Myosin Crossbridge Activation of Cardiac Thin Filaments: Implications for Myocardial Function in Health and Disease

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Abstract—At the level of the myofibrillar proteins, activation of myocardial contraction is thought to involve switch-like regulation of crossbridge binding to the thin filaments. A central feature of this view of regulation is that \( \text{Ca}^{2+} \) binding to the low-affinity \( (\approx 3 \, \mu\text{mol/L}) \) site on troponin C alters the interactions of proteins in the thin filament regulatory strand, which leads to movement of tropomyosin from its blocking position on the thin filament and binding of crossbridges to actin. Although \( \text{Ca}^{2+} \) binding is a critical step in initiating contraction, this event alone does not account for the activation dependence of contractile properties of myocardium. Instead, activation is a highly cooperative process in which initial crossbridge binding to the thin filaments recruits additional crossbridge binding to actin as well as increased \( \text{Ca}^{2+} \) binding to troponin C. This review addresses possible roles of thin filament cooperativity in myocardium as a process that modulates the activation dependence of force and the rate of force development and also possible mechanisms by which cooperative signals are transmitted along the thick filament. Emerging evidence suggests that such mechanisms could contribute to the regulation of fundamental mechanical properties of myocardium and alterations in regulation that underlie contractile disorders in diseases such as cardiomyopathies. (Circ Res. 2004;94:1290-1300.)

Key Words: \( \text{Ca}^{2+} \) regulation \[ \text{cardiac contractility} \] \[ \text{cardiomyopathy} \] \[ \text{cooperativity} \]

Mammalian hearts are capable of remarkable beat-to-beat variations in both the force and power of contraction, which ensures that venous blood is pumped forward as quickly as it returns to the heart. Matching cardiac contractility to hemodynamic load is a critical design feature in assuring the effectiveness and efficiency of the heart as a pump. The in vivo control systems that regulate contractility are multilayered and complex—contractility is in large part determined by nervous outflow to the heart and circulating chemical messengers, but mechanisms intrinsic to the myocardium contribute to these control systems to a surprising degree. For example, increases in venous return to an isolated working heart result in concomitant increases in stroke volume, a classical observation that describes the Frank-Starling relationship.1

At resting lengths, the amount of \( \text{Ca}^{2+} \) released from intracellular stores during a myocardial twitch is typically insufficient to saturate thin filament sites, so force and power are submaximal. As a consequence, increases in twitch force and power can be achieved by increasing the likelihood of
crossbridge binding to actin, eg, increased delivery of Ca\(^{2+}\) to the myoplasm, acceleration of crossbridge cycling kinetics, or increased responsiveness of the thin filaments to the activating effects of Ca\(^{2+}\) or crossbridge binding.

At the level of the myofilaments, activation of contraction is typically thought to involve switch-like regulation of crossbridge binding to the thin filaments (Figure 1). A central feature of this view of regulation is that Ca\(^{2+}\) binding to the low-affinity (\(\approx 3 \, \text{mmol/L}\)) site on troponin C (TnC) alters the interactions of proteins in the thin filament regulatory strand, which leads to movement of troponyosin from its blocking position on the thin filament and binding of crossbridges to actin. Although Ca\(^{2+}\) binding is a critical step in initiating contraction, this event alone does not account for the activation dependence of contractile properties of myocardium or the variations in contractile kinetics that are observed in vivo depending on sympathetic tone or in diseases such as end-stage heart failure. A simple regulatory switch would control only the number of crossbridges and not the kinetics of their interaction with actin. Thus, increases in myoplasmic [Ca\(^{2+}\)] would increase the force of contraction but would not affect the rates of force development or relaxation. In recent years, a growing body of work has shown that protein function within the thin filament is modulated by several factors, including the binding of myosin to actin, phosphorylation of myofibrillar proteins, and near-neighbor cooperative interactions extending from active into inactive regions of the thin filament. None of these mechanisms is obviously required for myocardial contraction, but all of them contribute to the force and speed of contraction in ways that serve to match contractility to ventricular workload.

This Review addresses the mechanisms of myocardial regulation beyond the initial binding of Ca\(^{2+}\) to TnC. The discussion emphasizes the activating effects of crossbridge binding to actin and the resulting changes in force and the rates of force development and relaxation.

**Steady-State Force Development**

The isometric force-generating capability of myocardium varies as a function of myoplasmic [Ca\(^{2+}\)] (reviewed by Gordon et al\(^2\)), which is evident in the relationship between force and pCa (\(-\log[\text{Ca}^{2+}]\)) in skinned myocardial preparations (Figure 1). The force-pCa relationship is sigmoidal (Figure 2) and is typically well fit with the Hill equation\(^3\):

\[
P = P_0 \frac{1}{1 + 10^{n(pCa-pCa_{50})}},
\]

where \(P\) is force, \(P_0\) is maximum force, \(n\) is the Hill coefficient, and \(pCa_{50}\) is the pCa of the activating solution at half-maximal activation. The Hill coefficient provides an estimate of the minimum number of binding sites involved in the regulation of force and is typically \(>3\) in cardiac muscle. The apparent discrepancy between the Hill coefficient and the presence of just one regulatory Ca\(^{2+}\) binding site on TnC has been taken to mean that there is positive cooperativity in the activation process,\(^2\) such that force at any [Ca\(^{2+}\)] is greater than would be predicted on the basis of a single binding site. Cooperativity could arise in Ca\(^{2+}\) binding to TnC or crossbridge binding to the thin filament or crossbridge-induced increases in the Ca\(^{2+}\) binding affinity of TnC.\(^4,5\)

