The Positional Stability of Thick Filaments in Activated Skeletal Muscle Depends on Sarcomere Length: Evidence for the Role of Titin Filaments

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Abstract. Electron microscopy was used to study the positional stability of thick filaments in isometrically contracting skinned rabbit psoas muscle as a function of sarcomere length at 7°C. After calcium activation at a sarcomere length of 2.6 μm, where resting stiffness is low, sarcomeres become nonuniform in length. The dispersion in sarcomere length is complete by the time maximum tension is reached. A-bands generally move from their central position and continue moving toward one of the Z-discs after tension has reached a plateau at its maximum level. The lengths of the thick and thin filaments remain constant during this movement. The extent of A-band movement during contraction depends on the final length of the individual sarcomere. After prolonged activation, all sarcomeres between 1.9 and 2.5 μm long exhibit A-bands that are adjacent to a Z-disc, with no intervening I-band. Sarcomeres 2.6 or 2.7 μm long exhibit a partial movement of A-bands. At longer sarcomere lengths, where the resting stiffness exceeds the slope of the active tension–length relation, the A-bands remain perfectly centered during contraction. Sarcomere symmetry and length uniformity are restored upon relaxation. These results indicate that the central position of the thick filaments in the resting sarcomere becomes unstable upon activation. In addition, they provide evidence that the elastic titin filaments, which join thick filaments to Z-discs, produce almost all of the resting tension in skinned rabbit psoas fibers and act to resist the movement of thick filaments away from the center of the sarcomere during contraction.

THE two-filament cross-bridge model of muscle contraction predicts that the structural arrangement of the sarcomere, with the thick filaments located in the center, is unstable upon activation. This is because the amount of force on each half of a thick filament is proportional to the fraction of its cross-bridge bearing length which overlaps with thin filaments. Any initial imbalance would thus be amplified as the thick filament is actually pulled toward one end of the sarcomere. This phenomenon has been observed by several authors who published electron micrographs of activated frog skeletal muscles which showed A-bands adjacent to one Z-line, with no intervening I-band (Page and Huxley, 1963; Eisenberg and Eisenberg, 1982; Bergman, 1983). However, several other electron microscopic studies of activated skeletal muscle have failed to demonstrate large-scale movement of thick filaments during isometric contraction (Carlsen et al., 1961; Galey, 1964; Sjostrand and Jagendorf-Elfvin, 1967). One possible explanation for this is that thick filament movement does occur during isometric contraction, but on a slower time scale than tension development. Muscle fibers could then be fixed after maximum tension development but before noticeable translation of thick filaments has occurred.

A second possible explanation is that some structure is present which, under certain conditions, stabilizes the position of the thick filament during contraction. Indeed, some stabilizing mechanism must exist if the thick filaments are to be recentered after a contraction. Obvious candidates for structures that might perform this function are the elastic filaments that link the thick filaments to the Z-disc. Structural studies have demonstrated the existence in skeletal muscle of filaments other than the two composed of actin and myosin. When sarcomeres are stretched so that a gap appears between thick and thin filaments, additional filaments are evident in the intervening space (Carlsen et al., 1961; Huxley and Peachey, 1961; Sjostrand, 1962; Locker and Leet, 1975; Magid et al., 1984). These "gap filaments" contain the megadalton-sized protein titin (also called connectin) which is the third most abundant protein in muscle (Maruyama et al., 1984; Wang, 1985; Gassner, 1986). The titin-containing filaments have been shown to span the entire region between thick filaments and Z-lines (Maruyama et al., 1985). Nebulin may also be a constituent of these filaments (Wang, 1985). When thick filaments are isolated they contain smaller filaments at their ends which are morphologically similar to isolated titin (Trinick et al., 1984). It was recently shown by immunoelectron microscopy that these end filaments also contain titin (Gassner, 1986; Hill and Weber, 1986). Therefore, the evidence accumulated from several laboratories now conclusively shows that filaments containing titin, and possibly nebulin, link thick filaments to Z-discs in skeletal muscle.

It was recently demonstrated that the degradation of titin and nebulin peptides by ionizing radiation (Horowits et al., 1986) or of titin alone by enzymatic digestion (Yoshioka et al., 1986) decreases the tension exerted by resting skinned skeletal muscle cells. From this it was concluded that it is stretch of the titin filaments that produces the resting tension. It follows that these filaments could be a stabilizing influence on the sarcomere, resisting movement of the thick filaments away from their central position. In the present study we found evidence for such an effect in skinned rabbit muscle fibers by observing the influence of sarcomere length on both resting tension and the positional stability of thick filaments at the center of the sarcomere during activation.

