

REVIEW

Novel insights into sarcomere regulatory systems control of cardiac thin filament activation

 Christopher Solís  and R. John Solaro 

Our review focuses on sarcomere regulatory mechanisms with a discussion of cardiac-specific modifications to the three-state model of thin filament activation from a blocked to closed to open state. We discuss modulation of these thin filament transitions by Ca^{2+} , by crossbridge interactions, and by thick filament-associated proteins, cardiac myosin-binding protein C (cMyBP-C), cardiac regulatory light chain (cRLC), and titin. Emerging evidence supports the idea that the cooperative activation of the thin filaments despite a single Ca^{2+} triggering regulatory site on troponin C (cTnC) cannot be considered in isolation of other functional domains of the sarcomere. We discuss long- and short-range interactions among these domains with the regulatory units of thin filaments, including proteins at the barbed end at the Z-disc and the pointed end near the M-band. Important to these discussions is the ever-increasing understanding of the role of cMyBP-C, cRLC, and titin filaments. Detailed knowledge of these control processes is critical to the understanding of mechanisms sustaining physiological cardiac state with varying hemodynamic load, to better defining genetic and acquired cardiac disorders, and to developing targets for therapies at the level of the sarcomeres.

Introduction: Major functional domains of the sarcomere

Recent progress requires reconsideration of thin filament activation and de-activation mechanisms in the long- and short-term control of systolic and diastolic function of the heart. In this review, we summarize findings that reinforce the necessity to consider the functional domains along the length of the cardiac thin filament from barbed to pointed ends, the Z-disc (Z-disk, Z-band), and the M-band. One rationale for including these functional domains is the seminal finding that mutant genes expressing proteins of the sarcomere are largely responsible for the inherited disorders of hypertrophic cardiomyopathy (HCM; Geisterfer-Lowrance et al., 1990) and linked to dilated cardiomyopathy (DCM; reviewed in Yotti et al., 2019). This linkage provided the foundations of a necessary interest in the details of a signaling cascade involving mechanotransduction resulting in alterations in transcription, translation, and assembly of sarcomere proteins and induction of cardiac remodeling. A second rationale is the quest to target sarcomere proteins and their signaling cascades with small molecules as therapies for HCM, DCM, and acquired heart failure (HF) with both preserved ejection fraction (HFpEF) and reduced ejection fraction (HFrEF). Building on earlier work (Solaro and Ruegg, 1982; reviewed in Arteaga et al., 2002; Alsulami and Marston, 2020; Alves et al., 2017; Kass and Solaro, 2006) identifying

sarcomere proteins as targets for inotropic agents, there has been recent progress and successes in the development of small molecules directly modifying sarcomere function as a therapeutic approach in HCM (Green et al., 2016) and HFrEF (Malik et al., 2011; Planelles-Herrero et al., 2017a; Teerlink et al., 2021). Small-molecule treatments for HFpEF are lacking to date; however, recent advances in animal models of HFpEF should catalyze the therapeutic development of drug candidates (Schiattarella et al., 2019). Despite these advances, gaps in our understanding of these therapeutic approaches as well as understanding of detailed pathological mechanisms require further understanding of the molecular mechanisms and how sarcomere function plays a role in homeostasis of cardiac function. We next introduce these mechanisms in the context of a current view of sarcomere domains.

Fig. 1 illustrates the sarcomere with domains of thin, thick, and titin filaments with emphasis on their connectivity and functional integration with the Z-disc and M-Band. Fig. 2 A depicts current concepts of regulation of tension and dynamics of a series of sarcomere regulatory units in the C-zone of the A-band, where cardiac myosin-binding protein C (cMyBP-C) locates. The myosin molecule in working ventricular myocytes is a hexamer with two heavy chains (myosin heavy chain [MHC]) each having a globular motor domain (S1) that splits

University of Illinois at Chicago, College of Medicine, Department of Physiology and Biophysics and Center for Cardiovascular Research, Chicago, IL.

Correspondence to R. John Solaro: solarorj@uic.edu.

© 2021 Solís and Solaro. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms/>). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at <https://creativecommons.org/licenses/by-nc-sa/4.0/>).

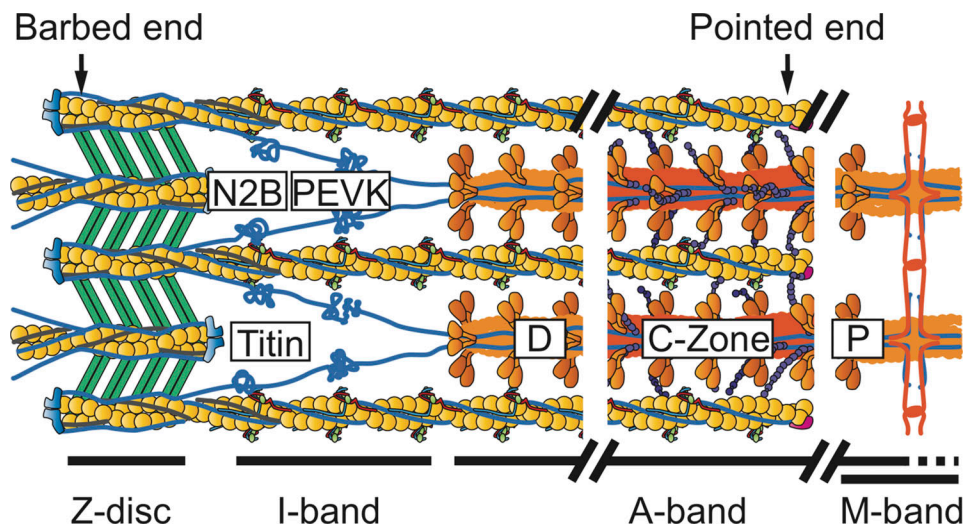


Figure 1. **The cardiac ventricular myocyte thin filament landscape in a half sarcomere.** The half sarcomere ultrastructure is divided into the Z-disc where the thin filament barbed ends are located, the I-band composed of thin filaments and the titin N2B and PEVK regions, the A-band that includes thin and thick filaments, and the M-band that contains thick filaments. The thick filaments are further divided into the D-zone, the C-zone that contains cMyBP-C, and the P-zone.

ATP and binds to actin. Downstream of each of the S1 heads is an extended domain (S2) and a terminating coiled-coil domain known as light meromyosin (LMM), which self-assembles to form the thick filament core. Attached to each of the motor domains below the ATPase site are two sets of light chains (LCs): one essential for function (cardiac essential LC) and one cardiac

regulatory LC (cRLC). In controlling activation state, other regulatory proteins interact with myosin and the thin filament, including cMyBP-C and titin. Fig. 2 A shows cross-bridges with the globular heads folded back in a so-called super-relaxed state (SRX) and in an extended state called the disordered relaxed state (DRX). Below, we discuss the

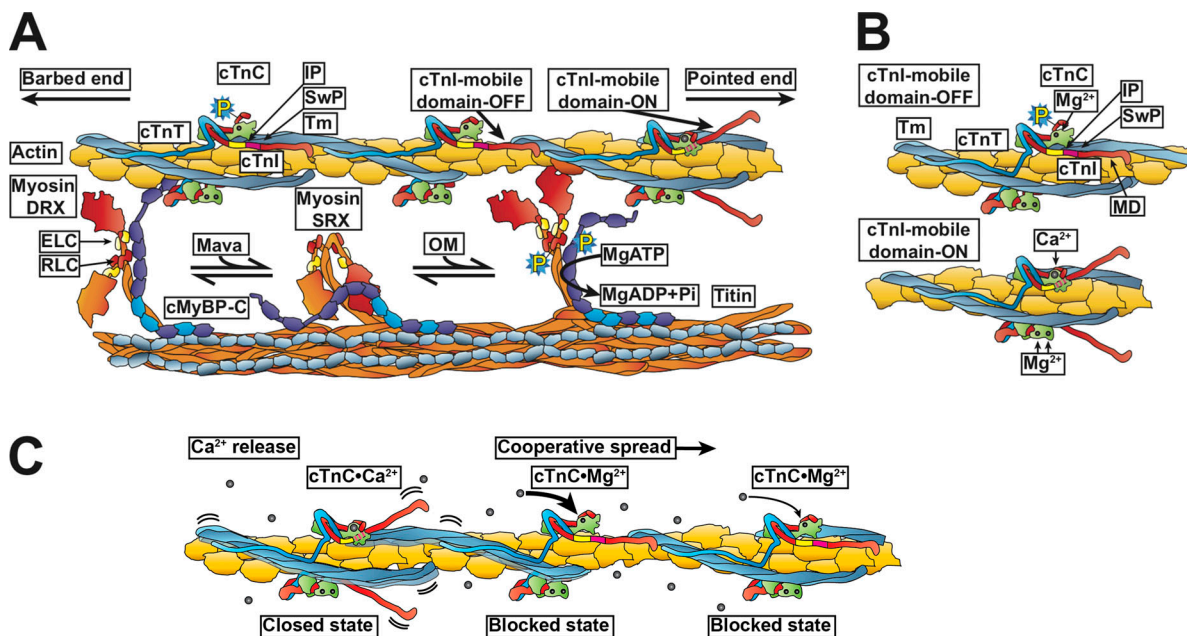


Figure 2. **The thin filament actin interfaces relevant to contractile regulation.** (A) The thin and thick filament interaction interfaces include the myosin motor domains (which adopt DRX, SRX, and crossbridge conformations) and cMyBP-C. Therapeutic agents and mutations linked to HCM have been shown to modify the population of crossbridges in the SRX and DRX. Illustrated here is Mava, which leads to an increased population of SRX myosin motor domains from a DRX population, whereas OM leads to an increased population in DRX together with a prolongation of crossbridge duty cycle. (B) The Ca^{2+} -dependent conformational transitions in the cTn complex illustrate the cTnI inhibitory peptide (IP; yellow, aa 138–149), the switch peptide (SwP; magenta, aa 150–161), and the mobile domain (MD; red, aa 162–210). (C) The cooperative spread of activation between neighbor Tn-Tm units increases cTnC binding affinity for Ca^{2+} . See text for further discussion. ELC, essential LC.

relevance of these dispositions of the crossbridges in relation to thin filament states.

Fig. 2, A and B illustrates inhibited and activated states of thin filament regulatory units consisting of cardiac troponin C (cTnC), cardiac troponin T (cTnT), cardiac troponin I (cTnI), tropomyosin (Tm), and actin in a 1:1:1:1:7 ratio. Below, we discuss the distinction between the regulatory units and functional units controlling contraction and relaxation. Switching on the sarcomere occurs by Ca^{2+} binding to a single regulatory site in the N-lobe of the thin filament protein cTnC. Transduction of this signal promotes actin–crossbridge reactions generating force (pressure) and shortening (ejection). Advances in the understanding of cardiac thin filament on and off structures have been provided by Yamada et al. (2020), using cryo-EM of cardiac thin filaments (reviewed in Solaro and Solaro, 2020; Tobacman, 2021). The transduction mechanism requires the reversal of an inhibited state imposed on the thin filament by the inhibitory protein (cTnI). As shown in Fig. 2 B, Ca^{2+} binding induces exposure of a hydrophobic patch on cTnC that binds to a switch peptide of cTnI, thereby releasing the hold of both an inhibitory peptide of cTnI on actin and the mobile C-terminal domain of cTnI on actin-Tm. These altered molecular interactions also generate downstream signaling to cTnT. cTnT is not only a Tm-binding protein but also interacts with cTnI, cTnC, and actin (Madan et al., 2020; Tobacman, 1996; Yamada et al., 2020) in aiding release and movement of Tm removing a steric block of the actin–crossbridge reaction. There is long-standing and compelling evidence for a cooperative spread of the activation of near-neighbor regulatory units along the thin filament strand as depicted in Fig. 2 C. This spread of activation may be (1) intrinsic to the thin filament or (2) modulated by processes involving domains outside the thin filament regulatory units. As illustrated in Fig. 3, A and B, ample evidence supports the distribution of the regulatory units into three states in which Ca^{2+} and myosin allosterically affect the net distribution of the regulatory units into these states. Below, we consider recent developments in the question of the relative role of these two mechanisms in the context of promotion of thin filament activation.

Fig. 4 also includes domains at the barbed (Z-disc; Fig. 4 A), the I-band (Fig. 4 B), and the pointed ends (Fig. 4 C) of the thin filament. Moreover, there are proteins capping the barbed ends (actin-capping protein Z [CapZ]) and at the pointed ends (tropomodulin [Tmod]). Although thin filaments do not extend into the M-band, we consider this domain together with other domains at the ends of thin filaments as integral to thin filament function. In an earlier review, we further developed the concept of remote control by thin filament functional state of kinases and phosphatases docked at the Z-disc. Our discussion here builds on this and other earlier reviews (Fowler and Dominguez, 2017; Gautel and Djinović-Carugo, 2016; Lange et al., 2020; Pyle and Solaro, 2004; Solaro, 2005).

The three-state model of thin filament activation as a framework for discussion

The durable three-state model conceptualized by McKillop and Geeves (1993) continues to provide a focal point and framework for discussion of emerging insights into thin filament regulation.

In response to a critique by Heeley et al. (2019) of the three-state model, Geeves et al. (2019) provided a detailed recapitulation of the model and its support in the literature. As illustrated in Fig. 3, the essence of the hypothesis is that full understanding of thin filament activation requires three states: a blocked B-state with Tm impeding the actin–crossbridge reaction in low Ca^{2+} , a closed C-state following Ca^{2+} binding to cTnC characterized by incomplete thin filament activation with Tm only partially mobile and not fully positioned to remove the steric block, and an open O-state or M-state with myosin reacting with the thin filament, repositioning Tm in a patch of the thin filament, and promoting a cooperative spread fully activating a stretch of regulatory units forming an extended functional unit (Fig. 3, A and B). Evidence for this process comes largely from studies of fast skeletal muscle proteins showing that Ca^{2+} binding alone cannot fully activate the thin filament and that strongly bound crossbridges can cooperatively activate the thin filament independently of Ca^{2+} (Bremel et al., 1973; Fitzsimons and Moss, 2007; Geeves et al., 2011). Evidence for the crossbridge-dependent activation of the thin filament also came from the disconnection between the dynamics of the Ca^{2+} transient, especially in the relaxation phase in which tension lags the fall in Ca^{2+} (Hinken and Solaro, 2007; Locher et al., 2011; Poggesi et al., 2005). Apart from discussions of evidence for a role of crossbridge detachment rates in relaxation, our review adds weight to the utility of the three-state model specific to heart sarcomeres with discussion of the relative role of Ca^{2+} binding and strong crossbridges in switching to the open state, the mechanisms of cooperative spread of activation. Also discussed is modulation of thin filament activation by protein interactions with cMyBP-C and cRLC dictated by the prevailing chemical, mechanical, and structural states of sarcomeres. Important questions are: what is the applicability of the concepts engrained in the three-state model to our understanding of cardiac pressure development and contraction/relaxation dynamics? How will this understanding help to define the homeostatic state of the heart, its pathology, and ways to approach therapies targeting the sarcomeres? It is apparent that we are closer to this long-range goal with application of state-of-the-art techniques using genetic and fluorescent reporters of sarcomere states in skinned fibers and living muscle, cryo-EM, high-speed atomic force microscopy (HS-AFM), and x-ray diffraction. It is evident that in some cases, the application of these approaches can be improved by incorporation of information identifying cardiac-specific tuning of the three-state model by DRX and SRX crossbridge states, cMyBP-C, cRLC, and titin in thin filament activation.

Crossbridge entry into and exit from force-generating states and myocardial dynamics

A discussion of existing approaches and important findings provides a backdrop for our consideration of recent progress in understanding sarcomere control mechanisms with focus on the three-state model. These approaches benefited from an ability to study sarcomeres isolated from membranes and thus permitting experimental control over Ca^{2+} interactions with cTnC (Fabiato and Fabiato, 1975; Fabiato and Fabiato, 1978; Solaro et al., 1971; Veksler et al., 1988). It has been 50 yr since Solaro et al. (1971)

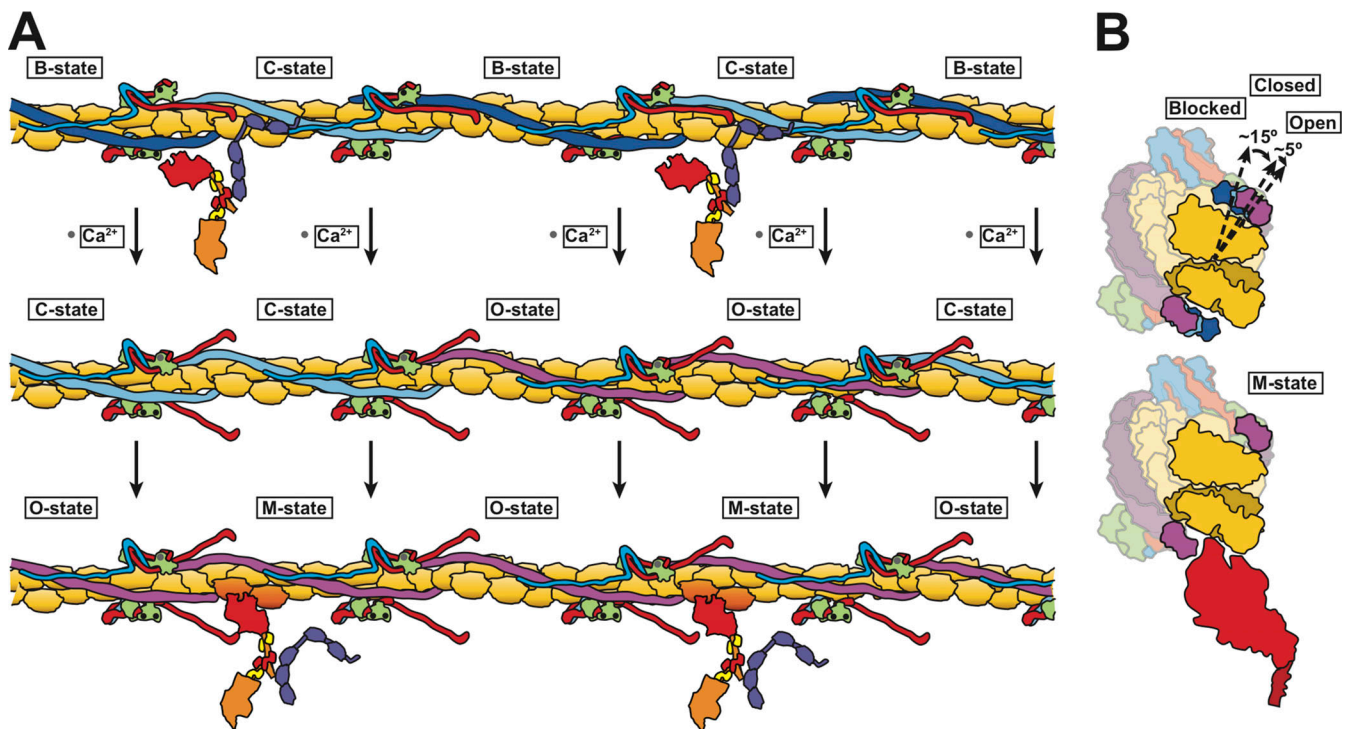


Figure 3. **The three-state model of thin filament activation.** (A) Top: At subsaturating Ca^{2+} levels, the thin filament primarily occupies the B- and C-states. Middle: Ca^{2+} promotes the increased occupancy of the C- and the O-state. Bottom: Myosin crossbridges promote further displacement of regulatory units into the O-state (or M-state). (B) Top: The cross-sectional view of the thin filament axis shows that the largest contribution to the azimuthal rotation of Tm is from the B- to the C-state transition relative to the C- to the O-state (or M-state) transition. Bottom: Superimposition of the strongly bound myosin conformation with the O-state (or M-state) shows unhindered interaction of myosin with actin by Tm. See text for further discussion.

published a method describing an approach to separation of the myofilaments of heart muscle from the mesh of membranes and mitochondria obscuring the fundamental and specific biochemical and mechanical properties of sarcomere response to Ca^{2+} and energy turnover. The simple method of removal of the membranes with Triton X-100 has been a durable approach applied worldwide. Having the ability to mimic the biochemical

environment of the sarcomere lattice and determine steady- and pre-steady-state functions permitted a remarkable evolution in understanding and appreciation of the relative role of sarcomere control mechanisms in cardiac function not only as a sensor/translator of Ca^{2+} signals and activator of tension but also as an integral participant in control of cardiac systolic elastance and dynamics downstream of electrochemical coupling. We discuss

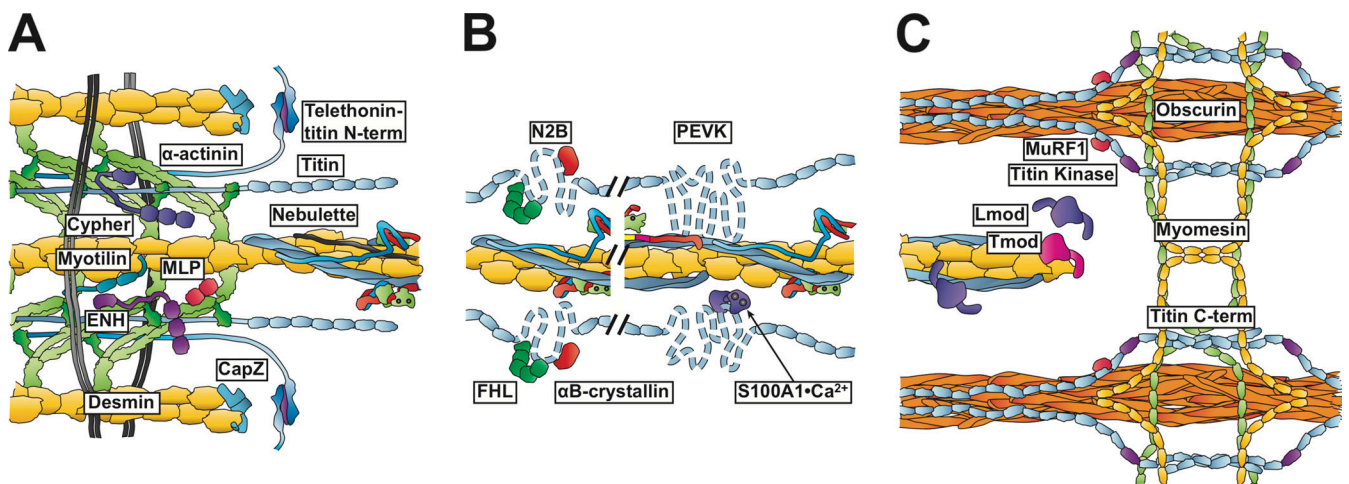


Figure 4. **Other sarcomeric domains that influence crossbridge performance.** (A–C) The main protein constituents of the Z-disc (A), the I-band (B), and the M-band and pointed ends (C) that play a significant role in maintaining sarcomere structure and function are illustrated. See text for further details.

this evolution with examples because an exhaustive discussion is beyond the scope of our review.