Thus, whereas myocardial contraction is initiated by Ca\(^{2+}\) binding to TnC, any description of the activation of force and the kinetics of force development requires inclusion of activating effects because of crossbridge binding to actin.\(^2,6–8\)

Binding studies of skeletal muscle proteins\(^9\) suggest that activation of a thin filament functional group (estimated to be \(\pm 10\) to 14 actin monomers\(^10,11\)) by Ca\(^{2+}\) or strong-binding crossbridges influences the activation of neighboring functional groups.\(^6\) When only a few functional groups are activated within a thin filament, which is the case at low [Ca\(^{2+}\)], force at each [Ca\(^{2+}\)] would be greater than predicted on the basis of a single Ca\(^{2+}\) binding site for each 7 actin monomers and the Hill coefficient would be \(>1\). Such a mechanism has gained support from experiments in which
Increasing the strength of either one of these interactions will increase the value of the Hill coefficient. However, it is possible in the model to simultaneously adjust the strength of these interactions in opposite directions, so that the slope of the force-pCa relationship is unchanged even though the underlying cooperative processes have been altered. Such effects are most likely mediated by interactions between adjacent troponymosins as well as other elements of the regulatory strand, such as effects of crossbridge and Ca$^{2+}$ binding on interactions between troponin and tropomyosin.$^{7,15}$ The Hill coefficient could be unchanged if, for example, the strength of interactions between adjacent functional groups is increased while the cooperative activation of functional groups by strongly bound crossbridges is reduced.

**Cooperativity in Ca$^{2+}$ Binding to Cardiac TnC**

Greater than expected forces at submaximal Ca$^{2+}$ concentrations could be attributable to an increase in the affinity of TnC binding sites for Ca$^{2+}$ resulting from an increase in either the number of crossbridges bound to actin or the amount of Ca$^{2+}$ bound to regulatory sites along the thin filament. Experimental evidence suggests that these phenomena occur in cardiac muscles and to a lesser degree in fast- or slow-twitch skeletal muscles.$^{16}$ Bremel and Weber$^{17}$ were the first to propose that crossbridge binding increases Ca$^{2+}$ binding affinity based on solution experiments in which crossbridge binding to skeletal muscle–regulated thin filaments was found to increase Ca$^{2+}$ binding. Subsequent work showed that strong-binding crossbridges also increased the apparent Ca$^{2+}$ binding affinity of TnC in skinned cardiac muscle.$^{18}$ Consistent with these results, several studies have reported conformational changes in TnC in response to strong crossbridge binding to the thin filaments of both cardiac and skeletal muscles.$^{19–21}$

Considerable support for the physiological relevance of crossbridge-induced increases in Ca$^{2+}$ binding affinity of TnC has emerged from experiments in which intracellular [Ca$^{2+}$]i is measured with Ca$^{2+}$ indicators. For example, in invertebrate striated muscles, shortening of the muscle during the relaxation phase was found to give rise to an extra Ca$^{2+}$ transient, which the authors interpreted as dissociation of Ca$^{2+}$ from TnC subsequent to the shortening-induced reduction in numbers of strongly bound crossbridges (Gordon and Ridgway$^{22}$ and references therein). Similarly, extra Ca$^{2+}$ appeared in the myoplasm of ferret ventricular muscle when the muscle was allowed to shorten, thereby reducing force and the number of crossbridges bound to the thin filament.$^{23}$

Of course, the mechanism for shortening-induced release of Ca$^{2+}$ from thin filament sites could involve the length change per se or occur as a result of the decrease in numbers of strongly bound crossbridges. This issue was addressed by Hofmann and Fuchs$^{24}$ in a critical series of studies showing that decreases in force induced by vanadate reduced the Ca$^{2+}$ sensitivity of force in cardiac muscle independent of changes in length. Hofmann and Fuchs used these and other findings to account for the length dependence of Ca$^{2+}$ sensitivity observed in cardiac muscle, i.e., Ca$^{2+}$ sensitivity of force increases at long lengths, as does Ca$^{2+}$ binding at any given free [Ca$^{2+}$]. Importantly, skeletal muscle fibers seem to have lesser length dependence of Ca$^{2+}$ sensitivity than cardiac
Crossbridge Activation of Cardiac Contraction

Cooperativity in Crossbridge Binding to Actin

Positive cooperativity in crossbridge binding was first shown in biochemical experiments in which myosin binding to regulated actin was facilitated by the presence of rigor complexes. Cooperative binding also occurs in the intact filament lattice, which has been demonstrated in a variety of ways, including direct fluorescence imaging of myofibrils in the presence of rhodamine-labeled myosin S1. The importance of this process to force development in striated muscles has been shown in experiments in which variations in the concentrations of MgADP or inorganic phosphate were used to manipulate the number of strongly bound crossbridges and thereby change the Ca binding affinity of TnC. Increased [MgADP] increases the Ca2+ sensitivity of force, presumably by increasing the number of strongly bound crossbridges at each [Ca2+] and thereby facilitating cooperative recruitment of additional crossbridges to force-generating states. Conversely, an increase in [P] reduces the Ca2+ sensitivity of force by reversal of the powerstroke and the resultant decrease in the number of strongly bound crossbridges.