Materials and Methods

Fiber Preparation and Mechanical Measurements

Strips of rabbit psoas muscle were chemically skinned by the method of Wood et al. (1975) in a 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 5°C. Small bundles of fibers ~0.25 mm in diameter or single fibers were dissected and connected to a leafspring force transducer (Thames et al., 1974) and a fixed end using T-shaped aluminum foil clips (Goldman and Simmons, 1984). The solution bathing the fiber could be rapidly changed and its temperature thermoelectrically controlled using a bath similar to that described by Hellam and Podolsky (1969). All mechanical experiments were conducted at 7°C. Resting tensions were measured in a 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. Active tensions were measured in an activating solution consisting of 3.5 mM $CaCl_2$ added to the relaxing solution to yield a final pCa of 4.5. Sarcomere length was monitored by laser light diffraction. Upon activation at sarcomere lengths less than 2.8 μm the laser diffraction pattern became broader and less intense; at longer sarcomere lengths the pattern was unaffected by activation.

Electron Microscopy

Bundles of skinned fibers ~0.25 mm in diameter were held isometrically in the mechanical apparatus as described above and fixed for 2 h at 7°C in 2.5% glutaraldehyde added to either the relaxing or activating solution. The laser diffraction pattern was unchanged by fixation, which indicated that the sarcomere length distribution was unaffected. Bundles fixed during activation had reached maximum tension before fixation. The time to reach half-maximal tension ranged from 0.3 to 0.8 min, and the time to peak tension ranged from 1.2 to 2.2 min.

After fixation the bundles were removed from the mechanical apparatus. They were then postfixed for 1 h with 2% osmium tetroxide, dehydrated with ethanol, and embedded in a mixture of Araldite and Epon. 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 either a Philips EM 300 or a Philips EM 400 microscope. For each experiment at least one bundle fixed at rest was processed in parallel with activated bundles. Sarcomeres in the resting samples were always extremely uniform in length, as judged both by laser diffraction and by electron microscopy. The average sarcomere length measured in electron micrographs of these resting muscles was taken to be equal to their length measured by laser diffraction immediately before and during fixation. This same scale was then applied to the micrographs of activated fibers. In this way micrographs of resting muscles were used to calibrate micrographs of activated fibers.

For each sarcomere in an electron micrograph, the position of the thick filaments was quantitated by measuring the distance between each end of the A-band and the center of the nearest Z-line, these distances being termed I_1 and I_2. The extent of A-band movement from the center of the sarcomere expressed as a fraction of its maximum possible movement was then calculated as $|I_1 - I_2|/(I_1 + I_2)$. This calculation yields a quantitative measure of sarcomere asymmetry that can be used to compare sarcomeres of different lengths. The mean width of the A-band in preparations fixed at rest was 1.68 ± 0.07 μm (±SD; n = 3 preparations) and was unchanged by activation. The thin filaments in resting preparations were 1.10 ± 0.04 μm long. In addition, a bare zone of 0.15 ± 0.02 μm width was observed at the center of the A-band. These measurements are in close agreement with previous reports of filament lengths in rabbit psoas muscle (Huxley, 1963; Trinick and Elliot, 1979). The close correspondence between the length of individual thick filaments and A-band width indicates that the measurement of thick filament position is not complicated by misalignment of filaments or by superposition of filaments within the section.

In some cases myofibrils were observed entering and leaving the plane of the section. However, this introduced only a small error in measurement. For example, given an extreme case where in a 1-μm diameter myofibril only three 2-μm-long sarcomeres lie in the section plane, the error in length measurements due to section obliqueness is ~1%.

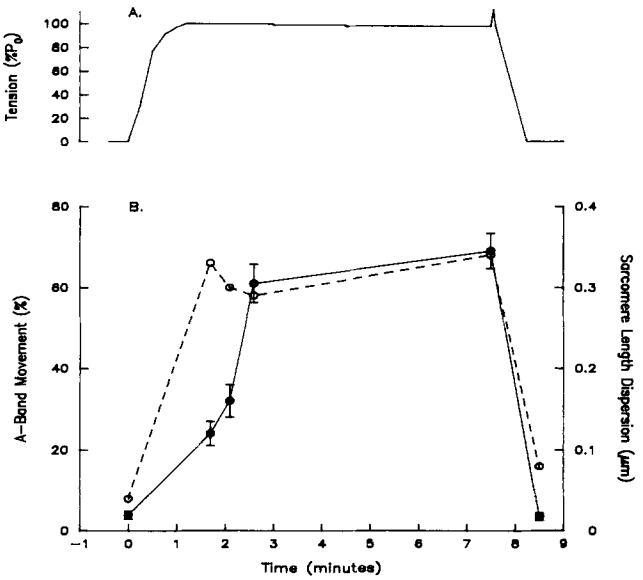

Figure 1. (*A*) Typical tension record from a small bundle of fibers. The sarcomere length of the resting bundle was adjusted to 2.6 μm. The bundle was then transferred from relaxing solution to activating solution at 0 time and returned to relaxing solution after 7.5 min. Tension is expressed as a percentage of the maximum active force (P_o), which was 454 mg. The original tension record was sampled and redrawn by computer. (*B*) The time course of sarcomere length dispersion (○) and A-band movement (●) during contraction. Small bundles of fibers were stretched to a sarcomere length of 2.6 μm and activated as illustrated in *A*. The bundles were transferred at the times indicated from either relaxing solution or activating solution to 2.5% glutaraldehyde dissolved in the same solution. Each bundle that was fixed during contraction had reached maximum force before fixation. Sarcomere length dispersion and A-band movement were measured from electron micrographs as detailed in the text. Each point represents the mean value obtained from 25 to 30 sarcomeres in a single resting preparation or from 60 to 110 sarcomeres in a single activated preparation. Error bars are ± 1 SEM.