Triton extraction of membranes (Solaro et al., 1971), other permeabilization approaches (Veksler et al., 1988), and physical removal of the surface membrane of myocardium (Fabiato and Fabiato, 1975; Fabiato and Fabiato, 1978), all referred to here as “skinned fibers,” aided determinations of both steady-state and dynamic properties of the sarcomeres and provided insights into understanding the integrative biology of relaxation of the myocardium. These insights were developed by making these measurements with isoform switching, post-translational modifications, mutations, or proteolysis; changes in the environment, such as pH, [p], and [nucleotides]; and variations in mechanical state, such as altered sarcomere length (for reviews see Gordon et al., 2000; Kobayashi et al., 2008; Solaro and Rarick, 1998; van der Velden and Stienen, 2019). Steady-state investigations with skinned fibers determining the dependence of tension, ATPase rate, and Ca^{2+} -binding on free Ca^{2+} identified a cooperative mechanism of activation despite control by a single regulatory site on cTnI. Moreover, simultaneous determination of tension and ATPase rate in skinned fibers provided a measure of crossbridge kinetics and of the energetic cost of tension under a particular set of conditions (Rundell et al., 2004). Flash photolysis of compounds surrounding the sarcomeres either rapidly releasing Ca^{2+} (DM-nitrophen) or rapidly removing Ca^{2+} (diazo-2) provide an approach, extending the studies of kinetics derived from the tension cost analysis. These studies using flash photolysis of caged compounds supported evidence from tension cost that skinned fibers predominantly regulated by β -MHC in place of α -MHC demonstrate significantly slower rates of relaxation (Fitzsimons et al., 1998). This finding has relevant implications in the context of HF. Nonfailing human ventricles exhibit an $\sim 7\%$ α/β -MHC protein ratio or $\sim 35\%$ α/β -MHC mRNA ratio, while ventricles from patients with HF have $\sim 0\%$ of protein and mRNA α/β -MHC ratios (Miyata et al., 2000). Measurement using the caged Ca^{2+} chelator diazo-2 also demonstrated that PKA-dependent phosphorylation of cTnI and cMyBP-C in skinned fibers increases the rate of relaxation in fibers controlled by cTnI but not in fibers expressing slow skeletal TnI (Kentish et al., 2001). Kentish et al. (2001) also measured dynamic stiffness in tetanized fibers to determine an increase in crossbridge kinetics induced by PKA phosphorylation. In demonstrating that relaxation of the heart depends strongly on the exit of crossbridges from their force-generating state, these findings are examples of how dynamic and steady-state data constrain theoretical models that relaxation of the myocardium depends on control of Ca^{2+} removal from sarcoplasm by fluxes into the sarcoplasmic reticulum at the early stages of diastole, but the rate-limiting step of crossbridge detachment is imposed by sarcomere protein states, particularly the α/β -MHC isoform population and post-translational modifications.

Complementing and extending these approaches are dynamic measurements of sarcomere function that impose a quick-release/quick-stretch method (Brenner and Eisenberg, 1986; Wolff et al., 1995) and quick stretch/quick release (Huxley and Simmons, 1968; Steiger, 1971) to reveal crossbridge kinetics. These studies are

often employed together with steady-state measurements to determine rates of crossbridge entry into and exit from force-generating states. In quick-release/quick-stretch measurements, skinned fibers developing tension at a given Ca^{2+} are made slack by reducing length $\sim 20\%$ within ~ 2 ms and then quickly returning to the initial muscle length. Tension returns as a single exponential, with a rate k_{tr} reflecting the kinetics of the population of crossbridges entering and exiting from the force-generating state. To provide measurements of crossbridges entering the force-generating state, k_{tr} is determined together with the measurements of steady-state tension cost, which is dominated by crossbridge detachment rates. For example, a report by de Tombe and Stienen (2007) used Triton-skinned fibers for high-fidelity determination of the temperature dependence of tension and ATPase rate in skinned cardiac fibers as an approach to measuring crossbridge kinetics and the energetic cost of steady-tension development. This study also used quick-release/quick-stretch protocols to investigate the kinetics of crossbridge entry into the force-generating states. An important conclusion was that tension cost depends on crossbridge detachment rate. In studies with porcine myocardium, a report by Locher et al. (2011) provided another example of the combined measurements of steady-state tension and ATPase rate together with k_{tr} measurements. In these studies, a small increase of $\sim 10\%$ of the fast α -MHC isoform in porcine myocardium induced a significant enhancement of crossbridge kinetics. Modeling this shift demonstrated how sarcomere myosin crossbridge kinetics, especially detachment rates, dominate twitch dynamics, with relaxation of tension slower than a simulated fall in the Ca^{2+} transient. Hinken and Solaro (2007) emphasized and extended this discussion of a dominant role of crossbridge kinetics in the time course of relaxation of isovolumic pressure. Lovelock et al. (2012) also applied these techniques to investigate mechanisms of cardiac dysfunction in a mouse model of HFpEF and the palliative effects of the anti-ischemic medication ranolazine. These studies demonstrated that in oxidative stress associated with HFpEF, there is a slowing of crossbridge kinetics and enhanced Ca^{2+} sensitivity associated with Ca^{2+} -independent slowing of cellular relaxation. Studies of the strain dependence of myocyte relaxation support a view of relaxation mechanisms in which strain is a more important controller of myocyte end-systolic lengthening than afterload (Chung et al., 2017). There is also evidence that in a population of myocytes with different dynamics that the integrated rate of relaxation is dominated by myocytes with faster thin filament activation by cTnI phosphorylation, faster release of crossbridges by cMyBP-C phosphorylation, and faster rate of stretch at end systole (Clark et al., 2021). These findings fit with data indicating that relaxation rates depend more on sarcomere properties than on the decay of the Ca^{2+} transient (Monasky et al., 2008).

Studies with bundles of myofibrils developing force in an environment in which rapid solution switching instantaneously adds and removes Ca^{2+} with little diffusional barriers provide a novel view of the role of sarcomeres in the kinetics of the isometric twitch (Colomo et al., 1997). Vitale et al. (2019) provided an excellent summary of the advantages of the use of myofibril-sized preparations over other techniques. This approach avoids the use of photolysis, which may lead to artifacts associated with

conditions required to release caged compounds. Following steady-tension development, rapid chelation of Ca^{2+} induces two phases of relaxation: a slow linear phase described as a rate constant k_{rel} interpreted to reflect the rate of crossbridge detachment. Following this phase there is an exponential decay interpreted to reflect the give of a few relaxed sarcomeres, leading to a collapse of tension. By making measurements in a variety of cardiac muscle types, including human samples together with determinations of tension cost, Vitale et al. were able to conclude a close correspondence between k_{rel} and tension cost, providing strong support for the role of crossbridge detachment rate as a significant factor in isometric relaxation. Studies with myofibrils have provided insights not revealed in steady-state and some pre-steady-state measurements, as exemplified by the effect of pseudo-phosphorylation of Tm (Tm-S283D) to significantly slow k_{rel} and Ca^{2+} dissociation rate from cTnC (Nixon et al., 2013).

Insights into control of cardiac dynamics at the level of the sarcomeres have also come from studies at the level of organization of single myocytes and multicellular preparations with simultaneous determinations of intracellular Ca^{2+} and mechanics. One example is the early work from the David Allen laboratory using aequorin to measure intracellular Ca^{2+} in multicellular preparations. These studies revealed that the inhibition of sarcomere response to Ca^{2+} is a dominant mechanism in the fall in tension with a drop in pH (Allen and Orchard, 1983; Solaro et al., 1988). Moreover, Solaro et al. (1988) reported that neonatal muscle preparations were protected against the fall in tension with acidic pH. A role for cTnI in this effect was confirmed in studies with isoform switching to the slow skeletal TnI, the embryonic and neonatal isoform in transgenic (TG) mouse hearts and in isolated myocytes (Urboniene et al., 2005; Westfall and Metzger, 2007). Advancements in the development of fluorescent probes permitted extension of the interrogation of the relative role of changes in Ca^{2+} fluxes and sarcomere response to Ca^{2+} as controllers of cardiac dynamics (Bers et al., 1996; Wier et al., 1988). Among these studies, measurements of Ca^{2+} and tension in multicellular papillary muscle preparations by Tong, Moss and collaborators supported the concept that phosphorylation of sarcomere proteins, especially cMyBP-C, are dominant in modifying twitch dynamics in response to frequency and adrenergic stimulation (Rosas et al., 2015; Tong et al., 2008; Tong et al., 2015). In summary, evidence from diverse approaches provides support for the conclusion that together with control of Ca^{2+} fluxes, rates of entry and exit of crossbridges to and from force-generating states contribute significantly to the cardiac dynamics. We next consider the issue of the relative role of force-generating crossbridges and Ca^{2+} binding to cTnC in promoting the transition to the thin filament open state.

Crossbridge-dependent and intrinsic cooperative interactions signaling via cTnC Ca^{2+} binding in activation of the thin filament

Cooperative interactions among sarcomere proteins in the response to Ca^{2+} remains an active area of investigation about control of steps in the three-state model of the thin filament

activation. There are gaps in understanding of detailed mechanisms and their role as a determinant of the level of cardiac activation. Quantification of the cooperativity factor or Hill coefficient n_H derived from Hill equation fits of the relation between pCa ($-\log_{10}[\text{Ca}^{2+}]_{free}$) and steady-state tension development of skinned fibers provides one common means of comparing the cooperativity, but the nature of the cooperative activation is a complex function of the interactions among thin filament regulatory units. In the C-zone of the A-band, activation also involves the N terminus of cMyBP-C interacting with regulatory units. Despite control of activation by a single Ca^{2+} -binding site on cTnC, the increase in steady-state tension occurs commonly over one pCa unit in which values of $n_H \geq 2-3$ are generally found. This finding indicates that either Ca^{2+} binding to one site promotes Ca^{2+} binding to a near-neighbor site or activation spreads intrinsically along the thin filament strand by promoting the release of the thin filament-inhibited state without Ca^{2+} bound to cTnC. Early investigations of Tawada and Tawada (1975) implicated end-to-end interactions of contiguous Tm units in this spread of activation. This idea has been updated by Geeves et al. (2011). Seminal studies of Bremel and Weber (1972) led to the widely studied theory that strongly bound crossbridges feed back to activate the thin filament. This theory makes sense when based on the consideration of detailed balance in which the interaction energies along the cascade of protein-protein interactions between cTnC and actin are not one way but occur from the actin-crossbridge reaction through Tm to the cTn complex. In modeling the relation between Ca^{2+} and steady-state tension and in a commentary on the use of the Hill equation for curve fitting, Shiner and Solaro (1984) emphasized the importance of energies of activation as a determinant of Hill n_H values not necessarily predicting the number of sites involved in the cooperative process, in this case, Ca^{2+} binding to cTnC.

Many studies have tested the effect of crossbridges on thin filament cooperative responses, but most have employed non-cycling rigor crossbridges (Fitzsimons and Moss, 2007; Kobayashi and Solaro, 2005; Tobacman, 1996). Indeed, when thin filaments are populated with rigor myosin heads, there is an increase in the Ca^{2+} affinity and a decrease in cooperativity of Ca^{2+} binding to thin filament cTnC (Davis et al., 2007; Pan and Solaro, 1987). Activation of a small population of regulatory units with rigor S1 induces an increase in force and ATPase rate in the cycling crossbridges remaining available (Engel et al., 2007; Fitzsimons and Moss, 2007). Desai et al. (2015) visualized the interactions of fluorescently labeled skeletal myosin S1 heads onto reconstituted single thin filaments suspended between posts, permitting a 3-D reaction. By varying Ca^{2+} -dependent activation of the thin filaments at relatively low MgATP concentrations, Desai et al. were able to demonstrate clustering of crossbridges interacting in a functional unit extending over several regulatory units. Cryo-EM revealed a potential interaction of crossbridge heads with the thin filament at a myosin S1-Tm interface in regions of actin-Tm interactions (Doran et al., 2020). Using molecular docking approaches, the authors concluded that this forms a basis of myosin-induced thin filament activation. As with many of these studies, the relevance of

these detailed atomic models to crossbridges cycling in the myofilament lattice and in situ remains to be determined. To test the Ca^{2+} -binding properties of cTnC in the myofilament lattice, we performed simultaneous determinations of steady-state-bound Ca^{2+} and tension in skinned fibers (Pan and Solaro, 1987). Although tension rose steeply with increasing free Ca^{2+} , binding of Ca^{2+} to the cTnC regulatory site was only weakly cooperative. This indicated that in steady-state conditions, the cooperative interaction among near-neighbor regulatory units may occur without an increase in cooperativity of Ca^{2+} binding but with cooperative spread of structural changes.

More recent use of techniques to probe the transduction of the cTnC Ca^{2+} -binding signal using fluorescent probes has indicated that thin filament structural changes do occur in a cooperative activation process. Studies pioneered by the laboratory of Wen-Ji Dong investigated thin filament activation as reported by FRET of cTnC in cardiac skinned fibers with different crossbridge states (Li et al., 2019; Rieck et al., 2013). Fluorescent probes placed on cTnC and exchanged into skinned fibers reported the cTnC–cTnI interaction by measurements of the opening of the cTnC hydrophobic patch that interacts with the switch peptide of cTnI to activate contraction (Fig. 2). pCa–tension relations were the same in skinned fibers controlled either by wild-type cTnC or fluorescently labeled cTnC. One significant finding was that FRET, as determined using lifetime measurements, tracked the steep rise of tension in the pCa–tension relation. Activation of tension and promotion of the cTnC–cTnI interaction had nearly identical pCa_{50} and n_H values. These studies confirmed that the open state of the hydrophobic cleft on cTnC senses steady-state tension development. In view of our data presented above showing only weak cooperation in steady-state Ca^{2+} binding to cTnC, these results indicate a near-neighbor spread of activation induced in concert with tension and relatively independent of cTnC Ca^{2+} binding. FRET measurements made in a strong binding crossbridge state induced by MgADP, which increased tension to maximum levels independent Ca^{2+} , demonstrated a stabilization of the interaction of the cTnC hydrophobic patch with the cTnI switch peptide. This is consistent with FRET experiments in reconstituted, Ca^{2+} -saturated thin filaments in which rigor myosin further stabilizes cTnI mobile domain proximity to cTnC relative to ATP- γ -S or blebbistatin bound to myosin (Solís et al., 2018). Experiments inhibiting tension with vanadate, however, did not support an exclusive role for force-generating crossbridges in this effect on the cTnC–cTnI interaction. In 1 mM vanadate, although tension was inhibited nearly 100%, there was a substantial activation of the cTnC–cTnI interaction over the same range of pCa values activating tension in the absence of vanadate (Rieck et al., 2013). There was no evidence of extraction of cTnI by 1 mM vanadate as previously reported (Strauss et al., 1992). There was a small effect of vanadate treatment on the slope of the pCa–FRET relation, but a significant rightward shift of the pCa–cTnC structural transition compared with controls. Similar results were obtained by Bell et al. (2006) using a rhodamine-labeled cTnC and fluorescence polarization to report probe angle in skinned fibers. They found that alteration of probe angle persisted in the presence of vanadate and was induced at pCa 9.0 in rigor. These experiments

indicate that mechanisms other than crossbridge-dependent activation may dominate in thin filament sarcomere activation. As developed below, a likely mechanism is the interaction of the N-terminal domain of cMyBP-C with actin-Tm.

Other studies probing structural changes in cTnC with bifunctional rhodamine probes have also demonstrated cooperative activation of the thin filament in cardiac skinned fibers independent of force-generating crossbridges. The probes used by Sun et al. (2009) reported changes in helical orientation of peptides near the IT arm (Fig. 2 B) consisting of the regulatory Ca^{2+} binding site and cTnC–C-terminal lobe interacting with cTnI. Probe orientation and force had similar n_H values, but when force was inhibited by blebbistatin, there was no change in the n_H values or pCa_{50} of the structural transition in the IT arm. Induction of the rigor state induced the structural transition even at pCa 9.0. Analysis of the data using double Hill equations that separate effects at the I-band and A-band showed that whereas cycling crossbridges affected the IT arm only in the A-band, rigor crossbridges promoted the structural transition in the IT arm in both the A-band and the I-band. In view of these findings, Sun et al. (2009) concluded a minimal role for cycling crossbridges in thin filament activation. One related set of much earlier experiments that requires interpretation are studies showing that upon a quick transient release of cardiac muscle in a twitch contraction, there is a transient rise “bump” in the cellular Ca^{2+} transient as measured with the aequorin technique (Allen and Kurihara, 1982). Sun et al. (2009) indicated that this is due to a small component of tension on cTnC Ca^{2+} affinity that is not a major regulator of thin filament activation. However, other data also indicate that some states of the thick filament can affect thin filament activation.

A study probing the mechanism of effects of a sarcomere activator targeted to myosin provided evidence that a small population of crossbridges reacting with actin may activate the filament. These studies with omecamtiv mecarbil (OM), a small-molecule inotropic agent, screened for its ability to activate myosin ATPase provide an insight into the possible role of a small proportion of crossbridges reacting with the thin filament with an increased lifetime of attachment to affect thin filament activation. Woody et al. (2018) tested the effect of OM using single myosin optical trapping experiments for measurements of the lifetime of the crossbridge attachment to actin filaments, as well as the size of the working stroke, and modeling data from in vitro motility assays with regulated actin filaments gliding on cardiac myosin. Surprisingly, Woody et al. discovered that despite the reported effect of OM to increase cardiac function (Teerlink et al., 2020), OM with a half-maximal effective concentration of 0.10 μM inhibited the working stroke size 10-fold and inhibited gliding of actin filaments by 30-fold while at the same time increasing the duration of the lifetime of the strong, force-generating state (duty cycle; Woody et al., 2018). Models employed for interpreting these results indicated that a relatively small population of crossbridges with this increase in duty cycle modify the thin filament to react more easily with OM-free crossbridges, thereby increasing tension and response to Ca^{2+} despite the effect of OM to decrease the working stroke. Detachment of OM crossbridges from actin was independent of

ATP concentration and proposed to occur off the pathway of the kinetic cycle without binding of ATP. It is important to point out that the preparations used in the [Woody et al. \(2018\)](#) study did not contain cMyBP-C, which has been reported to be important in the activation of sarcomeres by OM ([Mamidi et al., 2015](#)). Nevertheless, in related experiments using skinned fiber and testing whether a small population of rigor crossbridges may activate the thin filament, Engel and colleagues used titration of skinned fibers with rigor myosin S1 heads to determine the effect of small populations of rigor crossbridges on activation of tension ([Engel et al., 2009](#); [Engel et al., 2007](#)). In this case, there was no effect of the rigor bridges on activation of tension at pCa 9.0, but there was an increased response to Ca²⁺, depending on the state of the thin filament as revealed in skinned fibers with phosphorylation of cTnI or expressing the slow skeletal isoform of TnI. As discussed below, there is further structural evidence for a small population of crossbridges in the C-zone of cardiac sarcomeres as determinants of peak tension in a twitch contraction ([Brunello et al., 2020](#)).

Integration of recent advances in structural states of the thin filament as controlled by SRX and DRX thick filament states, interactions of cMyBP-C, and the cRLC

There is recent interest and focus on variations in thick filament control mechanisms in which the crossbridges are in a continuum of states with myosin heads either folded back on themselves and sequestered in an SRX or in an extended state called the DRX ([Alamo et al., 2017b](#); [Anderson et al., 2018](#); [McNamara et al., 2015](#); [Toepfer et al., 2020](#)). [Fig. 2 A](#) illustrates these states in the C-zone of the A-band. Considerations of the SRX and DRX are commonly focused only on the rates of crossbridge ATP hydrolysis and as a mechanism to vary cardiac reserve by shifts in the population of crossbridges available for contraction ([Anderson et al., 2018](#); [McNamara et al., 2015](#)). Yet, the role of these crossbridges beyond the thick filament has emerged as an important mechanism related to cooperative activation of the thin filament. The roles of these states in disorders of the heart and in therapy for hypercontractile states, such as HCM and HFpEF, and hypocontractile states, such as DCM and HFrEF, have added significance and interest in the detailed understanding of control mechanisms involving SRX and DRX. There is a maladaptive tilt in population of SRX and DRX associated with mutations in thick filament proteins linked to HCM ([Anderson et al., 2018](#)) and DCM ([Planelles-Herrero et al., 2017a](#)). Moreover, small molecules such as mavacamten (Mava), a sarcomere inhibitor, and OM, a sarcomere activator (described above), are thought to work in part by modifying the SRX and DRX ([Fig. 2 A](#)). As an example, porcine sarcomeres regulated by the HCM myosin mutant R403Q demonstrate small-angle x-ray diffraction-elevated levels of DRX, which is restored to control levels by Mava ([Anderson et al., 2018](#)). In the case of HFrEF, elevated levels of SRX are evident and reduced by OM ([Planelles-Herrero et al., 2017b](#)). [Alamo et al. \(2017b\)](#) provided another example of the molecular basis of HCM and DCM specific to myosin mutations. While HCM-causing mutations concentrate in the converter domain that may affect relaxation via conversion to the SRX state, DCM-causing mutations are enriched in the nucleotide-binding site but not in the converter domain.

The existence and designation of the SRX, which is structurally related to the interacting head motif of myosin, and DRX in striated muscle originated from pioneering work in tarantula muscle (reviewed in [Alamo et al., 2017a](#); [Toepfer et al., 2020](#)) have been demonstrated in multiple muscle types, including heart. SRX was identified to be associated with a slow rate of ATP hydrolysis monitored with N-methylanthraniloyl-ATP (mant-ATP; [Toepfer et al., 2020](#)), which has been convincingly correlated with crossbridge structural states using both small-angle x-ray diffraction ([Anderson et al., 2018](#)), and conformational probes of thick filament proteins ([Brunello et al., 2020](#); [Kampourakis et al., 2016](#)). Although SRX is evident in myosin alone, as described in two recent cryo-EM structures ([Scarff et al., 2020](#); [Yang et al., 2020](#)), investigations in the intact myofilament lattice have indicated the necessity of including the influence of other sarcomeric proteins on the integrated signaling mechanisms associated with SRX and DRX crossbridges. Early electron microscopic investigations of thick filament bundling by [Levine et al. \(2001\)](#) indicated that cMyBP-C phosphorylation induced an unpacking of the thick filaments, resulting in the crossbridges moving closer to the thin filament and interacting as weak crossbridges inducing the closed state. Studies by [Farman et al. \(2011\)](#) using small-angle x-ray diffraction demonstrated that increasing diastolic length induces a change in orientation of the crossbridges to a position more perpendicular to the thick filament. This finding indicates that external strain applied to cardiac muscle affects the population of SRX and DRX. In our x-ray diffraction investigation of effects of PKA phosphorylation on structural changes with changes in sarcomere length of cardiac trabeculae, there was a change in lattice spacing in the absence of cTnI phosphorylation attributable to cMyBP-C phosphorylation ([Konhilas et al., 2003](#)). We indicated that this was due to movement of mass away from the thick filament, which could now be interpreted to be like a shift from the SRX to DRX discovered much later. Along this line, there is evidence that PKA phosphorylation of cMyBP-C alters length-dependent activation ([Mamidi et al., 2016](#)), suggesting a modification of SRX and DRX. As discussed below, a major influence is the state of cMyBP-C, which interacts with the myosin S2, myosin LMM, myosin cRLC ([Ratti et al., 2011](#)), thin filament, and titin. Others are the state of the cRLC, which interacts with the myosin head, and interactions of titin with crossbridges and the thin filament.

