-line (Wang, 1985; Furst et al., 1988; Itoh et al., 1988; Whiting et al., 1989), suggesting that a single connectin molecule extends axially throughout the half-sarcomere. When relaxed muscle is stretched to various lengths, the connectin epitopes in the A-band region of the sarcomere generally behave as if they are rigidly bound to the thick filaments; in contrast, connectin epitopes in the I-band region generally move away from both the Z-line and the thick filaments when the sarcomere is stretched, as if they are part of an elastic structure (Wang et al., 1985; Furst et al., 1988; Itoh et al., 1988; Whiting et al., 1989). When connectin filaments are fragmented by ionizing radiation (Horowits et al., 1986) or enzymatic digestion (Yoshioka et al., 1986), the tension exerted by resting skinned skeletal muscle cells is greatly reduced. This indicates that significant resting tension is produced by stretching the elastic domains of connectin.

As elastic elements linking the thick filaments to the Z-lines, connectin filaments should exert a centering force on the thick filaments. Because the sliding filament model of muscle contraction predicts that the central position of the thick filaments within the sarcomere becomes unstable upon activation (Horowits et al., 1986; Horowits and Podolsky, 1987; 1988), this centering mechanism is a critical component of the contractile apparatus. We have recently demonstrated that thick filaments can move from the center to the side of the sarcomere during prolonged isometric contraction, but that they return to the center of the sarcomere upon relaxation (Horowits and Podolsky, 1987). We also showed that this movement does not occur in sarcomeres stretched to lengths where the resting stiffness originating in the connectin is high enough in theory to prevent thick filament movement during activation (Horowits and Podolsky, 1987, 1988). This formulation assumed that the properties of the elastic and inelastic domains of connectin that are observed in relaxed fibers do not change upon activation of the muscle. Al-

though this assumption is strongly supported by the dependence of thick filament movement on sarcomere length as well as by the constant tension observed during thick filament movement (Horowits and Podolsky, 1987; 1988), direct evidence concerning the disposition of connectin epitopes during activation is lacking.

In this study, we use an mAb that binds to an epitope in the elastic I-band domain of connectin to directly study the behavior of connectin during muscle activation and thick filament movement. We demonstrate that calcium activation does not change the elastic behavior of connectin, and that thick filament movement leads to stretch of connectin on one side of the sarcomere and shortening of connectin on the other side, as assumed in our previous model (Horowits and Podolsky, 1987, 1988).

Materials and Methods

mAb

An mAb against connectin was prepared by Shimizu et al. (1988) from a BALB/c mouse immunized with a crude myosin preparation. This monoclonal antibody, previously termed SM1-36-2 (Shimizu et al., 1988), or simply SM1 (Itoh et al., 1988), was kindly donated for this study by Dr. Teruo Shimizu of the University of Tokyo.

Gel Electrophoresis and Immunoblotting

Freshly excised rabbit psoas muscle was gently homogenized in 2 vol of buffer containing 10% SDS, 40 mM DTT, 10 mM EDTA, and 100 mM Tris-HCl, pH 8.0. The sample was boiled for 3 min and clarified by centrifugation at 15,000 *g* for 20 min. The supernatant proteins were separated by SDS-PAGE using a 2–12% gradient of polyacrylamide in the buffer system of Fairbanks et al. (1971). The separated proteins were electrophoretically transferred to a sheet of nitrocellulose (Towbin et al., 1979) and then exposed to the monoclonal anti-connectin. Bound antibody was detected after treatment with alkaline phosphatase-conjugated anti-mouse IgG (Cappel Laboratories, West Chester, PA).

Fiber Preparation and Immunoelectron Microscopy

Strips of rabbit psoas muscle were chemically skinned by the method of Wood et al. (1975) in a skinning solution containing 150 mM potassium propionate, 5 mM KH_2PO_4, 3 mM magnesium acetate, 5 mM EGTA, and 3 mM Na_2ATP, pH 7.0 at 4°C. Single fiber segments ~5–10 mm long were dissected and attached to T-shaped aluminum foil clips (Goldman and Simmons, 1984). The fibers were pinned near slack length, via the foil clips, to the bottom of a Petri dish coated with Sylgard (Dow Corning Corporation, Midland, MI). To ensure complete permeability of the muscle fibers to antibody, the pinned single fibers were incubated overnight in skinning solution with the addition of 0.5% Triton X-100. The detergent was then removed by three 5-min washes with skinning solution.