Although the use of chemical interventions such as changes in [MgADP] or [P] to alter the distributions of crossbridges between weakly and strongly bound states has provided insights into the cooperative activating effects of strongly bound crossbridges, they do not provide quantitative information about cooperative activation of force because of the fact that each of these interventions also has direct effects on force, the regulated variable being measured. The N-ethylmaleimide derivative of myosin S-1, NEM-S1, overcomes this complication; NEM-S1 binds strongly to the thin filament but does not develop force. In biochemical experiments, NEM-S1 facilitates crossbridge binding to regulated thin filaments and potentiates ATPase activity in the presence of Ca2+, resulting in the activation of force. The increased Ca2+ sensitivity of force is presumably a manifestation of positive cooperativity in crossbridge binding, as predicted by current models of regulation. The mechanism of this cooperation is not known in detail, but the results of various studies (reviewed by Gordon et al and Tobacman) suggest that crossbridge binding has allosteric effects on the regulatory strand (tropomyosin plus troponin) such that there is a spread of activation within and between regulatory units of 7 actins plus the associated tropomyosin and troponin molecules. Importantly, NEM-S1 has virtually no effect on the maximum force developed in the presence of saturating [Ca2+], which implies that the combined activating effects of Ca2+ and strong-binding crossbridges are maximal under these conditions. Thus, in terms of force development, the thin filaments of cardiac muscles are fully switched on at saturating [Ca2+]. Isometric force also increases in skeletal muscle when the number of strongly bound crossbridges is increased, but the increase in Ca2+ sensitivity induced by a given amount of NEM-S1 is much greater in cardiac muscle. Thus, both muscle types exhibit positive cooperativity in crossbridge binding, but skeletal muscle is the more cooperative of the two, because it requires a greater number of strongly bound crossbridges to achieve similar potentiation of force. Viewed another way, cardiac thin filaments are much more responsive to the activating effects of even a small number of crossbridges, which presumably contribute to fine gradations in twitch force observed on a beat-to-beat basis in living myocardium.

NEM-S1 also activates force at Ca2+ concentrations where virtually no Ca2+ is likely to be bound to the regulatory sites of TnC. This activating effect of strong-binding crossbridges is evident as active force at pCa 9.0 (Figure 3). This force is almost certainly active because these muscles actively shorten when slackened and redevelop force when released to a shorter length. Even though regulation seems to be a synergistic process involving both Ca2+ and crossbridge binding, this result indicates that it is possible to activate the thin filament in the absence of Ca2+ if the number of strong-binding crossbridges is sufficiently high. Such a phenomenon is nonphysiological, because this number of strongly bound crossbridges is never achieved in vivo, at least at the subsaturating concentrations of Ca2+ observed in living myocardium.

The effects of NEM-S1 to increase force in the absence of Ca2+ are also much greater in myocardium than in skeletal muscle. At 6 µmol/L NEM-S1, the force developed in skinned myocardium at pCa 9.0 was ~20% of the maximum active force at pCa 4.5, whereas in skeletal muscle under the same conditions, force was ~5% of maximum. This difference in crossbridge activation of force is consistent with the greater responsiveness of myocardium to the activating effects of strong-binding crossbridges.

The fact that contraction can be activated by strong-binding crossbridges in the absence of Ca2+ implies that the cardiac thin filament is not completely switched off in the absence of Ca2+. In the activation model proposed by McKillop and Geeves, the thin filament without Ca2+ bound is in a blocked state in which it cannot bind crossbridges, but Ca2+ binding converts the filament to a closed state in which it can bind crossbridges. The
effects of NEM-S1 on resting force suggest that the cardiac muscle thin filament is not fully blocked in the absence of Ca$^{2+}$.

Activation of force by strong-binding crossbridges at low levels of Ca$^{2+}$ may have important implications for diastolic function in beating myocardium. The observation that Ca$^{2+}$ and crossbridge binding to the thin filament synergistically activates force$^{13}$ suggests that small variations in diastolic [Ca$^{2+}$] could produce disproportionate changes in diastolic wall stress, owing to cooperative activation of force as [Ca$^{2+}$] increases and to cooperative inactivation of force as [Ca$^{2+}$] decreases.

**Rate of Rise of Force**

The primary determinants of the rate of rise of twitch force in living cardiac muscle seem to be the rate of delivery of Ca$^{2+}$ to the myoplasm and the rate of crossbridge cycling during the twitch. Because the focus of this Review is myofibrillar mechanisms, the present discussion will consider only the factors that influence crossbridge cycling kinetics in myocardium. There is a great deal of evidence that the rate of rise of force in myocardium varies with the expression of myosin heavy and light chain isoforms, which in turn varies between species, within species depending on developmental stage, and in diseases such as heart failure or altered thyroid status.$^{42}$

On a twitch-to-twitch basis, variations in the kinetics of force development seem to be related to the phosphorylation states of a range of myofibrillar proteins (regulatory light chain, troponin I, and possibly myosin binding protein-C) and changes in the activation state of the thin filament. Other Reviews in this series will focus on these phosphoproteins, and, thus, the emphasis here will be on thin filament regulation of the rate of rise of force.

