

REVIEW

Sarcomere mechanics in striated muscles: from molecules to sarcomeres to cells

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Rassier DE. Sarcomere mechanics in striated muscles: from molecules to sarcomeres to cells. *Am J Physiol Cell Physiol* 313: C134–C145, 2017. First published May 24, 2017; doi:10.1152/ajpcell.00050.2017.—Muscle contraction is commonly associated with the cross-bridge and sliding filament theories, which have received strong support from experiments conducted over the years in different laboratories. However, there are studies that cannot be readily explained by the theories, showing 1) a plateau of the force-length relation extended beyond optimal filament overlap, and forces produced at long sarcomere lengths that are higher than those predicted by the sliding filament theory; 2) passive forces at long sarcomere lengths that can be modulated by activation and Ca^{2+} , which changes the force-length relation; and 3) an unexplained high force produced during and after stretch of activated muscle fibers. Some of these studies even propose “new theories of contraction.” While some of these observations deserve evaluation, many of these studies present data that lack a rigorous control and experiments that cannot be repeated in other laboratories. This article reviews these issues, looking into studies that have used intact and permeabilized fibers, myofibrils, isolated sarcomeres, and half-sarcomeres. A common mechanism associated with sarcomere and half-sarcomere length nonuniformities and a Ca^{2+} -induced increase in the stiffness of titin is proposed to explain observations that derive from these studies.

cross-bridges; myofibril; myosin; sarcomere; titin

MUSCLE CONTRACTION is historically associated with the sliding filament (42, 45) and the cross-bridge (41, 44) theories. The sliding filament theory proposes that shortening of sarcomeres during activation is accomplished by the relative sliding of actin filaments over myosin filaments. The cross-bridge theory proposes that the sliding of actin filaments is caused by the rotation of cross-bridges. Ultimately, there are observations suggesting that the sliding of actin filaments is actually caused by changes in the orientation of the lever arm of attached myosin cross-bridges (40, 59). Together, these findings predict that force should be proportional to the number of cross-bridges attached to actin, and therefore proportional to the degree of overlap between myosin and actin filaments. Such prediction was confirmed in the classic study performed by Gordon et al. (33), who showed that the active force produced by single muscle fibers was directly related to the average sarcomere length, and consequently the degree of filament overlap.

The cross-bridge theory and the sliding filament theory have been accepted by the scientific community and became a paradigm in the muscle field. However, there are several studies that show results that cannot be readily explained by the

theories, showing 1) a plateau of the force-length relation extended beyond optimal filament overlap, and forces produced at long sarcomere lengths that are higher than those predicted by the sliding filament theory, 2) passive forces that are present at long lengths that can be modulated by activation and Ca^{2+} , which changes the force-length relation, and 3) an unexplained high force that is produced during and after a stretch is imposed to activated muscle fibers. Some of these studies even propose new models or a “new paradigm” of muscle contraction [e.g., (37–39, 72, 90)]; in one example, the original sliding filament theory is said to “fail miserably” when explaining forces produced after a stretch is imposed to muscles (37). While some of these observations deserve a careful evaluation, most studies proposing new models of contraction present data lacking rigorous control and that have not been repeated in other laboratories.

With the development of new techniques for muscle experimentation in recent years, it is timely to assess some of these contradictory observations. This article will review these issues using studies looking into muscle mechanics that range from intact and permeabilized fibers, myofibrils, isolated sarcomeres, and half-sarcomeres. Studies using whole animal muscles and human muscles will not be used in this review. Although these studies may provide important insights into physiological muscle functions, they cannot adequately inves-

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rigate cellular/molecular mechanisms that involve sarcomere mechanics during active and passive force generation.

The Sarcomere and the Myofibrils

A muscle fiber is composed of many myofibrils, which are formed by sarcomeres arranged in series. The sarcomere comprises several proteins organized in a three-dimensional lattice, optimally designed for active and passive force generation (Fig. 1, A–B). The bright area (I-band) of myofibrils comprises the length of the actin filaments that do not overlap with the thick filaments. The dark region (A-band) comprises the length of the thick filaments formed mostly by myosin molecules (92). The thick filament connects with the Z-disks through titin molecules, which also bind to specific sites of actin and other sarcomeric proteins (Fig. 1, A–B).

Myosin and active force generation. The molecular motor myosin II is the main component of the thick filaments in the sarcomere (Fig. 2, A and B). The molecule contains four subdomains linked by flexible connectors: the NH₂-terminal subdomain, the upper and lower 50-kDa subdomains (U50 and L50), and the converter subdomain. The domains are connected by the switch II, the strut, the relay, and the SH1 helix (Fig. 2C). Movements of the four domains are coupled by the connectors, allowing for communication between the various parts of the motor domain.

The NH₂ terminus in the motor domain—the catalytic domain—contains the adenosine triphosphate (ATP)-binding site and the actin-binding site (87, 95). Rotations of the converter and lever arm are responsible for amplifying smaller conformational changes within the rest of the motor domain (Fig. 2D). A 50-kDa cleft and the switch II in the motor domain separate the U50 and L50 domains. Switch I and switch II are especially sensitive to the presence of γ -phosphate in the active site and change configuration in response to the nucleotide state of the motor. The actin-binding interface comprises portions from both the U50 and L50 domains. Closure of the 50-kDa cleft, which is largely dictated by the conformations of the strut and switch II joints, results in an increased affinity and strong binding of myosin to actin, which changes the position of the lever arm.