Data reported by [Brunello et al. \(2020\)](#) demonstrated the potential influence and complex variations of SRX and DRX in the contraction and relaxation of the heart. Using synchrotron small-angle x-ray diffraction and interference x-ray techniques, [Brunello et al.](#) were able to analyze the disposition of the population of SRX and DRX crossbridges in local single-sarcomere domains during development and decay of tension in a twitch of cardiac muscle. These studies demonstrated that early and late in the twitch contraction, myosin heads outside the C-zone are activated as reported by the movement of these crossbridges radially from a conformation folded back onto the thick filament to an extended conformation closer to the thin filament. At peak tension, the data were interpreted to indicate that ~10% of the crossbridges restricted to the C-zone are extended and engaged

in sustaining peak tension. Signaling mechanisms for the generation of these states of the thick filament are yet to be clearly determined but may involve the coordinated control of proteins of the thin filament and proteins of the thick filament states and modulation by post-translational modifications. One likely candidate is cMyBP-C, which has been reported, as discussed below, to have inhibitory effects on the thick filaments but activating effects on the thin filaments (Craig et al., 2014; Kampourakis et al., 2014) by competing for Tm-binding sites on actin at low levels of Ca^{2+} . The absence of cMyBP-C in the D-zone could explain why crossbridges outside the C-zone are activated and inactivated out of phase with C-zone crossbridges.

Important evidence related to mechanisms of OM and Mava is the demonstration of a role for other sarcomere domains in the effects of these agents targeted to control myosin states. Measurements in skinned fibers from hearts of mice with ablated cMyBP-C used k_{tr} to determine effects of OM and Mava on entry of crossbridges into force-generating states and stretch activation to determine k_{rel} as a measure of rates of crossbridge detachment (Mamidi et al., 2015; Mamidi et al., 2018). Both k_{tr} and k_{rel} were inhibited by OM in controls, but the effect was greater in the skinned fibers lacking cMyBP-C. The result was that in the presence of OM, there are no differences in crossbridge kinetics between controls and the fibers lacking cMyBP-C. The authors suggested that OM may be a drug of choice for improving the inefficiency of hearts with increased crossbridge kinetics because of reduction in expression of cMyBP-C. In the case of Mava, there was the predicted depression in tension at relatively low Ca^{2+} , but the effect was greater in controls than in fibers lacking cMyBP-C (Mamidi et al., 2018). Moreover, Mava depressed k_{rel} only in the fibers lacking cMyBP-C. These results further emphasize the need for understanding the effects of other sarcomere proteins in the use of agents directed at modulating the SRX and DRX. Studies using similar techniques with OM that were performed comparing skinned fibers from donors and from hearts at the terminal stages of HF (Mamidi et al., 2017) support this statement. Although these studies reported an increase in tension generation by OM in control and HF samples, they also found a greater effect of OM in HF samples in slowing the time course of crossbridge transitions to states of activation. This raised the possibility that OM may increase systolic function but with an untoward effect on diastolic filling by prolongation of systolic ejection time. These data indicate that the effect of OM depends on an altered state of the HF myofilaments, which may be levels of phosphorylation of regulatory proteins such as cMyBP-C, cTnI, and/or cRLC. The influence of variations in the balance between SRX and DRX may also come into play.

Like cTnI, cMyBP-C has properties unique to the cardiac variant. Excellent reviews (Carrier et al., 2015; Flashman et al., 2004; Harris et al., 2011; Sadayappan and de Tombe, 2014; Wang et al., 2018) have described the domain structure of cMyBP-C consisting of Ig and fibronectin-like domains similar to titin and pointing out the existence of a unique C0 Ig-like domain at the N terminus together with a unique Pro-Ala domain linked to a unique motif called the M-domain located between the C1 and C2 domains that contains sites of phosphorylation. N-terminal domains bind to both actin and myosin in a phosphorylation-

dependent manner. At low levels of thin filament Ca^{2+} activation, binding of cMyBP-C to actin activates the thin filament and appears to do so in synergy with Tm. Phosphorylation of the M-domain by a variety of kinases, most notably PKA, inhibits the binding of cMyBP-C to actin and to the S2 region of myosin. This release of cMyBP-C from S2 stimulates actomyosin ATP hydrolysis and the shortening velocity of the sarcomere. Several studies provided the initial idea that cMyBP-C may regulate both thick and thin filament activation. Kampourakis et al. (2014) employed probes on cTnC and cRLC to test this hypothesis. The approach involved adding various concentrations of the N-terminal peptide (C1-m-C2) to skinned fiber preparations. In the absence of Ca^{2+} , addition of the peptides promoted a cooperative spread of an off state of the thick filaments as monitored by the cRLC probe. Addition of blebbistatin in the absence of Ca^{2+} did not modify the effect of the peptide on thick filament state. However, at low levels of activating Ca^{2+} , addition of small amounts of the C1-m-C2 peptide induced an activation of cTnC as monitored with probes at the IT domain. The activation of the cTnC structural change was highly cooperative and retained in the presence of blebbistatin, which reduced active tension to zero. Binding of cMyBP-C C0-C3 N-terminal fragments to reconstituted thin filaments enhanced myosin S1 decoration of thin filaments at low calcium levels but not at high calcium levels where the C0-C3 fragment acted as a myosin blocker (Inchingolo et al., 2019). Mechanistically, one can describe cMyBP-C as a myosin analogue that lacks ATPase activity, competes with myosin for thin filament binding and activation, and interacts with myosin to promote relaxation.

Although these data provide evidence that the N terminus of cMyBP-C is a major determinant of thin filament cooperative response, a desirable experiment for in situ FRET experiments is to selectively excise the cMyBP-C N terminus as described by Napierski et al. (2020) or by using cMyBP-C mice knockouts (KOs; Harris et al., 2002; Napierski et al., 2020). By inserting a cleavable region in the protein, Napierski et al. describe a “cut and paste” approach to selectively and rapidly cleave C0-C7 of cMyBP-C and to restore the peptide in permeabilized myocytes. The advantage is that the cleavage was correctly localized, which is not assured in methods involving adding peptides to permeabilized preparations. Napierski et al. reported that cleavage of C0-C7 induces a desensitization in force- Ca^{2+} relations in skinned myocytes with no change in maximum force or n_H . In k_{tr} measurements, cleavage of C0-C7 induced an enhanced rate of recovery of tension and an overshoot of tension above preslack levels. Cleavage also induced spontaneous oscillatory contractions (SPOC) originally described in skinned heart muscle by Fukuda et al. (1996). Restoring the nonphosphorylated cleaved peptide stopped the SPOC, but a phosphorylated peptide damped but did not stop the SPOC. The authors suggested that this effect of phosphorylation may be an aspect of the enhanced crossbridge kinetics that occurs with cMyBP-C PKA phosphorylation. Interestingly, Fukuda et al. speculated that SPOC represent an autonomous oscillator at the level of the sarcomere, aiding the response of the heart to the oscillation imposed by varying frequencies of the pacemaker.

In considering thin filament states at different levels of Ca^{2+} , Kampourakis et al. (2014) presumed that in the resting state a

fraction of cMyBP-C is extended and interacting with actin-Tm, thus stabilizing an active state that synergizes with the release of Tm signaled by initial binding of Ca^{2+} . A similar conclusion was also hypothesized by [Previs et al. \(2015\)](#) in which cMyBP-C rectifies troponin (Tn)-Tm activation at the D- and C-zones during the uneven rise in the Ca^{2+} from the Z-disc to the M-line during Ca^{2+} release from the sarcoplasmic terminal cisternae. With higher levels of Ca^{2+} , cTnC signals the promotion of the release of Tm, thereby competing with cMyBP-C actin binding, which causes dragging forces that oppose actomyosin sliding ([Previs et al., 2012](#)). To understand such cMyBP-C and thin filament interactions, [Risi et al. \(2018\)](#) used kinetic studies together with cryo-EM to elucidate the molecular mechanisms of the interaction of the N terminus of cMyBP-C with and activation of cardiac thin filaments. They reported that the C0 and C1 domains act cooperatively to activate thin filaments and demonstrated that a positively charged C1 region interacts with Tm and is stabilized by C0. Phosphorylation, most likely of cTnI and cMyBP-C, adds a layer of control on these processes. Specifically, cMyBP-C phosphorylation decreases cMyBP-C N terminus binding to thin filaments, causing desensitization of thin filaments at subsaturating Ca^{2+} levels ([Ponnam et al., 2019](#)).

In the C5 Ig domain, there is a cardiac-specific stretch of 27 amino acids (aa) that appears to act as a hinge in the structural rearrangements of cMyBP-C. At the C-terminal end, C8-C10 of cMyBP-C constitutively interact with titin and LMM ([Al-Khayat et al., 2013](#); [Zoghbi et al., 2008](#)). An interesting observation demonstrated in studies of a heterozygous cMyBP-C KO mouse model of HCM is evidence that there may be an interaction of the four-and-a-half LIM protein 1 (Fhl1) associated with the C terminus C6-C10 domains ([Carrier et al., 2015](#); [Vignier et al., 2014](#)). In the HCM model, Fhl1 was downregulated but restored by blockade of the angiotensin-1 receptor, which also restored structure and function. Although there are multiple protein-protein interactions involving Fhl1, an interesting and important high-affinity interaction is with the spring-like Pro-Glut-Val-Lys (PEVK) and N2B region of titin in which there is a modification of titin compliance in muscle cells either by a direct interaction or via an ability of LIM proteins to scaffold mitogen-activated protein kinases and modify titin phosphorylation ([Liang et al., 2018](#); [Sheikh et al., 2008](#)). We also reported a possibly related finding that signaling promoted by biased ligand signaling at the angiotensin-1 receptor induced a translocation of β -arrestin to the sarcomere colocalizing with LMM and increasing tension ([Ryba et al., 2017](#)). It would be of interest to know the potential for these signaling proteins to react with cMyBP-C and affect function. We next discuss a further complexity associated with oxidative-related post-translational modifications of cysteines in cMyBP-C.

Oxidative stress, commonly associated with many disorders of the heart, induces a post-translational modification of cMyBP-C by S-glutathionylation and provides further evidence of the complexity of cMyBP-C regulation of thin filament activation state. Our studies of myofilaments isolated from a mouse model of HFpEF provided the initial finding that cMyBP-C could be S-glutathionylated ([Lovelock et al., 2012](#)). This modification induced increased myofilament response to Ca^{2+} and slowing of

crossbridge kinetics that were closely correlated with depressed diastolic function ([Lovelock et al., 2012](#)). Suppression of the oxidative stress response reversed the S-glutathionylation and the diastolic dysfunction ([Jeong et al., 2013](#)). Follow-up studies in samples of human HF confirmed an increase in S-glutathionylation compared with controls likely to occur in cysteine residues in or near the C1-m-C2 domain interacting with myosin-S2 ([Stathopoulou et al., 2016](#)). Moreover, S-glutathionylation impeded PKA-dependent phosphorylation of the unique M-region. Mass spectrometry of cMyBP-C treated with oxidized glutathione identified Cys-249 (near the phosphorylation domain; [Stathopoulou et al., 2016](#)), Cys-655 and Cys-479 (near regions anchoring cMyBP-C to the thick filament), and Cys-627 (in C5 that contains a 27-aa peptide unique to the heart; [Patel et al., 2013](#)). It is apparent that the mechanism by which S-glutathionylation of cMyBP-C decreases Ca^{2+} -activated actomyosin ATPase rate is by its binding to myosin-S2, but how it increases myofilament response to Ca^{2+} requires further investigation. It is apparent that the oxidation induces a state of cMyBP-C different from phosphorylation, which increases actomyosin ATPase and decreases thin filament activation. Although the suppression of phosphorylation of cMyBP-C by this oxidation also requires more thorough investigation in cardiac disorders, there is evidence that the cross talk between cMyBP-C S-glutathionylation and PKA-dependent phosphorylation is an important determinant of altered cardiac function in stressful exercise in which the oxidation is increased and the phosphorylation is decreased ([Chakouri et al., 2018](#)). A similar effect has been reported in doxorubicin-induced cardiac toxicity ([Chakouri et al., 2020](#)).

There is evidence that interactions of cRLC with thin filaments provide a signaling mechanism in addition to cMyBP-C-actin interactions for coordinated control of thin filament activation by proteins of the thick filament. The development of an understanding of the role of cRLC in controlling cardiac function has been extensively reviewed ([Scruggs and Solaro, 2011](#); [Sheikh et al., 2015](#); [Sitbon et al., 2020](#); [Yadav and Szczesna-Cordary, 2017](#)). A consensus view is that cRLC regulates myofilament response to Ca^{2+} and the actomyosin ATPase rate that varies with phosphorylation state. In studies of a TG mouse model expressing a nonphosphorylatable cRLC, TG-cRLC-(P-), we ([Scruggs et al., 2009](#)) reported that the loss of cRLC phosphorylation was associated with a depression of tension cost (ratio of unit of tension developed/unit of ATPase activity). Hearts of the TG mice also demonstrated a reduction in phosphorylation of cTnI and cMyBP-C compared with controls. These data indicated that interactions among these proteins affected functional state. The response to β -1-adrenergic stimulation in TG-cRLC-(P-) hearts was blunted, whereas phosphorylation of cTnI and cMyBP-C remained the same as in controls. This result indicated that PKA signaling to these proteins was not affected. In general support and advancement of this concept, [Kampourakis et al. \(2016\)](#) reported that phosphorylation of the cRLC generates a transition of crossbridge state from SRX to DRX that modifies thin filament activation. [Kampourakis et al.](#) exchanged native proteins with cRLC or cTnC labeled with bifunctional probes to report cRLC changes, indicating a modification of the folded

state of crossbridges and thin filament cTnC structural changes with and without cRLC phosphorylation. They reported that the increase in Ca^{2+} sensitivity associated with cRLC phosphorylation induced a movement of the cRLC away from the thick filament proper into a more perpendicular orientation, indicating a transition to the DRX. Moreover, this phosphorylation-induced structural change spread in a cooperative manner from phosphorylated cRLC crossbridges to nonphosphorylated cRLC crossbridges in the thick filament. cRLC phosphorylation also induced a significantly increased Ca^{2+} sensitivity of cTnC structural changes, which persisted with blebbistatin treatment and complete inhibition of force generation. Increases in sarcomere length also induced cooperative structural changes in the thick filaments. These effects involved modifications in the N terminus of cRLC, which houses the phosphorylation site Ser-15. As indicated above, phosphorylation at this site increased myofilament Ca^{2+} sensitivity; deletion of the N-terminal peptide of cRLC mimicked these effects of phosphorylation. It is apparent from these results that the dephosphorylated N terminus of cRLC interacts with the thin filament or with myosin-suppressing activation, which is released by cRLC phosphorylation, mimicked by truncation of the N terminus. This mechanism is reminiscent of the effect of phosphorylation of cTnI at Ser-22,23 in a unique N-terminal peptide, which releases it from binding to cTnC and promotes increased Ca^{2+} -release kinetics from cTnC (Solaro et al., 2013). This effect is mimicked by truncation of the Ser-22,23 N-terminal peptide (Tachampa et al., 2008). A related finding is evidence that the human isoform of cardiac myosin light kinase is able to phosphorylate both cRLC as well as Ser-23 of cTnI (Sevrieva et al., 2020). These data reveal an important role for cRLC in signaling pathways between myosin heads and between myosin heads and the thin filament. Detailed mechanisms remain to be elucidated, but involvement of interactions of cRLC with cMyBP-C with the thin filament may be one route of signaling from the thick to the thin filaments. Together with in vitro data on skinned fibers showing integrated effects of cRLC and cMyBP-C, data on their effects on systolic elastance indicate that alterations in cMyBP-C and cRLC are integrated and important in intact cardiac function. Palmer et al. (2004) reported that among a variety of modifications on cardiac muscle regulatory mechanisms, only hearts of a mouse model expressing a homozygous truncated cMyBP-C (t/t) in which cMyBP-C protein could not be detected demonstrated an abbreviation of systolic elastance time course. Similarly, Scruggs et al. (2009) reported that hearts of TG-cRLC-(P-) mice showed a prolonged ejection duration with an abbreviation of the time to left ventricular (LV) elastance.

Advances in understanding the three-state model in cardiac thin filament activation

In addition to the use of small-angle x-ray diffraction, three recent approaches provide insights into the three-state model in cardiac thin filament activation. These include cryo-EM and sensors of structural changes and Ca^{2+} binding in sarcomeres functioning in situ. Risi et al. (2017) used cryo-EM and single-particle analysis to reconstruct the position of Tm in frozen,

hydrated samples and compared their findings with measurements reported in the same paper using negative staining as traditionally applied (Pirani et al., 2005). Compared with the negative staining images, they reported a smaller extent of azimuthal movement of Tm induced by the transition from the B- to C-states and to the M-state with rigor crossbridges (Fig. 3 A). In other words, whereas the M-state position is unchanged, the cardiac B- and C-states are shifted closer to the M-state compared with the canonical B- and C-states. Moreover, in relaxing conditions, they found not only portions of the thin filaments in a blocked state like the canonical B-state but also portions of the thin filament in an open state like the canonical C-state (Fig. 3 B). Ca^{2+} binding induced the open state to a greater extent than weakly bound crossbridge interactions. The step to full activation of the thin filament thus resulted more from Ca^{2+} -binding-induced release from cTnI inhibition than from strong crossbridge interactions. Thus, these findings fit generally with conclusions from kinetic measurements of actomyosin interactions, indicating a stronger role for Ca^{2+} binding than strong crossbridges in cardiac versus skeletal muscle thin filament activation. They also fit with the findings reported in dynamic measurement of thin filament activation using HS-AFM (Matusovsky et al., 2019) and FRET reporters of the cTnI-cTnC interaction (Solís et al., 2018; Vetter et al., 2020). Given that these cardiac thin filament state distributions were found in native thin filaments, further structural work should address how these distributions change in the presence of strongly bound myosin and cMyBP-C. A direct comparison of the cardiac and skeletal thin filament states using these structural techniques will be beneficial to better understand the specific regulatory differences between the two kinds of striated muscle thin filaments.

HS-AFM provides a technique to image movements of Tm and actin monomer structure transitions in the nanometer range in real time under conditions close to the physiological state. As an interrogation of the three-state model, Matusovsky et al. (2019) deposited cardiac thin filaments on lipid layers and monitored movements of Tm in response Ca^{2+} and crossbridges with and without MgATP. As detailed in the data presented, the results demonstrated that the fully relaxed and fully activated thin filaments are not in a fully blocked or activated state as pointed out above (Pirani et al., 2005) but exist in a combination of states with the balance determined by the prevailing Ca^{2+} and crossbridge states. Important conclusions include a demonstration that weak binding can shift the position of Tm locally on the thin filament in a manner like strong binding and that strong binding crossbridges can activate adjacent regulatory units to a greater extent than weakly bound crossbridges. Furthermore, saturating Ca^{2+} shifts Tm by $\sim 17.6^\circ$ in agreement with the $\sim 15^\circ$ Ca^{2+} -induced shift from prior single-particle cryoelectron reconstructions of cardiac thin filaments (Risi et al., 2017). Other cryoelectron structures of cardiac thin filaments have reported Ca^{2+} -induced azimuthal shifts of the Tm-pseudo repeats, ranging from 18° that fit with the C-state to 28° that fit with the M-state (Paul et al., 2017). These data provide support for and a new perspective on the three-state model and set the stage for more studies to fully understand modulation of these states as discussed below.

Further support for the conclusion from the data of [Risi et al. \(2017\)](#) comes from the use of FRET sensors attached to the cTnC C-lobe and cTnI C-terminal domain ([Solís et al., 2018](#)). By immobilizing myosin monomers to a glass surface akin to an *in vitro* motility assay setup, [Solís et al. \(2018\)](#) applied fluorescence lifetime imaging FRET to the labeled cTn bound to thin filaments in contact with myosin. Their data demonstrated that most of the cTn activation is from Ca²⁺ binding compared with the additional activating contribution of rigor myosin with a concomitant gain of the cTnI mobile domain conformational flexibility. This observation is consistent with the recent cryo-EM structure of cardiac thin filaments that showed the TnI mobile domain anchored to the actin-Tm surface in the Ca²⁺-free state, while this region is missing in the Ca²⁺-bound state possibly because of a lack of a defined structural state ([Yamada et al., 2020](#)). It was also reported that the nonhydrolysable myosin substrate ATP- γ -S has no further effects on cTn activation at saturating and subsaturating Ca²⁺ compared with rigor myosin. The effect of rigor myosin activation was more pronounced at subsaturating Ca²⁺ compared with saturating Ca²⁺. On the other hand, blebbistatin in the presence of ADP and phosphate had a modest effect on cTn activation at subsaturating Ca²⁺ possibly because of the existence of strongly bound myosin species. However, other reports suggested that a myosin-ADP-blebbistatin complex may capture myosin into a prepower stroke state, which is a strongly bound conformation of myosin ([Takács et al., 2010](#)). This illustrates how the complex equilibrium distribution of myosin may influence cTn response to Ca²⁺.

Another approach reported used a FRET conformational sensor attached to cTnC with the goal of monitoring the activation state of thin filament in real time in intact muscle preparations generating force under conditions expected to modify myofilament activation. On the basis of development of this type of FRET probe by [Badr et al. \(2016\)](#), [Vetter et al. \(2020\)](#) incorporated a donor and an acceptor fluorescent protein at the N- and C-lobes of cTnC and exchanged the modified cTnC in studies of permeabilized fibers. Also studied were heart preparations from TG mice expressing modified cTnC. Studies in intact papillary muscles and myocytes showed that the FRET sensor does not report cytoplasmic Ca²⁺ transients. Important findings included a demonstration that the thin filament senses β -adrenergic stimulation by a faster rise time and decay of the signal. This fits with earlier predictions that phosphorylation of cTnI influences decay of relaxation ([Kentish et al., 2001](#); [Solaro et al., 2013](#)). The authors acknowledged that the response to β -adrenergic stimulation on the tension transient is likely to occur because of integrated effects of other myofilament proteins, especially cMyBP-C. A surprising finding was that unloaded myocytes showed no change in the reporter signal when tension was promoted by OM or inhibited by a blebbistatin analogue. In contrast, in loaded papillary muscles, developing tension isometrically, the FRET sensor had a longer duration and slower decay compared with unloaded myocytes. Changes in tension imposed by OM correlated with the signals from the FRET sensor. The conclusion was that cycling crossbridges promote activation of the thin filaments. Studies in preparations in which cTnI was replaced with mutants linked to HCM

supported these general conclusions. No studies were performed with effects of β -adrenergic stimulation on loaded preparations. This would be of interest because earlier work showed that the effects of cTnI phosphorylation on relaxation kinetics are enhanced in auxotonically loaded ejecting hearts ([Layland et al., 2004](#); [Layland et al., 2005](#)). [Vetter et al. \(2020\)](#) concluded from their studies that the results fit well with the cryo-EM structural data, indicating a dominant role of Ca²⁺ and its interactions with the cTnI-cTnC complex in moving Tm to the open M-state.