The detergent-treated fibers were pinned to small pieces of styrofoam for ease of manipulation during fixation, antibody treatment, and dehydration. Each piece of styrofoam was notched so that a laser beam could be passed through the pinned fibers and their sarcomere lengths were measured by laser light diffraction. Fibers to be fixed at rest were pinned at sarcomere lengths ranging from 2.1 to 3.8 μm, whereas fibers to be activated were pinned at a sarcomere length of ~2.6 μm. After pinning each fiber at the desired length, the fibers were incubated for several minutes in relaxing solution containing 100 mM potassium propionate, 3 mM EGTA, 25 mM imidazole, 7 mM $MgCl_2$, 5 mM Na_2ATP, 15 mM Na_2-creatine phosphate, and 20 U/ml creatine kinase (Sigma Chemical Co., St. Louis, MO), pH 7.0, at 4°C. Some fibers were then maximally activated for 5 min at 4°C in a solution containing 3.5 mM $CaCl_2$ added to the relaxing solution. Relaxed and activated fibers were lightly fixed for 15 min at 4°C in 0.2% glutaraldehyde added to either the relaxing or activating solution. In one case, an activated fiber was fixed using 0.1% glutaraldehyde, yielding identical results. After fixation, fibers were washed once in PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 1.47 mM KH_2PO_4, pH 7.4) at 4°C. Fibers were activated and fixed at low temperature, as detailed above, in order to be consistent with our previous studies on thick filament movement (Horowits and Podolsky, 1987; 1988). All subsequent incubations were performed at room temperature on a rotary shaker.

After further washing in PBS (three changes in 1.5 h), any free aldehyde groups were quenched by incubating the fibers for 30 min in PBS containing 20 mM $NaBH_4$. The fibers were washed in PBS (three changes in 1.5 h) and then exposed to the anti-connectin mAb in PBS overnight. Control fibers were incubated in PBS without the anti-connectin antibody. Unbound primary antibody was removed by extensive washing in PBS (four changes in 2 h). The fibers were then exposed to FITC-conjugated rat anti-mouse IgG (Boehringer Mannheim, Indianapolis, IN) diluted 1:10 with PBS. After removing unbound secondary antibody by washing in PBS (four changes in 2 h), the fibers were fixed in 2.5% glutaraldehyde in PBS for 2 h. Fibers were postfixed for 30 min with 1% osmium tetroxide, dehydrated with ethanol, and embedded in Spurr (Polysciences, Inc., Warrington, PA).

Gold or silver longitudinal sections were cut with a diamond knife edge oriented parallel to the long axis of the fibers. The sections were stained with uranyl acetate and lead citrate. They were viewed and photographed using a microscope (EM400; Philips Electronic Instruments, Mahwah, NJ). In each experiment several fibers fixed at rest were processed in parallel with activated fibers. The mean A-band width in the relaxed fibers was taken to be equal to 1.6 μm (Huxley, 1963; Page and Huxley, 1963; Trinick and Elliot, 1979; Horowits and Podolsky, 1987), and this same scale was then applied to the micrographs of activated fibers. The thin filaments in relaxed fibers were 1.14 ± 0.07 μm long (±SD; $n = 7$ fibers). This value agrees closely with the length of thin filaments in unfixed rabbit psoas myofibrils (Huxley, 1963), indicating that the relative dimensions of the A-band and I-band regions were well preserved by the chemical fixation technique used.

Results

Antibody Specificity

As shown in Fig. 1, the antibody used in this study specifically binds to connectin in an SDS extract of whole rabbit psoas muscle. The diffuse band of antibody staining immediately below connectin is probably due to a small amount of degraded connectin in the sample, which is a common result of boiling muscle extracts in SDS for several minutes (Wang, 1985). The antibody did not cross-react with nebulin, myosin, or any other rabbit psoas muscle protein. The specificity of this antibody for connectin has previously been demonstrated in skeletal muscles of the frog and chicken (Itoh et al., 1988), as well as the rabbit (Shimizu et al., 1988).

Immunolocalization of Connectin in Relaxed Sarcomeres

Previous localization studies using mAbs have demonstrated that the connectin molecule contains many different epitopes located in various parts of the sarcomere and arranged symmetrically about the M-line (Wang, 1985; Furst et al., 1988; Itoh et al., 1988; Whiting et al., 1989). In skinned rabbit psoas fibers fixed at rest, the anti-connectin mAb used here produces a single-electron dense stripe in the I-band of each half sarcomere (Fig. 2 *A*). Control fibers exposed only to the secondary antibody do not exhibit these atypical striations within the I-band (Fig. 2 *B*). The antibody stripes in Fig. 2 are ~50 nm wide, which is two to three times that found by Furst et al. (1988) using a similar double labeling technique and approximately five times the length of a single antibody molecule. This may indicate the presence of closely spaced repetitive epitopes of connectin, as previously demonstrated with the use of this antibody in frog skeletal muscle (Itoh et al., 1988).