Myosin-actin interactions and the active force-length relation. The cyclical interaction between myosin and actin—the cross-bridge cycle—is dependent on ATP hydrolysis, which liberates energy for the mechanical work to be produced. The cross-bridge model describes three states of myosin (Fig. 2D): 1) cross-bridge weakly bound to actin (pre-powerstroke), 2) cross-bridge strongly bound to actin (post-powerstroke), and 3) detached state. The cross-bridge cycle can be generally described as follows (Fig. 2E). 1) One ATP molecule binds to the motor domain of myosin (subfragment 1, S1), and changes the lever configuration, forcing the dissociation from the actin filament. 2) The ATPase in S1 cleaves ATP into adenosine diphosphate (ADP) and inorganic phosphate (P_i). At this step, ADP and P_i are held into the myosin head, which changes its conformation again, so that the lever increases its angle and points the myosin head toward the actin filament. 3) After myosin-actin binding, P_i is liberated from the S1, triggering the powerstroke; the myosin head moves and slides the actin filament towards the M-line of the sarcomere. 4) ADP is

released and the myosin S1 goes back to its initial configuration.

When several cross-bridges cooperatively interact with the actin in a random fashion, they slide the actin filament over the thick filament towards the center of the sarcomeres. Many sarcomeres contracting in series contract the myofibrils, causing shortening of the whole muscle fiber. Assuming that 1) myosin molecules work independently and bind to actin in a cyclical and random fashion, and that 2) the filaments of actin and myosin are mostly inextensible, the active force should be directly proportional to the degree of filament overlap within the sarcomeres. In a landmark study that is commonly used as a reference for the sliding filament theory, Gordon et al. (33) performed experiments with single muscle fibers from the frog to derive a force-length relation. The authors measured the average sarcomere length in a central segment of the fibers containing ~50,000 sarcomeres and used a feedback system to maintain the segment isometric during contractions. When the segment shortened or stretched during contractions, the entire fibers would respond by elongating or shortening, respectively. As a result, the fiber length changed during contractions, and the force would not achieve a steady state. Instead, force first rose rapidly, and then more slowly, creating the “creep” phase of the contraction. To avoid the creep phase, Gordon et al. (33) used an extrapolated force before maximal force was achieved to derive the classic force-length relation. The extrapolated force was maximal across sarcomere lengths of 2.00 μm –2.25 μm , a region where the overlap between the thick and thin filaments is optimal. At longer lengths, force decreased linearly with the decrease in filament overlap and reached zero in a sarcomere length of 3.65 μm , where overlap ceases.

Titin and the passive forces. Titin is the largest sarcomeric protein (3–4 MDa) (6) and spans from the Z-line to the M-line of the sarcomere. The titin in the A-band of the sarcomere is arranged in a highly conserved repeating pattern (34) (Fig. 1C). The structure of titin in skeletal muscles is composed of a distal and a proximal segment of tandem Ig residues, one PEVK (proline, glutamate, valine, lysine) domain separating the two Ig domains, and a N2A segment (up to 2,200 residues) between the end of the proximal Ig domain and the PEVK domain. Both Ig and PEVK domains are longer in skeletal muscles than in cardiac muscles (52). Skeletal muscle N2A titin isoforms are classified in slow and fast, according to the muscle fiber type, but the majority of the skeletal muscle fibers express just one isoform of titin. Slow muscles express the longest isoform of titin, whereas the fast muscles express a shorter isoform of titin (52).

At a given sarcomere length, the passive force is inversely proportional to length of the titin isoform. In skeletal muscles, titin Ig-segments control passive force development from the slack length of 2.0 μm to the extended length of 2.7 μm , at which length PEVK extension starts to predominate (31, 58). Several short domains of the I-band titin behave as molecular springs. They are organized in a tandem fashion, forming long segments that respond to sarcomere length changes and develop passive force.

While the PEVK domain accounts for most of the titin extension (57, 58, 103), Ig-segments are able to adjust their length in physiological sarcomere lengths (63). Recent work performed by Rivas-Pardo et al. (88) showed directly that Ig-segments unfold and refold under low forces (6–8 pN) in