The development of genetically encoded Ca²⁺ indicators is a significant advance in the tools to understand interactions of Ca²⁺ with the microdomains of the cardiac sarcomere. The probes are known as red genetically encoded Ca²⁺ indicator for optical imaging (RGECO; [Sparrow et al., 2019](#)). [Sparrow et al. \(2019\)](#) conjugated the probe with cTnI or cTnT and exchanged the labeled proteins with native proteins using viral vectors. The exchanged protein localized correctly, and the modified proteins did not interfere with Ca²⁺-dependent myofilament ATPase activity. To compare myofilament-bound Ca²⁺ to bulk sarcoplasmic Ca²⁺, Sparrow et al. performed measurements of fluorescence dynamics of the unbound RGECO and the bound RGECO in single cardiac myocytes. Important findings include the demonstration that local kinetics of Ca²⁺ at the sarcomeres sensed by RGECO show faster on and slower off rates than predicted by cytoplasmic unconjugated RGECO. Whereas OM had no effect, Mava significantly reduced the peak heights of both sarcoplasm- and sarcomere-localized Ca²⁺ as well as increased the rate of Ca²⁺ binding to and release from sarcomeres. The unconjugated probes reported variable changes in the rate of rise and fall of the sarcoplasmic Ca²⁺ transient with Mava treatment. Other important findings demonstrated differences in dynamics of the sarcoplasmic Ca²⁺ transient and Ca²⁺ at the sarcomere compartment in myocytes regulated by cTnI mutants linked to HCM. Issues related to use of RGECO include an inability to quantify the Ca²⁺ levels and to determine changes in diastolic Ca²⁺ ([Campbell et al., 2019](#)). Moreover, [Sparrow et al. \(2019\)](#) did not determine mechanical readouts of myocyte function. The measurements were performed in unloaded myocytes, which as pointed out in earlier studies have effects on thin filament signaling ([Ait Mou et al., 2008](#); [Layland et al., 2004](#); [Monasky et al., 2010](#); [Vetter et al., 2020](#)). Nevertheless, RGECO represents a significant step toward determinations relating sarcomere function and signaling *in situ*.

How the Z-disc influences the actin-crossbridge reaction and other sarcomere domains

Ends of the thin filaments comprise domains essential to the assembly, disassembly, and stability of the thick and thin filaments. An important area of investigation related to our review is how the same signals intervening in the phosphorylation of the thin and thick filament proteins also integrate into the Z-disc protein network that affects sarcomere structure and function. Evidence indicates that the barbed and pointed ends of the thin filaments are dynamic domains that ultimately influence cardiac contractility.

Barbed ends of thin filaments are tightly anchored to sarcomeres at the Z-discs and appear to have a monolithic appearance

relative to the thin and thick filament participation in cross-bridge reactions with actin. Yet, Z-discs also experience wide structural dynamics and harbor several kinases and phosphatases that modify activity at the overlap regions by a process we previously described as “remote control” (Solaro, 2005). The Z-discs also support thick filaments indirectly via titin and link the sarcomere to the extracellular matrix through a series of the adaptor proteins that constitute the costameres (Samarel, 2005). Solaro and Solaro (2020) reviewed the connections of sarcomere domains to the cytoskeleton in the context of the instabilities of the myocyte membrane inducing a release of cTn into serum.

The Z-discs of cardiac muscles experience reversible deformation of their lattice structure from relaxed to actively shortening muscle fibers that increase the interspace distance between thin filaments (Goldstein et al., 1989; Oda and Yanagisawa, 2020). This structural versatility is remarkable when considering that the Z-disc is the most rigid region in the sarcomere when measuring the transverse stiffness of myofibrils by atomic force microscopy (Yoshikawa et al., 1999). At a molecular level, this particular flexibility may be a result of α -actinin behaving as a “knob and rod” structure that facilitates reverse deformability of the Z-disc lattice (Gautel and Djinović-Carugo, 2016; Ribeiro et al., 2014). Perz-Edwards and Reedy (2011) suggested that the reversible structural transition of Z-discs is in part influenced by Tm rotation on thin filaments during muscle contraction, pointing to a direct mechanical communication occurring at the I-Z-I interface.

Cardiac thin filaments are also linked to the Z-discs by nebulin. The C terminus of nebulin is integrated into the Z-discs and interacts with several structural proteins, including myopalladin, palladin, titin, zyxin, N-WASP/WASL, XIRP1 and XIRP2, and α -actinin (Bang and Chen, 2015; Chu et al., 2016). The nebulin repeats are shown to interact with actin, Tm, Tn, and desmin, and deletions of these repeats compromise sarcomeric stability (Bonzo et al., 2008; Hernandez et al., 2016). Nebulin mouse KO mice exhibited normal cardiac function under normal and increased afterload conditions except for widening of the Z-disc structure, suggesting a role of nebulin in stabilizing the Z-discs (Mastrototaro et al., 2015).

Another key sarcomere structural feature is the titin N-terminal anchorage to the Z-disc depicted in Fig. 4 A. Near the titin Z terminus a series of Z repeats are the docking site of the α -actinin calmodulin-like domains, which are sensitive to conditional binding of phosphatidylinositol 4,5-bisphosphate (PIP₂) to α -actinin (Young and Gautel, 2000). At the tip of the titin N terminus, telethonin (or T-cap) links two parallel titin N termini in a palindromic arrangement (Zou et al., 2006). This telethonin–titin linkage appears to work as a “safety belt” because ablation of telethonin does not induce sarcomere disarray or dysfunction unless the myocardium is stressed by pressure overload (Knöll et al., 2011b). One intriguing interaction is between the Ser/Thr titin kinase found at the M-line and telethonin serving as the substrate during myofibrillogenesis (Mayans et al., 1998). Telethonin is also a target of PKD and CaMKII phosphorylation, and telethonin also stabilizes the interactions between the E3 ubiquitin ligase MuRF1 and the E2 ligases necessary for protein degradation (Candasamy et al.,

2014; Polge et al., 2018). The broad functional consequences of these modifications need to be further explored in the context of sarcomere assembly and maintenance.

CapZ is involved in multiple interactions affecting both myofilament function and structure. Studies of mouse hearts expressing a slightly reduced level of CapZ at the Z-discs revealed altered positioning of PKC ϵ and PKC β together with an increase in the myofilament Ca²⁺ sensitivity of tension (Pyle et al., 2002; Pyle et al., 2006). De novo thin filament assembly begins with CapZ positioning to the Z-discs that follow actin assembly (Schafer et al., 1993). TnT and Tm subsequently decorate nascent filaments to stabilize thin filament elongation (Ferrante et al., 2011; Huang et al., 2009). An interesting and important aspect of the mechanism by which CapZ may control thin filament function is by its continuous exchange with actin at the barbed ends of the thin filament. The exchange is augmented by mechanical and neurohumoral signals converging in the Z-disc. One example is that PIP₂ binding to CapZ leads to reduced binding to actin barbed ends (Hartman et al., 2009; Heiss and Cooper, 1991). This mechanism has been proposed to act via steric inhibition of the actin- and CapZ-binding interface by PIP₂ (Kuhn and Pollard, 2007). However, in silico and in vitro studies indicate that PIP₂ may interact with CapZ bound to actin barbed ends (Cooper and Pollard, 1985; Kim et al., 2007; Schafer et al., 1996; Solís and Russell, 2019). CapZ phosphorylation by PKC ϵ and acetylation via histone deacetylase 3 increase CapZ protein exchange at the barbed ends as a result of adenosine receptor activation, cyclic mechanical strain, and increased stiffness of the microenvironment (Li and Russell, 2013; Lin et al., 2015; Lin et al., 2016; Solís and Russell, 2019). Similarly, actin exchange rates at the barbed ends increased in response to β -adrenergic signaling, angiotensin II, and cyclic mechanical strain (Lin et al., 2013b; Li et al., 2014; Mkrtschjan et al., 2018). The long-standing hypothesis here is that increasing CapZ dynamics destabilize the Z-disc to promote filament remodeling and hypertrophy (Hartman et al., 2009).

Other proteins at the Z-disc have the general function of serving as adaptors for kinases via their LIM domains while anchoring to α -actinin through their PDZ domains (Li et al., 2012). PKC positioning at the Z-discs may be mediated in part by members of the actinin-associated LIM protein/enigma subfamily of PDZ-LIM-domain proteins. Members of this subfamily include cardiac actinin-associated LIM protein; enigma homologue (ENH), also known as PDZ and LIM domain 5; LIM domain-binding protein 3 (Cypher, ZASP, Oracle, or LDB3); and Enigma (LMP1; Knöll et al., 2011a). All these proteins have one PDZ and three LIM domains, with the number of LIM domains varying in some isoforms. Cypher has been identified to work as an A-kinase anchoring protein for PKA activity and as a receptor for activated kinases for conventional and novel PKC isoforms (Lin et al., 2013a; Zhou et al., 1999). It is composed by a ZASP motif flanked by a PDZ domain at its N terminus and three LIM domains at its C terminus. It was determined that the Cypher cardiac-specific region encoded by exon 4 (aa 108–227) interacts with the RII α -regulatory subunit of PKA and the Ser/Thr phosphatase calcineurin. Mice lacking the long form of Cypher that lacks the three C-terminal LIM domains involved in kinase

anchoring exhibit pressure overload that precedes DCM (Cheng et al., 2011). This shows the modular function of Cypher as a structural and signaling adaptor protein in the heart.

Another key regulator of the Z-disc structural assembly is ENH, which has been shown to work as a PKC β I and PKD1 anchoring protein (Kuroda et al., 1996; Maturana et al., 2008). Deletion of all ENH isoforms in mice exhibits impaired cardiac contraction, DCM, decreased levels of Cypher short isoform and calsarcin-1, and decreased phosphorylation levels of sarcomeric proteins (Cheng et al., 2010; Gregorich et al., 2019). In contrast, overexpression of the ENH1 isoform in rat neonatal ventricular myocytes leads to hypertrophy (Yamazaki et al., 2010). ENH1 was found to be upregulated in the normal embryonic heart. However, adult hearts exhibit ENH1 upregulation during pressure overload-induced hypertrophy. Given that PKD phosphorylation by PKC leads to hypertrophy, one hypothesis is that ENH1 enables progression of this signaling pathway by providing anchoring to both kinases at the Z-disc (Iwata et al., 2005; Rozengurt et al., 2005). Recent work by Tucholski et al. (2020) revealed that ENH and TnI phosphorylation may be part of the same PKA activation pathway. The phosphorylation state of several sarcomeric proteins assessed in healthy and HCM hearts determined that both TnI and ENH2 isoform phosphorylation decrease in HCM ventricles relative to healthy samples. It was also confirmed that PKA phosphorylates ENH2 in vitro at Ser-118, which is part of a consensus PKA substrate sequence. Although the effects of TnI phosphorylation by PKA are well established, the functional implications of ENH2 phosphorylation warrant further study. It is remarkable that phosphorylation signaling may target not only the A- and I-bands of the sarcomeres but also the Z-discs and that these modifications also vary with disease states.

The Z-disc also harbors many other structural proteins essential for maintaining sarcomere function, assembly, and protein quality control (Henderson et al., 2017; Martin and Kirk, 2020; Sanger and Sanger, 2008). These include intermediate filaments, such as desmin (Capetanaki et al., 2007); members of the LIM domain proteins (Li et al., 2012); the nebulin family, which includes nebulin and LASP-2 (Bang and Chen, 2015; Chu et al., 2016); and the Ig domain myopalladin/palladin/myotilin family of proteins (Otey et al., 2009). An in-depth description of these protein families, the isoforms being expressed in the heart, and their accompanying signaling pathways are out of the scope of this review. However, readers are encouraged to consult the dedicated literature.

The I-band as a regulator of muscle compliance

Despite lacking thick filaments, the I-band is a signaling destination that fine-tunes the elastic response of sarcomeres during contraction. The main constituents of the I-band are thin filaments and the titin spring or extensible I-band region illustrated in Fig. 4 B. Here, a brief discussion is given on how the I-band domain affects the crossbridge performance. Readers are encouraged to consult specialized reviews for additional information (Hidalgo and Granzier, 2013; LeWinter and Granzier, 2010).

One of the constituents of the I-band is the titin spring region in which its domain composition varies with each titin isoform

(Hidalgo and Granzier, 2013). Three cardiac titin isoform classes exist that include the N2B (~3 MDa), N2BA (~3.3–3.5 MDa), and the fetal-neonatal (~3.6–3.8 MDa) of which the N2B and N2BA are the most relevant in the developed heart (LeWinter and Granzier, 2010). The proportion between the shorter N2B and the longer N2BA isoform classes largely defines the force-extension relationship developed when extending relaxed muscles known as the passive tension. In addition to differential splicing, phosphorylation of titin also affects the passive tension profiles. Specifically, PKC activation leads to increased passive tension, while PKA and PKG attenuate the passive tension (Hidalgo and Granzier, 2013).

Chung and Granzier (2011) reported that titin contributes to nearly 80% of the passive tension in the heart when operating at normal sarcomere lengths of 1.8–2.2 μ m. By comparing the passive tension of permeabilized hearts and hearts with the majority of the myofibrillar proteins being extracted, including titin, it was established that the contribution of the extracellular matrix to the passive tension only becomes relevant at sarcomere lengths longer than ~2.2 μ m. This highlights the chief contribution of cardiomyocytes, relative to other histological components of the myocardium, to diastolic function.

As described in the previous paragraph, the composition of the titin I-band region is variable. However, three distinctive elements can be identified, including the tandem Ig segments, the spring-like PEVK element, and the spring-like N2B element specific to the cardiac titin isoforms (Hidalgo and Granzier, 2013).

Chung et al. (2013) tested the physiological function of the tandem Ig segments in a mouse model in which nine Ig-like domains (Ig3–Ig11) near the Z-disc were deleted. The myocytes from the TG mice exhibited increased passive tension in addition to increased extension of the other titin spring elements (i.e., N2B, PEVK) as demonstrated by cryo-EM and immunolabeling. These mice also exhibited increased diastolic stiffness and reduced tolerance to exercise.

The importance of the spring-like N2B element for normal heart function was established by generating mice lacking the N2B spring element (Radke et al., 2007). N2B KO mice showed normal systolic function, albeit heart sizes were smaller and mice presented diastolic dysfunction accompanied by increased diastolic wall stress and increased passive tension. Similar to the Ig segment deletion, the N2B deletion led to increased extension of the other titin spring elements. Mouse KO mice showed downregulation of FHL2 (a LIM domain family member) but not of α B-crystallin (a heat shock protein), which correspond to two proteins that interact with the N2B spring.

The physiological function of the spring-like PEVK element has also been studied by deletion of the C-terminal region of the PEVK domain in mice (Granzier et al., 2009). The PEVK mouse KO mice exhibited diastolic dysfunction, hypertrophy, and increased passive tension but only mild or null effects on systolic parameters. Cryo-EM images revealed a more extended N2B element, and Western blots demonstrated upregulation of FHL1, FHL2, and α B-crystallin. Another regulatory feature of the titin PEVK derives from its capacity to interact with F-actin. Yamazaki et al. (2001) demonstrated that the cardiac PEVK interaction with

F-actin causes dragging forces that contribute to the development of passive tension. It was also established that physiological levels of systolic Ca^{2+} disrupt such F-actin-PEVK interactions *in vivo* by the cytosolic EF-hand calcium binding protein S100A1 that interacts with the PEVK element. Yamasaki et al. proposed that the functional role of this Ca^{2+} -dependent regulatory element is to reduce the passive force during active contraction. Further studies should be guided to establish the physiological advantage of the F-actin and titin PEVK domain interactions for normal myofibrillar function.

Due to the high structural interconnectivity of sarcomeres, distal mechanical interventions can affect the activation of thin filaments remotely. This is exemplified by the findings of Cazorla et al. (2001) showing that by increasing the passive tension in skinned single cardiac muscle cells, the Ca^{2+} sensitivity of the isometric force development correspondingly increased. By shifting sarcomere lengths from 2.0 to 2.3 μm and with varying levels of passive tension, it was established that length-dependent activation has a component that is independent of the passive tension and another that increases proportionally with the passive tension. Titin PEVK elements are sensitive to trypsin cleavage, and treating skinned cardiomyocytes with trypsin attenuated the shift in pCa_{50} caused by increasing the passive tension. It was also reported that the rise in passive tension decreased the cell width and interfilament spacing. One consideration from these observations is that thin filament sensitivity is conditioned by the extent of overlap with thick filaments. One example of this is that thin filaments at the A-band are more sensitive to Ca^{2+} than filaments at the I-band. Under resting sarcomere lengths, about a third of the thin filament length resides in the I-band region and lacks the influence of thick filament interactions. This causes the Tn to exhibit distinct activation responses with pCa_{50} estimates of 5.3 at the I-band and 6.6 at the A-band (Sun et al., 2009). These considerations will be highly relevant when dissecting the properties of structural proteins in the I-band and across the sarcomere.

The pointed ends fine-tune thin filament length and stability

Given that regulation of thin filaments is critical in optimal functioning of the actin-crossbridge reaction, a long-standing question has been how thin filament assembly is maintained in the beating heart (Michele et al., 1999). This appears to occur at the pointed ends and involves interplay between Tmod capping and leiomodin (Lmod) pointed end growth, of which Tmod1 and Lmod2 are the prevalent isoforms in cardiac thin filaments (Fowler and Dominguez, 2017). Developmental studies show that Lmod2 expression lags Tmod1 expression in chicken embryo cardiomyocytes, which suggests that Lmod2 is not involved in myofibrillogenesis but, rather, in maintenance of thin filament assembly and protein turnover (Tsukada et al., 2010).

Tmod caps thin filaments pointed ends by simultaneously interacting with actin and two parallel Tm chains (Rao et al., 2014). Lmod also localizes near the pointed ends and to a lesser extent across thin filaments (Boczkowska et al., 2015; Skwarek-Maruszewska et al., 2010; Tsukada et al., 2010). Lmod recruits three actin monomers and Tm to actin pointed ends to promote

actin pointed-end elongation (Chereau et al., 2008). Functionally, Lmod and Tmod can bind to pointed ends, albeit Tmod exhibits a greater binding affinity for pointed ends, whereas Lmod preferentially binds G-actin to initiate nucleation (Boczkowska et al., 2015; Fowler and Dominguez, 2017; Tsukada et al., 2010).

The antagonistic equilibrium between Tmod and Lmod is demonstrated in mice overexpressing Tmod1 that display DCM, myofibrillar degradation, and compromised contractile function (Sussman et al., 1998). Inhibition of Tmod capping activity *in vitro* leads to elongation of actin filaments at their pointed ends (Gregorio et al., 1995). Conversely, Lmod2^{-/-} KO mice exhibit a similar phenotype to Tmod1 overexpression with shorter cardiac thin filaments, reduced actin incorporation at the pointed ends, and decreased contractile force with development of DCM (Li et al., 2016; Pappas et al., 2015). Strikingly, TG mice overexpressing Lmod2 exhibit reduced contractile force with enlarged atrial and ventricular chambers and extensive interstitial fibrosis despite having longer thin filaments (Mi-Mi et al., 2020). The first Lmod2 mutation in a human was recently identified as a mutation encoding a premature stop codon (Ahrens-Nicklas et al., 2019). This mutation led to the absence of Lmod2 expression, shorter thin filaments, and disorganized myofibrils in the explanted heart and a mouse disease avatar.

Tmod and Lmod may regulate muscle contractility in two ways: by controlling thin filament length and by interfering with interactions of force generating crossbridges. First, shorter thin filaments in Lmod2^{-/-} KO mice exhibit reduced contractile force that may be caused by a reduced number of force-generating crossbridges from decreased thin-thick filament overlap (Pappas et al., 2015). Second, the implications of Lmod binding laterally to thin filaments and interference with crossbridge interactions were explored in ATPase activity assays. Lmod2 exhibited inhibition of ATPase activity between F-actin and heavy meromyosin at Lmod2 concentrations $\geq 1 \mu\text{M}$ (Szatmári et al., 2017). To observe an effect on crossbridge interactions *in vivo* with appreciable levels of lateral thin filament binding, it is necessary to induce Lmod2 overexpression (Tsukada et al., 2010). Thus, it remains unclear whether native *in vivo* Lmod2 concentrations are sufficient to affect crossbridge function. Taken together, substantial evidence supports the hypothesis that Tmod and Lmod control thin filament protein turnover and fine-tune length at the pointed ends rather than being involved in myofibrillogenesis. Many physiological outcomes from Tmod/Lmod dysregulation, such as decreased contractile force from shorter thin filaments that cause decreased thin and thick filament overlap, are mechanistically sound. However, other outcomes from disrupting the Tmod/Lmod regulatory balance remain less understood.

The M-band: the less explored domain in control of sarcomere function

If the Z-disc is seen as the anchor point of thin filaments, the M-band is the reciprocal for the thick filaments. This region is probably the least studied in the sarcomeres, and many of the M-band protein constituents have been discovered in the current century (Lange et al., 2020). As with the Z-disc, the M-band

also harbors proteins that influence the control of sarcomere function and maintenance.

The major constituents of the M-band include the structural proteins myomesin, obscurin, and the titin C terminus. Myomesin is one of the last proteins that incorporate to the sarcomeres, which is dependent on myosin thick filaments and the titin C terminus (Prill et al., 2019). Myomesin is a highly compliant protein that provides dampening capacity to thick filaments. Obscurin appears to localize to multiple regions outside the M-line like the sarcoplasmic reticulum and the dystrophin-sarcoglycan complex via ankyrin-B attachment (Cunha and Mohler, 2008). The titin C terminus is a hotspot of signaling proteins like MuRF1 that bind to titin and trigger protein ubiquitination for subsequent degradation via the proteasome (LeWinter and Granzier, 2010). Downstream of the MuRF1 binding site is the titin kinase domain that has an important role in muscle hypertrophy. Unfolding of the titin kinase domain via mechanical strain promotes the association of nbr1, p62, and MuRF2 that ultimately lead to protein synthesis and hypertrophy via serum response factor-dependent gene expression (Lange et al., 2005).

In addition to the main structural proteins described, other signaling proteins also interact at the M-line. Fhl DRAL/FHL-2 facilitates the targeting of creatine kinase, adenylate kinase, and phosphofructokinase-involved ATP synthesis to the N2B and M-band regions of titin (Lange et al., 2002). This exemplifies the intense signaling exchange that the M-band triggers via mechanical cues, which have a profound effect on sarcomere contractile performance and assembly.