Fig. 3 shows relaxed sarcomeres of varying lengths stained with monoclonal anti-connectin. As the sarcomere is stretched from its rest length, the spacing between the Z-line

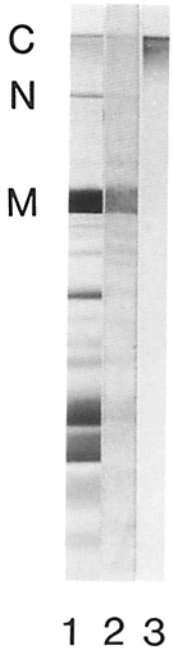

Figure 1. Immunoblot analysis of mAb binding to rabbit psoas muscle proteins. Lane *1*, Coomassie brilliant blue-stained gel pattern of whole muscle proteins. Lane *2*, amido black-stained proteins after transfer from the gel to nitrocellulose. Lane *3*, antibody-stained proteins. *C*, connectin, *M*, myosin heavy chain; *N*, nebulin. Note that the antibody is specific for connectin.

and the labeled connectin epitope increases, as does the spacing between the connectin epitope and the thick filaments. Clearly, this epitope of connectin is not rigidly attached to either the Z-line, thin filaments, or thick filaments, but is located in the elastic domain of the molecule (Wang et al., 1985; Furst et al., 1988; Itoh et al., 1988; Whiting et al., 1989), which links the thick filaments to the Z-line (Maruyama et al., 1985; Furst et al., 1988).

In sarcomeres shorter than ~3.5 μm, the antibody labeling is clear and well localized (Fig. 3, *A* and *B*), indicating a precise axial alignment of connectin filaments across the width of the sarcomere. However, in longer sarcomeres the antibody labeling becomes much more diffuse, and hence less apparent (Fig. 3 *C*). This apparent axial misalignment of connectin epitopes upon extreme stretch has been previously reported for both A-band and I-band domains of the molecule (Itoh et al., 1988), and may either reflect axial misalignment of thick filaments or a small amount of random slippage of connectin molecules from their binding sites along the thick filaments in response to excessive strain.

Immunolocalization of Connectin in Activated Sarcomeres

The sites on connectin that bind antibodies can be readily visualized in relaxed fibers, because they can be reacted with anti-connectin antibodies before fixation (Furst et al., 1988) or after only brief fixation with formaldehyde (Maruyama et al., 1985). However, immunolabeling active sarcomeres presented the problem of preserving the unique structural features associated with isometric contraction, while simultaneously preserving the ability of a particular epitope of connectin to bind antibody. We found that an initial 15-min exposure to 0.1 or 0.2% glutaraldehyde at 4°C was sufficient to preserve the structural features of interest without impairing antibody binding (see Figs. 2 and 4), provided that the