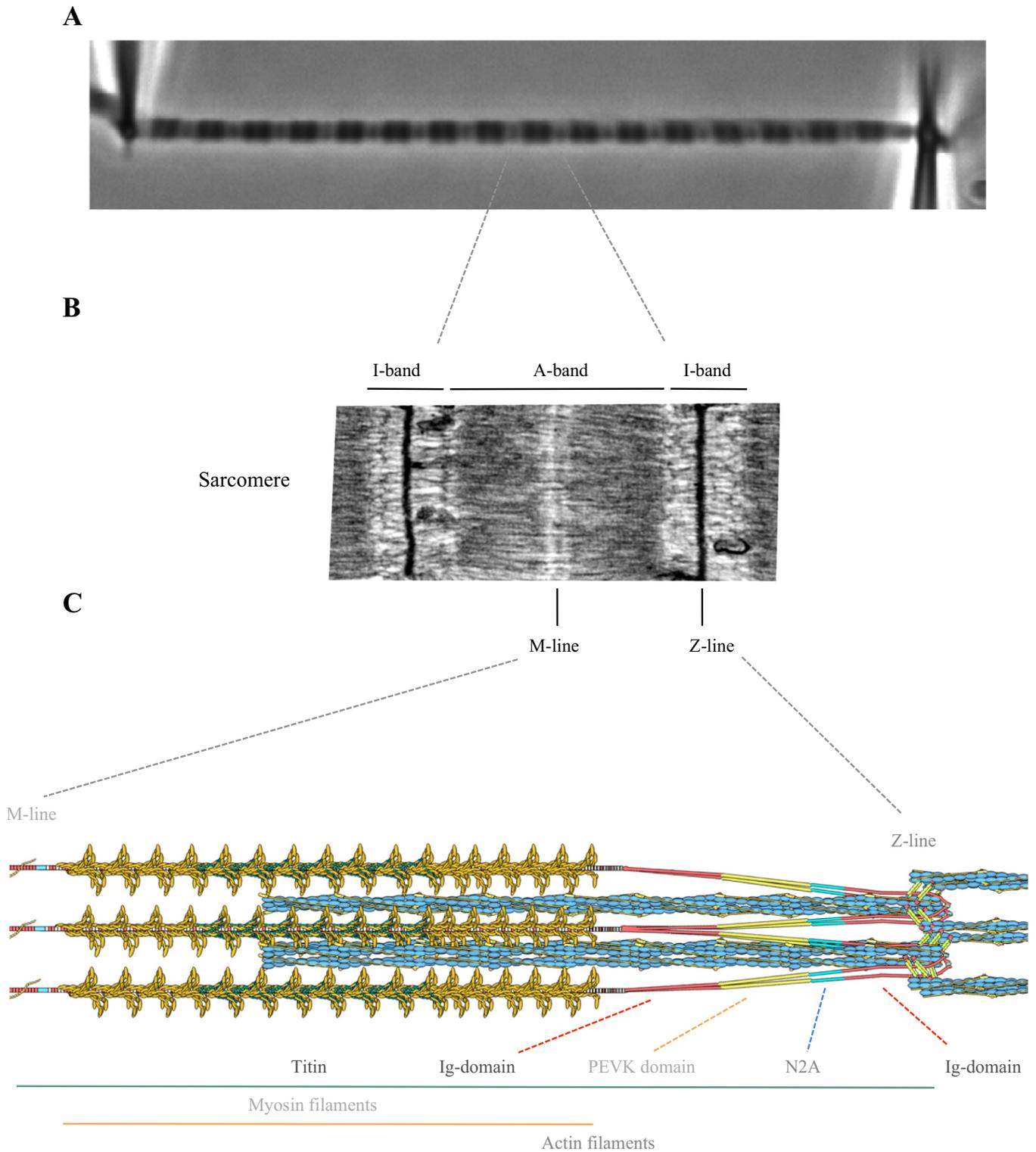


Fig. 1. *A*: a skeletal muscle myofibril suspended between two microneedles. The myofibril is formed by sarcomeres arranged in series. There is a striation pattern due to the thick and thin filaments. *B*: close view of one sarcomere under electron microscopy. *C*: schematic representation of the main protein within the sarcomere: myosin (thick filaments), actin (thin filaments), and titin composed of several different domains.

the I-band region of intact myofibrils at physiological sarcomere lengths. The authors also observed that segments of the titin proximal Ig-domain unfold and refold under forces of 6 pN, consistent with the results observed in myofibrils (88).

Of special importance for this review, titin has Ca^{2+} binding sites in the PEVK domain. Tatsumi and colleagues (96, 97) used ^{45}Ca autoradiography technique and observed that titin (then called α -connectin) had Ca^{2+} binding sites in the area