Results

A-Band Movement

Initial Sarcomere Length = 2.6 μm. Bundles of skinned rabbit psoas fibers were stretched to a sarcomere length of 2.6 μm and fixed at various times after immersion in activating solution. A typical tension record of a 7.5-min activation is shown in Fig. 1 *A*. Fig. 2 shows electron micrographs of fibers fixed at rest and at varying times after the start of con-

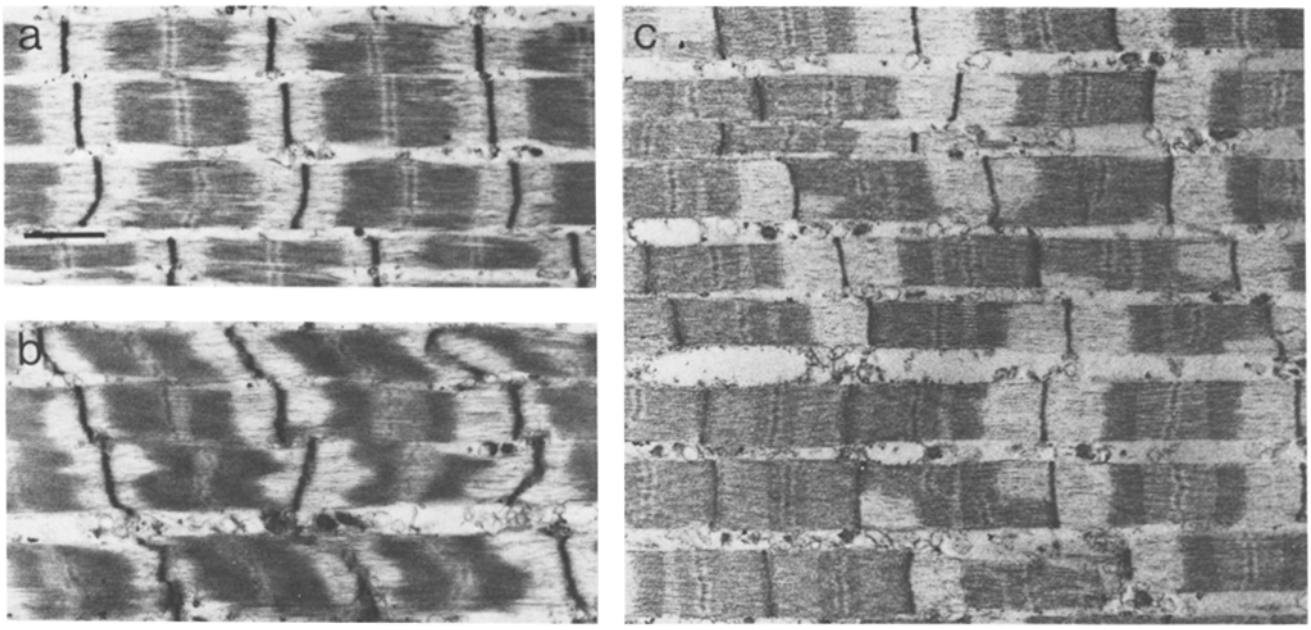

Figure 2. Electron micrographs of fibers used in the experiment of Fig. 1. The bundle in *a* was fixed at rest. Those in *b* and *c* were fixed 1.7 and 7.5 min after the start of activation, respectively. Bar, 1 μm.

traction. The relaxed fibers exhibit sarcomeres of uniform length with centrally located A-bands (Fig. 2 *a*). In contrast, the activated samples show considerable nonuniformity in sarcomere length. The sample fixed 1.7 min after the start of activation exhibits many uncentered A-bands, but very rarely have they moved completely to one side of the sarcomere (Fig. 2 *b*). However, after 7.5 min of activation many sarcomeres are observed in which the A-band as a whole is dramatically displaced from its central position (Fig. 2 *c*). In many cases the A-band is located adjacent to a Z-line, having moved completely to one side of the sarcomere. Longer sarcomeres in the same field exhibit A-bands that are centrally located between the Z-lines. The lengths of thick and thin filaments are unchanged after the A-bands have moved completely to one side of the sarcomere when compared to centered A-bands in the same preparation or in resting preparations. This indicates that A-band movement involves a simple translation of the thick filament array.