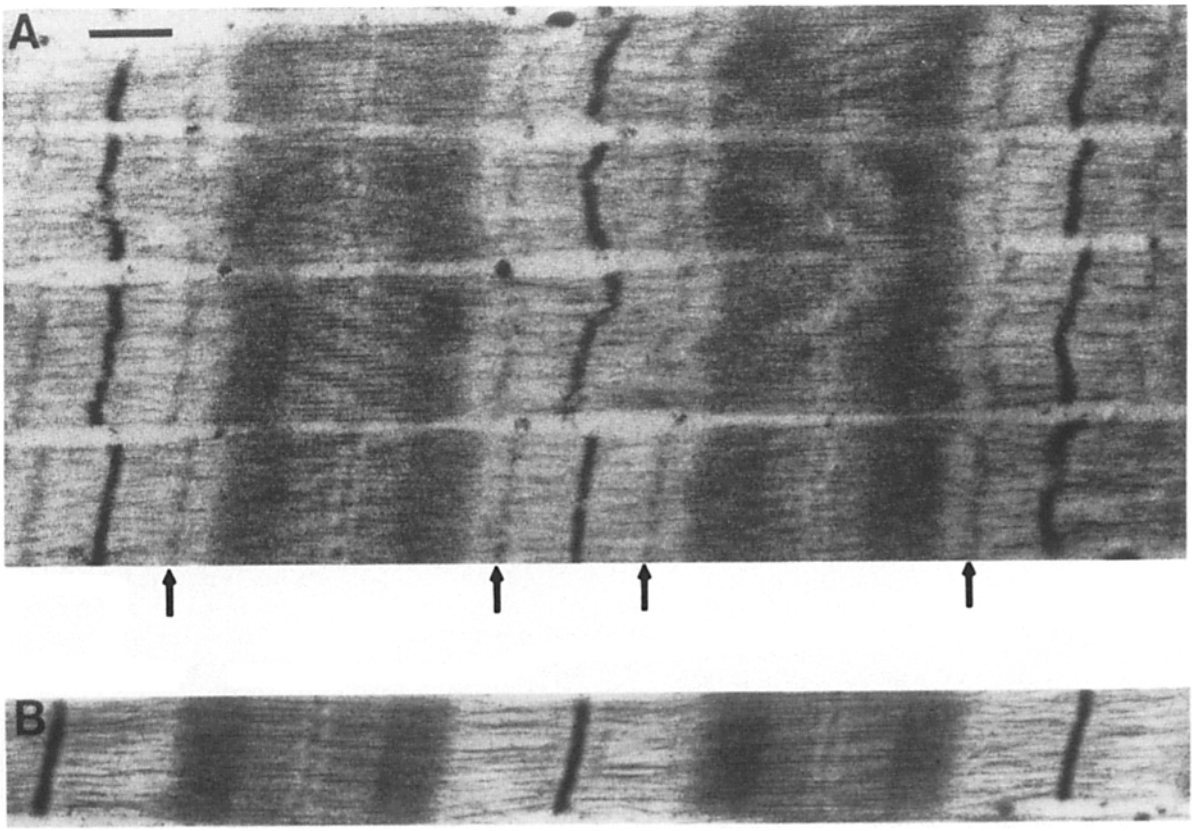

Figure 2. (*A*) Relaxed sarcomeres stained with anti-connectin. Note the presence of two antibody stripes per sarcomere (*arrows*). (*B*) Relaxed control sarcomeres not exposed to anti-connectin. Bar, 0.5 μm.

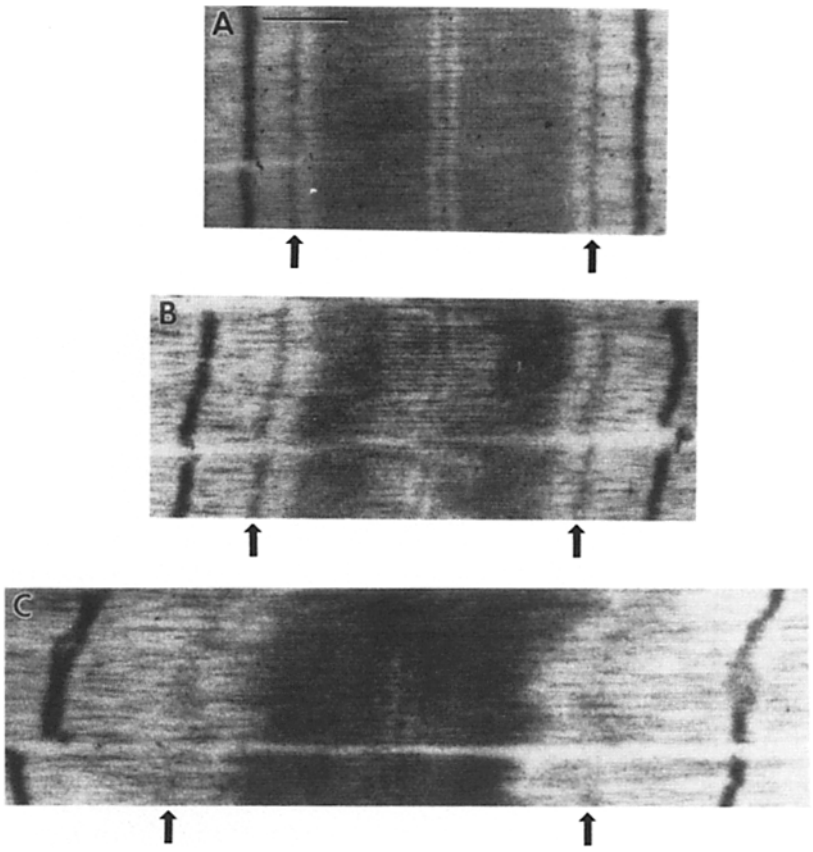

Figure 3. Relaxed sarcomeres of different lengths stained with anti-connectin. Antibody density, indicated by the arrows, is sharply defined in sarcomeres that are 2.3 (*A*) and 2.8 (*B*) μm long, but is more diffuse in the sarcomere stretched to 4.0 μm (*C*). Note that the antibody increases its distance from both the Z-line and the thick filaments as the sarcomere is stretched. Calibration bar: 0.5 μm.

muscle fiber was held isometric throughout the subsequent treatment with antibodies and final fixation. However, preliminary experiments indicated that increasing the glutaraldehyde concentration to 0.4% during the initial fixation results in greatly diminished antibody binding to connectin (data not shown).