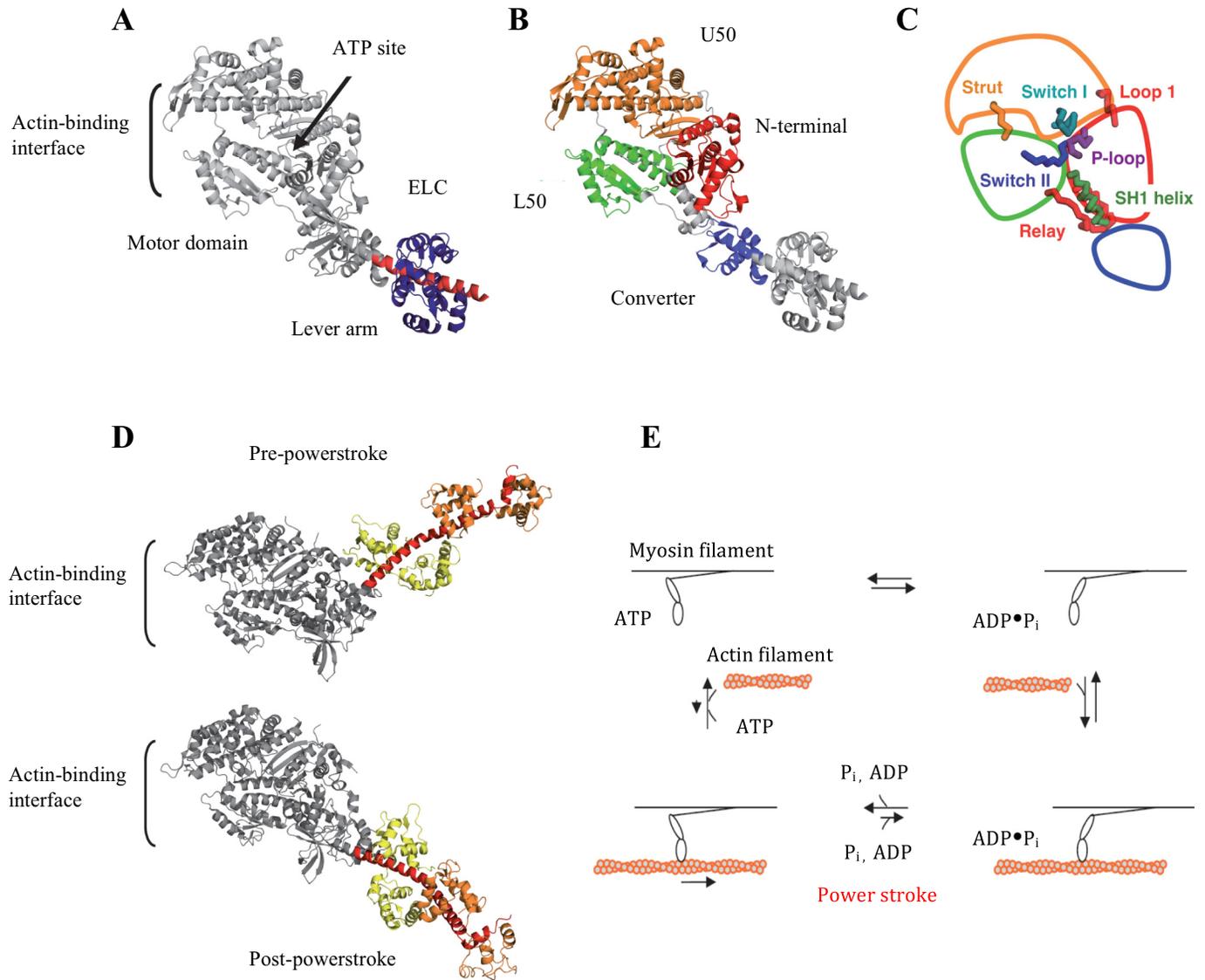


Fig. 2. Structure of the myosin molecule and the cross-bridge cycle. *A*: the motor domain of myosin is shown in gray, the neck domain is shown in red, and the essential light chain (ELC) is shown in blue. *B*: the four subdomains of the motor domain are the NH₂-terminal subdomain (red), the upper 50-kDa subdomain (U50, orange), the lower 50-kDa subdomain (L50, green), and the converter subdomain (blue). *C*: the four joints in the myosin molecule are the switch II (blue), the strut (orange), the relay (red), and the SH1 helix (green). The P-loop (purple), the Loop 1 (orange), and the switch I (teal) are also shown in this representation. *D*: two conformations of the myosin lever arm [based on *Agropecten irradians* (bay scallop) myosin II]. The motor domain is gray, the neck domain is red, and the two light chains are orange and yellow. Consistent with the lever arm model, the pre-powerstroke (pdb: IQVI) and post-rigor (pdb: 1SR6) states of myosin show small conformational changes in the motor domain coupled to a large change in the position of the lever arm. *E*: myosin chemomechanical cycle as described in the text.

spanning from the N2A segment to the M-line (then called β -connectin portion). These findings led the authors to suggest that the main Ca²⁺ binding region of titin was the PEVK segment. Subsequently they showed that circular dichroic spectra of a 400-kDa fragment of titin, which constitutes the NH₂-terminal elastic region of β -connectin in the PEVK region, were changed by the binding of Ca²⁺ ions (98). Consequently, Labeit et al. (51) observed that a minimal titin fragment containing a central E-rich domain with glutamates flanked by PEVK repeats changes its conformation in response to Ca²⁺ binding. Since skeletal muscle titin contains a variable number of PEVK repeats and E-rich motifs (6), any Ca²⁺ effect on the conformation of the PEVK is significant. An elevation in intracellular Ca²⁺ concentration and Ca²⁺ binding

to the PEVK region of the titin causes a decrease in its persistence length, which is associated with an increase in stiffness, and consequently passive force production (51).

The Active Force-Length Relation and Sarcomere Length Nonuniformity

The original force-length relation presented by Gordon et al. (33) has been largely accepted and repeated by other investigators (3, 26, 35), despite the fact that force was not always directly quantified. However, there are also controversial results in the literature. Several studies have plotted the force-length relation using the maximal force obtained during the contractions instead of the extrapolated force and found dif-

ferent results. Besides, studies that measured the force and sarcomere length during contractions where the fibers were allowed to freely shorten, without clamping a population of sarcomeres, also found contrasting results. Most of these studies were conducted with fibers from the frog and observed a force-length relation with maximal forces between sarcomere lengths of 1.6 μm and 3.0 μm (10, 28, 29, 35, 62, 101). The force varies little between maximal overlap and half-maximal overlap and falls to only ~50% of the maximum force in an average sarcomere length of 3.4 μm where only ~10% of the available cross-bridges should overlap with the actin filament (Fig. 3A). Forces of ~20% of maximal values were observed at sarcomere lengths of 3.8 μm –4.0 μm , where conceptually force should not be produced. Studies performed with mammalian muscle fibers repeated the same basic observation, and although the sarcomere length ranges differ owing to the varying lengths of the filaments, an extended plateau in the force-length relation was observed (85, 102). Finally, studies with *Limulus* muscles, in which the filaments are longer than in vertebrates, repeat the same observation that does not fit the classic force-length relation (104).

Mechanism. After much debate, the results observed in studies in which the force-length relation differs from the original study by Gordon et al. (33) were attributed to sarcomere length nonuniformity, which develops when fibers are allowed to shorten before reaching maximal force (24–26). Such redistribution of segment lengths has also been associated with the creep observed during tetanic contractions (33).

There is evidence that sarcomere lengths are shorter near the ends compared with the middle of highly stretched fibers (10, 43, 48). Edman and Reggiani (25, 26) showed that the majority of the sarcomeres situated near the ends of the fibers shorten, whereas the majority of the sarcomeres in central parts of the fiber elongate during contraction at long lengths (25, 26). As a result, small differences in sarcomere lengths can lead to large changes in the force upon activation of muscle fibers. Assuming that each sarcomere follows an individual force-length relation, strong sarcomeres will shorten at the expense of the weaker sarcomeres, which will lengthen. The average velocity of all sarcomeres will be equilibrated. Since the slope of the elongating side of the force-velocity relation is steeper than that of the shortening side, the force transmitted across these two groups of sarcomeres in series will lie closer to the isometric force of the shorter (stronger) sarcomere than to the force produced by the “average” isometric sarcomere length (70, 71). Such mechanism assumes that individual sarcomeres will continue to change length upon activation, resulting in increased inhomogeneity and an enhanced force during a tetanic contraction. Evidence supporting this hypothesis was provided in a study performed in our laboratory conducted with mechanically isolated sarcomeres (75). The force-length relation obtained in this study was similar to a theoretical curve based on filament overlap and similar to the relation derived by Gordon et al. (33). The plateau of the force-length relation was observed between 2.0 μm and 2.4 μm , where filament overlap is optimal for the psoas muscle, and the descending limb was fitted with a straight line between 2.4 μm and 3.5 μm , which provided an abscissa extrapolating to 3.87 μm .

The Passive Force-Length Relation

When skeletal muscles are stretched without activation, there is an increase in passive forces that is developed mostly by titin molecules. In skeletal muscles, the increase in passive forces does not start until sarcomeres are stretched along the descending limb of the force-length relation, and its point of inflection depends on the titin isoform (81). When evaluating the predictions of the sliding filament theory, investigators commonly discard the passive forces from the total force to isolate the active components of the force-length relation [e.g., (26, 33, 101)]. Such procedure is correct to evaluate myosin-actin interaction and assumes that activation does not change the passive force. However, there is mounting evidence that Ca^{2+} affects the force produced by non-cross-bridges structures, changing the passive force-length relation.