Most studies of the regulation of the rate of force development have been done using skinned myocardium, in which force develops after photolysis of caged Ca$^{2+}$ (reviewed by Gordon et al$^{2}$) or redevelops at constant [Ca$^{2+}$]$_{in}$ after a mechanical maneuver to reduce force to nearly zero.$^{43}$ At 15°C, maximum rate constant of force development ($k_w$) is $\approx 10$ s$^{-1}$ in rat skinned myocardium (Figure 4), compared with $\approx 15$ s$^{-1}$ in fast-twitch skeletal muscle fibers.$^{41}$ $k_w$ is taken to be a direct measure of the rate of crossbridge cycling$^{43}$ and has been found to be activation dependent in skinned myocardium,$^{13,44,45}$ increasing $\approx$10-fold as Ca$^{2+}$ is increased from threshold to maximal concentrations (Figure 4). These results imply that the rate of force development in living muscle would increase simply as a consequence of increased delivery of Ca$^{2+}$ to the myoplasm. However, the relationship between $k_w$ and pCa differs from the force-pCa relationship in that the pCa$_{50}$ for $k_w$ is less than the pCa$_{50}$ for force, ie, a higher [Ca$^{2+}$] is required to achieve half-maximal activation of $k_w$. The implication of these differing Ca$^{2+}$ dependencies of force and the kinetics of force development is that increases in [Ca$^{2+}$] will increase isometric force before significant increases in $k_w$ are observed.

The basis for activation dependence of $k_w$ is an important issue because of its potential role in determining the rate of rise of force in living myocardium. One possibility is that variations in [Ca$^{2+}$] directly mediate the activation dependence of $k_w$, which would be expected if crossbridge cycling kinetics were directly regulated by Ca$^{2+}$ binding to thick filament (regulatory light chain) or thin filament (TnC) proteins. Another possibility is that crossbridge kinetics are regulated by strong binding of crossbridges to the thin filament, such that cycling kinetics are cooperatively accelerated by increasing numbers of crossbridges bound to actin. In the latter case, the activation dependence of $k_w$ would be an indirect consequence of alterations in the amount of activator Ca$^{2+}$ delivered to the myoplasm.

**Mechanisms of Activation of Crossbridge Cycling Kinetics**

The 10-fold increase in $k_w$ as [Ca$^{2+}$] is increased has been interpreted in terms of a mechanism in which Ca$^{2+}$ binding to myofibrillar regulatory proteins directly modulates crossbridge cycling kinetics,$^{46}$ ie, a direct effect of Ca$^{2+}$ on the rate constant of crossbridge attachment ($f_{app}$). Subsequent modeling$^{47}$ accounted for the activation dependence of $k_w$ by treating Ca$^{2+}$ binding to TnC as a rate-determining step in the activation of contraction. In such a scheme, the rate of Ca$^{2+}$ binding to TnC would be a second-order determinant of $f_{app}$, such that $f_{app}$ increases as [Ca$^{2+}$] is increased. This mechanism could certainly contribute to the increase in $k_w$ at high levels of activation, but it does not account for the effects of strong-binding crossbridges on $k_w$, especially the NEM-S1-mediated acceleration of $k_w$ at very low levels of Ca$^{2+}$.$^{12,13,41}$

There is growing evidence that strong binding of myosin crossbridges to the thin filament cooperatively accelerates the rate of $k_w$ in both cardiac and skeletal muscles. For example, increasing the concentration of MgADP in skinned skeletal muscle fibers increases $k_w$ at low and intermediate levels of Ca$^{2+}$ activation,$^{30,31}$ presumably by increasing the number of strongly bound MgADP crossbridges. Conversely, reducing the number of strongly bound crossbridges with vanadate$^{48}$ slows $k_w$ at submaximal Ca$^{2+}$ concentrations (Z. Lu, R. Moss, and J. Walker, 1992, unpublished data). NEM-S1 has been
used to assess possible cooperative activating effects of strong-binding crossbridges on the rate of force development. NEM-S1 accelerates \( k_n \) at submaximal \( \text{Ca}^{2+} \) concentrations (Figure 4), which is also the case in skeletal muscle, and thereby increases the \( \text{Ca}^{2+} \) sensitivity of \( k_n \). NEM-S1 has no effect on maximal \( k_n \) (Figure 4), which implies that the cardiac thin filament is fully activated at saturating \( \text{Ca}^{2+} \) concentrations with respect to the kinetics of force development. As might be expected from results such as these, crossbridge effects on the rate of force development comprise a key element of current models of contraction.