Fig. 4 shows a field of sarcomeres fixed after prolonged activation and stained with anti-connectin antibody. As previously reported (Horowits and Podolsky, 1987), sarcomeres longer than 2.8 μm have centered thick filaments, whereas shorter sarcomeres exhibit movement of the thick filaments completely to one side of the sarcomere. The sarcomeres with centered thick filaments exhibit two anti-connectin stripes symmetrically spaced about the center of the sarcomere. This pattern is indistinguishable from that seen in relaxed sarcomeres (compare Figs. 2 and 4).

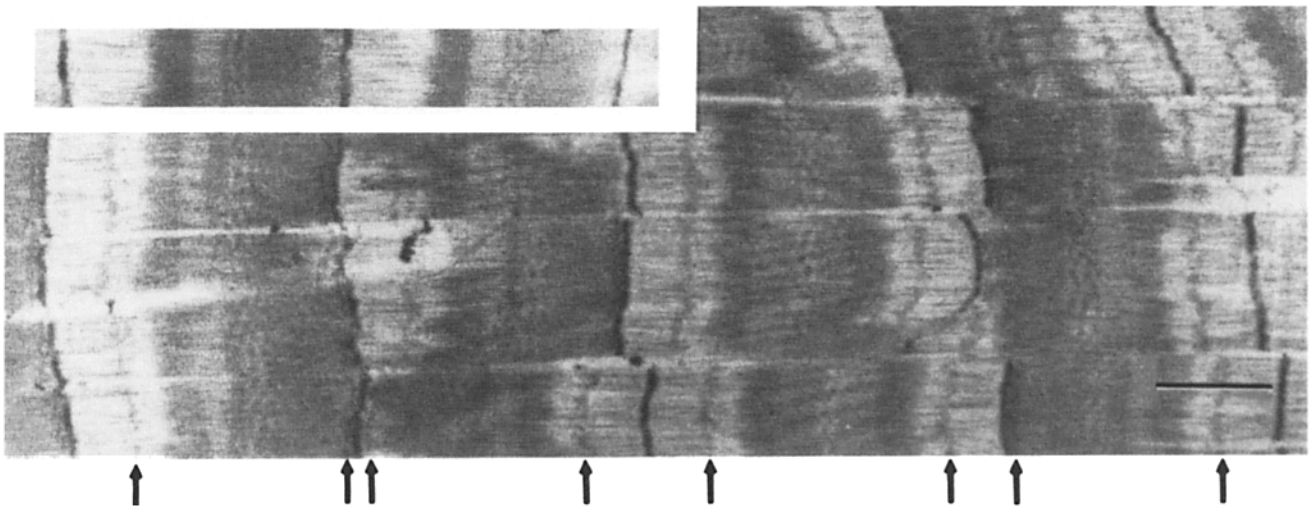

Figure 4. Active sarcomeres stained with anti-connectin. After prolonged activation, sarcomeres longer than 2.8 μm have centered thick filaments, whereas shorter sarcomeres have thick filaments adjacent to a Z-line. Note that the location of antibody staining within the sarcomere, indicated by arrows, varies with the location of the thick filaments. The inset shows two active sarcomeres with displaced thick filaments that have not been exposed to the anti-connectin antibody. Bar, 1.0 μm.