To obtain a quantitative measure of the extent of movement of the A-band from its central position, the distance between each end of the A-band and the center of the nearest Z-line was measured in each sarcomere, these quantities being termed I_1 and I_2, respectively. The quantity $|I_1 - I_2|/(I_1 + I_2)$ is thus the extent of A-band movement from the center of the sarcomere expressed as a fraction of its maximum possible movement. The standard deviation of the sarcomere length was taken as a measure of the dispersion, or nonuniformity, of sarcomere lengths and was determined from electron micrographs of each bundle. Sarcomere length dispersion increases after activation and is maximal by the time maximal tension is reached (Fig. 1 *B*, open circles). The measurements of A-band position are most simply interpreted as a unidirectional movement of the A-band away from the center of the sarcomere and toward one of the Z-discs. This movement lags tension development and sarcomere

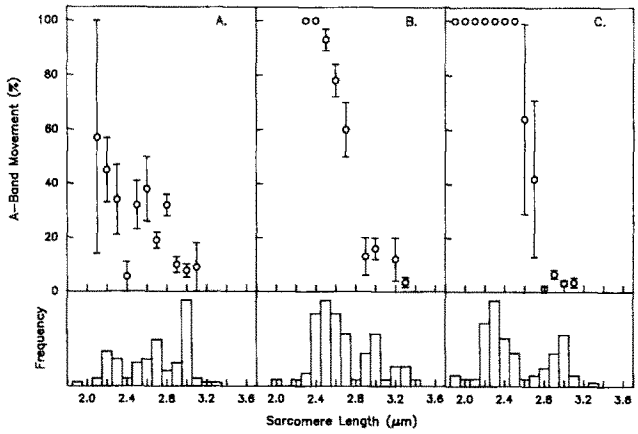

Figure 3. A-band movement vs. sarcomere length at different times after the start of activation. *A*, *B*, and *C* show the sarcomere length distribution and the relationship between A-band movement and sarcomere length in a single bundle activated for 1.7, 2.6, and 7.5 min, respectively. The sarcomere length of each bundle was set at 2.6 μm before activation. Frequencies in the sarcomere length distributions were normalized to the maximum frequency in each preparation; the total areas under the histograms in *A*, *B*, and *C* correspond to 83, 69, and 107 sarcomeres, respectively. Each point represents the mean ± SEM for A-band movement in sarcomeres of a certain length. A-band movement is not plotted at lengths at which only one sarcomere was observed.

length dispersion by ~1 min, and then A-band position remains constant until the bundle is relaxed (Fig. 1 *B*, closed circles). These changes are fully reversed upon relaxation. After 1 min in calcium-free solution the bundle is totally relaxed, sarcomere length dispersion has returned to its initial value, and the A-bands have all returned to the center of the sarcomere (Fig. 1 *B*).

Fig. 3 shows the sarcomere length distribution and the de-

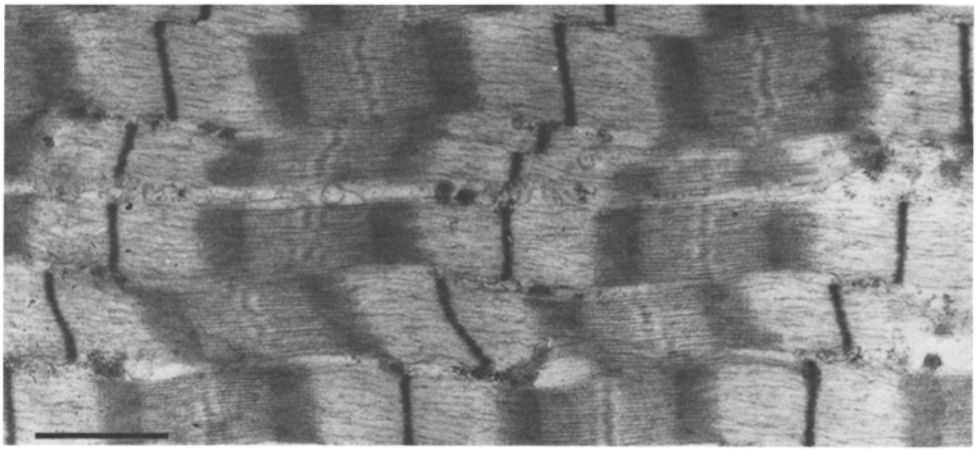

Figure 4. Electron micrograph of a fiber from a bundle that was stretched to a sarcomere length of 3.2 μm and then fixed 5 min after the start of activation. Sarcomeres are uniformly long and have centrally located A-bands. Bar, 1 μm.

