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Editorial

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An Elastic Link Between Length and Active Force Production in Myocardium

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Studies by Frank, Starling, and colleagues demonstrated that elevations of end-diastolic volume increase cardiac output in working hearts,[1] a phenomenon referred to as the Frank-Starling (FS) relationship. It stands as one of earliest descriptions of the importance of diastolic dimensions to systolic function, yet how alterations in sarcomere length (SL) influence myofilament Ca^{2+} sensitivity, and hence the inotropic state of the heart, is not completely understood. The FS relationship in the intact ventricle is qualitatively similar to the underlying length-tension relationship in single cells. As length is increased from short SLs ($\approx$1.7 μm), the force developed by a myocardial cell increases to a peak at an SL of $\approx$2.3 μm, corresponding to the maximum SL in working hearts. Similarly, when end-diastolic volume is increased, cardiac work during systole also increases. It is clear, however, that the FS relationship in cardiac muscle cannot be explained as straightforwardly as the length-tension relationship in skeletal muscle, ie, based solely on changes in overlap of the thick and thin filaments. In skinned myocardium, the length-tension relationship obtained during maximal activation with Ca^{2+} looks very much like that seen in maximally activated skeletal muscle. At submaximal concentrations of Ca^{2+}, however, the relationship becomes much steeper, so that force increases rapidly as SL is increased from 1.7 to $\approx$2.3 μm. Thus, the steepness of the FS relationship appears to be a predictable consequence of submaximal levels of Ca^{2+} activation during a typical cardiac twitch. Consistent with this interpretation, there is compelling evidence that length-dependent increases in contraction involve an increased sensitivity of the myofilaments to activation by Ca^{2+}, ie, there is a leftward shift in the force-pCa relationship.

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This shift of the force-pCa relationship requires a structural element(s) capable of detecting and transducing changes in SL into either an increase in the affinity of the thin-filament regulatory protein, troponin C (TnC), for Ca^{2+} or an increase in the number of force-generating cross-bridges formed for a given level of Ca^{2+} binding to TnC. There is convincing evidence that both conditions occur as SL is increased from 1.7 to 2.3 μm. Measurements of Ca^{2+} binding and of intracellular Ca^{2+} transients have demonstrated that TnC exhibits a greater affinity for Ca^{2+} at longer SLs.[1,2] Although the mechanism underlying this change in affinity is unclear, it appears to be a positive feedback response to increases in cross-bridge–generated force.[2] Furthermore, increases in SL result in generation of greater force at given Ca^{2+} concentrations. The following hypothesis is the most widely favored explanation for this latter effect. Because of the opposing effects of negative surface charges on the thick and thin filaments and constraints of the surface membrane, the volume of a cardiac cell remains constant when it is lengthened, resulting in a decreased cell diameter. As a consequence, the lateral spacing between the thick and thin filaments, the "filament lattice spacing," is decreased such that the myosin heads are brought closer to the actin monomers in the thin filaments. This increase in the effective concentrations of the reacting species leads to the formation of a greater number of cross-bridges during a subsequent contraction. The finding that neither the length-dependent leftward shift of the force-pCa relationship nor the increase in Ca^{2+} binding to TnC was observed when lattice spacing was held constant by osmotic compression[3,4] suggests that changes in filament spacing constitute an important step in the FS relationship and that increases in SL result sequentially in decreased filament lattice spacing and an increase in the number of cross-bridges bound to actin. An increase in cross-bridge number should increase force proportionally; however, increased cross-bridge binding also appears to increase force indirectly by cooperative recruitment of additional cross-bridges and increasing Ca^{2+} binding to TnC.[5] Consistent with this hypothesis, osmotic compression of skinned cardiac preparations decreases filament lattice spacing and produces a leftward shift of the force-pCa curve independent of changes in SL.[4]

Given the preceding hypothesis, an important question concerns the identity of the structural element(s) that senses changes in SL and induces downstream alterations in cross-bridge state and/or TnC Ca^{2+} sensitivity. Although it might be assumed that mechanical and hydrostatic forces across the cell membrane are sufficient to reduce filament spacing at long lengths, recent studies by Cazorla et al[6] and Fukuda et al[7] suggest that this is not the case, at least not entirely. Data from both groups confirm and extend earlier suggestions[8] that alterations in passive stress mediated by the extensible sarcomeric protein titin qualify as one sensor of SL in the heart. The molecular mechanism(s) through which changes in titin-mediated passive stress affect Ca^{2+} activation of force

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remain unclear, however, because the 2 groups suggest that different mechanisms are involved. Cazorla et al find that titin-mediated passive stress at longer SLs results in decreased lattice spacing and a leftward shift of the force-pCa curve. In contrast, Fukuda et al suggest that the influences of titin on length-dependent activation require neither changes in filament spacing nor alteration of the force-pCa relationship.