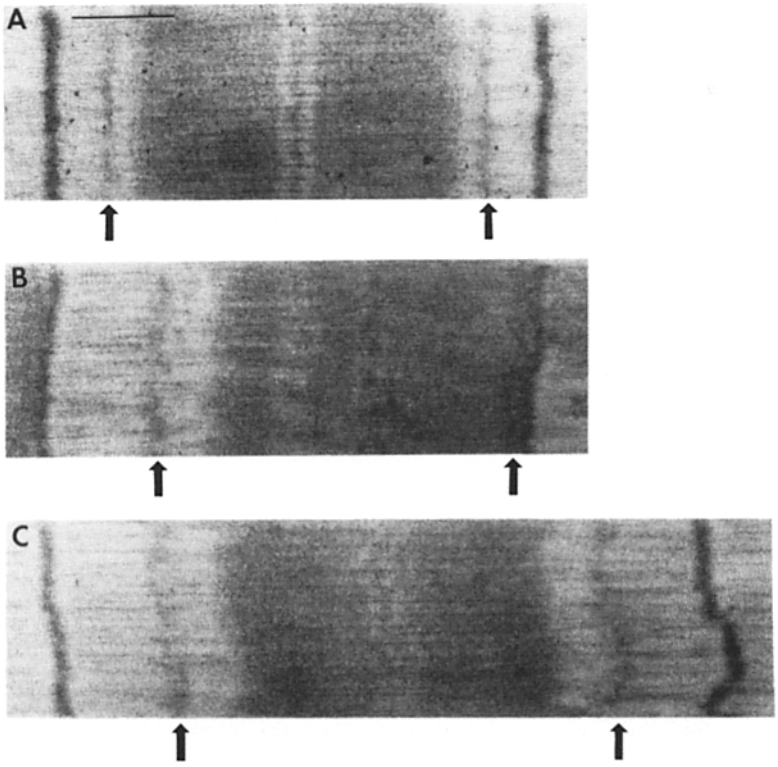

Figure 5. Relaxed (*A*) and active (*B* and *C*) sarcomeres stained with anti-connectin. Antibody density is indicated by arrows. Comparison of *A* and *B* shows that translocation of thick filaments from the center to the side of the sarcomere is accompanied by movement of the site on connectin that binds mAb. Comparison of *B* and *C* shows that the location of antibody binding relative to the Z-line and thick filaments is the same in two half sarcomeres with identical I-band lengths, even though the sarcomere in B is ~0.8 μm shorter than the one in *C*. Bar, 0.5 μm.

Sarcomeres in which the thick filaments have moved to the Z-line also exhibit two anti-connectin stripes per sarcomere (Fig. 4). However, these stripes are no longer symmetrically arranged about the center of the sarcomere. Nor are they equidistant from the M-line, which remains at the center of the translocated A-band. One anti-connectin stripe in these asymmetric sarcomeres is located in the enlarged I-band approximately two-thirds of the way between the Z-line and the A-band; the second anti-connectin stripe is located within the translocated A-band, almost adjacent to the other Z-line (Fig. 4). Although this second antibody stripe is sometimes difficult to observe against the high background mass of the A-band, it is clearly visible in most cases. Sarcomeres with A-bands adjacent to one Z-line but which have not been treated with anti-connectin antibody do not exhibit these extra striations (Fig. 4, *inset*).

Table I. Antibody Position in Relaxed Symmetric and Active Asymmetric Sarcomeres 2.40 ± 0.05 μm Long

		Active spacings	
	Relaxed spacings	Longer half-sarcomere	Shorter half-sarcomere
		μm	
I-band length	0.38 ± 0.01	0.73 ± 0.01	0.00 ± 0.00
Antibody to Z-line	0.27 ± 0.01	0.50 ± 0.01	0.06 ± 0.01
Antibody to A-band	0.11 ± 0.01	0.23 ± 0.01	−0.06 ± 0.01

Data are the means ± SEM for five relaxed and eight active sarcomeres. I-band length represents the distance from the center of the Z-line to the edge of the nearest A-band. Antibody to Z-line and antibody to A-band represent the distances from the center of the antibody stripe to the center of the nearest Z-line and to the edge of the nearest A-band, respectively.

Movement of Thick Filaments within the Sarcomere Is Accompanied by Movement of Elastic Domains of Connectin

Fig. 5, *A* and *B* show a direct comparison between a relaxed sarcomere with centered thick filaments and an activated sarcomere of identical length but in which the thick filaments have moved to the Z-line. As the A-band moves from the center of the relaxed sarcomere (Fig. 5 *A*) to the right hand side of the sarcomere with prolonged activation (Fig. 5 *B*), the antibody stripe on the left side of the sarcomere also moves to the right (Fig. 5, *A* and *B, left arrows*). As this occurs, there is an increase in the separation between the antibody and the Z-line, as well as between the antibody and the thick filaments. The labeled connectin epitope on the right hand side of these sarcomeres also changes its location as the A-band moves; it is compressed against the right hand side of the sarcomere as the A-band moves to the right (Fig. 5, *A* and *B, right arrows*).

We quantitated the variation of antibody position with A-band position in sarcomeres of constant length, and the results are shown in Table I. Antibody position was measured in all relaxed and active sarcomeres between 2.35 and 2.45 μm long. In relaxed fibers, the thick filaments are centered and the distance from their ends to the nearest Z-lines (I-band length) is the same on both sides of the sarcomere. However, during prolonged activation one I-band doubles in length (longer half-sarcomere) while the other one disappears (shorter half-sarcomere). In the longer half sarcomere, the spacing between the antibody stripe and the Z-line also doubles, whereas in the shorter half sarcomere it decreases to almost 0. Similar changes occur in the spacing between the antibody stripe and the A-band (Table I and Fig. 5 *B*).