Labeit et al. (51) demonstrated that permeabilized fibers, in which events linked to Ca^{2+} release/uptake are not involved, produce an increase in the passive force-length relation in the presence of Ca^{2+} and in the absence of myosin-actin interactions. The results were repeated by our group, which investigated the regulation of the passive force-length relation in fibers depleted from regulatory proteins and thin filaments (19). We observed an upward shift in the force-length curve, similar to Labeit et al. (51). We also observed a small but significant upward shift in the passive force-length relation when isolated myofibrils were treated with EDTA, which depletes the preparation from troponin C, gelsolin, which depletes the preparation from thin filaments, and blebbistatin, which eliminates the possibility that myosin-actin interaction was activated in pCa 4.5 (18). Myofibrils exclude any possibility that the increase in passive forces is due to structures outside the sarcomeres, and allow direct measurements of sarcomere length during activation. The increase in force in the presence of Ca^{2+} was directly associated with the muscle types (soleus, psoas, and ventricle), which have different titin isoforms. Cardiac myofibrils did not show any increase in the passive force upon Ca^{2+} activation, while the increase was larger in psoas myofibrils than in soleus myofibrils (18). The results suggest that the increase in force with Ca^{2+} is directly associated with titin isoforms.

The studies investigating the increase in passive forces with Ca^{2+} show a notable repeatability across experimental conditions (Fig. 3B), making this observation a general phenomenon to be taken into account when investigating the force-length relation. There is one study that shows values that are remarkably different from others (54) (Fig. 3C). In this study, the authors observed an increase in force of ~350% in a sarcomere length of 6 μm after myofibrils are stretched in a pCa of 4.5 when compared with a pCa of 9.0. They observed that these forces reached levels ~700% above the active forces developed in the plateau of the force-length relation. Their results have been used for the proposal of a “new paradigm” of muscle contraction (38, 39) or a “winding filament theory” of contraction (72). It is well known that muscle fibers stretched by as little as 20% from the plateau of the force-length relation get irreversibly damaged [e.g., (5, 8, 61, 74, 107)]. Furthermore, Linke et al. (58) showed that myofibrils from psoas muscles yield and the A-band titin is dislodged from the thick filament at a sarcomere length of ~3.6 μm . Such observation was confirmed in a subsequent study that showed a yield point for