Thin filament dynamics in a heartbeat

Integration of the dynamics of molecular events in sarcomeres with pressure and volume dynamics during a heartbeat provides a summary of the recent insights into thin filament function summarized here. Fig. 5 A relates sarcomere molecular mechanisms at time points in the rise and fall of LV pressure (LVP), LV volume, sarcomere length, and intracellular Ca^{2+} in a physiological beat of an adult human heart in a resting neurohumoral environment steady state. The schemes in Fig. 5 B show the disposition of sarcomere proteins in the C-zone at critical time points in the beat. In diastole, the binding of Ca^{2+} to cTnC is below the threshold for activation of thin filament regulatory units; regions of the thin filaments are mostly in a blocked state, with Tm impeding the actin-crossbridge reaction, but some regions of the thin filament are poised to transfer to an open force-generating state. These regions are in a closed state with cMyBP-C interactions and weak myosin head interactions. In the D-zone, the ratio of SRX/DRX crossbridge states are largely determined by the relative levels of cRLC phosphorylation. In the C-zone, Fig. 5 B indicates that this ratio depends on whether cMyBP-C is interacting with the thin filament to promote the closed state or reacting with myosin-S2, which depends on the proportion of cMyBP-C phosphorylated at the PKA sites. We presume that in a basal state, the fraction of phosphorylated cMyBP-C is relatively low and reflects cardiac contraction and relaxation reserve. On the other hand, it is apparent that the phosphorylation of cRLC may be more constant with ~40% of

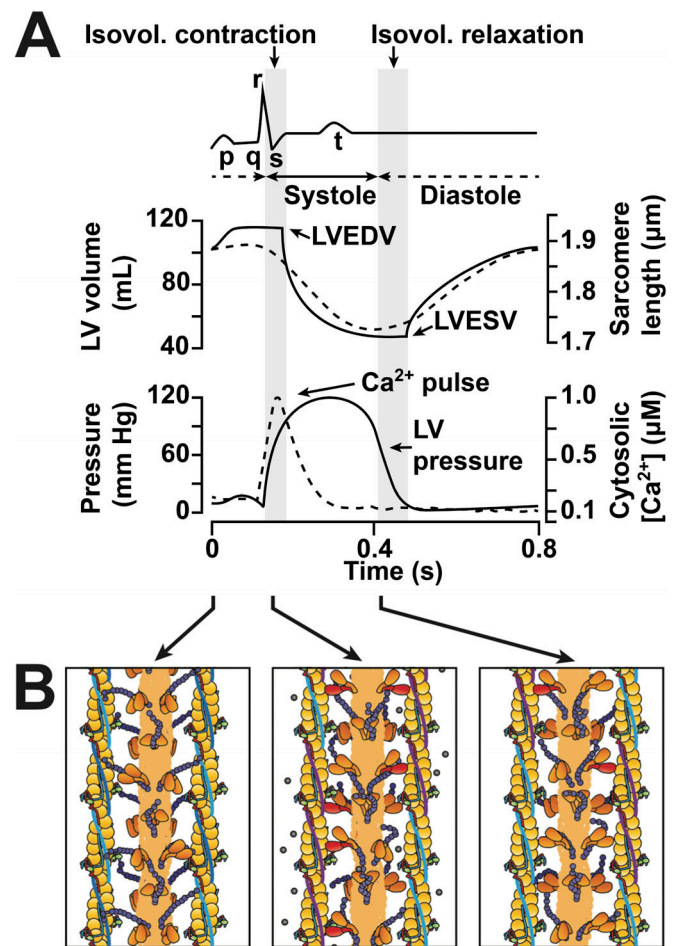


Figure 5. Dynamics of myocyte sarcomere activation and intracellular Ca^{2+} during a heartbeat. (A) Top: An electrocardiogram summarizes the depolarization of the atria (p) and ventricles (qrs) and the repolarization of the ventricles (t). Middle: The solid line shows volumes during the beat in which the LV ejects a stroke volume equal to the difference between the LV end diastolic volume (LVEDV) and the LV end systolic volume (LVESV). The dashed line shows an estimate of the changes in sarcomere length during the changes in LV volume. Bottom: The dashed line shows the Ca^{2+} release in the sarcoplasm coupled to the depolarization wave. Ca^{2+} rises, binds to cTnC triggering sarcomere tension and shortening, and then falls with reuptake. The solid line shows the associated rise and fall in LVP. (B) C-zone molecular interactions among sarcomere proteins during the heartbeat. Left: In diastole before Ca^{2+} release, crossbridges are predominantly in SRX, with a small population interacting with the thin filament in a weakly binding state. Thin filament regulatory units are predominantly in the B-state with some in a C-state promoted by interactions with the N terminus of cMyBP-C at the actin-Tm interface. Middle: Upon Ca^{2+} release, and Ca^{2+} binding to cTnC promotes the C-state in a cooperative process among thin filament regulatory units. Cycling crossbridges become active, thin filaments populate the O-state (or M-state), and tension and pressure rise. Right: Tension and pressure are sustained by force-generating crossbridges during ejection. With deactivation of the thin filaments, tension and pressure fall, but the rate of detachment of the ensemble of myosins lags the Ca^{2+} -reuptake kinetics, leading to residually active regulatory units in thin filaments. A is adapted with permission from RadioGraphics (Sheth et al., 2015), with the addition of the sarcomere length (Aguirre et al., 2014) and the Ca^{2+} pulse (Allen and Kurihara, 1982) time series. See text for further discussion. Isovolumic, isovolumic.

the heads phosphorylated (Scruggs and Solaro, 2011). Unphosphorylated cMyBP-C is expected to be in a relatively extended state interacting with S2 as well as with actin-Tm. It appears likely that in the C-zone, there are interactions between cRLC and cMyBP-C that also determine the SRX/DRX ratio.

The onset of systole is triggered as indicated by the electrocardiogram trace in Fig. 5 A. A wave of depolarization induces a rise in sarcoplasmic Ca^{2+} , which in turn triggers the rise in sarcomere tension and the isovolumic rise of LVP at constant sarcomere length. Ca^{2+} released to the sarcomeres first contacts the D-zone regulatory unit microdomains activating the actin-myosin interaction, increasing tension and pressure. As the transient rise in Ca^{2+} continues, the thin filaments in the C-zone switch to closed state, which rapidly promotes active tension/pressure in a cooperative process among near-neighbor regulatory units aided dominantly not only by their interactions with cMyBP-C at actin-Tm but also by interactions with the force-generating crossbridges. The C-zone actin-crossbridge interactions sustain peak tension and pressure as the sarcomeres shorten.

As Ca^{2+} falls in the sarcoplasmic space, Ca^{2+} is released from cTnC, but the fall in Ca^{2+} in the cTnC microdomain may be slower and may be faster in the D-zone than in the C-zone. As the regulatory units are deactivated, the fall in tension and pressure is not coincident with the release of cTnC Ca^{2+} . The return to the diastolic state is dominated by an initial relatively slow fall in tension and isovolumic pressure, rate limited by the detachment of force-generating crossbridges and the strain associated with stretch. The tension and pressure fall to a point where they collapse rapidly because of intersarcomere interactions with rapid lengthening of the weakly activated sarcomeres.

Ejection ends with an isovolumic fall in LVP to diastolic levels. Also illustrated is the fall in LV volume with ejection and filling during diastole and the pulse and ventricular pressure development. This is correlated with the molecular events summarized in Fig. 4 B. Here, at subactivating Ca^{2+} levels, both the thin and thick filaments are at a minimal level of activation. At activating Ca^{2+} levels, both thin and thick filaments are activated. After Ca^{2+} reuptake, the relative long-lasting myosin detachment rate delays the inactivation of thin and thick filaments. This sequence of events attempts to show how all the regulatory domains described in detail in the previous paragraphs integrate into the elegant mechanism of cardiac muscle contraction. More details are still missing in this model, but this illustrates the state of the art of the scientific corpus of knowledge.

The dynamics described above and illustrated in Fig. 5 are modified during exercise to tune the heartbeat to increased hemodynamic/metabolic demands and increased heart rate. In the short term, elevations in neurohumoral signaling (e.g., adrenergic stimulation of the heart) increase phosphorylation of cMyBP-C, cTnI, and cRLC. Pressure and tension increase as more Ca^{2+} is made available to activate the thin filament regulatory units. A critical aspect is a reduction in overall cycle time of the beat. This occurs as the extent of phosphorylation of cMyBP-C and cTnI increases. It is evident that the phosphorylation of cMyBP-C may be dominant in enhancing the rate of crossbridge

kinetics in contraction and relaxation, but evidence exists that this occurs in concert with the promotion of the thin filament off state by phosphorylation of cTnI. Moreover, the phosphorylation levels of both cMyBP-C and cRLC may increase the population of crossbridges in the DRX. Complex structural modifications occur as cMyBP-C bends away from the thin filament in response to phosphorylation as the N-terminal domain becomes more compact and appears unlikely to interact with actin-Tm. However, there are in vitro data that with elevations in Ca^{2+} , this effect of phosphorylation is reversed with non-canonical interactions of Ca^{2+} with cMyBP-C (Previs et al., 2016). This has led to the hypothesis that even with phosphorylation, there is an interaction of cMyBP-C with actin-Tm, promoting the cooperative activation of the thin filament. Other data indicate that at relatively high levels of Ca^{2+} , the effect of cTn to activate regulatory units competes with cMyBP-C for interactions with actin-Tm. It is unknown whether both effects could occur as Ca^{2+} is elevated in the cell, and the nature of Ca^{2+} interactions with cMyBP-C is unknown. The observation of a role for SPOC induced by phosphorylation of cMyBP-C has also been proposed to be a factor modulating crossbridge kinetics and in using the stored energy in the oscillatory behavior to improve efficiency (Harris, 2021). In addition to exercise-induced modifications in the heartbeat, there are multiple modifications that may be understood by considerations of modifications in the context of the basic physiological mechanisms described above.

Thin filament signaling in long-term modifications of the heartbeat

Ample evidence reviewed here indicates the sensing and integrated effects of mechanosignaling initiated at the C- and D-zones of the sarcomere to the Z-disc and M-band regions. One view of this process relates to the effects of intermittent exercise, which engages the altered thin filament dynamics described above. Over long periods of activation of these signaling cascades, it is apparent that the thin filaments importantly function in homeostatic control of sarcomere protein quality control and levels of phosphorylation. A novel twist on these ideas has been presented in the finding of a role of SPOC associated with phosphorylation of the N-terminal domain of cMyBP-C. Napierski et al. (2020) suggested that the SPOC may be sensed across sarcomeres and contribute to signaling at the Z-discs.

Challenges and future directions

Despite the significant advances in understanding the role of cardiac myocyte thin filaments in long- and short-term regulatory networks, major challenges remain. As stressed in reviews of these challenges, it is critical to integrate findings at the molecular level with the major elements in the contraction and relaxation mechanisms in the heart, including Ca^{2+} fluxes, and the state of activation of thin and thick filaments (Biesiadecki et al., 2014; Solaro et al., 2013). The complexities of activation and relaxation of cardiac sarcomeres have led to a perplexing array of possibilities for physiological and pathological modulation. An outcome is that therapies directed at sarcomeres in improving cardiac disorders may rely on focused studies lacking

integration or on intuition and guesswork and not on predictions leading to efficient, effective, and safe approaches. We take as an example the necessity for a more complete understanding of the role of cMyBP-C, which has multiple sites of interaction as an adaptor protein at actin-Tm, at the MHC and cRLC and is subject to redox and phosphorylation modifications that need further investigation. cMyBP-C has phosphorylation sites that are substrates for multiple kinase/phosphatase pairs. As emphasized by Birtwistle (2015) in other contexts, steady-state levels and flux through the phosphorylation sites, and most likely the oxidatively modified sites, are likely to be important in sarcomere regulation. A practical example of this concept is presented by Kampourakis et al. (2018) who showed that pseudo-phosphorylation of cMyBP-C fails to mimic the changes in the cMyBP-C and thin filament interaction seen with phosphorylation. A description of the mathematical approach, which is described by Birtwistle with relevant examples, is beyond the scope of this review; our purpose here is to highlight this as a significant impediment to productive progress in the field of sarcomere control mechanisms. Induction of SPOC and interactions of cMyBP-C with titin make it part of the protein network regulating Z-disc dynamics, mechanotransduction, and signaling to and from the sarcomeres. There is a need for increased and improved understanding of the role of cMyBP-C and its mutations as determinants of the relative population of SRX and DRX. Presently, in design of therapeutic agents for use in HCM, DCM, and HF, there is a general lack of consideration of interactions of cMyBP-C and its variants with thin, thick, and titin filaments.

An important future direction is the continued evolution of the application of computational methods related to various mechanisms that determine the level of activity and dynamics of the heart in short- and long-term physiological and disordered states. Multiscale modeling with the heart operating in a vascular network provides an approach to “bookkeeping” of the elements and processes involved and for constraining theories to fit the overt function of the heart and the circulation (Campbell et al., 2020; Sewanan et al., 2016). A related challenge and future direction is the implementation and application of “omics” to understanding sarcomere function and signaling. An example is the current momentum in precision medicine as applied to HCM (Goldspink et al., 2020; Maron et al., 2019). A primary challenge is to make sure that the approach is not only precise but also accurate. Data interpretation and pathway analysis rely heavily on bioinformatic approaches that must be performed with rigor. Moreover, investigators must keep up with the improvement and evolution of databases that modify conclusions. New directions in omics approaches offer the promise of cell-specific transcriptional profiling that can be combined with proteomic profiling of the newly transcribed proteins (Doroudgar et al., 2019; Goldspink et al., 2020).

Epigenetic control mechanisms involving thin filament proteins located in the nuclear compartment are an important and challenging future direction (Asumda and Chase, 2012; Chase et al., 2013; Cole et al., 2016; Wu et al., 2015). There is evidence that epigenetic control by thin filament proteins affects enzymes regulating adrenergic signaling and thus feeding back

to exacerbate dysregulation of sarcomere function and maladaptive remodeling. Chase et al. (2013) and Asumda and Chase (2012) have summarized evidence published for a role of Tm and Tn in the nucleus. The review indicated that SUMOylation may be an important post-translational modification promoting nuclear translocation of Tm. Moreover, their prediction that mutations in thin filament proteins may alter nuclear function has been demonstrated by Wu et al. (2015), who reported that altered adrenergic control of contractility in a TNNT2 DCM (cTnT-R173W) mutation occurred because of the mutant cTnT entering the nucleus and epigenetically upregulating phosphodiesterase genes. Similar results were obtained in a restrictive cardiomyopathy mutation in which a mutant nuclear cTnI interacted with histone deacetylase 1, inducing down-regulation of phosphodiesterase-4 (Zhao et al., 2020).

While the study of the classic proteins involved in the crossbridge cycle and regulation is paramount to understanding cardiac function and disease, studies on the Z-disc, M-line, and thin filament pointed-end proteins have shown that they also play crucial roles in maintaining assembly and muscle work. This evidence points to a mechanistic role of the barbed ends in regulating myofibrillogenesis while the pointed ends are responsible for protein turnover and maintenance, both of which require full integration with understanding of cardiac contractile state.

Acknowledgments

Henk L. Granzier served as editor.

The authors acknowledge the support of National Institutes of Health, National Heart, Lung, and Blood Institute grants K99 HL 151825 (to C. Solís), PO1 HL 062426 (to R.J. Solaro), and RO1 HL 119199 (to R.J. Solaro).

R.J. Solaro is a member of the scientific advisory board of Cytokinetics and a consultant to Pfizer and Edgewise Therapeutics. The remaining author declares no competing financial interests.

Author contributions: R.J. Solaro established the general outline of the review. C. Solís and R.J. Solaro wrote the manuscript. C. Solís prepared the figures.

Submitted: 29 September 2020

Accepted: 23 February 2021

References

- Aguirre, A.D., C. Vinegoni, M. Sebas, and R. Weissleder. 2014. Intravital imaging of cardiac function at the single-cell level. *Proc. Natl. Acad. Sci. USA.* 111:11257–11262. <https://doi.org/10.1073/pnas.1401316111>
- Ahrens-Nicklas, R.C., C.T. Pappas, G.P. Farman, R.M. Mayfield, T.M. Larrinaga, L. Medne, A. Ritter, I.D. Krantz, C. Murali, K.Y. Lin, et al. 2019. Disruption of cardiac thin filament assembly arising from a mutation in *LMOD2*: A novel mechanism of neonatal dilated cardiomyopathy. *Sci. Adv.* 5:eaa2066. <https://doi.org/10.1126/sciadv.aax2066>
- Ait Mou, Y., J.Y. le Guennec, E. Mosca, P.P. de Tombe, and O. Cazorla. 2008. Differential contribution of cardiac sarcomeric proteins in the myofibrillar force response to stretch. *Pflugers Arch.* 457:25–36. <https://doi.org/10.1007/s00424-008-0501-x>
- Al-Khayat, H.A., R.W. Kensler, J.M. Squire, S.B. Marston, and E.P. Morris. 2013. Atomic model of the human cardiac muscle myosin filament.

- Proc. Natl. Acad. Sci. USA. 110:318–323. <https://doi.org/10.1073/pnas.1212708110>
- Alamo, L., N. Koubassova, A. Pinto, R. Gillilan, A. Tsaturyan, and R. Padrón. 2017a. Lessons from a tarantula: new insights into muscle thick filament and myosin interacting-heads motif structure and function. *Biophys. Rev.* 9:461–480. <https://doi.org/10.1007/s12551-017-0295-1>
- Alamo, L., J.S. Ware, A. Pinto, R.E. Gillilan, J.G. Seidman, C.E. Seidman, and R. Padrón. 2017b. Effects of myosin variants on interacting-heads motif explain distinct hypertrophic and dilated cardiomyopathy phenotypes. *eLife.* 6:e24634. <https://doi.org/10.7554/eLife.24634>
- Allen, D.G., and S. Kurihara. 1982. The effects of muscle length on intracellular calcium transients in mammalian cardiac muscle. *J. Physiol.* 327: 79–94. <https://doi.org/10.1113/jphysiol.1982.sp014221>
- Allen, D.G., and C.H. Orchard. 1983. The effects of changes of pH on intracellular calcium transients in mammalian cardiac muscle. *J. Physiol.* 335: 555–567. <https://doi.org/10.1113/jphysiol.1983.sp014550>
- Alsulami, K., and S. Marston. 2020. Small molecules acting on myofilaments as treatments for heart and skeletal muscle diseases. *Int. J. Mol. Sci.* 21: 9599. <https://doi.org/10.3390/ijms21249599>
- Alves, M.L., C.M. Warren, J.N. Simon, R.D. Gaffin, E.M. Montminy, D.F. Wiczorek, R.J. Solaro, and B.M. Wolaska. 2017. Early sensitization of myofilaments to Ca²⁺ prevents genetically linked dilated cardiomyopathy in mice. *Cardiovasc. Res.* 113:915–925. <https://doi.org/10.1093/cvr/cvx068>
- Anderson, R.L., D.V. Trivedi, S.S. Sarkar, M. Henze, W. Ma, H. Gong, C.S. Rogers, J.M. Gorham, F.L. Wong, M.M. Morck, et al. 2018. Deciphering the super relaxed state of human β -cardiac myosin and the mode of action of mavacamten from myosin molecules to muscle fibers. *Proc. Natl. Acad. Sci. USA.* 115:E8143–E8152. <https://doi.org/10.1073/pnas.1809540115>
- Arteaga, G.M., T. Kobayashi, and R.J. Solaro. 2002. Molecular actions of drugs that sensitize cardiac myofilaments to Ca²⁺. *Ann. Med.* 34:248–258. <https://doi.org/10.1080/078538902320322510>
- Asumda, F.Z., and P.B. Chase. 2012. Nuclear cardiac troponin and tropomyosin are expressed early in cardiac differentiation of rat mesenchymal stem cells. *Differentiation.* 83:106–115. <https://doi.org/10.1016/j.diff.2011.10.002>
- Badr, M.A., J.R. Pinto, M.W. Davidson, and P.B. Chase. 2016. Fluorescent protein-based Ca²⁺ sensor reveals global, divalent cation-dependent conformational changes in cardiac troponin C. *PLoS One.* 11:e0164222. <https://doi.org/10.1371/journal.pone.0164222>
- Bang, M.-L., and J. Chen. 2015. Roles of nebulin family members in the heart. *Circ. J.* 79:2081–2087. <https://doi.org/10.1253/circj.CJ-15-0854>
- Bell, M.G., E.B. Lankford, G.E. Gonye, G.C. Ellis-Davies, D.A. Martyn, M. Regnier, and R.J. Barsotti. 2006. Kinetics of cardiac thin-filament activation probed by fluorescence polarization of rhodamine-labeled troponin C in skinned guinea pig trabeculae. *Biophys. J.* 90:531–543. <https://doi.org/10.1529/biophysj.105.072769>
- Bers, D.M., J.W. Bassani, and R.A. Bassani. 1996. Na-Ca exchange and Ca fluxes during contraction and relaxation in mammalian ventricular muscle. *Ann. N. Y. Acad. Sci.* 779:430–442. <https://doi.org/10.1111/j.1749-6632.1996.tb44818.x>
- Biesiadecki, B.J., J.P. Davis, M.T. Ziolo, and P.M.L. Janssen. 2014. Tri-modal regulation of cardiac muscle relaxation; intracellular calcium decline, thin filament deactivation, and cross-bridge cycling kinetics. *Biophys. Rev.* 6:273–289. <https://doi.org/10.1007/s12551-014-0143-5>
- Birtwistle, M.R. 2015. Analytical reduction of combinatorial complexity arising from multiple protein modification sites. *J. R. Soc. Interface.* 12: 20141215. <https://doi.org/10.1098/rsif.2014.1215>
- Boczkowska, M., G. Rebowski, E. Kremneva, P. Lappalainen, and R. Dominguez. 2015. How Leiomodulin and Tropomodulin use a common fold for different actin assembly functions. *Nat. Commun.* 6:8314. <https://doi.org/10.1038/ncomms9314>
- Bonzo, J.R., A.A. Norris, M. Esham, and C.L. Moncman. 2008. The nebulin repeat domain is necessary for proper maintenance of tropomyosin with the cardiac sarcomere. *Exp. Cell Res.* 314:3519–3530. <https://doi.org/10.1016/j.yexcr.2008.09.001>
- Bremel, R.D., and A. Weber. 1972. Cooperation within actin filament in vertebrate skeletal muscle. *Nat. New Biol.* 238:97–101. <https://doi.org/10.1038/newbio238097a0>
- Bremel, R.D., J.M. Murray, and A. Weber. 1973. Manifestations of cooperative behavior in the regulated actin filament during actin-activated ATP hydrolysis in the presence of calcium. *Cold Spring Harb. Symp. Quant. Biol.* 37:267–275. <https://doi.org/10.1101/SQB.1973.037.01.037>
- Brenner, B., and E. Eisenberg. 1986. Rate of force generation in muscle: correlation with actomyosin ATPase activity in solution. *Proc. Natl. Acad. Sci. USA.* 83:3542–3546. <https://doi.org/10.1073/pnas.83.10.3542>
- Brunello, E., L. Fusi, A. Ghisleni, S.J. Park-Holohan, J.G. Ovejero, T. Narayanan, and M. Irving. 2020. Myosin filament-based regulation of the dynamics of contraction in heart muscle. *Proc. Natl. Acad. Sci. USA.* 117: 8177–8186. <https://doi.org/10.1073/pnas.1920632117>
- Campbell, S.G., Y. Qyang, and J.T. Hinson. 2019. Sarcomere-directed calcium reporters in cardiomyocytes. *Circ. Res.* 124:1151–1153. <https://doi.org/10.1161/CIRCRESAHA.119.314877>
- Campbell, K.S., B.S. Chrisman, and S.G. Campbell. 2020. Multiscale modeling of cardiovascular function predicts that the end-systolic pressure volume relationship can be targeted via multiple therapeutic strategies. *Front. Physiol.* 11:1043. <https://doi.org/10.3389/fphys.2020.01043>
- Candasamy, A.J., R.S. Haworth, F. Cuello, M. Ibrahim, S. Aravamudan, M. Krüger, M.R. Holt, C.M. Terracciano, M. Mayr, M. Gautel, and M. Avkiran. 2014. Phosphoregulation of the titin-cap protein telethonin in cardiac myocytes. *J. Biol. Chem.* 289:1282–1293. <https://doi.org/10.1074/jbc.M113.479030>
- Capetanaki, Y., R.J. Bloch, A. Kouloumenta, M. Mavroidis, and S. Psarras. 2007. Muscle intermediate filaments and their links to membranes and membranous organelles. *Exp. Cell Res.* 313:2063–2076. <https://doi.org/10.1016/j.yexcr.2007.03.033>
- Carrier, L., G. Mearini, K. Stathopoulou, and F. Cuello. 2015. Cardiac myosin-binding protein C (MYBPC3) in cardiac pathophysiology. *Gene.* 573: 188–197. <https://doi.org/10.1016/j.gene.2015.09.008>
- Cazorla, O., Y. Wu, T.C. Irving, and H. Granzier. 2001. Titin-based modulation of calcium sensitivity of active tension in mouse skinned cardiac myocytes. *Circ. Res.* 88:1028–1035. <https://doi.org/10.1161/hh1001.090876>
- Chakouri, N., C. Reboul, D. Boulghobra, A. Kleindienst, S. Nottin, S. Gayraud, F. Roubille, S. Matecki, A. Lacampagne, and O. Cazorla. 2018. Stress-induced protein S-glutathionylation and phosphorylation crosstalk in cardiac sarcomeric proteins - Impact on heart function. *Int. J. Cardiol.* 258:207–216. <https://doi.org/10.1016/j.ijcard.2017.12.004>
- Chakouri, N., C. Farah, S. Matecki, P. Amedro, M. Vincenti, L. Saumet, L. Vergely, N. Sirvent, A. Lacampagne, and O. Cazorla. 2020. Screening for in-vivo regional contractile defaults to predict the delayed Doxorubicin Cardiotoxicity in Juvenile Rat. *Theranostics.* 10:8130–8142. <https://doi.org/10.7150/thno.47407>
- Chase, P.B., M.P. Szczypinski, and E.P. Soto. 2013. Nuclear tropomyosin and troponin in striated muscle: new roles in a new locale? *J. Muscle Res. Cell Motil.* 34:275–284. <https://doi.org/10.1007/s10974-013-9356-7>
- Cheng, H., K. Kimura, A.K. Peter, L. Cui, K. Ouyang, T. Shen, Y. Liu, Y. Gu, N.D. Dalton, S.M. Evans, et al. 2010. Loss of enigma homolog protein results in dilated cardiomyopathy. *Circ. Res.* 107:348–356. <https://doi.org/10.1161/CIRCRESAHA.110.218735>
- Cheng, H., M. Zheng, A.K. Peter, K. Kimura, X. Li, K. Ouyang, T. Shen, L. Cui, D. Frank, N.D. Dalton, et al. 2011. Selective deletion of long but not short Cypher isoforms leads to late-onset dilated cardiomyopathy. *Hum. Mol. Genet.* 20:1751–1762. <https://doi.org/10.1093/hmg/ddr050>
- Chereau, D., M. Boczkowska, A. Skwarek-Maruszewska, I. Fujiwara, D.B. Hayes, G. Rebowski, P. Lappalainen, T.D. Pollard, and R. Dominguez. 2008. Leiomodulin is an actin filament nucleator in muscle cells. *Science.* 320:239–243. <https://doi.org/10.1126/science.1155313>
- Chu, M., C.C. Gregorio, and C.T. Pappas. 2016. Nebulin, a multi-functional giant. *J. Exp. Biol.* 219:146–152. <https://doi.org/10.1242/jeb.126383>
- Chung, C.S., and H.L. Granzier. 2011. Contribution of titin and extracellular matrix to passive pressure and measurement of sarcomere length in the mouse left ventricle. *J. Mol. Cell. Cardiol.* 50:731–739. <https://doi.org/10.1016/j.yjmcc.2011.01.005>
- Chung, C.S., K.R. Hutchinson, M. Methawasini, C. Saripalli, J.E. Smith III, C.G. Hidalgo, X. Luo, S. Labeit, C. Guo, and H.L. Granzier. 2013. Shortening of the elastic tandem immunoglobulin segment of titin leads to diastolic dysfunction. *Circulation.* 128:19–28. <https://doi.org/10.1161/CIRCULATIONAHA.112.001268>
- Chung, C.S., C.W. Hoopes, and K.S. Campbell. 2017. Myocardial relaxation is accelerated by fast stretch, not reduced afterload. *J. Mol. Cell. Cardiol.* 103:65–73. <https://doi.org/10.1016/j.yjmcc.2017.01.004>
- Clark, J.A., L.R. Sewanan, J. Schwan, J. Kluger, K.S. Campbell, and S.G. Campbell. 2021. Fast-relaxing cardiomyocytes exert a dominant role in the relaxation behavior of heterogeneous myocardium. *Arch. Biochem. Biophys.* 697:108711. <https://doi.org/10.1016/j.abb.2020.108711>
- Cole, L.A., J.H. Dennis, and P.B. Chase. 2016. Commentary: epigenetic regulation of phosphodiesterases 2A and 3A underlies compromised β -adrenergic signaling in an iPSC model of dilated cardiomyopathy. *Front. Physiol.* 7:418. <https://doi.org/10.3389/fphys.2016.00418>
- Colomo, F., N. Pirroddi, C. Poggesi, G. te Kronnie, and C. Tesi. 1997. Active and passive forces of isolated myofibrils from cardiac and fast skeletal