pendence of A-band movement on sarcomere length in three individual bundles activated for different amounts of time. Analysis of electron micrographs indicates that the mean sarcomere length of the preparations did not change significantly upon activation. However, as indicated in Fig. 1 *B* the dispersion of sarcomere lengths increases dramatically upon activation at this length. Sarcomere lengths in the micrographs were measured to the nearest 0.1 μm, along with the extent of A-band movement. After 1.7 min of activation there is a significant negative correlation between A-band movement and sarcomere length within a single preparation (Fig. 3 *A*). A-bands in sarcomeres longer than 2.8 μm tend to remain centered, while significant movement from the center is observed in shorter sarcomeres. After 2.6 or 7.5 min of activation the rough correlation between A-band movement and sarcomere length has changed to a sharp dependence (Fig. 3, *B* and *C*). Sarcomeres between 1.9 and 2.5 μm long exhibit A-bands that have moved completely to one side. Therefore, at these sarcomere lengths the A-bands appear to move from the center of the sarcomere to the Z-disc on a time scale of minutes. Sarcomeres 2.6 or 2.7 μm long exhibit partial movement of A-bands, while longer sarcomeres exhibit perfectly centered A-bands. The position of the A-band is therefore completely unstable at sarcomere lengths below 2.5 μm. A-band stability increases between 2.5 and 2.8 μm, and is maximal at longer sarcomere lengths.

As shown in Fig. 1 *B*, A-bands continue moving between 1.7 and 2.6 min after the start of activation, even though the sarcomere length dispersion remains constant during this period. This observation, along with the sharp dependence of A-band position on sarcomere length after 2.6 min of activation, suggests that the maximum extent of A-band movement that can occur is effectively determined by the length of the individual sarcomere.

Initial Sarcomere Length ≥ 2.8 μm. The time course of tension development was similar to that illustrated in Fig. 1 *A* at all sarcomere lengths studied. When a fiber is fixed after stretching to a sarcomere length greater than 2.8 μm and activation for 5 min, the sarcomeres tend to be more uniform in length (Fig. 4). In addition, most of these sarcomeres contain centrally located A-bands. Fig. 5, *A–C*, shows histograms illustrating the sarcomere length distributions of three bundles stretched to average sarcomere lengths of 2.8, 3.0, and 3.2 μm, respectively, and fixed after 5 min of activation. The mean extent of A-band movement at each sarcomere length is also illustrated for each preparation. The dependence of A-band movement on sarcomere length is approximately the same for each of these bundles, indicating that the extent of A-band movement is determined by the final length of the sarcomere and is independent of its initial length before activation. At each sarcomere length the mean values of A-band movement from all three preparations were averaged to yield the curve shown in Fig. 5 *D*. This relation is similar to that seen after 2.6 or 7.5 min of activation at shorter overall sarcomere lengths (cf. Fig. 5 *D* with Fig. 3, *B* and *C*). In ev-

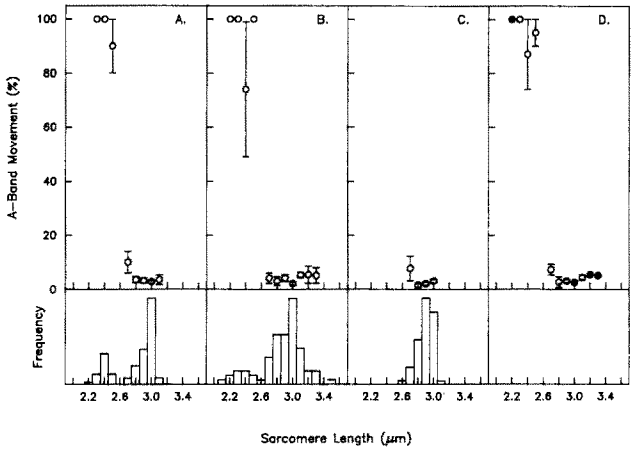

Figure 5. Dependence of A-band movement on sarcomere length in bundles stretched to sarcomere lengths of 2.8 (*A*), 3.0 (*B*), or 3.2 (*C*) μm before activation. *A–C* each show the sarcomere length distribution and the relationship between A-band movement and sarcomere length in a single bundle after 5 min of activation. Frequencies in the sarcomere length distributions were normalized to the maximum frequencies in each preparation; the total areas under the histograms in *A–C* correspond to 100, 74, and 66 sarcomeres, respectively. Each point represents the mean ± SEM for A-band movement in sarcomeres of a certain length. A-band movement is not plotted at lengths at which only one sarcomere was observed. *D* shows the relation between A-band movement and sarcomere length that is obtained by averaging the mean values from *A–C*. Each open circle represents the mean ± SEM of the average values. Solid circles are the average values obtained from a single bundle.

