Perspectives on: SGP Symposium on Muscle in Health and Disease

Regulation of contraction in mammalian striated muscles—the plot *thick*-ens

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Contraction of vertebrate striated muscles is regulated via switch-like activation of the thin filament due to Ca^{2+} binding to the troponin C (TnC) subunit of troponin, which together with tropomyosin comprise the thin filament regulatory strand. This mechanism has been known for nearly 50 years, dating to the initial publication by Ebashi and Endo (1968) on this topic, and is widely accepted and taught in the field as an obligatory step in the activation of muscle under physiological (as opposed to pathophysiological) conditions. The simple elegance of this mechanism and the obligatory role of Ca^{2+} in activation have contributed to the perception that Ca^{2+} binding to TnC composes the entirety of regulation in vertebrate skeletal and cardiac muscles, and yet, some properties of regulation cannot be explained without invoking additional processes. As an example, the variation of isometric force with $[Ca^{2+}]$ in permeabilized muscle preparations suggests the presence of cooperative processes in activation, which occur to differing degrees in myocardium and in fast- and slow-twitch skeletal muscles. There is also a nearly 10-fold acceleration of the rate constant of force development as Ca^{2+} concentration is increased from threshold to saturating levels with respect to steady-state force. Thus, either Ca^{2+} binding to TnC in these muscles is something more than a simple switch, and/or additional processes contribute to the activation of contraction. These issues were discussed at the 63rd Symposium of the Society of General Physiologists on "Muscle in Health and Disease" held in September 2009 at Woods Hole, Massachusetts, and are the subject of this Perspective.

By the 1980s, experimental evidence began to suggest that thick filament–based mechanisms contributed to the regulation of contraction. Work by Stull et al. (for review see Sweeney et al., 1993) showed that the posttetanic potentiation of twitch force in skeletal muscles was associated with stimulus frequency–dependent phosphorylation of myosin regulatory light chain. For example, return of peak twitch force to pre-tetanus levels followed the same time course as the posttetanic decrease in phosphorylation of regulatory light chain. This was the first evidence in vertebrate striated muscles that a process other than Ca^{2+} binding to TnC contributed to the regulation of contraction, although unlike Ca^{2+} binding to TnC, phosphorylation of the light chain is not required for the activation of contraction. Subsequent work showed that phosphorylation of regulatory light chain in rabbit skeletal muscle accelerated the rate of force development (Metzger et al., 1989), which could account for posttetanic increases in twitch force. It is now evident from x-ray diffraction studies (Colson et al., 2010) that phosphorylation of the regulatory light chain causes the myosin head to move closer to the thin filament, presumably due to charge repulsion with the surface charge of the thick filament, thereby increasing the probability of cross-bridge binding to actin. In other muscle types, such as many smooth muscles, phosphorylation of the regulatory light chain is an obligatory step in activating force development, although here, too, the force and speed of contraction are modulated by a range of signaling processes targeting both the thick and thin filaments. Further, evidence from some invertebrate muscles indicates that contraction in these muscles is regulated by Ca^{2+} binding to myosin, rather than to thin filament proteins, but there is as yet little evidence for regulation of vertebrate striated muscle contraction via Ca^{2+} binding to myosin. Although this remains an intriguing possibility, vertebrate skeletal muscle myosin lacks the regulatory high-affinity Ca^{2+}-binding site that is formed in some invertebrate muscles by the confluence of the regulatory and essential light chains with the myosin heavy chain (Szent-Györgyi, 1996).

In the past several years, considerable attention has focused on the modulation of Ca^{2+}-activated contraction in vertebrate striated muscles. Although current work involves both skeletal and cardiac muscles, the evolutionary elaboration of secondary regulatory processes is most

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Abbreviations used in this paper: cMyBP-C, myosin-binding protein C; NEM-S1, *N*-ethylmaleimide-modified myosin S1; TnC, troponin C; TnI, troponin I.

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evident in myocardium, in which the in vivo tuning of contraction varies considerably from beat to beat depending on circulatory load and sympathetic tone. In contrast, contractions of skeletal muscle fibers tend to be all-or-none events that are principally modulated by the delivery of Ca^{2+} to the myoplasm during excitation–contraction coupling and phosphorylation of the regulatory light chain of myosin.