- muscle of the frog. *J. Physiol.* 500:535–548. <https://doi.org/10.1113/jphysiol.1997.sp022039>
- Cooper, J.A., and T.D. Pollard. 1985. Effect of capping protein on the kinetics of actin polymerization. *Biochemistry.* 24:793–799. <https://doi.org/10.1021/bi00324a039>
- Craig, R., K.H. Lee, J.Y. Mun, I. Torre, and P.K. Luther. 2014. Structure, sarcomeric organization, and thin filament binding of cardiac myosin-binding protein-C. *Pflugers Arch.* 466:425–431. <https://doi.org/10.1007/s00424-013-1426-6>
- Cunha, S.R., and P.J. Mohler. 2008. Obscurin targets ankyrin-B and protein phosphatase 2A to the cardiac M-line. *J. Biol. Chem.* 283:31968–31980. <https://doi.org/10.1074/jbc.M806050200>
- Davis, J.P., C. Norman, T. Kobayashi, R.J. Solaro, D.R. Swartz, and S.B. Tikunova. 2007. Effects of thin and thick filament proteins on calcium binding and exchange with cardiac troponin C. *Biophys. J.* 92:3195–3206. <https://doi.org/10.1529/biophysj.106.095406>
- de Tombe, P.P., and G.J. Stienen. 2007. Impact of temperature on cross-bridge cycling kinetics in rat myocardium. *J. Physiol.* 584:591–600. <https://doi.org/10.1113/jphysiol.2007.138693>
- Desai, R., M.A. Geeves, and N.M. Kad. 2015. Using fluorescent myosin to directly visualize cooperative activation of thin filaments. *J. Biol. Chem.* 290:1915–1925. <https://doi.org/10.1074/jbc.M114.609743>
- Doran, M.H., E. Pavada, M.J. Rynkiewicz, J. Walklate, E. Bullitt, J.R. Moore, M. Regnier, M.A. Geeves, and W. Lehman. 2020. Cryo-EM and molecular docking shows myosin loop 4 contacts actin and tropomyosin on thin filaments. *Biophys. J.* 119:821–830. <https://doi.org/10.1016/j.bpj.2020.07.006>
- Doroudgar, S., C. Hofmann, E. Boileau, B. Malone, E. Riechert, A.A. Gorska, T. Jakobi, C. Sandmann, L. Jürgensen, V. Kmietczyk, et al. 2019. Monitoring cell-type-specific gene expression using ribosome profiling in vivo during cardiac hemodynamic stress. *Circ. Res.* 125:431–448. <https://doi.org/10.1161/CIRCRESAHA.119.314817>
- Engel, P.L., T. Kobayashi, B. Biesiadecki, J. Davis, S. Tikunova, S. Wu, and R.J. Solaro. 2007. Identification of a region of troponin I important in signaling cross-bridge-dependent activation of cardiac myofilaments. *J. Biol. Chem.* 282:183–193. <https://doi.org/10.1074/jbc.M512337200>
- Engel, P.L., A. Hinken, and R.J. Solaro. 2009. Differential effects of phosphorylation of regions of troponin I in modifying cooperative activation of cardiac thin filaments. *J. Mol. Cell. Cardiol.* 47:359–364. <https://doi.org/10.1016/j.yjmcc.2009.04.016>
- Fabiato, A., and F. Fabiato. 1975. Effects of magnesium on contractile activation of skinned cardiac cells. *J. Physiol.* 249:497–517. <https://doi.org/10.1113/jphysiol.1975.sp011027>
- Fabiato, A., and F. Fabiato. 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J. Physiol.* 276:233–255. <https://doi.org/10.1113/jphysiol.1978.sp012231>
- Farman, G.P., D. Gore, E. Allen, K. Schoenfelt, T.C. Irving, and P.P. de Tombe. 2011. Myosin head orientation: a structural determinant for the Frank-Starling relationship. *Am. J. Physiol. Heart Circ. Physiol.* 300:H2155–H2160. <https://doi.org/10.1152/ajpheart.01221.2010>
- Ferrante, M.I., R.M. Kiff, D.A. Goulding, and D.L. Stemple. 2011. Troponin T is essential for sarcomere assembly in zebrafish skeletal muscle. *J. Cell Sci.* 124:565–577. <https://doi.org/10.1242/jcs.071274>
- Fitzsimons, D.P., and R.L. Moss. 2007. Cooperativity in the regulation of force and the kinetics of force development in heart and skeletal muscles: cross-bridge activation of force. *Adv. Exp. Med. Biol.* 592:177–189. https://doi.org/10.1007/978-4-431-38453-3_16
- Fitzsimons, D.P., J.R. Patel, and R.L. Moss. 1998. Role of myosin heavy chain composition in kinetics of force development and relaxation in rat myocardium. *J. Physiol.* 513:171–183. <https://doi.org/10.1111/j.1469-7793.1998.171by.x>
- Flashman, E., C. Redwood, J. Moolman-Smook, and H. Watkins. 2004. Cardiac myosin binding protein C: its role in physiology and disease. *Circ. Res.* 94:1279–1289. <https://doi.org/10.1161/01.RES.0000127175.21818.C2>
- Fowler, V.M., and R. Dominguez. 2017. Tropomodulins and leiomodins: actin pointed end caps and nucleators in muscles. *Biophys. J.* 112:1742–1760. <https://doi.org/10.1016/j.bpj.2017.03.034>
- Fukuda, N., H. Fujita, T. Fujita, and S. Ishiwata. 1996. Spontaneous tension oscillation in skinned bovine cardiac muscle. *Pflugers Arch.* 433:1–8. <https://doi.org/10.1007/s004240050241>
- Gautel, M., and K. Djinić-Carugo. 2016. The sarcomeric cytoskeleton: from molecules to motion. *J. Exp. Biol.* 219:135–145. <https://doi.org/10.1242/jeb.124941>
- Geeves, M., H. Griffiths, S. Mijailovich, and D. Smith. 2011. Cooperative [Ca²⁺]-dependent regulation of the rate of myosin binding to actin: solution data and the tropomyosin chain model. *Biophys. J.* 100:2679–2687. <https://doi.org/10.1016/j.bpj.2011.04.020>
- Geeves, M.A., S.S. Lehrer, and W. Lehman. 2019. The mechanism of thin filament regulation: Models in conflict? *J. Gen. Physiol.* 151:1265–1271. <https://doi.org/10.1085/jgp.201912446>
- Geisterfer-Lowrance, A.A., S. Kass, G. Tanigawa, H.P. Vosberg, W. McKenna, C.E. Seidman, and J.G. Seidman. 1990. A molecular basis for familial hypertrophic cardiomyopathy: a beta cardiac myosin heavy chain gene missense mutation. *Cell.* 62:999–1006. [https://doi.org/10.1016/0092-8674\(90\)90274-I](https://doi.org/10.1016/0092-8674(90)90274-I)
- Goldspink, P.H., C.M. Warren, J. Kitajewski, B.M. Wolska, and R.J. Solaro. 2020. A perspective on personalized therapies in hypertrophic cardiomyopathy. *J. Cardiovasc. Pharmacol.* Publish Ahead of Print. <https://doi.org/10.1097/FJC.0000000000000968>
- Goldstein, M.A., L.H. Michael, J.P. Schroeter, and R.L. Sass. 1989. Two structural states of Z-bands in cardiac muscle. *Am. J. Physiol.* 256:H552–H559. <https://doi.org/10.1152/ajpheart.1989.256.2.H552>
- Gordon, A.M., E. Homsher, and M. Regnier. 2000. Regulation of contraction in striated muscle. *Physiol. Rev.* 80:853–924. <https://doi.org/10.1152/physrev.2000.80.2.853>
- Granzier, H.L., M.H. Radke, J. Peng, D. Westermann, O.L. Nelson, K. Rost, N.M. King, Q. Yu, C. Tschöpe, M. McNabb, et al. 2009. Truncation of titin's elastic PEVK region leads to cardiomyopathy with diastolic dysfunction. *Circ. Res.* 105:557–564. <https://doi.org/10.1161/CIRCRESAHA.109.200964>
- Green, E.M., H. Wakimoto, R.L. Anderson, M.J. Evanichik, J.M. Gorham, B.C. Harrison, M. Henze, R. Kawas, J.D. Oslob, H.M. Rodriguez, et al. 2016. A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. *Science.* 351:617–621. <https://doi.org/10.1126/science.1243456>
- Gregorich, Z.R., J.R. Patel, W. Cai, Z. Lin, R. Heurer, D.P. Fitzsimons, R.L. Moss, and Y. Ge. 2019. Deletion of Enigma Homologue from the Z-disc slows tension development kinetics in mouse myocardium. *J. Gen. Physiol.* 151:670–679. <https://doi.org/10.1085/jgp.201812214>
- Gregorio, C.C., A. Weber, M. Bondad, C.R. Pennise, and V.M. Fowler. 1995. Requirement of pointed-end capping by tropomodulin to maintain actin filament length in embryonic chick cardiac myocytes. *Nature.* 377:83–86. <https://doi.org/10.1038/377083a0>
- Harris, S.P. 2021. Making waves: A proposed new role for myosin-binding protein C in regulating oscillatory contractions in vertebrate striated muscle. *J. Gen. Physiol.* 153:e202012729. <https://doi.org/10.1085/jgp.202012729>
- Harris, S.P., C.R. Bartley, T.A. Hacker, K.S. McDonald, P.S. Douglas, M.L. Greaser, P.A. Powers, and R.L. Moss. 2002. Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. *Circ. Res.* 90:594–601. <https://doi.org/10.1161/01.RES.0000012222.70819.64>
- Harris, S.P., R.G. Lyons, and K.L. Bezold. 2011. In the thick of it: HCM-causing mutations in myosin binding proteins of the thick filament. *Circ. Res.* 108:751–764. <https://doi.org/10.1161/CIRCRESAHA.110.231670>
- Hartman, T.J., J.L. Martin, R.J. Solaro, A.M. Samarel, and B. Russell. 2009. CapZ dynamics are altered by endothelin-1 and phenylephrine via PIP2- and PKC-dependent mechanisms. *Am. J. Physiol. Cell Physiol.* 296:C1034–C1039. <https://doi.org/10.1152/ajpcell.00544.2008>
- Heeley, D.H., H.D. White, and E.W. Taylor. 2019. Investigation into the mechanism of thin filament regulation by transient kinetics and equilibrium binding: Is there a conflict? *J. Gen. Physiol.* 151:628–634. <https://doi.org/10.1085/jgp.201812198>
- Heiss, S.G., and J.A. Cooper. 1991. Regulation of CapZ, an actin capping protein of chicken muscle, by anionic phospholipids. *Biochemistry.* 30:8753–8758. <https://doi.org/10.1021/bi00100a006>
- Henderson, C.A., C.G. Gomez, S.M. Novak, L. Mi-Mi, and C.C. Gregorio. 2017. Overview of the muscle cytoskeleton. *Compr. Physiol.* 7:891–944. <https://doi.org/10.1002/cphy.c160033>
- Hernandez, D.A., C.M. Bennett, L. Dunina-Barkovskaya, T. Wedig, Y. Capetanaki, H. Herrmann, and G.M. Conover. 2016. Nebulette is a powerful cytolinker organizing desmin and actin in mouse hearts. *Mol. Biol. Cell.* 27:3869–3882. <https://doi.org/10.1091/mbc.E16-04-0237>
- Hidalgo, C., and H. Granzier. 2013. Tuning the molecular giant titin through phosphorylation: role in health and disease. *Trends Cardiovasc. Med.* 23:165–171. <https://doi.org/10.1016/j.tcm.2012.10.005>
- Hinken, A.C., and R.J. Solaro. 2007. A dominant role of cardiac molecular motors in the intrinsic regulation of ventricular ejection and relaxation. *Physiology (Bethesda).* 22:73–80. <https://doi.org/10.1152/physiol.00043.2006>
- Huang, W., R. Zhang, and X. Xu. 2009. Myofibrillogenesis in the developing zebrafish heart: A functional study of tnnt2. *Dev. Biol.* 331:237–249. <https://doi.org/10.1016/j.ydbio.2009.04.039>