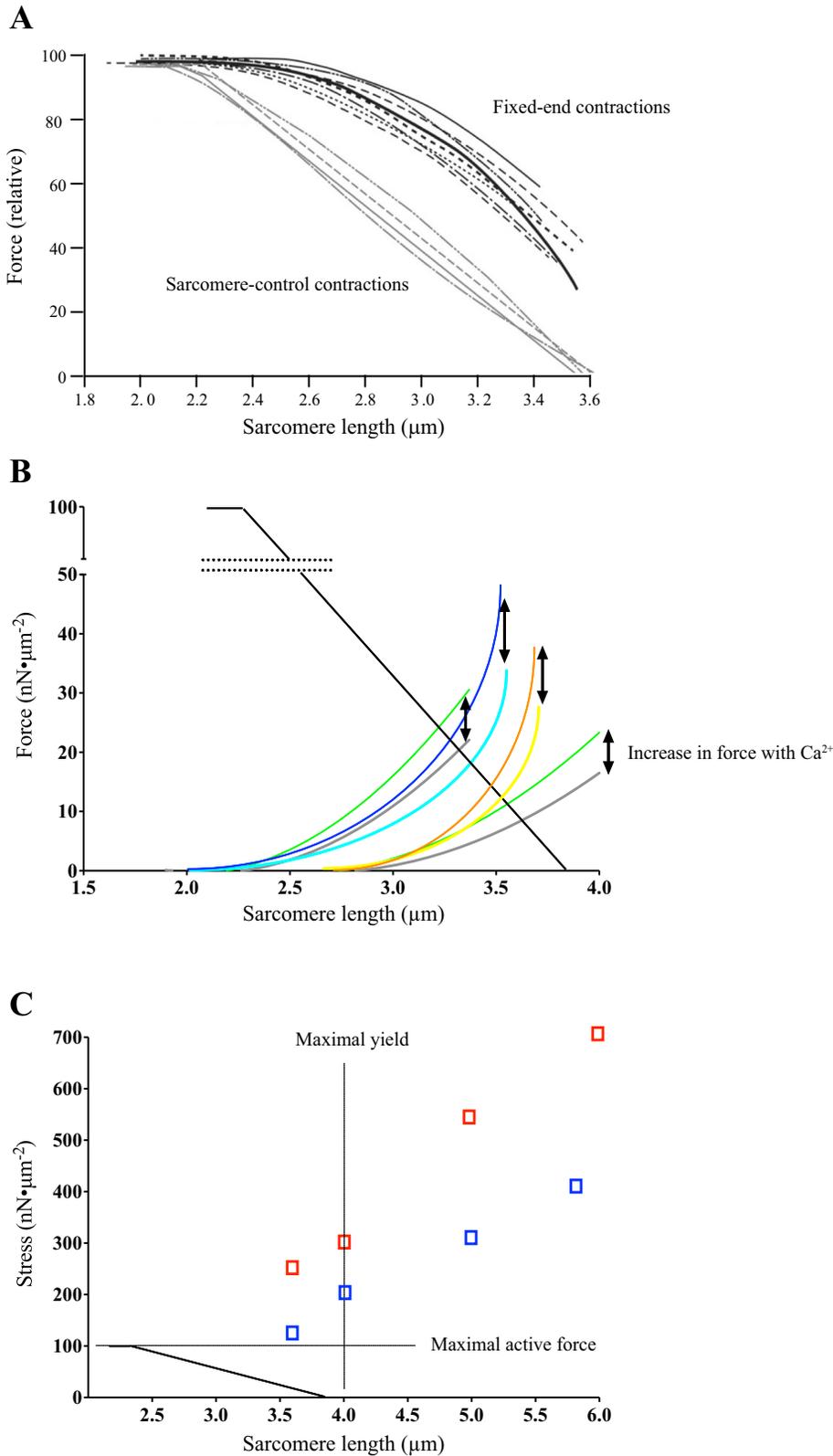


Fig. 3. *A*: force-length relation when contractions are developed with sarcomere length clamping (3, 26, 33, 35) and without sarcomere length clamping, when the maximal force is used instead of the extrapolated force (10, 11, 28, 29, 62, 101). The graph was adapted from Pollack (80). Note that the plateau is extended to long lengths, and the descending limb of the force-length relation is deviated to the right. *B*: the passive-force length relation before and after an increase Ca^{2+} concentration. The graph is based on results from three separate studies [green lines for the psoas and soleus fibers (18); orange lines for psoas fibers (19); blue lines for soleus fibers (51)]. The graph shows a small but consistent increase in the force in the presence of Ca^{2+} and absence of myosin-actin interactions, as indicated by the arrows; the upper line is always in the presence of Ca^{2+} . *C*: figure based on results from one study (54) that is highly different from other laboratories and cannot be explained by current models of contraction. The solid lines represent the theoretical force-length relation based on Gordon et al. (33). In this study (54) the force was measured with (blue squares) or without (red squares) myosin-actin inhibition in sarcomere lengths longer than $4 \mu\text{m}$. The graph shows the point in which myofibrils yield and titin is dislodged from the A-band (traced vertical line). The passive force in this case is significantly higher than the maximal active force (traced horizontal line).

myofibrils at $\sim 4 \mu\text{m}$, and beyond this point the force did not increase further, likely because of titin damage (105). Therefore, the results showing very large forces upon stretch of myofibrils to $6.0 \mu\text{m}$ cannot be reconciled with well-known

properties of skeletal muscle (54). They have not been repeated in any other laboratory.

Mechanism. There is a hypothesis to explain the increase in passive forces upon muscle activation that is supported indi-

rectly by several studies conducted independently: a Ca^{2+} -induced regulation of titin that increases the passive forces upon muscle activation. The first evidence for such mechanism arises from studies showing a "static" stiffness and tension in skeletal muscle fibers (2, 4, 14, 19, 73). When muscle fibers are activated in the presence of different myosin inhibitors that block myosin-actin interactions and then are stretched, the force increases sharply. This static tension remains elevated for as long as activation persists after the stretch (2, 4, 19). The static tension increases with the amplitude of stretch and initial sarcomere length but is independent of the velocity of stretch, characteristics that fit a titin-based mechanism of force regulation. A recent study conducted with intact fibers isolated from the mouse showed that the static stiffness is greater in extensor digitorum longus (fast) muscle than in soleus (slow) muscle (73). This muscle type dependence strengthens the possibility that static stiffness is caused by titin.

Another mechanism by which Ca^{2+} could regulate titin mechanics is by increasing the binding to actin, consequently increasing the overall stiffness of the sarcomere. It has been shown that the binding of the PEVK domain to actin can be modulated by S100A1, a member of the S100 family of EF-hand Ca^{2+} binding proteins (106). However, while one study showed that titin inhibited the sliding of the actin filaments on in vitro motility essays in the presence of Ca^{2+} , suggesting a strong actin-titin affinity (49), subsequent studies using titin fragments failed to detect binding between the tandem Ig segments of titin and actin (50, 106). In fact, one study showed that S100A1-PEVK interaction reduces the force that arises when F-actin slides relative to the PEVK domain, alleviating the PEVK-based inhibition of F-actin motility (106).

The Effects of Increasing the Load and Stretching the Muscles

If muscles are stretched while activated, they produce a substantial increase in force (1, 20, 32, 60, 76) while the rate of ATP hydrolysis is decreased (56). After stretch, force decays and reaches a steady state, which is higher than the force obtained at the corresponding length during purely isometric contractions, i.e., there is a residual force enhancement (23, 27, 86, 91, 94). Traditional cross-bridge models cannot easily fit the increase on force developed during stretch, and the residual force enhancement departs from the traditional force-length relation and predictions of the sliding filament theory.

Force increase during stretch. When the stretch is performed at slow velocities [i.e., rate of stretch < 2 optimal lengths (L_0) per second], the force enhancement has two components, 1) a steep phase, in which force increases significantly over a few nanometers per half-sarcomere, and 2) a slow phase, in which force increases less steeply or remains unchanged (20, 22, 32, 66, 67, 79). The transition between these phases is associated with the mechanical detachment of cross-bridges after they reach a critical extension (32, 83), between 8 nm and 10 nm of stretch (32, 60). The force obtained at the transition point increases as a function of the velocity of stretch, to reach a maximum of $\sim 2.0 P_0$ at $1.0 \mu\text{m}\cdot\text{s}^{-1}\cdot\text{half-sarcomere}^{-1}$ (20, 21, 30, 60, 77).

Mechanically detached cross-bridges must reattach rapidly after they detach so the force can be maintained during the

stretch (30, 32). The detachment rate must also be small to keep the range of cross-bridges populated at high velocities of stretch (17). When these ideas are implemented in cross-bridge models, the rapid attachment needed to maintain force during stretch at high velocities is inconsistent with the decline in cross-bridge number during shortening. Harry et al. (36) circumvented such difficulty assuming that the force during stretch is maintained by cross-bridges extended to extreme lengths, but they would exceed the repeat distance between actin sites.

Mechanism. Force enhancement during stretch has been attributed primarily to 1) an increased in the mean cross-bridge force or changes in the configuration of the attached cross-bridges (15, 16, 32); 2) an increase in the number of cross-bridges attached to actin (9, 55); or a combination of both.