Before considering these reports further in greater detail, it is useful to review how titin's molecular and physical properties, localization within the sarcomere, and interactions with other sarcomeric proteins position it to serve as a sensor of SL. Titin is a very large protein consisting of a single ≈3-MDa polypeptide. With a length greater than 1 μm, a single titin polypeptide extends across a half-sarcomere, with its N- and C-termini crossing the Z disc and M line, respectively. Titin contains multiple immunoglobulin domains and a PEVK (enriched in proline [P], glutamine [E], valine [V], and lysine [K]) domain within the I band. These domains reversibly unfold and can be viewed as 2 springs connected in series. Titin interacts with other sarcomeric proteins, including (1) in the Z disc, a protein called the "titin cap," or T cap, and α-actinin (which connects thin filaments and the overlapping ends of titin molecules from adjacent sarcomeres); (2) in the I band, actin and the muscle-specific calpain protease p94; (3) in the A band, the tail region of myosin and myosin-binding protein C (protein C); and (4) in the M line, myomesin and the p94 calpain protease. Although some of these interactions are inferred from in vitro studies, the intersarcomeric connections between titin molecules and intrasarcomeric ones between titin, actin, and myosin provide the physical basis for sensing changes in SL. In addition, multiple phosphorylation sites exist near the N- and C-termini, and a serine/threonine kinase domain is present in the N-terminal (M line) region of titin. Additional details concerning the structure and function of titin can be found in 2 recent reviews.[9,10]

Consistent with its elastic nature and its position within the sarcomere, titin (together with collagen) is a primary determinant of passive stress and stiffness in cardiomyocytes,[11,12] with contributions by titin dominating at shorter SLs.[12] Titin also appears to provide at least part of the force responsible for restoring resting length after a contraction and assisting ventricular filling.[13] Two alternatively spliced titin isoforms are expressed in the heart, perhaps within the same cardiomyocyte.[11] Various ratios of these isoforms, which differ in the length of the extensible I-band region, correlate with differences in the stiffness of cardiomyocytes from different species.[11]

As introduced above, recent work by Cazorla et al and Fukuda et al provide additional and differing insights into the role(s) of titin-mediated passive stress in cardiac muscle. Cazorla et al investigated this issue in skinned mouse myocyte and myocardial preparations by measuring force-pCa relationships at SLs that normally have low (2.0 μm, $SL_{2.0}$) and high (2.3 μm, $SL_{2.3}$) passive forces. Titin-dependent passive stress was varied over a range of intermediate values before activation at $SL_{2.3}$ by use of the relaxation of passive stress that occurs after rapid increases in preactivation SL or exposure to trypsin (which selectively degrades titin in striated muscles.[6,7]) Filament spacing was altered independently of changes in SL by osmotic compression with dextran T-500.[4] The results are generally consistent with the hypothesis that sequential changes in SL, titin-mediated passive stress, and filament spacing lead to increased cross-bridge formation and increased Ca^{2+} binding to TnC. (1) The leftward shift of force-pCa curves obtained at $SL_{2.3}$ (relative to $SL_{2.0}$) was directly related to the passive stress imposed at $SL_{2.3}$. (2) Filament spacing (inferred from measurements of cell width) was inversely related to changes in passive stress. (3) Decreased filament spacing due to osmotic compression also left-shifted the force-pCa curve at both SLs (relative to that obtained without dextran). A caveat noted by the authors, however, concerns the finding that although degradation of titin reduced passive force by ≈80% and increased filament spacing (measured directly from x-ray patterns in skinned muscle preparations), the force-pCa relationship at $SL_{1.9}$ was unchanged. This suggests that the Ca^{2+} sensitivity of force development is not influenced solely by changes in titin-mediated passive stress and filament spacing.

Fukuda et al also conclude that titin-mediated passive stress is an important element in length-dependent activation of cardiac muscle. These workers studied this phenomenon in rat skinned right ventricular trabeculae and also used trypsin to selectively degrade titin and dextran to osmotically alter filament spacing. The data obtained by these groups, however, differ in several significant ways. (1) Fukuda et al found that exposure to trypsin reduced maximal active force at SLs ≥1.9 μm. In contrast, in an earlier study, Cazorla et al[8] found that maximal force developed by isolated rat cardiomyocytes at SLs of 1.9 and 2.3 μm was unchanged after trypsin. (2) In the studies by Fukuda et al, filament spacing (estimated from changes in fiber diameter) was not altered by trypsin. In contrast, Cazorla et al[6] observed that trypsin increased filament spacing (measured directly from x-ray patterns from mouse myocardium) at all SLs studied. (3) The leftward shift of the force-pCa curve with increased SL ($SL_{2.3}$ versus $SL_{1.9}$) observed by Fukuda et al was unaffected by trypsin, whereas trypsin partially reversed the shift observed by Cazorla et al.