A variety of models or a combination of models can be used to explain the \( \text{Ca}^{2+} \) activation dependence of \( k_n \) and the changes in this relationship caused by the addition of NEM-S1. The activation dependence of \( k_n \) in skeletal muscle was explained with a model in which \( \text{Ca}^{2+} \) influences the rate constant of crossbridge attachment to actin, \( k_{\text{app}} \); \( k_{\text{app}} \) decreases when \( [\text{Ca}^{2+}] \) is lowered, and this has been extended in more recent work. As discussed above, such a mechanism could certainly be operative in cardiac muscle, but it does not account for the effect of strong-binding crossbridges (NEM-S1) to accelerate \( k_n \). Because of this, we favor a model developed by Campbell, which is a variation of one developed earlier by McKillop and Geeves. According to Campbell, the \( \text{Ca}^{2+} \) dependence of \( k_n \) is attributable to cooperativity-induced slowing of force development during submaximal activation. In this model, crossbridges are distributed between cycling and noncycling populations. Cycling crossbridges undergo repeated transitions between non-force-bearing and force-bearing states under the influence of the rate constants \( f_{\text{app}} \) and \( g_{\text{app}} \); whereas noncycling crossbridges are recruited to the cycling population as a result of \( \text{Ca}^{2+} \) binding to troponin or cooperative effects of strong-binding crossbridges to enhance activation of the thin filament. At lower levels of \( \text{Ca}^{2+} \), a small fraction of crossbridges is initially recruited into the cycling population as a direct result of \( \text{Ca}^{2+} \) binding to the thin filament, so that most crossbridges are in the noncycling pool and are thus available for cooperative recruitment to the cycling pool. Progressive recruitment of crossbridges from the noncycling pool would then slow the rate of force development. In contrast, at high levels of \( \text{Ca}^{2+} \), the rate constant of force development is much greater because most crossbridges are recruited to the cycling pool when \( \text{Ca}^{2+} \) binds to troponin, which leaves few crossbridges available in the noncycling pool for subsequent cooperative recruitment. At a saturating \( [\text{Ca}^{2+}] \) of pCa 4.5, the rate constant of force development would thus approach the sum of the forward and reverse rate constants for the force-generating transition, \( f_{\text{app}} + g_{\text{app}} \).

In the context of this model, the effects of NEM-S1 to accelerate \( k_n \) at submaximal \( \text{Ca}^{2+} \) concentrations can be explained as a cooperative activation of the thin filament by the strong-binding NEM-S1. The observation that NEM-S1 increases \( k_n \) to maximal or supramaximal values at the very lowest levels of \( \text{Ca}^{2+} \) activation implies that NEM-S1 eliminates the cooperativity in crossbridge binding in this activation range, perhaps because of reduced interactions between neighboring regulatory units. The decrease in \( k_n \) as \( [\text{Ca}^{2+}] \) is raised to intermediate levels would then be explained on the basis of an increase in cooperativity in crossbridge binding, and the increase in \( k_n \) at the highest \( \text{Ca}^{2+} \) concentrations would be attributable to reduced cooperativity due to saturation of \( \text{Ca}^{2+} \) and crossbridge binding to the thin filaments.

It is likely that the cooperative activation of the thin filament by NEM-S1 is greater than the effects of crossbridge binding in cardiac muscle in vivo, because \( k_n \) measured in control myocardium in the absence of NEM-S1 does not increase substantially until \( \text{Ca}^{2+} \)-activated isometric forces are greater than half-maximal.

Although NEM-S1 dramatically accelerates \( k_n \), strong-binding crossbridges do not entirely account for the activation of crossbridge kinetics, because NEM-S1 alone is insufficient at intermediate levels of activation to increase \( k_n \) to maximal values or to completely eliminate the activation dependence of \( k_n \). Instead, elimination of activation dependence required both NEM-S1 and partial extraction of TnC from the thin filament. Because partial extraction of TnC disrupts near-neighbor communication between functional groups in the thin filament, the activation dependence of \( k_n \) involves effects of strongly bound crossbridges to cooperatively recruit additional crossbridges within the same and neighboring regions of the thin filament. In fact, a modification of Campbell’s model to include near-neighbor interactions that cooperatively recruit crossbridges to strongly bound states predicts these results. By extracting TnC, these interactions were presumably disrupted and cooperative recruitment of crossbridges from neighboring functional groups was reduced or eliminated, thereby speeding the rate of force development.

Much work remains to be done to determine the relative contributions of \( \text{Ca}^{2+} \) and crossbridge binding to the regulation of force development in myocardium. Despite the technical difficulties of working with myocardium, the potential importance of such work is great, because either of these mechanisms might be altered in diseases such as heart failure in which the rate of force (or pressure) development (+\( \text{dP/} \text{dt}_{\text{max}} \)) can be substantially lower than in healthy tissue. Furthermore, the findings of familial hypertrophic cardiomyopathy mutations in thin filament regulatory proteins indicate that it is plausible to propose that the mechanisms contributing to the cooperative activation of the thin filament are altered or disrupted.

Mechanisms of Cooperative Signaling Within the Thin Filament

Functional studies discussed in earlier sections of this Review have provided compelling evidence that crossbridge binding generally enhances the activation state of the thin filament in both cardiac and skeletal muscles. The mechanisms of these effects have been studied for several decades, but the molecular events underlying cooperative activation are only beginning to emerge. Three-dimensional reconstructions of regulated thin filaments studied by electron cryo-microscopy have shown that there are multiple positions of tropomyosin on the thin filament corresponding to the absence of \( \text{Ca}^{2+} \), \( \text{Ca}^{2+} \), bound to TnC, and both \( \text{Ca}^{2+} \) and crossbridges bound to the thin filaments. Such reconstructions provide a compelling structural context for regulation in which \( \text{Ca}^{2+} \) and cross-
bridge binding act synergistically to effect full activation of the thin filament and are consistent with ideas about thin filament regulation that are emerging from biochemical and biophysical studies (reviewed by Gordon et al2 and Tobacman7). Such studies are the focus of another Review in this series, and thus the emphasis here will be on the components of the regulatory strand that most likely confer the unique sensitivity of the cardiac thin filament to the activating effects of strong-binding crossbridges. There is evidence from several studies implicating several thin filament proteins as having possible roles, most notably tropomyosin and troponin T.