- Huxley, A.F., and R.M. Simmons. 1968. A capacitance-gauge tension transducer. *J. Physiol.* 197:12P.
- Inchingolo, A.V., S.B. Previs, M.J. Previs, D.M. Warshaw, and N.M. Kad. 2019. Revealing the mechanism of how cardiac myosin-binding protein C N-terminal fragments sensitize thin filaments for myosin binding. *Proc. Natl. Acad. Sci. USA.* 116:6828–6835. <https://doi.org/10.1073/pnas.1816480116>
- Iwata, M., A. Maturana, M. Hoshijima, K. Tatematsu, T. Okajima, J.R. Vandenhede, J. Van Lint, K. Tanizawa, and S. Kuroda. 2005. PKCepsilon-PKD1 signaling complex at Z-discs plays a pivotal role in the cardiac hypertrophy induced by G-protein coupling receptor agonists. *Biochem. Biophys. Res. Commun.* 327:1105–1113. <https://doi.org/10.1016/j.bbrc.2004.12.128>
- Jeong, E.M., M.M. Monasky, L. Gu, D.M. Taglieri, B.G. Patel, H. Liu, Q. Wang, I. Greener, S.C. Dudley Jr., and R.J. Solaro. 2013. Tetrahydrobiopterin improves diastolic dysfunction by reversing changes in myofibrillar properties. *J. Mol. Cell. Cardiol.* 56:44–54. <https://doi.org/10.1016/j.yjmcc.2012.12.003>
- Kampourakis, T., Z. Yan, M. Gautel, Y.B. Sun, and M. Irving. 2014. Myosin binding protein-C activates thin filaments and inhibits thick filaments in heart muscle cells. *Proc. Natl. Acad. Sci. USA.* 111:18763–18768. <https://doi.org/10.1073/pnas.1413922112>
- Kampourakis, T., Y.B. Sun, and M. Irving. 2016. Myosin light chain phosphorylation enhances contraction of heart muscle via structural changes in both thick and thin filaments. *Proc. Natl. Acad. Sci. USA.* 113: E3039–E3047. <https://doi.org/10.1073/pnas.1602776113>
- Kampourakis, T., S. Ponnamp, Y.B. Sun, I. Sevrieva, and M. Irving. 2018. Structural and functional effects of myosin-binding protein-C phosphorylation in heart muscle are not mimicked by serine-to-aspartate substitutions. *J. Biol. Chem.* 293:14270–14275. <https://doi.org/10.1074/jbc.AC118.004816>
- Kass, D.A., and R.J. Solaro. 2006. Mechanisms and use of calcium-sensitizing agents in the failing heart. *Circulation.* 113:305–315. <https://doi.org/10.1161/CIRCULATIONAHA.105.542407>
- Kentish, J.C., D.T. McCloskey, J. Layland, S. Palmer, J.M. Leiden, A.F. Martin, and R.J. Solaro. 2001. Phosphorylation of troponin I by protein kinase A accelerates relaxation and crossbridge cycle kinetics in mouse ventricular muscle. *Circ. Res.* 88:1059–1065. <https://doi.org/10.1161/hh1001.091640>
- Kim, K., M.E. McCully, N. Bhattacharya, B. Butler, D. Sept, and J.A. Cooper. 2007. Structure/function analysis of the interaction of phosphatidylinositol 4,5-bisphosphate with actin-capping protein: implications for how capping protein binds the actin filament. *J. Biol. Chem.* 282: 5871–5879. <https://doi.org/10.1074/jbc.M609850200>
- Knöll, R., B. Buyandelger, and M. Lab. 2011a. The sarcomeric Z-disc and Z-discopathies. *J. Biomed. Biotechnol.* 2011:569628. <https://doi.org/10.1155/2011/569628>
- Knöll, R., W.A. Linke, P. Zou, S. Miocic, S. Kostin, B. Buyandelger, C.H. Ku, S. Neef, M. Bug, K. Schäfer, et al. 2011b. Telethonin deficiency is associated with maladaptation to biomechanical stress in the mammalian heart. *Circ. Res.* 109:758–769. <https://doi.org/10.1161/CIRCRESAHA.111.245787>
- Kobayashi, T., and R.J. Solaro. 2005. Calcium, thin filaments, and the integrative biology of cardiac contractility. *Annu. Rev. Physiol.* 67:39–67. <https://doi.org/10.1146/annurev.physiol.67.040403.114025>
- Kobayashi, T., L. Jin, and P.P. de Tombe. 2008. Cardiac thin filament regulation. *Pflugers Arch.* 457:37–46. <https://doi.org/10.1007/s00424-008-0511-8>
- Konhilas, J.P., T.C. Irving, B.M. Wolska, E.E. Jweied, A.F. Martin, R.J. Solaro, and P.P. de Tombe. 2003. Troponin I in the murine myocardium: influence on length-dependent activation and interfilament spacing. *J. Physiol.* 547:951–961. <https://doi.org/10.1113/jphysiol.2002.038117>
- Kuhn, J.R., and T.D. Pollard. 2007. Single molecule kinetic analysis of actin filament capping. Polyphosphoinositides do not dissociate capping proteins. *J. Biol. Chem.* 282:28014–28024. <https://doi.org/10.1074/jbc.M705287200>
- Kuroda, S., C. Tokunaga, Y. Kiyohara, O. Higuchi, H. Konishi, K. Mizuno, G.N. Gill, and U. Kikkawa. 1996. Protein-protein interaction of zinc finger LIM domains with protein kinase C. *J. Biol. Chem.* 271:31029–31032. <https://doi.org/10.1074/jbc.271.49.31029>
- Lange, S., D. Auerbach, P. McLoughlin, E. Perriard, B.W. Schäfer, J.C. Perriard, and E. Ehler. 2002. Subcellular targeting of metabolic enzymes to titin in heart muscle may be mediated by DRAL/FHL-2. *J. Cell Sci.* 115: 4925–4936. <https://doi.org/10.1242/jcs.00181>
- Lange, S., F. Xiang, A. Yakovenko, A. Vihola, P. Hackman, E. Rostkova, J. Kristensen, B. Brandmeier, G. Franzen, B. Hedberg, et al. 2005. The kinase domain of titin controls muscle gene expression and protein turnover. *Science.* 308:1599–1603. <https://doi.org/10.1126/science.1110463>
- Lange, S., N. Pinotsis, I. Agarkova, and E. Ehler. 2020. The M-band: The underestimated part of the sarcomere. *Biochim. Biophys. Acta Mol. Cell Res.* 1867:118440. <https://doi.org/10.1016/j.bbamer.2019.02.003>
- Layland, J., D.J. Grieve, A.C. Cave, E. Sparks, R.J. Solaro, and A.M. Shah. 2004. Essential role of troponin I in the positive inotropic response to isoprenaline in mouse hearts contracting autotonically. *J. Physiol.* 556: 835–847. <https://doi.org/10.1113/jphysiol.2004.061176>
- Layland, J., R.J. Solaro, and A.M. Shah. 2005. Regulation of cardiac contractile function by troponin I phosphorylation. *Cardiovasc. Res.* 66:12–21. <https://doi.org/10.1016/j.cardiores.2004.12.022>
- Levine, R., A. Weisberg, I. Kulikovskaya, G. McClellan, and S. Winegrad. 2001. Multiple structures of thick filaments in resting cardiac muscle and their influence on cross-bridge interactions. *Biophys. J.* 81:1070–1082. [https://doi.org/10.1016/S0006-3495\(01\)75764-5](https://doi.org/10.1016/S0006-3495(01)75764-5)
- LeWinter, M.M., and H. Granzier. 2010. Cardiac titin: a multifunctional giant. *Circulation.* 121:2137–2145. <https://doi.org/10.1161/CIRCULATIONAHA.109.860171>
- Li, J., and B. Russell. 2013. Phosphatidylinositol 4,5-bisphosphate regulates CapZβ1 and actin dynamics in response to mechanical strain. *Am. J. Physiol. Heart Circ. Physiol.* 305:H1614–H1623. <https://doi.org/10.1152/ajpheart.00477.2013>
- Li, A., F. Ponten, and C.G. dos Remedios. 2012. The interactome of LIM domain proteins: the contributions of LIM domain proteins to heart failure and heart development. *Proteomics.* 12:203–225. <https://doi.org/10.1002/pmic.201100492>
- Li, J., E.J. Tanhehco, and B. Russell. 2014. Actin dynamics is rapidly regulated by the PTEN and PIP2 signaling pathways leading to myocyte hypertrophy. *Am. J. Physiol. Heart Circ. Physiol.* 307:H1618–H1625. <https://doi.org/10.1152/ajpheart.00393.2014>
- Li, S., K. Mo, H. Tian, C. Chu, S. Sun, L. Tian, S. Ding, T.R. Li, X. Wu, F. Liu, et al. 2016. Lmod2 piggyBac mutant mice exhibit dilated cardiomyopathy. *Cell Biosci.* 6:38. <https://doi.org/10.1186/s13578-016-0101-y>
- Li, K.L., M. Methawasini, B.C.W. Tanner, H.L. Granzier, R.J. Solaro, and W.J. Dong. 2019. Sarcomere length-dependent effects on Ca²⁺-troponin regulation in myocardium expressing compliant titin. *J. Gen. Physiol.* 151:30–41. <https://doi.org/10.1085/jgp.201812218>
- Liang, Y., W.H. Bradford, J. Zhang, and F. Sheikh. 2018. Four and a half LIM domain protein signaling and cardiomyopathy. *Biophys. Rev.* 10: 1073–1085. <https://doi.org/10.1007/s12551-018-0434-3>
- Lin, C., X. Guo, S. Lange, J. Liu, K. Ouyang, X. Yin, L. Jiang, Y. Cai, Y. Mu, F. Sheikh, et al. 2013a. Cypher/ZASP is a novel A-kinase anchoring protein. *J. Biol. Chem.* 288:29403–29413. <https://doi.org/10.1074/jbc.M113.470708>
- Lin, Y.-H., J. Li, E.R. Swanson, and B. Russell. 2013b. CapZ and actin capping dynamics increase in myocytes after a bout of exercise and abates in hours after stimulation ends. *J. Appl. Physiol.* (1985). 114:1603–1609. <https://doi.org/10.1152/jappphysiol.01283.2012>
- Lin, Y.-H., E.R. Swanson, J. Li, M.A. Mkrtchjan, and B. Russell. 2015. Cyclic mechanical strain of myocytes modifies CapZβ1 post translationally via PKCε. *J. Muscle Res. Cell Motil.* 36:329–337. <https://doi.org/10.1007/s10974-015-9420-6>
- Lin, Y.H., C.M. Warren, J. Li, T.A. McKinsey, and B. Russell. 2016. Myofibril growth during cardiac hypertrophy is regulated through dual phosphorylation and acetylation of the actin capping protein CapZ. *Cell. Signal.* 28:1015–1024. <https://doi.org/10.1016/j.cellsig.2016.05.011>
- Locher, M.R., M.V. Razumova, J.E. Stelzer, H.S. Norman, and R.L. Moss. 2011. Effects of low-level α;-myosin heavy chain expression on contractile kinetics in porcine myocardium. *Am. J. Physiol. Heart Circ. Physiol.* 300: H869–H878. <https://doi.org/10.1152/ajpheart.00452.2010>
- Lovelock, J.D., M.M. Monasky, E.M. Jeong, H.A. Lardin, H. Liu, B.G. Patel, D.M. Taglieri, L. Gu, P. Kumar, N. Pokhrel, et al. 2012. Ranolazine improves cardiac diastolic dysfunction through modulation of myofibrillar calcium sensitivity. *Circ. Res.* 110:841–850. <https://doi.org/10.1161/CIRCRESAHA.111.258251>
- Madan, A., M.C. Viswanathan, K.C. Woulfe, W. Schmidt, A. Sidor, T. Liu, T.H. Nguyen, B. Trinh, C. Wilson, S. Madathil, et al. 2020. TNNT2 mutations in the tropomyosin binding region of TNT1 disrupt its role in contractile inhibition and stimulate cardiac dysfunction. *Proc. Natl. Acad. Sci. USA.* 117:18822–18831. <https://doi.org/10.1073/pnas.2001692117>
- Malik, F.I., J.J. Hartman, K.A. Elias, B.P. Morgan, H. Rodriguez, K. Brejc, R.L. Anderson, S.H. Sueoka, K.H. Lee, J.T. Finer, et al. 2011. Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science.* 331:1439–1443. <https://doi.org/10.1126/science.1200113>

- Mamidi, R., K.S. Gresham, A. Li, C.G. dos Remedios, and J.E. Stelzer. 2015. Molecular effects of the myosin activator omecamtiv mecarbil on contractile properties of skinned myocardium lacking cardiac myosin binding protein-C. *J. Mol. Cell. Cardiol.* 85:262–272. <https://doi.org/10.1016/j.yjmcc.2015.06.011>
- Mamidi, R., K.S. Gresham, S. Verma, and J.E. Stelzer. 2016. Cardiac myosin binding protein-C phosphorylation modulates myofilament length-dependent activation. *Front. Physiol.* 7:38. <https://doi.org/10.3389/fphys.2016.00038>
- Mamidi, R., J. Li, K.S. Gresham, S. Verma, C.Y. Doh, A. Li, S. Lal, C.G. Dos Remedios, and J.E. Stelzer. 2017. Dose-dependent effects of the myosin activator omecamtiv mecarbil on cross-bridge behavior and force generation in failing human myocardium. *Circ. Heart Fail.* 10:e004257. <https://doi.org/10.1161/CIRCHEARTFAILURE.117.004257>
- Mamidi, R., J. Li, C.Y. Doh, S. Verma, and J.E. Stelzer. 2018. Impact of the myosin modulator mavacamten on force generation and cross-bridge behavior in a murine model of hypercontractility. *J. Am. Heart Assoc.* 7:e009627. <https://doi.org/10.1161/JAHA.118.009627>
- Maron, B.J., M.S. Maron, B.A. Maron, and J. Loscalzo. 2019. Moving beyond the sarcomere to explain heterogeneity in hypertrophic cardiomyopathy: JACC review topic of the week. *J. Am. Coll. Cardiol.* 73:1978–1986. <https://doi.org/10.1016/j.jacc.2019.01.061>
- Martin, T.G., and J.A. Kirk. 2020. Under construction: The dynamic assembly, maintenance, and degradation of the cardiac sarcomere. *J. Mol. Cell. Cardiol.* 148:89–102. <https://doi.org/10.1016/j.yjmcc.2020.08.018>
- Mastrototaro, G., X. Liang, X. Li, P. Carullo, N. Piroddi, C. Tesi, Y. Gu, N.D. Dalton, K.L. Peterson, C. Poggesi, et al. 2015. Nebulette knockout mice have normal cardiac function, but show Z-line widening and up-regulation of cardiac stress markers. *Cardiovasc. Res.* 107:216–225. <https://doi.org/10.1093/cvr/cvv156>
- Maturana, A.D., S. Wälchli, M. Iwata, S. Ryser, J. Van Lint, M. Hoshijima, W. Schlegel, Y. Ikeda, K. Tanizawa, and S. Kuroda. 2008. Enigma homolog 1 scaffolds protein kinase D1 to regulate the activity of the cardiac L-type voltage-gated calcium channel. *Cardiovasc. Res.* 78:458–465. <https://doi.org/10.1093/cvr/cvn052>
- Matusovsky, O.S., A. Mansson, M. Persson, Y.S. Cheng, and D.E. Rassier. 2019. High-speed AFM reveals subsecond dynamics of cardiac thin filaments upon Ca²⁺ activation and heavy meromyosin binding. *Proc. Natl. Acad. Sci. USA.* 116:16384–16393. <https://doi.org/10.1073/pnas.1903228116>
- Mayans, O., P.F. van der Ven, M. Wilm, A. Mues, P. Young, D.O. Fürst, M. Wilmanns, and M. Gautel. 1998. Structural basis for activation of the titin kinase domain during myofibrillogenesis. *Nature.* 395:863–869. <https://doi.org/10.1038/27603>
- McKillop, D.F., and M.A. Geeves. 1993. Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament. *Biophys. J.* 65:693–701. [https://doi.org/10.1016/S0006-3495\(93\)81110-X](https://doi.org/10.1016/S0006-3495(93)81110-X)
- McNamara, J.W., A. Li, C.G. Dos Remedios, and R. Cooke. 2015. The role of super-relaxed myosin in skeletal and cardiac muscle. *Biophys. Rev.* 7: 5–14. <https://doi.org/10.1007/s12551-014-0151-5>
- Mi-Mi, L., G.P. Farman, R.M. Mayfield, J. Strom, M. Chu, C.T. Pappas, and C.C. Gregorio. 2020. In vivo elongation of thin filaments results in heart failure. *PLoS One.* 15:e0226138. <https://doi.org/10.1371/journal.pone.0226138>
- Michele, D.E., F.P. Albayya, and J.M. Metzger. 1999. Thin filament protein dynamics in fully differentiated adult cardiac myocytes: toward a model of sarcomere maintenance. *J. Cell Biol.* 145:1483–1495. <https://doi.org/10.1083/jcb.145.7.1483>
- Miyata, S., W. Minobe, M.R. Bristow, and L.A. Leinwand. 2000. Myosin heavy chain isoform expression in the failing and nonfailing human heart. *Circ. Res.* 86:386–390. <https://doi.org/10.1161/01.RES.86.4.386>
- Mkrtshchjan, M.A., C. Solis, A.Y. Wondmagegn, J. Majithia, and B. Russell. 2018. PKC epsilon signaling effect on actin assembly is diminished in cardiomyocytes when challenged to additional work in a stiff micro-environment. *Cytoskeleton (Hoboken).* 75:363–371. <https://doi.org/10.1002/cm.21472>
- Monasky, M.M., K.D. Varian, J.P. Davis, and P.M. Janssen. 2008. Dissociation of force decline from calcium decline by preload in isolated rabbit myocardium. *Pflügers Arch.* 456:267–276. <https://doi.org/10.1007/s00424-007-0394-0>
- Monasky, M.M., B.J. Biesiadecki, and P.M. Janssen. 2010. Increased phosphorylation of tropomyosin, troponin I, and myosin light chain-2 after stretch in rabbit ventricular myocardium under physiological conditions. *J. Mol. Cell. Cardiol.* 48:1023–1028. <https://doi.org/10.1016/j.yjmcc.2010.03.004>
- Napierski, N.C., K. Granger, P.R. Langlais, H.R. Moran, J. Strom, K. Touma, and S.P. Harris. 2020. A novel “cut and paste” method for in situ replacement of cMyBP-C reveals a new role for cMyBP-C in the regulation of contractile oscillations. *Circ. Res.* 126:737–749. <https://doi.org/10.1161/CIRCRESAHA.119.315760>
- Nixon, B.R., B. Liu, B. Scellini, C. Tesi, N. Piroddi, O. Ogut, R.J. Solaro, M.T. Ziolo, P.M. Janssen, J.P. Davis, et al. 2013. Tropomyosin Ser-283 pseudo-phosphorylation slows myofibril relaxation. *Arch. Biochem. Biophys.* 535: 30–38. <https://doi.org/10.1016/j.abb.2012.11.010>
- Oda, T., and H. Yanagisawa. 2020. Cryo-electron tomography of cardiac myofibrils reveals a 3D lattice spring within the Z-discs. *Commun. Biol.* 3:585. <https://doi.org/10.1038/s42003-020-01321-5>
- Otey, C.A., R. Dixon, C. Stack, and S.M. Goicoechea. 2009. Cytoplasmic Ig-domain proteins: cytoskeletal regulators with a role in human disease. *Cell Motil. Cytoskeleton.* 66:618–634. <https://doi.org/10.1002/cm.20385>
- Palmer, B.M., D. Georgakopoulos, P.M. Janssen, Y. Wang, N.R. Alpert, D.F. Belardi, S.P. Harris, R.L. Moss, P.G. Burgon, C.E. Seidman, et al. 2004. Role of cardiac myosin binding protein C in sustaining left ventricular systolic stiffening. *Circ. Res.* 94:1249–1255. <https://doi.org/10.1161/01.RES.0000126898.95550.31>
- Pan, B.S., and R.J. Solaro. 1987. Calcium-binding properties of troponin C in detergent-skinned heart muscle fibers. *J. Biol. Chem.* 262:7839–7849. [https://doi.org/10.1016/S0021-9258\(18\)47644-2](https://doi.org/10.1016/S0021-9258(18)47644-2)
- Pappas, C.T., R.M. Mayfield, C. Henderson, N. Jamilpour, C. Cover, Z. Hernandez, K.R. Hutchinson, M. Chu, K.H. Nam, J.M. Valdez, et al. 2015. Knockout of Lmod2 results in shorter thin filaments followed by dilated cardiomyopathy and juvenile lethality. *Proc. Natl. Acad. Sci. USA.* 112: 13573–13578. <https://doi.org/10.1073/pnas.1508273112>
- Patel, B.G., T. Wilder, and R.J. Solaro. 2013. Novel control of cardiac myofilament response to calcium by S-glutathionylation at specific sites of myosin binding protein C. *Front. Physiol.* 4:336. <https://doi.org/10.3389/fphys.2013.00336>
- Paul, D.M., J.M. Squire, and E.P. Morris. 2017. Relaxed and active thin filament structures; a new structural basis for the regulatory mechanism. *J. Struct. Biol.* 197:365–371. <https://doi.org/10.1016/j.jsb.2017.01.004>
- Perz-Edwards, R.J., and M.K. Reedy. 2011. Electron microscopy and x-ray diffraction evidence for two Z-band structural states. *Biophys. J.* 101: 709–717. <https://doi.org/10.1016/j.bpj.2011.06.024>
- Pirani, A., C. Xu, V. Hatch, R. Craig, L.S. Tobacman, and W. Lehman. 2005. Single particle analysis of relaxed and activated muscle thin filaments. *J. Mol. Biol.* 346:761–772. <https://doi.org/10.1016/j.jmb.2004.12.013>
- Planelles-Herrero, V.J., J.J. Hartman, J. Robert-Paganin, F.I. Malik, and A. Houdusse. 2017a. Mechanistic and structural basis for activation of cardiac myosin force production by omecamtiv mecarbil. *Nat. Commun.* 8:190. <https://doi.org/10.1038/s41467-017-00176-5>
- Planelles-Herrero, V.J., J.J. Hartman, J. Robert-Paganin, F.I. Malik, and A. Houdusse. 2017b. Mechanistic and structural basis for activation of cardiac myosin force production by omecamtiv mecarbil. *Nat. Commun.* 8:190. <https://doi.org/10.1038/s41467-017-00176-5>
- Poggesi, C., C. Tesi, and R. Stehle. 2005. Sarcomere determinants of striated muscle relaxation kinetics. *Pflügers Arch.* 449:505–517. <https://doi.org/10.1007/s00424-004-1363-5>
- Polge, C., S. Cabantous, C. Deval, A. Claustrate, A. Hauvette, C. Bouchenot, J. Amiot, D. Béchet, L. Combaret, D. Attaix, and D. Taillandier. 2018. A muscle-specific MuRF1-E2 network requires stabilization of MuRF1-E2 complexes by telethonin, a newly identified substrate. *J. Cachexia Sarcopenia Muscle.* 9:129–145. <https://doi.org/10.1002/jcsm.12249>
- Ponnamp, S., I. Sevrieva, Y.B. Sun, M. Irving, and T. Kampourakis. 2019. Site-specific phosphorylation of myosin binding protein-C coordinates thin and thick filament activation in cardiac muscle. *Proc. Natl. Acad. Sci. USA.* 116:15485–15494. <https://doi.org/10.1073/pnas.1903033116>
- Previs, M.J., S. Beck Previs, J. Gulick, J. Robbins, and D.M. Warshaw. 2012. Molecular mechanics of cardiac myosin-binding protein C in native thick filaments. *Science.* 337:1215–1218. <https://doi.org/10.1126/science.1223602>
- Previs, M.J., B.L. Prosser, J.Y. Mun, S.B. Previs, J. Gulick, K. Lee, J. Robbins, R. Craig, W.J. Lederer, and D.M. Warshaw. 2015. Myosin-binding protein C corrects an intrinsic inhomogeneity in cardiac excitation-contraction coupling. *Sci. Adv.* 1:e1400205. <https://doi.org/10.1126/sciadv.1400205>
- Previs, M.J., J.Y. Mun, A.J. Michalek, S.B. Previs, J. Gulick, J. Robbins, D.M. Warshaw, and R. Craig. 2016. Phosphorylation and calcium antagonistically tune myosin-binding protein C's structure and function. *Proc. Natl. Acad. Sci. USA.* 113:3239–3244. <https://doi.org/10.1073/pnas.1522236113>
- Prill, K., C. Carlisle, M. Stannard, P.J. Windsor Reid, and D.B. Pilgrim. 2019. Myomesin is part of an integrity pathway that responds to sarcomere