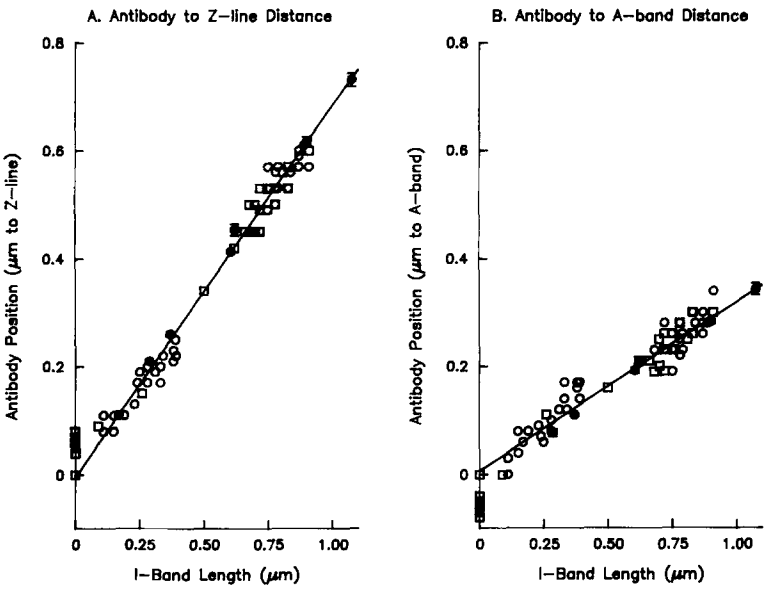

Figure 6. Antibody position relative to the Z-line (*A*) and the A-band (*B*) as a function of I-band length. (●) Mean antibody position ± SEM measured in 6–10 sarcomeres from a single relaxed fiber. (□) Antibody position measured in active sarcomeres with thick filaments adjacent to a Z-line. (○) Antibody position measured in active sarcomeres in which the thick filaments are not adjacent to a Z-line. Each open symbol represents results from a single half sarcomere. A total of 56 relaxed sarcomeres from 6 single fibers and 106 active half-sarcomeres from 4 single fibers were analyzed. Many points overlay others and are not visible in the figure. The lines were fit to the data at I-band lengths >0.3 μm, as described in the text.

As in the relaxed muscle, these observations show that the labeled connectin epitope is not rigidly attached to either the Z-line, the thin filaments, or the thick filaments. However, in this case, translocation of the connectin epitopes occurs as a result of internal movement of the thick filaments, without any change in sarcomere length.

The Disposition of the Elastic Connectin Domains Depends Uniquely on the Z-line to Thick Filament Spacing

The results presented in Fig. 5, *A* and *B* and quantitated in Table I clearly demonstrate that the disposition of elastic domains of connectin within the sarcomere does not depend only on sarcomere length. These observations instead show that the elastic domains in each half sarcomere behave independently, and suggest that the position of the antibody depends on the distance between the Z-line and the thick filaments. Fig. 5, *B* and *C* show two active sarcomeres that have identical Z-line to thick filament spacings on the left side of the sarcomere. Note that the antibody positions within these half-sarcomeres are also identical, even though the completely asymmetric sarcomere in Fig. 5 *B* is more than 0.8 μm shorter than the symmetric sarcomere in Fig. 5 *C*.

We measured the position of the antibody stripe in each relaxed and active half sarcomere relative to both the nearest Z-line and the thick filaments. Fig. 6 shows that these spacings increase roughly linearly with increasing I-band length. Furthermore, the relation between antibody spacing and I-band length is identical in relaxed and activated sarcomeres (Fig. 6, *closed* and *open symbols*, respectively). Finally, the dependence of antibody position on I-band length is unchanged by movement of the thick filaments completely to one side of the sarcomere (Fig. 6, *open squares*).

We analyzed the dependence of antibody position on I-band length by linear regression. The data in Fig. 6 clearly deviate from linearity at I-band lengths <0.1 μm. Therefore, we fit only the data in I-bands >0.3 μm, which is the length of I-bands in relaxed, slack muscle fibers. The slopes calculated from these data, which include measurements from 166 half-sarcomeres, are 0.69 ± 0.01 and 0.31 ± 0.01 for Fig. 6, *A* and *B*, respectively. The corresponding ordinate intercepts calculated from the data are −5 ± 6 nm for Fig. 6 *A* and 7 ± 6 nm for Fig. 6 *B*. These intercepts are not significantly different from 0, indicating that the connectin filaments are anchored at the Z-line and at the ends of the thick filaments. Furthermore, this analysis shows that, within experimental error, the entire length of connectin that spans the distance from the Z-line to the thick filaments behaves elastically when stretched.