**Tropomyosin**

The thin filament regulatory strand in striated muscles is comprised of tropomyosin and the Ca$^{2+}$ binding protein troponin. Within the regulatory strand, Tm occurs as a linear array of coiled-coil dimers in which adjacent dimers overlap and interact in head-to-tail fashion (reviewed by Wolska and Wieczorek58). Such interactions provide a plausible means for signaling activation events such as Ca$^{2+}$ or crossbridge binding from one region to others along the thin filament. As discussed earlier, present models of regulation propose three activation states of the thin filament, each corresponding to a different position of tropomyosin in relation to actin. Based on predominantly biochemical studies, McKillop and Geeves37 proposed a model in which the thin filament is in a blocked state in the absence of Ca$^{2+}$, a closed state in the presence of Ca$^{2+}$, and an open state in the presence of Ca$^{2+}$ and strongly bound crossbridge. Subsequent 3D reconstructions from electron cryo-microscopies of thin filaments in different regulatory states have provided support for the idea that Tm assumes different positions in the thin filament.56,57 The transitions from blocked to closed states and from closed to open suggest that there are conformational changes in the regulatory strand associated with Ca$^{2+}$ binding and cross-binding, respectively, to the thin filament. Based on these results, it seems reasonable to suppose that the interactions between linearly adjacent Tm molecules play a role in determining thin filament responsiveness to the activating effects of crossbridge binding. In this regard, Pan et al59 have shown that near-neighbor interactions are virtually abolished when the C-terminal 11 amino acids of Tm are deleted, in that binding of myosin S1 to such filaments is no longer cooperative.

Studies in skinned fibers support the idea that the spread of activation along the thin filament involves head-to-tail interactions between Tm molecules. Partial extraction of TnC from skeletal muscle fibers reduced the Ca$^{2+}$ sensitivity of force and the slope (Hill coefficient) of the force-pCa relationship.50,51 Recent studies by Regnier et al60 strongly support the notion that much of the cooperativity in the activation of force is attributable to interactions between adjacent functional groups along the thin filament, presumably via Tm-Tm interactions. Replacement of a fraction of endogenous TnC with a mutant TnC with much reduced Ca$^{2+}$ binding affinity at the regulatory site disrupted the spread of activation and reduced force, the Ca$^{2+}$ sensitivity of force, and the slope of the force-pCa relationship.

Additional studies61,62 have shown that Tm isoform content has significant effects on the contractile properties of both skinned and living myocardium. Transgenic expression increased β-Tm to $\approx$60% of total Tm, which enhanced the activation of thin filaments by strongly bound crossbridges; the Ca$^{2+}$ sensitivity of force was increased in skinned preparations and twitch relaxation was slowed in living myocardium. Such effects are presumably attributable to a change in Tm interactions within a functional group, alterations in end-to-end interactions of neighboring Tm molecules, or both. In addition, structural studies63 of thin filaments suggest that Tm localization is modulated (at least in the absence of troponin) when various isoforms of Tm are incorporated into thin filaments.

**Troponin T**

In cardiac muscle, the C-terminal region of cTnT forms a Ca$^{2+}$-modulated complex with cTnC, cTnI, and Tm, whereas the N-terminal region seems to anchor cardiac troponin to Tm through interactions with the C-terminus of Tm and extends over the end-to-end overlap regions of adjacent Tm molecules.64 When Ca$^{2+}$ binds to TnC, the interaction between TnT and Tm is weakened65 and the resulting movement of the regulatory strand drives the thin filament from the blocked state to an open state in which weakly bound crossbridges can make the transition to strongly bound force-generating states.37 The NH$_2$ terminus of cTnT also seems to have a regulatory role in controlling crossbridge binding to thin filaments—a tail peptide of cTnT slowed myosin ATPase activity in the presence of actin and Tm66,67 and also increased the number of myosin heads required for in vitro motility.67 Thus, it is plausible that thin filament responsiveness to the activating effects of strong-binding crossbridges might vary when cTnT is altered, for example, in tail domain mutations and observed in some familial hypertrophic cardiomyopathies and have been found to perturb the interactions of Tn with Tm.68–70 This in turn could influence the responsiveness of thin filaments to the activating effects of strongly bound crossbridges. Consistent with this idea, at least one mutation in cTnT (R141W) associated with dilated cardiomyopathy was found to stabilize the interaction between cTnT and Tm and reduce the Ca$^{2+}$ sensitivity of force.71