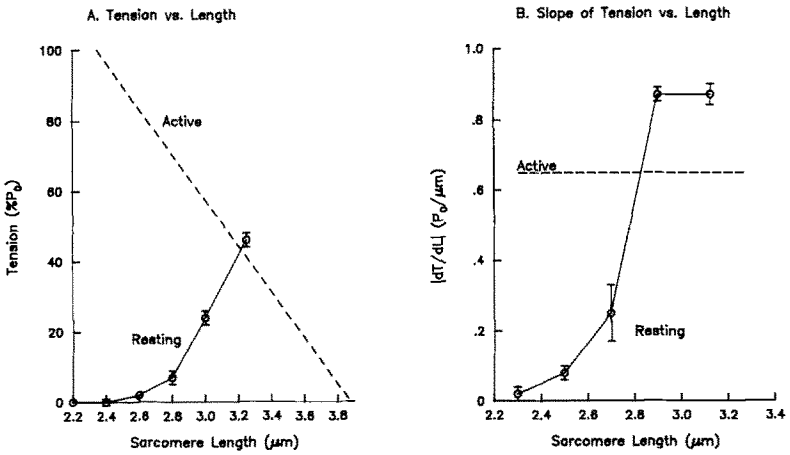

Figure 6. (*A*) Dependence of resting and active tensions on sarcomere length. Resting tension was measured 5 min after stretch and normalized to the maximum calcium-activated force measured at a sarcomere length of 2.4 μm (P_o). Each point is the mean resting tension ± SEM for three single fibers. The dashed line is the active tension that is predicted on the basis of filament lengths. (*B*) Dependence of the slopes of the resting and active tension–length relations ($|dT/dL|$) on sarcomere length. Resting stiffness for a given fiber was taken as the slope of its resting tension–length relation midway between two experimental points. Each point represents the mean resting stiffness ± SEM for the same three fibers used in *A*. The dashed line indicates the magnitude of the slope of the active tension–length relation that is predicted on the basis of filament lengths. It is exceeded by the resting stiffness at sarcomere lengths greater than 2.8 μm.

ery case, sarcomeres longer than 2.8 μm exhibit centrally located A-bands, while sarcomeres shorter than 2.5 μm exhibit maximum A-band movement.

Resting and Active Tension

The resting tension of single-skinned fibers was measured at various sarcomere lengths and expressed as a fraction of the maximum active force measured at a sarcomere length of 2.4 μm. The resting tension declines immediately after stretch, but is relatively stable after 5 min (Horowits et al., 1986). Resting tension is undetectably small at lengths less than 2.5 μm. Between 2.6 and 2.8 μm the resting tension–length relation curves upward, and becomes linear at higher sarcomere lengths (Fig. 6 *A*, solid line). The slope of the resting tension–length relation, which defines the resting stiffness, thus increases from 0 to a maximum value as the sarcomere is stretched from 2.4 to 2.8 μm (Fig. 6 *B*, solid line).

The active tension–length relation decreases linearly in stretched fibers as overlap between thick and thin filaments is reduced from a maximum to 0 (Gordon et al., 1966). The active tension–length relation calculated from the lengths of the thick and thin filaments (Fig. 6 *A*, dashed line) has a slope of 0.65 P_o per μm sarcomere length, where P_o is the maximum tension produced at full overlap (Fig. 6 *B*, dashed line). The slope of the resting tension–length relation exceeds this value at sarcomere lengths greater than 2.8 μm where, as described above, A-bands remain centered after prolonged activation.

Discussion

We find that A-bands can move from their central position in the sarcomere during isometric contraction, while the lengths of the thick and thin filaments remain constant. This confirms that the position of the thick filaments in the resting muscle becomes unstable upon activation, as predicted by the sliding filament theory. However, A-band movement is significantly slower than tension development. The lag between maximum tension development and the completion of A-band movement may account for the fact that this phenomenon was not always apparent in previous studies.

The Quantitative Relationship between Resting Tension and A-Band Movement

We have recently demonstrated that the selective degradation of titin and nebulin leads to a marked decrease in the tension exerted by the resting skinned muscle fiber; this effect is accompanied by a decrease in the positional stability of the thick filaments in the activated fiber (Horowits et al., 1986). These observations led to a model in which elastic titin filaments link thick filaments to Z-discs, are the source of all the resting tension, and tend to stabilize the position of the thick filaments at the center of the sarcomere during activation. This model predicts that when the positive slope of the resting tension–length relation is greater in magnitude than the negative slope of the active tension–length relation, no movement of thick filaments should occur during isometric activation (see Appendix). However, movement of thick filaments should occur during activation if this condition is not met. Thus, the relation of the mechanical properties of the resting and active muscle fiber to the positional stability of the thick filaments during activation constitutes a critical test of the proposed model.

The mechanical measurements indicate that at a sarcomere length of ~2.7 μm the resting stiffness is half of what is needed to hold the thick filaments in the center of the sarcomere, and reaches the level needed for complete stabilization at ~2.8 μm (Fig. 6 *B*). (Due to the steepness of the relation between resting stiffness and sarcomere length, the estimated critical length for A-band stability is relatively insensitive to the time scale over which resting tension and stiffness are measured; for resting stiffness measurements made on a time scale ranging from 10 s to 5 min after stretch of the muscle, the critical sarcomere length for A-band stability varies less than 0.1 μm). This result is in good agreement with the measurements of A-band movement made after a steady state has been reached; A-band movement is half maximal at a sarcomere length between 2.6 and 2.7 μm and is abolished at lengths greater than 2.8 μm (Figs. 3 and 5). Therefore, these data strongly support the hypothesis that the elastic titin filaments produce most of the resting tension and tend to stabilize the position of the thick filaments at the center of the sarcomere during contraction (Horowits et al., 1986).