As suggested by Fukuda et al, the preceding differences could be due to greater levels of collagen in rat trabeculae and the ability of this extracellular matrix component to control filament spacing independently of titin. In this regard, it would be interesting to compare the levels of collagen in rat and mouse hearts, because trypsin increased filament spacing in mouse skinned myocardium[6] and the effectiveness of the trypsin treatment used in the 2 studies. Cazorla et al[8] found previously that passive stress had to be decreased by >30% before Ca^{2+} sensitivity was altered by trypsin in rat cardiomyocytes. Although Fukuda et al obtained somewhat larger changes than this, differences in efficacy of trypsin treatment between the studies, perhaps due to the greater level of collagen present in rat trabeculae, might contribute to the differing results. In any case, it appears that titin-mediated passive stress may influence length-dependent activation through (at least) 2 mechanisms. The first involves the changes in filament spacing, cross-bridge forces, and TnC Ca^{2+} affinity described above. A second mechanism sug-

gested by Fukuda et al involves titin-mediated stress-induced conformational changes in myosin that increase thick-filament disorder and the number of weak binding cross-bridges, which have an increased probability of being activated at a given level of Ca^{2+}. This mechanism was proposed earlier for insect flight and mammalian skeletal muscles by Granzier and Wang[14] and was noted as a possibility by Cazorla et al.[6] It has also been suggested to underlie the influences of myosin light chain phosphorylation on skeletal muscle contraction.[15,16]

An interesting aspect of the data of Fukuda et al is that although trypsin decreased Ca^{2+}-activated force, it did not affect the force-pCa relationship. It might be anticipated that the latter relationship would be shifted to the right if the number of weak binding cross-bridges were decreased. For example, myosin light chain phosphorylation in skeletal muscle, which is proposed to increase the number of these cross-bridges, left-shifts the force-pCa curve.[15,16] Inspection of the results shown in Figure 4A of Fukuda et al reveals that trypsin decreased Ca^{2+}-activated force in a proportionate manner at all SLs greater than the slack length. Thus, trypsin-induced decreases in absolute active force disappear when the data are normalized against the maximal value obtained for each treatment group, as is performed to generate typical force-pCa curves (see Figure 8 of Fukuda et al). At least under the conditions used by Fukuda et al, changes in titin-mediated passive stress produce a Ca^{2+}-independent offset of the amplitude of Ca^{2+}-activated force. Alterations in Ca^{2+}-dependent variables, such as the Ca^{2+} affinity of TnC and the quantity of activating Ca^{2+} available, remain as the major players in determining the steepness of the ascending limb of the FS relationship. The results from Cazorla et al and Fukuda et al, however, indicate that changes in titin-mediated passive stress and attendant changes in cross-bridge properties also contribute to this relationship. The latter changes appear to be both Ca^{2+}-dependent (Cazorla et al) and Ca^{2+}-independent (Fukuda et al). These results also suggest that a multivariate approach is necessary to adequately assess the contributions of Ca^{2+}-dependent and passive force (titin, collagen, etc)-dependent factors to the FS relationship.

A final issue concerns the extent to which the FS relationship influences cardiac output under normal and pathophysiological conditions. As discussed by Lakatta,[17] the FS relationship, in concert with changes in contractility and heart rate, is an important determinant of performance in the intact heart. Moreover, although the extent of change is controversial, the length dependence of activation is altered in heart failure.[18–20] This may be due to alterations in the quantity of activating Ca^{2+} made available for a contraction[18]; the results obtained by Cazorla et al and Fukuda et al and the observation that the level of titin is decreased in at least some types of heart failure,[19] however, suggest that chronic changes in titin-mediated stress could also affect function in the failed heart. Moreover, the multiple phosphorylation sites and the kinase domain in titin, its interactions with other sarcomeric proteins, including a Ca^{2+}-dependent protease, and the expression of alternative splice variants provide the means for dynamic regulation of titin on short- as well as long-term time scales. In addition, if differences in level of collagen affect the functional consequences of changes in titin-mediated passive stress, as suggested by Fukuda et al, it will be important to consider alterations in the extracellular matrix, such as the fibrosis associated with cellular remodeling during heart failure, when analyzing the FS relationship.

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