Additional studies suggest that developmental changes in the cTnT isoforms expressed in myocardium modulate cardiac contraction. Multiple cTnT isoforms are expressed in mammalian heart, which arise because of alternative splicing and a hypervariable N-terminal domain.72–74 In rabbit cardiac muscle, developmental downregulation of neonatal cTnT and upregulation of adult cTnT are associated with reduced Ca$^{2+}$ sensitivity of force, ie, a rightward shift in the force-pCa relationship.75,76 Similarly, there is a shift in cTnT isoform expression from 30% cTnT$_1$ and 70% cTnT$_4$ in day 7 rat neonates to 100% cTnT$_4$ in the adult and a concomitant decrease in Ca$^{2+}$ sensitivity of force,77 as well as an increase in the slope of the force-pCa relationship. Complicating the interpretation of these results is the finding that TnI expression also changes in this same time frame, ie, from the slow skeletal isoform in neonates to the cardiac isoform in...
adults. Subsequent work found no differences in the apparent cooperativity of force development in transgenic myocardium expressing stTnI versus wild-type myocardium expressing cTnT. Thus, reduced cooperative activation of neonatal myocardium relative to adult myocardium is most likely attributable to developmental shifts in cTnT isoform expression.

More recently, Potter and colleagues showed that TnT, has reduced ability to inhibit ATPase activity of myosin-actin-Tm compared with TnT, and also has reduced ability to relax skinned myocardium. Huang et al. found that incorporation of fast skeletal TnT into mouse myocardium increases the apparently cooperativity of force development, seen as a marked increase in the steepness of the force-pCa relationship. These results strongly suggest that TnT isoforms contribute to the cooperative activation of the thin filament and may act as a molecular mechanism to tune myofilament function to meet the work demands on the ventricle. The mechanisms underlying these effects of cTnT isoforms on regulation of force are not yet understood. For example, do cTnT isoforms modulate the Ca$^{2+}$ affinity of TnC either directly or by changing thin filament responsiveness to the activating effects of strongly bound crossbridges? Alternatively, different cTnT isoforms might alter the regulation of crossbridge interaction kinetics by strong-binding crossbridges.

**Functional Implications of Activating Effects Attributable to Myosin Binding**

The cooperative activation of thin filaments attributable to crossbridge binding has significant implications for myocardial function. As discussed in earlier sections, these activating effects are the basis for the observed acceleration of the kinetics of force development as the level of activating Ca$^{2+}$ is increased and, conversely, the slowing of kinetics when [Ca$^{2+}$] is reduced. Cooperativity in crossbridge binding and thin filament activation is likely to influence or mediate other functional characteristics of myocardium, and derangements in cooperative processes may underlie contractile dysfunction in some myocardial diseases.

**Myocardial Relaxation**

Several factors contribute to the time course of myocardial relaxation, including Ca$^{2+}$ handling and reuptake processes, crossbridge cycling kinetics, and the presence or absence of inotropic agonists and blockers. Although each of these factors has predictable effects on relaxation rates, it is difficult to quantitatively relate the process of relaxation to definable transitions in the crossbridge interaction cycle. ADP release from the A · M · ADP complex is kinetically coupled to the dissociation of crossbridges from actin and the resulting decrease in force; however, the process is very likely complicated in living myocardium by regional variations in the onset of relaxation.

A recent study by Stehle et al. found that relaxation of cardiac myofibrils in response to rapid reductions in [Ca$^{2+}$] occurs in two distinct phases: an initial slow phase that is approximately linear, and a subsequent phase that is much faster. Videomicroscopy of the myofibrils revealed that the transition from the slow phase to the fast phase of relaxation was related to sudden lengthening of a single sarcomere. Previous studies on tetanically stimulated skeletal muscle fibers yielded similar results, ie, relaxation was biphasic and the initial slow phase of relaxation could be significantly prolonged by imposition of sarcomere length control. Thus, relaxation in the initial phase is likely to be determined primarily by crossbridge detachment rate, but much of relaxation is attributable to collapse of sarcomere order as a result of asynchronous relaxation along the length of the preparation.

Cooperativity in crossbridge binding also seems to influence the rate of myocardial relaxation. Wolska et al. showed that transgenic expression of the skeletal isoform of tropomyosin (β Tm) in mouse myocardium slowed relaxation in living myocytes, a phenomenon that the authors related to the greater cooperativity of force development (greater Hill coefficient) measured in skinned preparations compared with wild-type myocardium expressing α Tm. Greater cooperativity would prolong the linear phase of relaxation attributable to sustained activation of the thin filament by strongly bound crossbridges, even as [Ca$^{2+}$] decreases. Although it is evident from these results that changes in cooperativity can alter relaxation rates, the importance of this mechanism in living myocardium in health and disease remains to be determined. However, recent modeling of the myocardial twitch that includes near-neighbor cooperativity in the activation of myofilaments predicts similar results to those of Wolska et al. by varying the degree of interaction between adjacent tropomyosins in the thin filament.

Activation of force by strong-binding crossbridges at low levels of Ca$^{2+}$ might also have important implications for diastolic function in beating myocardium. The observation that Ca$^{2+}$ and crossbridge binding to the thin filament synergistically activates force suggests that small variations in diastolic [Ca$^{2+}$] could produce disproportionate changes in diastolic wall stress owing to cooperative activation of force as [Ca$^{2+}$] increases and to cooperative inactivation of force as [Ca$^{2+}$] decreases.