A-Band Movement and Muscle Mechanics

After activation, the movement of A-bands lags both the dispersion of sarcomere lengths and the development of tension (Fig. 1). Since sarcomere length dispersion and A-band movement were measured from the same sarcomeres, this observation cannot be an artifact caused by slow diffusion of fixative into the preparation. Thus there is a period of at least 30 s when A-bands are moving while both sarcomere length and tension are constant. The change in relative A-band position after full activation corresponds to a 0.14-μm translation occurring within ~30 s, or a velocity of 5 nm/s. The maximum velocity of shortening of skinned rabbit psoas fibers at low temperature is 0.5 muscle lengths per second (Brenner, 1980), corresponding to a relative filament velocity of 600 nm/s. Thus the A-bands move with a velocity that is less than 1% of maximum, implying that this movement occurs against an almost maximal load. This slow velocity of filament sliding is consistent with the observation that tension remains constant during A-band movement and does not change when this movement is completed.

The source of the load resisting A-band motion certainly includes the crossbridges on that half of the thick filament that is moving away from the nearest Z-line. Strained elastic filaments at this end of the thick filament may also be a restraining influence on thick filament movement. The data in Fig. 1 show that the continuing movement of A-bands after maximum tension has been reached causes the overlap region between thick and thin filaments to decrease by 25% at one end of the A-band. The observation that active tension remains constant even after A-band motion ceases indicates that titin filaments that have been strained by A-band movement are effective in transmitting active tension to the Z-disc.

The role of the elastic filaments can thus be schematically illustrated, as in Fig. 7. Upon activation at short sarcomere length, the unstable A-bands move from their central position in the sarcomere (left-hand panels, +Ca). This stretches the elastic filaments at one end of the sarcomere, which then transmit tension to the Z-disc. The component of active tension transmitted by the elastic filaments would otherwise be lost, never reaching the ends of the sarcomere. At longer sarcomere lengths the elastic filaments are relatively stiff in the relaxed fiber (right hand panels). In this case they effectively hold the A-bands at the center of the sarcomere during activation. Finally, upon relaxation the elastic filaments recenter any displaced A-bands (left-hand panels, −Ca). While A-band movement has been observed in intact muscle (Page and Huxley, 1963; Eisenberg and Eisenberg, 1982; Bergman, 1983), the extent to which these processes actually occur in the animal must ultimately be determined by careful measurement of the time course of A-band movement during neural stimulation under conditions found in vivo.

Appendix

Define the magnitude of the slope of the descending limb of the active tension–sarcomere length relation as S_a, and the slope of the resting tension–sarcomere length relation as S_r. Then for a displacement Δx of a thick filament from the center of the sarcomere, the overlap at one end of the thick filament increases by Δx. Hence the active force tending to pull the filament toward that end of the sarcomere increases by $2\Delta x S_a$. The elastic filament(s) at that end of the thick filament is (are) simultaneously shortened by Δx, and the passive force pulling the thick filament in that same direction decreases by $2\Delta x S_r$. Precisely opposite changes in active and passive forces occur at the other end of the thick filament. The position of the thick filament at the center of the sarcomere is stable when the sum of the forces resisting movement is greater than the sum of those acting in the direction of movement. This condition is met when $2\Delta x S_r - 2\Delta x S_a > 2\Delta x S_a - 2\Delta x S_r$, which simplifies to $S_r > S_a$.

Thus, the elastic filaments should be effective in holding the thick filaments at the center of the sarcomere at any length at which the slope of the passive tension–length relation is greater in magnitude than the negative slope of the active tension–length relation.

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References

Bergman, R. A. 1983. Ultrastructural configuration of sarcomeres in passive and contracted frog sartorius muscle. *Am. J. Anat.* 166:209–222.

Brenner, B. 1980. Effect of free sarcoplasmic Ca^{2+} concentration on maximum unloaded shortening velocity: measurements on single glycerinated rabbit psoas muscle fibers. *J. Muscle Res. Cell Motil.* 1:409–428.

Carlsen, F., G. G. Knappeis, and F. Buchthal. 1961. Ultrastructure of the resting and contracted striated muscle fiber at different degrees of stretch. *J. Biophys. Biochem. Cytol.* 11:95–117.

Eisenberg, B. R., and R. S. Eisenberg. 1982. The T-SR junction in contracting single skeletal muscle fibers. *J. Gen. Physiol.* 79:1–19.

Galey, F. R. 1964. Local contraction patterns of striated muscle. *J. Ultrastruct. Res.* 11:389–400.

Gassner, D. 1986. Myofibrillar interaction of blot immunoaffinity-purified antibodies against native titin as studied by direct immunofluorescence and immunogold staining. *Eur. J. Cell Biol.* 40:176–184.

Goldman, Y. E., and R. M. Simmons. 1984. Control of sarcomere length in skinned muscle fibres of Rana Temporaria during mechanical transients. *J. Physiol. (Lond.).* 350:497–518.