- damage and disease. *PLoS One*. 14:e0224206. <https://doi.org/10.1371/journal.pone.0224206>
- Pyle, W.G., and R.J. Solaro. 2004. At the crossroads of myocardial signaling: the role of Z-discs in intracellular signaling and cardiac function. *Circ. Res.* 94:296–305. <https://doi.org/10.1161/01.RES.0000116143.74830.A9>
- Pyle, W.G., M.C. Hart, J.A. Cooper, M.P. Sumandea, P.P. de Tombe, and R.J. Solaro. 2002. Actin capping protein: an essential element in protein kinase signaling to the myofilaments. *Circ. Res.* 90:1299–1306. <https://doi.org/10.1161/01.RES.0000024389.03152.22>
- Pyle, W.G., G. La Rotta, P.P. de Tombe, M.P. Sumandea, and R.J. Solaro. 2006. Control of cardiac myofilament activation and PKC-betaII signaling through the actin capping protein, CapZ. *J. Mol. Cell. Cardiol.* 41:537–543. <https://doi.org/10.1016/j.yjmcc.2006.06.006>
- Radke, M.H., J. Peng, Y. Wu, M. McNabb, O.L. Nelson, H. Granzier, and M. Gotthardt. 2007. Targeted deletion of titin N2B region leads to diastolic dysfunction and cardiac atrophy. *Proc. Natl. Acad. Sci. USA*. 104:3444–3449. <https://doi.org/10.1073/pnas.0608543104>
- Rao, J.N., Y. Madasu, and R. Dominguez. 2014. Mechanism of actin filament pointed-end capping by tropomodulin. *Science*. 345:463–467. <https://doi.org/10.1126/science.1256159>
- Ratti, J., E. Rostkova, M. Gautel, and M. Pfuhl. 2011. Structure and interactions of myosin-binding protein C domain C0: cardiac-specific regulation of myosin at its neck? *J. Biol. Chem.* 286:12650–12658. <https://doi.org/10.1074/jbc.M110.156646>
- Ribeiro, E.A. Jr., N. Pinotsis, A. Ghisleni, A. Salmazo, P.V. Konarev, J. Kostan, B. Sjöblom, C. Schreiner, A.A. Polyansky, E.A. Gkoukoulia, et al. 2014. The structure and regulation of human muscle α -actinin. *Cell*. 159:1447–1460. <https://doi.org/10.1016/j.cell.2014.10.056>
- Rieck, D.C., K.L. Li, Y. Ouyang, R.J. Solaro, and W.J. Dong. 2013. Structural basis for the in situ Ca^{2+} sensitization of cardiac troponin C by positive feedback from force-generating myosin cross-bridges. *Arch. Biochem. Biophys.* 537:198–209. <https://doi.org/10.1016/j.abb.2013.07.013>
- Risi, C., J. Eisner, B. Belknap, D.H. Heeley, H.D. White, G.F. Schröder, and V.E. Galkin. 2017. Ca^{2+} -induced movement of tropomyosin on native cardiac thin filaments revealed by cryoelectron microscopy. *Proc. Natl. Acad. Sci. USA*. 114:6782–6787.
- Risi, C., B. Belknap, E. Forgacs-Lonart, S.P. Harris, G.F. Schröder, H.D. White, and V.E. Galkin. 2018. N-terminal domains of cardiac myosin binding protein C cooperatively activate the thin filament. *Structure*. 26:1604–1611.e4. <https://doi.org/10.1016/j.str.2018.08.007>
- Rosas, P.C., Y. Liu, M.I. Abdalla, C.M. Thomas, D.T. Kidwell, G.F. Dusio, D. Mukhopadhyay, R. Kumar, K.M. Baker, B.M. Mitchell, et al. 2015. Phosphorylation of cardiac Myosin-binding protein-C is a critical mediator of diastolic function. *Circ. Heart Fail.* 8:582–594. <https://doi.org/10.1161/CIRCHEARTFAILURE.114.001550>
- Rozengurt, E., O. Rey, and R.T. Waldron. 2005. Protein kinase D signaling. *J. Biol. Chem.* 280:13205–13208. <https://doi.org/10.1074/jbc.R500002200>
- Rundell, V.L., D.L. Geenen, P.M. Buttrick, and P.P. de Tombe. 2004. Depressed cardiac tension cost in experimental diabetes is due to altered myosin heavy chain isoform expression. *Am. J. Physiol. Heart Circ. Physiol.* 287:H408–H413. <https://doi.org/10.1152/ajpheart.00049.2004>
- Ryba, D.M., J. Li, C.L. Cowan, B. Russell, B.M. Wolska, and R.J. Solaro. 2017. Long-term biased β -arrestin signaling improves cardiac structure and function in dilated cardiomyopathy. *Circulation*. 135:1056–1070. <https://doi.org/10.1161/CIRCULATIONAHA.116.024482>
- Sadayappan, S., and P.P. de Tombe. 2014. Cardiac myosin binding protein-C as a central target of cardiac sarcomere signaling: a special mini review series. *Pflugers Arch.* 466:195–200. <https://doi.org/10.1007/s00424-013-1396-8>
- Samarel, A.M. 2005. Costameres, focal adhesions, and cardiomyocyte mechanotransduction. *Am. J. Physiol. Heart Circ. Physiol.* 289:H2291–H2301. <https://doi.org/10.1152/ajpheart.00749.2005>
- Sanger, J.M., and J.W. Sanger. 2008. The dynamic Z bands of striated muscle cells. *Sci. Signal*. 1:pe37. <https://doi.org/10.1126/scisignal.132pe37>
- Scarff, C.A., G. Carrington, D. Casas-Mao, J.M. Chalovich, P.J. Knight, N.A. Ranson, and M. Peckham. 2020. Structure of the shutdown state of myosin-2. *Nature*. 588:515–520. <https://doi.org/10.1038/s41586-020-2990-5>
- Schafer, D.A., J.A. Waddle, and J.A. Cooper. 1993. Localization of CapZ during myofibrillogenesis in cultured chicken muscle. *Cell Motil. Cytoskeleton*. 25:317–335. <https://doi.org/10.1002/cm.970250403>
- Schafer, D.A., P.B. Jennings, and J.A. Cooper. 1996. Dynamics of capping protein and actin assembly in vitro: uncapping barbed ends by polyphosphoinositides. *J. Cell Biol.* 135:169–179. <https://doi.org/10.1083/jcb.135.1.169>
- Schiattarella, G.G., F. Altamirano, D. Tong, K.M. French, E. Villalobos, S.Y. Kim, X. Luo, N. Jiang, H.I. May, Z.V. Wang, et al. 2019. Nitrosative stress drives heart failure with preserved ejection fraction. *Nature*. 568:351–356. <https://doi.org/10.1038/s41586-019-1100-z>
- Scruggs, S.B., and R.J. Solaro. 2011. The significance of regulatory light chain phosphorylation in cardiac physiology. *Arch. Biochem. Biophys.* 510:129–134. <https://doi.org/10.1016/j.abb.2011.02.013>
- Scruggs, S.B., A.C. Hinken, A. Thawornkaiwong, J. Robbins, L.A. Walker, P.P. de Tombe, D.L. Geenen, P.M. Buttrick, and R.J. Solaro. 2009. Ablation of ventricular myosin regulatory light chain phosphorylation in mice causes cardiac dysfunction in situ and affects neighboring myofilament protein phosphorylation. *J. Biol. Chem.* 284:5097–5106. <https://doi.org/10.1074/jbc.M807414200>
- Sevrieva, I.R., B. Brandmeier, S. Ponnam, M. Gautel, M. Irving, K.S. Campbell, Y.B. Sun, and T. Kampourakis. 2020. Cardiac myosin regulatory light chain kinase modulates cardiac contractility by phosphorylating both myosin regulatory light chain and troponin I. *J. Biol. Chem.* 295:4398–4410. <https://doi.org/10.1074/jbc.RA119.011945>
- Sewanam, L.R., J.R. Moore, W. Lehman, and S.G. Campbell. 2016. Predicting effects of tropomyosin mutations on cardiac muscle contraction through myofilament modeling. *Front. Physiol.* 7:473. <https://doi.org/10.3389/fphys.2016.00473>
- Sheikh, F., A. Raskin, P.H. Chu, S. Lange, A.A. Domenighetti, M. Zheng, X. Liang, T. Zhang, T. Yajima, Y. Gu, et al. 2008. An FHL1-containing complex within the cardiomyocyte sarcomere mediates hypertrophic biomechanical stress responses in mice. *J. Clin. Invest.* 118:3870–3880. <https://doi.org/10.1172/JCI34472>
- Sheikh, F., R.C. Lyon, and J. Chen. 2015. Functions of myosin light chain-2 (MYL2) in cardiac muscle and disease. *Gene*. 569:14–20. <https://doi.org/10.1016/j.gene.2015.06.027>
- Sheth, P.J., G.H. Danton, Y. Siegel, R.E. Kardon, J.C. Infante Jr., E. Ghersin, and J.E. Fishman. 2015. Cardiac physiology for radiologists: review of relevant physiology for interpretation of cardiac MR imaging and CT. *Radiographics*. 35:1335–1351. <https://doi.org/10.1148/rg.2015140234>
- Shiner, J.S., and R.J. Solaro. 1984. The hill coefficient for the Ca^{2+} -activation of striated muscle contraction. *Biophys. J.* 46:541–543. [https://doi.org/10.1016/S0006-3495\(84\)84051-5](https://doi.org/10.1016/S0006-3495(84)84051-5)
- Sitbon, Y.H., S. Yadav, K. Kazmierczak, and D. Szczesna-Cordary. 2020. Insights into myosin regulatory and essential light chains: a focus on their roles in cardiac and skeletal muscle function, development and disease. *J. Muscle Res. Cell Motil.* 41:313–327. <https://doi.org/10.1007/s10974-019-09517-x>
- Skwarek-Maruszewska, A., M. Boczkowska, A.L. Zajac, E. Kremneva, T. Svitkina, R. Dominguez, and P. Lappalainen. 2010. Different localizations and cellular behaviors of leiomodin and tropomodulin in mature cardiomyocyte sarcomeres. *Mol. Biol. Cell*. 21:3352–3361. <https://doi.org/10.1091/mbc.e10-02-0109>
- Solaro, R.J. 2005. Remote control of A-band cardiac thin filaments by the I-Z-I protein network of cardiac sarcomeres. *Trends Cardiovasc. Med.* 15:148–152. <https://doi.org/10.1016/j.tcm.2005.04.007>
- Solaro, R.J., and H.M. Rarick. 1998. Troponin and tropomyosin: proteins that switch on and tune in the activity of cardiac myofilaments. *Circ. Res.* 83:471–480. <https://doi.org/10.1161/01.RES.83.5.471>
- Solaro, R.J., and J.C. Rüegg. 1982. Stimulation of Ca^{++} binding and ATPase activity of dog cardiac myofibrils by AR-L 115BS, a novel cardiotonic agent. *Circ. Res.* 51:290–294. <https://doi.org/10.1161/01.RES.51.3.290>
- Solaro, C.R., and R.J. Solaro. 2020. Implications of the complex biology and micro-environment of cardiac sarcomeres in the use of high affinity troponin antibodies as serum biomarkers for cardiac disorders. *J. Mol. Cell. Cardiol.* 143:145–158. <https://doi.org/10.1016/j.yjmcc.2020.05.010>
- Solaro, R.J., D.C. Pang, and F.N. Briggs. 1971. The purification of cardiac myofibrils with Triton X-100. *Biochim. Biophys. Acta.* 245:259–262. [https://doi.org/10.1016/0005-2728\(71\)90033-8](https://doi.org/10.1016/0005-2728(71)90033-8)
- Solaro, R.J., J.A. Lee, J.C. Kentish, and D.G. Allen. 1988. Effects of acidosis on ventricular muscle from adult and neonatal rats. *Circ. Res.* 63:779–787. <https://doi.org/10.1161/01.RES.63.4.779>
- Solaro, R.J., M. Henze, and T. Kobayashi. 2013. Integration of troponin I phosphorylation with cardiac regulatory networks. *Circ. Res.* 112:355–366. <https://doi.org/10.1161/CIRCRESAHA.112.268672>
- Solis, C., and B. Russell. 2019. CapZ integrates several signaling pathways in response to mechanical stiffness. *J. Gen. Physiol.* 151:660–669. <https://doi.org/10.1085/jgp.201812199>
- Solis, C., G.H. Kim, M.E. Moutsoglou, and J.M. Robinson. 2018. Ca^{2+} and myosin cycle states work as allosteric effectors of troponin activation. *Biophys. J.* 115:1762–1769. <https://doi.org/10.1016/j.bpj.2018.08.033>

- Sparrow, A.J., K. Sievert, S. Patel, Y.F. Chang, C.N. Broyles, F.A. Brook, H. Watkins, M.A. Geeves, C.S. Redwood, P. Robinson, and M.J. Daniels. 2019. Measurement of myofibrillar-localized calcium dynamics in adult cardiomyocytes and the effect of hypertrophic cardiomyopathy mutations. *Circ. Res.* 124:1228–1239. <https://doi.org/10.1161/CIRCRESAHA.118.314600>
- Stathopoulou, K., I. Wittig, J. Heidler, A. Piasecki, F. Richter, S. Diering, J. van der Velden, F. Buck, S. Donzelli, E. Schröder, et al. 2016. S-glutathiolation impairs phosphoregulation and function of cardiac myosin-binding protein C in human heart failure. *FASEB J.* 30:1849–1864. <https://doi.org/10.1096/fj.201500048>
- Steiger, G.J. 1971. Stretch activation and myogenic oscillation of isolated contractile structures of heart muscle. *Pflugers Arch.* 330:347–361. <https://doi.org/10.1007/BF00588586>
- Strauss, J.D., C. Zeugner, J.E. Van Eyk, C. Bletz, M. Troschka, and J.C. Ruegg. 1992. Troponin replacement in permeabilized cardiac muscle. Reversible extraction of troponin I by incubation with vanadate. *FEBS Lett.* 310:229–234. [https://doi.org/10.1016/0014-5793\(92\)81338-M](https://doi.org/10.1016/0014-5793(92)81338-M)
- Sun, Y.B., F. Lou, and M. Irving. 2009. Calcium- and myosin-dependent changes in troponin structure during activation of heart muscle. *J. Physiol.* 587:155–163. <https://doi.org/10.1113/jphysiol.2008.164707>
- Sussman, M.A., S. Welch, N. Cambon, R. Klevitsky, T.E. Hewett, R. Price, S.A. Witt, and T.R. Kimball. 1998. Myofibril degeneration caused by tropomodulin overexpression leads to dilated cardiomyopathy in juvenile mice. *J. Clin. Invest.* 101:51–61. <https://doi.org/10.1172/JCI1167>
- Szatmári, D., B. Bugyi, Z. Ujfalusi, L. Grama, R. Dudás, and M. Nyitrai. 2017. Cardiac leiomodulin binds to the sides of actin filaments and regulates the ATPase activity of myosin. *PLoS One.* 12:e0186288. <https://doi.org/10.1371/journal.pone.0186288>
- Tachampa, K., T. Kobayashi, H. Wang, A.F. Martin, B.J. Biesiadecki, R.J. Solaro, and P.P. de Tombe. 2008. Increased cross-bridge cycling kinetics after exchange of C-terminal truncated troponin I in skinned rat cardiac muscle. *J. Biol. Chem.* 283:15114–15121. <https://doi.org/10.1074/jbc.M801636200>
- Takács, B., N. Billington, M. Gyimesi, B. Kintszes, A. Málnási-Csizmadia, P.J. Knight, and M. Kovács. 2010. Myosin complexed with ADP and blebbistatin reversibly adopts a conformation resembling the start point of the working stroke. *Proc. Natl. Acad. Sci. USA.* 107:6799–6804. <https://doi.org/10.1073/pnas.0907585107>
- Tawada, Y., and K. Tawada. 1975. Co-operative regulation mechanism of muscle contraction: inter-tropomyosin co-operation model. *J. Theor. Biol.* 50:269–283. [https://doi.org/10.1016/0022-5193\(75\)90081-8](https://doi.org/10.1016/0022-5193(75)90081-8)
- Teerlink, J.R., R. Diaz, G.M. Felker, J.J.V. McMurray, M. Metra, S.D. Solomon, K.F. Adams, I. Anand, A. Arias-Mendoza, T. Biering-Sørensen, et al. GALACTIC-HF Investigators. 2021. Cardiac myosin activation with omecamtiv mecarbil in systolic heart failure. *N. Engl. J. Med.* 384:105–116. <https://doi.org/10.1056/NEJMoa2025797>
- Teerlink, J.R., R. Diaz, G.M. Felker, J.J.V. McMurray, M. Metra, S.D. Solomon, J.C. Legg, G. Büchele, C. Varin, C.E. Kurtz, et al. 2020. Omecamtiv mecarbil in chronic heart failure with reduced ejection fraction: rationale and design of GALACTIC-HF. *JACC Heart Fail.* 8:329–340. <https://doi.org/10.1016/j.jchf.2019.12.001>
- Tobacman, L.S. 1996. Thin filament-mediated regulation of cardiac contraction. *Annu. Rev. Physiol.* 58:447–481. <https://doi.org/10.1146/annurev.ph.58.030196.002311>
- Tobacman, L.S. 2021. Troponin revealed: uncovering the structure of the thin filament on-off switch in striated muscle. *Biophys. J.* 120:1–9. <https://doi.org/10.1016/j.bpj.2020.11.014>
- Toepfer, C.N., A.C. Garfinkel, G. Venturini, H. Wakimoto, G. Repetti, L. Alamo, A. Sharma, R. Agarwal, J.F. Ewoldt, P. Cloonan, et al. 2020. Myosin sequestration regulates sarcomere function, cardiomyocyte energetics, and metabolism, informing the pathogenesis of hypertrophic cardiomyopathy. *Circulation.* 141:828–842. <https://doi.org/10.1161/CIRCULATIONAHA.119.042339>
- Tong, C.W., J.E. Stelzer, M.L. Greaser, P.A. Powers, and R.L. Moss. 2008. Acceleration of crossbridge kinetics by protein kinase A phosphorylation of cardiac myosin binding protein C modulates cardiac function. *Circ. Res.* 103:974–982. <https://doi.org/10.1161/CIRCRESAHA.108.177683>
- Tong, C.W., X. Wu, Y. Liu, P.C. Rosas, S. Sadayappan, A. Hudmon, M. Muthuchamy, P.A. Powers, H.H. Valdivia, and R.L. Moss. 2015. Phosphoregulation of cardiac inotropy via myosin binding protein-C during increased pacing frequency or β -adrenergic stimulation. *Circ. Heart Fail.* 8:595–604. <https://doi.org/10.1161/CIRCHEARTFAILURE.114.001585>
- Tsukada, T., C.T. Pappas, N. Moroz, P.B. Antin, A.S. Kostyukova, and C.C. Gregorio. 2010. Leiomodulin-2 is an antagonist of tropomodulin-1 at the pointed end of the thin filaments in cardiac muscle. *J. Cell Sci.* 123:3136–3145. <https://doi.org/10.1242/jcs.071837>
- Tucholski, T., W. Cai, Z.R. Gregorich, E.F. Bayne, S.D. Mitchell, S.J. McIlwain, W.J. de Lange, M. Wrobbel, H. Karp, Z. Hite, et al. 2020. Distinct hypertrophic cardiomyopathy genotypes result in convergent sarcomeric proteoform profiles revealed by top-down proteomics. *Proc. Natl. Acad. Sci. USA.* 117:24691–24700.
- Urbaniene, D., F.A. Dias, J.R. Peña, L.A. Walker, R.J. Solaro, and B.M. Wolska. 2005. Expression of slow skeletal troponin I in adult mouse heart helps to maintain the left ventricular systolic function during respiratory hypercapnia. *Circ. Res.* 97:70–77. <https://doi.org/10.1161/01.RES.0000173849.68636.1e>
- van der Velden, J., and G.J.M. Stienen. 2019. Cardiac disorders and pathophysiology of sarcomeric proteins. *Physiol. Rev.* 99:381–426. <https://doi.org/10.1152/physrev.00040.2017>
- Veksler, V.I., R. Ventura-Clapier, P. Lechene, and G. Vassort. 1988. Functional state of myofibrils, mitochondria and bound creatine kinase in skinned ventricular fibers of cardiomyopathic hamsters. *J. Mol. Cell. Cardiol.* 20:329–342. [https://doi.org/10.1016/S0022-2828\(88\)80067-1](https://doi.org/10.1016/S0022-2828(88)80067-1)
- Vetter, A.D., A.A. Martin, B.R. Thompson, D.D. Thomas, and J.M. Metzger. 2020. Sarcomere integrated biosensor detects myofibrillar-activating ligands in real time during twitch contractions in live cardiac muscle. *J. Mol. Cell. Cardiol.* 147:49–61. <https://doi.org/10.1016/j.yjmcc.2020.07.012>
- Vignier, N., P. Le Corvoisier, C. Bland, L. Sambin, F. Azibani, S. Schlossarek, C. Delcayre, L. Carrier, L. Hittinger, and J.B. Su. 2014. AT1 blockade abolishes left ventricular hypertrophy in heterozygous cMyBP-C null mice: role of FHL1. *Fundam. Clin. Pharmacol.* 28:249–256. <https://doi.org/10.1111/fcp.12031>
- Vitale, G., C. Ferrantini, N. Piroddi, B. Scellini, J.M. Pioner, B. Colombini, C. Tesi, and C. Poggesi. 2019. The relation between sarcomere energetics and the rate of isometric tension relaxation in healthy and diseased cardiac muscle. *J. Muscle Res. Cell Motil.* <https://doi.org/10.1007/s10974-019-09566-2>
- Wang, L., J. Geist, A. Grogan, L.R. Hu, and A. Kontogianni-Konstantopoulos. 2018. Thick filament protein network, functions, and disease association. *Compr. Physiol.* 8:631–709. <https://doi.org/10.1002/cphy.c170023>
- Westfall, M.V., and J.M. Metzger. 2007. Single amino acid substitutions define isoform-specific effects of troponin I on myofibrillar Ca²⁺ and pH sensitivity. *J. Mol. Cell. Cardiol.* 43:107–118. <https://doi.org/10.1016/j.yjmcc.2007.05.017>
- Wier, W.G., D.J. Beckelmann, and L. Barcenas-Ruiz. 1988. [Ca²⁺]_i in single isolated cardiac cells: a review of recent results obtained with digital imaging microscopy and fura-2. *Can. J. Physiol. Pharmacol.* 66:1224–1231. <https://doi.org/10.1139/y88-202>
- Wolff, M.R., K.S. McDonald, and R.L. Moss. 1995. Rate of tension development in cardiac muscle varies with level of activator calcium. *Circ. Res.* 76:154–160. <https://doi.org/10.1161/01.RES.76.1.154>
- Woody, M.S., M.J. Greenberg, B. Barua, D.A. Winkelmann, Y.E. Goldman, and E.M. Ostap. 2018. Positive cardiac inotrope omecamtiv mecarbil activates muscle despite suppressing the myosin working stroke. *Nat. Commun.* 9:3838. <https://doi.org/10.1038/s41467-018-06193-2>
- Wu, H., J. Lee, L.G. Vincent, Q. Wang, M. Gu, F. Lan, J.M. Churko, K.I. Sallam, E. Matsa, A. Sharma, et al. 2015. Epigenetic regulation of phosphodiesterases 2A and 3A underlies compromised β -adrenergic signaling in an iPSC model of dilated cardiomyopathy. *Cell Stem Cell.* 17:89–100. <https://doi.org/10.1016/j.stem.2015.04.020>
- Yadav, S., and D. Szczeszna-Cordary. 2017. Pseudophosphorylation of cardiac myosin regulatory light chain: a promising new tool for treatment of cardiomyopathy. *Biophys. Rev.* 9:57–64. <https://doi.org/10.1007/s12551-017-0248-8>
- Yamada, Y., K. Namba, and T. Fujii. 2020. Cardiac muscle thin filament structures reveal calcium regulatory mechanism. *Nat. Commun.* 11:153. <https://doi.org/10.1038/s41467-019-14008-1>
- Yamasaki, R., M. Berri, Y. Wu, K. Trombitás, M. McNabb, M.S. Kellermayer, C. Witt, D. Labeit, S. Labeit, M. Greaser, and H. Granzier. 2001. Titin-actin interaction in mouse myocardium: passive tension modulation and its regulation by calcium/S100A1. *Biophys. J.* 81:2297–2313. [https://doi.org/10.1016/S0006-3495\(01\)75876-6](https://doi.org/10.1016/S0006-3495(01)75876-6)
- Yamazaki, T., S. Wälchli, T. Fujita, S. Ryser, M. Hoshijima, W. Schlegel, S. Kuroda, and A.D. Maturana. 2010. Splice variants of enigma homolog, differentially expressed during heart development, promote or prevent hypertrophy. *Cardiovasc. Res.* 86:374–382. <https://doi.org/10.1093/cvr/cvq023>
- Yang, S., P. Tiwari, K.H. Lee, O. Sato, M. Ikebe, R. Padrón, and R. Craig. 2020. Cryo-EM structure of the inhibited (I0S) form of myosin II. *Nature.* 588:521–525. <https://doi.org/10.1038/s41586-020-3007-0>

- Yoshikawa, Y., T. Yasuike, A. Yagi, and T. Yamada. 1999. Transverse elasticity of myofibrils of rabbit skeletal muscle studied by atomic force microscopy. *Biochem. Biophys. Res. Commun.* 256:13–19. <https://doi.org/10.1006/bbrc.1999.0279>
- Yotti, R., C.E. Seidman, and J.G. Seidman. 2019. Advances in the genetic basis and pathogenesis of sarcomere cardiomyopathies. *Annu. Rev. Genomics Hum. Genet.* 20:129–153. <https://doi.org/10.1146/annurev-genom-083118-015306>
- Young, P., and M. Gautel. 2000. The interaction of titin and alpha-actinin is controlled by a phospholipid-regulated intramolecular pseudoligand mechanism. *EMBO J.* 19:6331–6340. <https://doi.org/10.1093/emboj/19.23.6331>
- Zhao, W., X. Wu, Z. Wang, B. Pan, L. Liu, L. Liu, X. Huang, and J. Tian. 2020. Epigenetic regulation of phosphodiesterase 4d in restrictive cardiomyopathy mice with cTnI mutations. *Sci. China Life Sci.* 63:563–570. <https://doi.org/10.1007/s11427-018-9463-9>
- Zhou, Q., P. Ruiz-Lozano, M.E. Martone, and J. Chen. 1999. Cypher, a striated muscle-restricted PDZ and LIM domain-containing protein, binds to alpha-actinin-2 and protein kinase C. *J. Biol. Chem.* 274:19807–19813. <https://doi.org/10.1074/jbc.274.28.19807>
- Zoghbi, M.E., J.L. Woodhead, R.L. Moss, and R. Craig. 2008. Three-dimensional structure of vertebrate cardiac muscle myosin filaments. *Proc. Natl. Acad. Sci. USA.* 105:2386–2390. <https://doi.org/10.1073/pnas.0708912105>
- Zou, P., N. Pinotsis, S. Lange, Y.H. Song, A. Popov, I. Mavridis, O.M. Mayans, M. Gautel, and M. Wilmanns. 2006. Palindromic assembly of the giant muscle protein titin in the sarcomeric Z-disk. *Nature.* 439:229–233. <https://doi.org/10.1038/nature04343>