Discussion

Calcium Ions and Cross-Bridge Activity Do Not Affect Connectin Elasticity or Thick Filament Binding

Our observations indicate that thick filament sliding within the sarcomere stretches connectin filaments at one end of the sarcomere while simultaneously shortening connectin filaments at the other end of the sarcomere. Because movement of thick filaments within the activated sarcomere is accompanied by movement of an elastic epitope of connectin in the same direction, it follows that connectin remains bound to thick filaments during calcium activation.

Furthermore, we observed that the location of this connectin epitope within the half-sarcomere depends uniquely on the Z-line to thick filament spacing. This is true whether I-band length is varied by stretching relaxed sarcomeres or by the active sliding of thick filaments within a sarcomere of constant length. In all cases, the distances from the Z-line to the connectin epitope and from the connectin epitope to the thick filaments increased with I-band length in the same manner (Fig. 6). These results indicate that the elastic properties of connectin are not modulated by calcium ions or cross-bridge activity.

Shortening of Connectin Filaments below Their Equilibrium Length

The length of I-bands in relaxed, slack muscle fibers is ~0.3 μm. This is presumably the length at which the elastic domains of the connectin filaments are unstrained. As thick

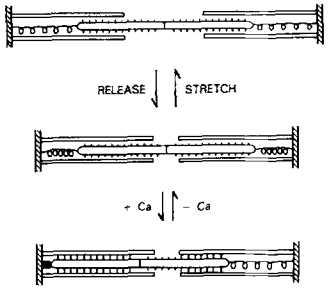

Figure 7. Schematic model of resting and active sarcomeres showing the disposition of the elastic domain of connectin, which links the thick filaments to the Z-discs. The lengths of the connectin filaments are altered by changes in sarcomere length or by movement of the thick filament within the sarcomere. The parts of connectin that are bound along the length of the thick filament are not shown.

filaments move from the center of the sarcomere to the Z-disc, the connectin filaments on one side of the sarcomere are compressed. We observe that as the I-band shortens from 0.3 to 0.1 μm, the position of the antibody stripe continues to depend linearly on I-band length, demonstrating that the connectin filament compresses elastically over this range (Fig. 6). However, as the I-band shortens further the antibody stripe actually enters the A-band (Figs. 5 *B* and 6; Table I), indicating that the connectin filaments stop compressing as ideal elastic elements in I-bands shorter than 0.1 μm. Because the connectin filaments remain attached to the thick filaments and the Z-line, the appearance of antibody labeling within the A-band as the I-band disappears indicates that the compressed connectin molecules first pile up in front of the Z-line and then fold back on themselves with further thick filament movement. This type of behavior may contribute to the apparent lack of movement of connectin epitopes located within 0.1 μm of the Z-line in sarcomeres close to or shorter than their equilibrium length (Furst et al., 1988).

Connectin Filaments as Elastic, Tension-bearing Structures

Fig. 7 schematically illustrates the mechanical role of connectin filaments in the sarcomere. Stretch of the resting sarcomere is accompanied by lengthening of the elastic domain of connectin, which produces the resting tension (Fig. 7, *stretch*). When the sarcomere is activated by calcium ions, the thick filaments tend to move from their initial position at the center of the sarcomere toward one of the Z-lines. In sarcomeres longer than 2.8 μm, the connectin filaments are stiff enough to prevent this movement (Horowits and Podolsky, 1987; 1988). However, in shorter sarcomeres the thick filaments can move completely to the Z-line (Fig. 7, $^+$Ca). In this case, the connectin filaments at one end of the thick filaments are stretched. The stretched connectin filaments transmit significant tension to the Z-disc and are responsible for maintaining the tension output of the sarcomere at a constant level during thick filament movement, even though the extent of overlap between thick and thin filaments is reduced on one side of the sarcomere (Horowits and Podolsky, 1988). Upon relaxation, the connectin filaments recenter any displaced thick filaments (Fig. 7, $-$Ca).

The model depicted in Fig. 7 is now supported by several lines of evidence. In addition to being consistent with structural evidence regarding the extensibility of connectin in relaxed muscle (this report; Wang et al., 1985; Furst et al., 1988; Itoh et al., 1988; Whiting et al., 1989), two independent lines of physiological evidence argue that the connectin filaments are both the source of resting tension and are important in positioning the thick filaments within the sarcomere. The first is that fragmentation of connectin is accompanied by loss of resting tension (Horowits et al., 1986; Yoshioka et al., 1986) and by misalignment of thick filaments (Horowits et al., 1986). The second is that the positional stability of thick filaments during prolonged isometric contraction depends on sarcomere length (Horowits and Podolsky, 1987), and that isometric tension output remains high during thick filament movement (Horowits and Podolsky, 1988). Using an mAb, we have now directly observed the stretching and shortening of the elastic domain of connectin that was predicted to accompany the movement of thick filaments within the activated sarcomere.

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