Gordon, A. M., A. F. Huxley, and F. J. Julian. 1966. The variation in isometric tension with sarcomere length in vertebrate muscle fibers. *J. Physiol. (Lond.).* 184:170–192.

Hellam, D. C., and R. J. Podolsky. 1969. Force measurements in skinned muscle fibres. *J. Physiol. (Lond.).* 200:807–819.

Hill, C., and K. Weber. 1986. Monoclonal antibodies distinguish titins from heart and skeletal muscle. *J. Cell Biol.* 102:1099–1108.

Horowits, R., E. S. Kempner, M. E. Bisher, and R. J. Podolsky. 1986. A physiological role for titin and nebulin in skeletal muscle. *Nature (Lond.).* 323: 160–164.

Huxley, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* 7:281–308.

Huxley, A. F., and L. D. Peachey. 1961. The maximum length for contraction in vertebrate striated muscle. *J. Physiol. (Lond.).* 156:150–165.

Locker, R. H., and N. G. Leet. 1975. Histology of highly-stretched beef muscle. I. The fine structure of grossly stretched single fibers. *J. Ultrastruct. Res.* 52:64–75.

Magid, A., H. P. Ting-Beall, M. Carvell, T. Kontis, and C. Lucaveche. 1984. Connecting filaments, core filaments, and side struts: a proposal to add three new load-bearing structures to the sliding filament model. *In* Contractile Mechanisms in Muscle. G. H. Pollack and H. Sugi, editors. Plenum Publishing Corp., New York. pp. 307–328.

Maruyama, K., H. Sawada, S. Kimura, K. Ohashi, H. Higuchi, and Y. Umazume. 1984. Connectin filaments in stretched skinned fibers of frog skeletal

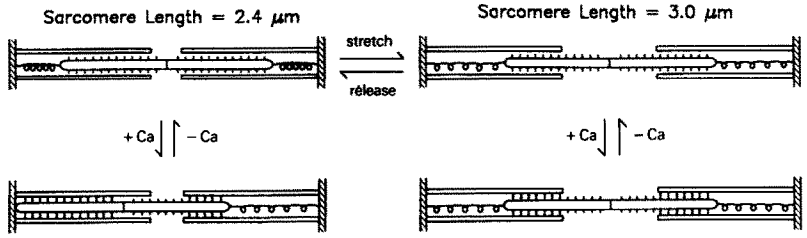

Figure 7. Schematic model of resting and active sarcomeres. Elastic filaments link the ends of thick filaments to Z-discs and keep the A-bands centered at rest. Activation by calcium ions leads to A-band movement at sarcomere lengths less than 2.8 μm. This stretches elastic filaments at one end of the sarcomere, which then transmit tension to the Z-disc. In sarcomeres longer than 2.8 μm, the elastic filaments are stiff enough to prevent A-band movement during activation.

muscle. *J. Cell Biol.* 99:1391–1397.
Maruyama, K., T. Yoshioka, H. Higuchi, K. Ohashi, S. Kimura, and R. Natori. 1985. Connectin filaments link thick filaments and Z-lines in frog skeletal muscle as revealed by immunoelectron microscopy. *J. Cell Biol.* 101: 2167–2172.
Page, S. G., and H. E. Huxley. 1963. Filament lengths in striated muscle. *J. Cell Biol.* 19:369–390.
Sjostrand, F. S. 1962. The connections between A- and I-band filaments in striated frog muscle. *J. Ultrastruct. Res.* 7:225–246.
Sjostrand, F. S., and M. Jagendorf-Elfvin. 1967. Ultrastructural studies of the contraction-relaxation cycle of glycerinated rabbit psoas muscle. I. The ultrastructure of glycerinated fibers contracted by treatment with ATP. *J. Ultrastruct. Res.* 17:348–378.
Thames, M. D., L. E. Teichholz, and R. J. Podolsky. 1974. Ionic strength and the contraction kinetics of skinned muscle fibers. *J. Gen. Physiol.* 63: 509–530.
Trinick, J., and A. Elliott. 1979. Electron microscope studies of thick filaments from vertebrate skeletal muscle. *J. Mol. Biol.* 131:133–136.
Trinick, J., P. Knight, and A. Whiting. 1984. Purification and properties of native titin. *J. Mol. Biol.* 180:331–356.
Wang, K. 1985. Sarcomere-associated cytoskeletal lattices in striated muscle. *Cell and Muscle Motil.* 6:315–369.
Wood, D. S., J. Zollman, J. P. Reuben, and P. W. Brandt. 1975. Human skeletal muscle: properties of the "chemically skinned" fiber. *Science (Wash. DC).* 187:1075–1076.
Yoshioka, T., H. Higuchi, S. Kimura, K. Ohashi, Y. Umazume, and K. Maruyama. 1986. Effects of mild trypsin treatment on the passive tension generation and connectin splitting in stretched skinned fibers from frog skeletal muscle. *Biomed. Res.* 7:181–186.