Investigators observed an increase in fiber stiffness between 10 and 20% during or just after stretch (15, 32). They calculated that such increase is not large enough to explain the increase in force. Instead, they suggest that the force enhancement is caused largely by an increase in the mean force produced by the cross-bridges, i.e., an increased strain during stretch would induce higher cross-bridges forces. Evidence for such hypothesis comes from a series of studies in which fast stretches ($>L_0$) were imposed to muscle fibers so a clear force transient could be detected, which was associated with a critical cross-bridge extension. Increasing the force produced by cross-bridges by elevating the experimental temperatures (16), lowering the ionic strength (13), or inducing slow stretches (15) decreases the critical cross-bridge extension needed for attaining the force transient, suggesting that strained cross-bridges resist lower strains before detaching from actin.

It has also been suggested that the increased force during stretch is caused by cross-bridges working in pre-powerstroke state that precedes phosphate release (12, 32, 66, 67, 79, 83). These cross-bridges would not produce substantial force during isometric contractions, but large forces when stretched. Studies that manipulated cross-bridges into pre-powerstroke states with different interventions, including *N*-benzyl-*p*-toluene sulfonamide (BTS) (79), high concentrations of phosphate (93), vanadate (Vi) together and aluminum fluoride (AlF_4) (12, 32), or blebbistatin (67), show a large decrease in isometric force with a small decrease in stretch forces, increasing the stretch-to-isometric force ratios. Two studies performed in our laboratory with isolated myofibrils support such a mechanism. One study showed that myofibrils treated with 2,3-butanedione monoxime (BDM) showed an increased stretch-to-isometric force ratio and also an increase in the critical sarcomere length extension (83). A subsequent study showed that myofibrils activated with MgADP, which biases cross-bridges into strong bound states, presented a reverse effect; the stretch force relative to the isometric force was decreased (64).

Although these studies suggest that the increase in force is caused by an increase in the force or actomyosin state of the cross-bridges, other investigators who have rigorously measured the X-ray diffraction arising from the myosin layers have shown increases in force accompanied by an increase in stiffness of 22–60%, without apparent changes in the cross-bridge mean force (9, 55). They suggest that stretch induces an increase in the number of cross-bridges attached to actin, which could increase force significantly above isometric levels. Such increase could be accommodated by the special rates of

myosin-actin attachment/detachment. The mechanism behind the increase in the number of cross-bridges attached to actin is unclear, but Linari et al. (55) and Brunello et al. (9) provided strong evidence that it may be accomplished by the engagement of the second cross-bridge that shared the myosin S2 segment. Accordingly, the attachment of a second cross-bridge to a binding site situated next to the actin already bound to a cross-bridge would be favored by the change in strain to the filament caused by the first head. Such increase in the number of attached cross-bridges could fit into a model that assumes a new population of cross-bridges present during the stretch. Lombardi and Piazzesi (60) and Piazzesi et al. (76, 78) modeled a rapid reattachment of mechanically detached cross-bridges that populate force-producing states. This state would be populated only during stretch. The authors obtained results that were consistent with experimental observations.

Residual force enhancement. After the decay of force after the stretch, there is residual force enhancement [e.g., (23, 27, 47, 82, 86, 94)] that cannot be readily explained by predictions of the sliding filament theory: the force is higher than that produced during isometric contractions in a similar average sarcomere length (and conceptually, a similar degree of filament overlap). An example of the force-length relation derived after stretch experiments performed by Edman et al. (23) is given in Fig. 4. Note that there is a clear deviation towards larger forces after stretch.

There are many studies investigating the residual force enhancement, and the results vary according to the experimental procedures (amplitudes of stretches, initial sarcomere length, among other factors). Such variability makes challenging to plot a unique force-length relation with the values obtained after stretch. Unfortunately, the majority of these studies are highly descriptive, with experiments that are not well controlled (e.g., no measurements of sarcomere lengths; absence of control contractions during experiments) and contribute little to a mechanistic understanding of the phenomenon. When experiments with fibers that used controlled conditions are selected, in which forces are compared at similar (measured) sarcomeres lengths, the number of studies to be evaluated becomes surprisingly low [e.g., (23, 27, 47, 94)]. The levels of force enhancement observed in these studies vary approximately between 10% and 40% above the corresponding average sarcomere length.

Our group has performed two studies with myofibrils and/or small groups of sarcomeres in which the sarcomere length was measured throughout the contractions (86, 91). First, we investigated segments of myofibrils and observed force enhancement levels between ~10 and 40%, consistent with most studies with single fibers (86). More recently, we developed a system to, for the first time, synchronize the sarcomere lengths in myofibrils during and after length changes for proper comparisons of force values (91). We observed that skeletal muscle myofibrils produced an increase in force of ~9% in sarcomere lengths ranging from 2.24 μm to 3.13 μm . Finally, we investigated the residual force enhancement in mechanically isolated sarcomeres (65, 86) and mechanically isolated half-sarcomeres (65), using protocols that are similar to what has been done in single fibers (i.e., comparing isometric contractions with stretch contractions). We observed that force enhancement was present in these preparations in levels of ~10% above the reference contractions (86), showing that the residual force enhancement is associated with a sarcomeric structure.

There are two studies investigating residual force enhancement in myofibrils/sarcomeres that show values incompatible with other studies in the field (46, 53). The authors observed an increase in force after stretch at levels of 285% (53) and 386% (46) when compared with the isometric reference forces. These studies make assumptions of sarcomere length measurements and forces that are not necessarily appropriate, such as comparing stretch forces with predicted (not measured) forces produced at isometric lengths (46) and a lack of contractions to assure that the preparations are viable throughout the experiments (53). Until these results can be repeated in other laboratories with well-controlled experiments, they cannot be conciliated into a general mechanism for the residual force enhancement.