**Frank-Starling Relationship**

The intrinsic ability of the cardiac pump to adjust stroke volume to compensate for variations in venous return, ie, the Frank-Starling mechanism, has been related to force-length relationship of myocardial strips or single cardiac myocytes. At the level of the myofilaments, increases in sarcomere length within the physiological range are associated with increases in the Ca$^{2+}$ sensitivity of force, such that at each submaximal [Ca$^{2+}$], force increases steeply as length is increased. In living hearts, this length dependence of Ca$^{2+}$ sensitivity would be expected to contribute to increases in stroke volume when end-diastolic volume increases.

The mechanism of length dependence of Ca$^{2+}$ sensitivity is not known for certain but is likely to be multifactorial. As discussed in an earlier section, cardiac muscle exhibits length-dependent binding of Ca$^{2+}$ to TnC, which could play a role in length dependence of contraction. Additional experiments showing that length dependence could be mimicked by osmotic compression of the filament lattice led to the idea
that the increase in Ca\textsuperscript{2+} sensitivity of force at long lengths is attributable to increased probability of crossbridge binding to actin as a consequence of reduced lateral separation of the thick and thin filaments. Subsequent work in which radio- graph diffraction was used to quantify changes in lattice dimensions as a function of length or osmotic compression lattice reported that these two variables can be adjusted to produce similar changes in Ca\textsuperscript{2+} sensitivity of force but with substantially different effects on filament lattice spacing.\textsuperscript{86,87} These results have led to a new hypothesis in which length dependence is a consequence of subtle changes in crossbridge orientation at long lengths, which increases the probability of crossbridge binding.

Regardless of the underlying mechanism for increases in crossbridge number at long lengths, it now seems likely that increased crossbridge attachment increases the Ca\textsuperscript{2+} sensitivity of force by cooperative recruitment of additional crossbridges to force-generating states. In support of this idea, application of NEM-S1 to skinned myocardium not only increased the Ca\textsuperscript{2+} sensitivity of force but abolished its length dependence.\textsuperscript{88} Such a finding suggests that interventions that change the cooperativity of force development will also change the nature of the Frank-Starling relationship. Wolska et al\textsuperscript{69} found that myocardium expressing an alternate isoform of Tm (β Tm) exhibited greater apparent cooperativity of force development and depression of the Frank-Starling relationship in skinned muscle strips. An intriguing possibility that emerges from this discussion is that the depressed Frank-Starling relationships that have been observed in diseases such as heart failure may be a consequence of alterations in the cooperativity of force development subsequent to altered expression of myofibrillar proteins.

### Stretch Activation

Recent work has suggested that stretch activation of myocardium plays an important role in sustaining myocardial power output during the systolic ejection phase of the cardiac cycle.\textsuperscript{89} Stretch activation occurs in cyclic muscles (such as myocardium) and is seen as a delayed increase in force after imposed stretch.\textsuperscript{90} Davis et al\textsuperscript{89} have proposed that epicardium develops greater specific force than endocardium during the early stages of systole, and as a result the endocardium is stretched. The resulting stretch activation of endocardium would then produce a delayed force that is envisioned by these authors to contribute to the generation of myocardial power during ejection. This intriguing idea is based on the authors' observations that epicardium exhibits greater phosphorylation of the myosin regulatory light chain, which they associated with greater force at submaximal [Ca\textsuperscript{2+}]; on the other hand, the lower levels of RLC phosphorylation in endocardium was associated with a greater stretch activation response.

The mechanism of stretch activation in heart muscle is not known for certain but may involve stretch-induced cooperative recruitment of crossbridges to force-generating states. Stretching myocardium could increase crossbridge number by changing the register of myosin heads with binding sites on actin or as a result of strain of the thick filament, possibly via its interactions with titin.\textsuperscript{91,95} The delayed tension transient would then be the sum of increasing force attributable to crossbridge recruitment and decreasing force attributable to detachment of crossbridges strained by the stretch. The importance of this mechanism is that any variation in the apparent cooperativity of force development, as discussed above in relation to expression of alternate isoforms of Tm, should have predictable effects on the stretch-activation response of myocardium and on systolic ejection. This idea needs to be investigated systematically in both healthy and diseased myocardium.

### Conclusions

The idea that there is molecular cooperation in the activation of myocardial contraction has emerged over the past two decades but might seem to the occasional observer to be of primarily academic interest. This brief Review suggests otherwise. Myocardial force and the rates of force development and relaxation are modulated by the activating effects of the strong binding of myosin to actin. At the subsaturating Ca\textsuperscript{2+} concentrations that pertain in living cardiac muscle, cooperative activation of the thin filament slows the rate of both the development and relaxation of force. In both cases, crossbridge binding promotes the activation of the thin filament and recruitment of additional crossbridges to force-generating states. Thus, cooperativity is potentially important in the working heart as a determinant of systolic and diastolic function. In turn, depressed function in myocardial diseases such as heart failure may in part be a manifestation of altered cooperativity attributable to changes in the expression or posttranslational modification of contractile or regulatory proteins.

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### References


52. Wolska BM, Keller RS, Evans CC, Palmer KA, Phillips RM, Muthuchamy M, Oehlenschlager J, Wieczorek DF, deTombe PP, Solaro RJ. Correlation between myofilament response to Ca2⁺ and altered dynamics
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