Mechanism. It has been proposed that the residual force enhancement is associated with sarcomere length nonuniformity that develops during muscle contraction (47, 68, 69). Stretch of an activated muscle would exacerbate the nonuniformity of sarcomere lengths present during fixed-end contractions, similar to what was described in a previous section. A slight difference in the proposed mechanism for the residual force enhancement is the presence of overstretched sarcomeres that would “pop” and be supported entirely by high passive forces (68, 69). However, several studies showed that sarco-

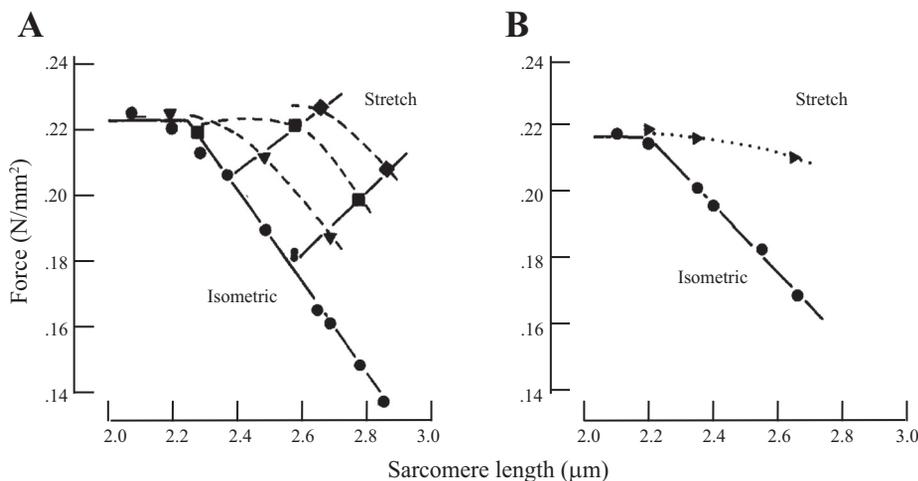


Fig. 4. Residual force enhancement following stretch of activate fibers of the frog (23). *A*: forces measured 4.2 s after the end of stretch compared with the isometric length-tension relation. *B*: forces measured in a single fiber 6 s after the end of stretch compared with the isometric length-tension relation. There is an increase in force after stretches of different magnitudes.

mere length nonuniformity alone could not explain the extra force after stretch (79, 82, 86, 89). Studies with myofibrils failed to observe popping sarcomeres, and force enhancement was observed in myofibrils without a large increase in sarcomere length dispersion (82). Most tellingly, force enhancement observed in single sarcomeres and half-sarcomeres (65) indicates that mechanisms independent of sarcomere length nonuniformity may be involved.

An alternative mechanism to explain force enhancement assumes the presence of two factors: half-sarcomere length nonuniformity that develops upon activation and after stretch, and an increase in the stiffness of titin (86). Nonuniformity in half-sarcomere lengths induced at the beginning of activation can increase throughout contractions, even in the absence of overstretch or popping sarcomeres. Half-sarcomere nonuniformities during isometric contractions have been directly observed in myofibrils (99, 100) and isolated sarcomeres (75). There are A-band displacements that follow a characteristic pattern that resembles the force-length relation. At long lengths when titin is stretched, there is less movement of A-bands during activation (75).

Half-sarcomere nonuniformity and displacements of A-bands would result in variable amounts of filament overlap. There would be more cross-bridges interacting with actin and thus more active force production in strong half-sarcomeres. Titin filaments would be stretched and become stiffer in the adjacent half-sarcomeres, increasing the sarcomere strain and balancing opposing forces from the strong halves. In fact, force enhancement increases when measurements are performed at sarcomere lengths up to ~20–30% longer than the plateau of the force-length relation (23, 27), a region where A-band displacement is significant (75) and passive forces start to play a role in most skeletal muscles. Furthermore, there is a strong correlation between force enhancement and A-band displacements (84). Simultaneously to the increase in passive strain and increase in filament overlap, A-band displacements would cause cross-bridges to constantly stretch while the half-sarcomeres are not stabilized (75, 99), which could add to the force enhancement by a mechanism similar to what happens during muscle fiber stretch.

Concurrent with the half-sarcomeres increasing the overlap in one-half of the sarcomere and the stiffness of titin in the other half, the “passive” force produced by titin can also be increased further owing to the augmented stiffness of the PEVK domain of titin with Ca^{2+} , which increases further the force after stretch. The mechanism explains well the observations described in the previous sections.

Finally, the finding that Ig-domains of titin spontaneously unfolds and refolds against small forces suggests that titin may play an important role during active force generation. Rivas-Pardo et al. (88) calculated that the refolding events deliver significant contractile energy during myofibril activation, even higher than that released by myosin motors. Such additional mechanism for active force generation is still controversial (7), but if confirmed in further studies, it could contribute to the residual force enhancement. If stretching activated muscles would provoke additional unfolding/refolding events in the Ig-domain, it could increase the force beyond the levels obtained during isometric contractions. Such mechanism needs to be evaluated in the future.

Conceptual Framework to Explain Deviations From the Force-Length Relation

On the basis of studies produced by single fibers, myofibrils, sarcomeres, and half-sarcomeres, the following sequence of mechanisms is proposed to explain the observations discussed in this review.

- 1) Nonuniformity of sarcomere lengths that happens naturally in single fibers increases upon activation and leads to an equilibrium state that produces a force that is larger than that predicted by the average filament overlap; strong sarcomeres are supported by passive sarcomeres through the stiffness of titin.
- 2) There is a stiffness of the PEVK segment of titin upon activation that increases the passive force and shifts the force-length relation upward; the increase in the passive force also balances the force produced by stretched sarcomeres with those with a higher active force produced due to an increased filament overlap.
- 3) If the muscles are stretched upon activation, there is an increase in the nonuniformity of half-sarcomere lengths, which changes the overlap between filaments. There is also an increase in the force produced by titin, which is stiffer due to the Ca^{2+} .

These proposed mechanisms would explain most observations made in studies that are well controlled. They are simple and have been consistently tested. Most importantly, they eliminate the need to evoke “new theories of contraction” that cannot fit the most common properties of muscle contraction that have been documented in the literature.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

D.E.R. prepared figures; drafted manuscript; edited and revised manuscript; approved final version of manuscript.

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