Thin filament cooperativity in the activation of force development

Ca^{2+} activation of contraction in vertebrate striated muscles is a highly cooperative process that is most clearly evident in the steepness of force–pCa (–log[Ca^{2+}]) relationships from both cardiac and skeletal muscles. Relationships from fast-twitch skeletal muscles exhibit Hill coefficients as great as 7–9, despite the presence of just two regulatory Ca^{2+}-binding sites on TnC. The relationships in cardiac and slow-twitch skeletal muscles are shallower, indicated by Hill coefficients of 2–6, but still greater than predicted by the single regulatory binding site for Ca^{2+} in cardiac/slow TnC. The molecular basis for cooperation is not well understood in any of these muscle types, although there is evidence for positive cooperativity in (a) Ca^{2+} binding to TnC and (b) myosin binding to actin, as well enhancement of Ca^{2+} binding as a consequence of myosin binding to the thin filament. In this regard, Grabarek et al. (1983) showed that Ca^{2+} binding to skeletal TnC in solution exhibited no apparent cooperativity, but the Hill coefficients derived from binding plots increased progressively when binding was measured in intact thin filaments and then in the presence of myosin S1. Thus, the greatest cooperativity in Ca^{2+} binding is observed in the intact thin filament with myosin strongly bound to actin. But even this demonstration does not account for all possible determinants of cooperativity in Ca^{2+} binding to TnC because Fuchs and Wang (1996) showed that Ca^{2+} binding to TnC in skinned myocardium varies with developed force; i.e., mechanical stress within the thin filament promotes Ca^{2+} binding to TnC. At present, although there is clearly positive cooperativity in the binding of Ca^{2+} to TnC, its contribution to the characteristics of regulation in living muscles or to possible differences in regulation between cardiac and skeletal muscles is not known. To the degree that cooperativity is operative in muscles, it seems probable that the process would be more dynamic in cardiac muscles because the amount of Ca^{2+} released to the myoplasm is variable and typically not sufficient to saturate the Ca^{2+}-binding sites on the thin filament, unlike the case in skeletal muscle. Thus, cooperative processes have the potential in cardiac muscle to increase the Ca^{2+}-binding affinity of cardiac TnC (cTnC).

Positive cooperativity in the binding of myosin heads to actin has been demonstrated in both cardiac and skeletal muscles, principally in the binding of myosin S1–ADP to regulated thin filaments from both muscle types. A mechanism for such cooperativity is simple to envision. Distortion of the regulatory strand by strong binding of myosin heads facilitates the binding of heads in adjacent regions of the thin filament. Such binding has a persistence length equivalent to at least 14 actin monomers in skeletal muscle (Swartz et al., 1990), although the corresponding number in cardiac muscle is not yet known. The intuitive nature of this concept is in some ways compelling, but experimental data demonstrating such a mechanism in contracting muscles have yet to be published. Nonetheless, the activation of fast-twitch skeletal muscle thin filaments is thought to be a more highly cooperative process than in cardiac thin filaments. Supporting this conclusion is the observation that the Hill coefficient from the force–pCa relationship is greater in skeletal muscle, and higher concentrations of a strong-binding derivative of myosin S1, i.e., *N*-ethylmaleimide-modified myosin S1 (NEM-S1), are required to activate force development in the absence of Ca^{2+} (Fitzsimons and Moss, 2007). Another way to view differences between skeletal and cardiac muscles is that cardiac thin filaments have greater sensitivity to the activating effects of cross-bridge strong binding, which is consistent with the observation that the threshold NEM-S1 concentration required to elicit force development is lower in cardiac than in skeletal muscles. It is important to note that cooperative processes play significant roles in the regulation of both types of muscle; i.e., cross-bridge binding to the thin filament increases activation (or force development) in both muscle types. The distinction to be made between cardiac and fast-twitch skeletal muscles is that fewer cross-bridges must be bound to actin to increase the activation state of the cardiac thin filament than are required to increase the activation state of skeletal muscle thin filaments.

In addition to influencing the steepness of the force–pCa relationship, and by extension the Ca^{2+} sensitivity of force, cooperative processes also modulate the rate of rise of force in vertebrate striated muscles. For example, NEM-S1 accelerates the rate of force development at submaximal but not at saturating levels of activator Ca^{2+} in skeletal (Swartz and Moss, 1992) and cardiac muscles (Fitzsimons et al., 2001). Infusion of micromolar concentrations of NEM-S1 increases the rate constant of force development at low levels of activation to near-maximal values. In this instance, also, cardiac myofilaments exhibit greater sensitivity to the activating effects of strong-binding myosin heads (Fitzsimons et al., 2001; Regnier et al., 2004). This difference between muscle types would be expected to contribute to the more explosive all-or-none nature of twitches in fast-twitch skeletal muscles as opposed to the dynamic gradation of twitch characteristics on a beat-to-beat basis in cardiac muscle.

Current models for the regulation of contraction suggest plausible mechanisms for the activation dependence of force and the kinetics of force development, although the underlying mechanisms are not mutually exclusive. The critical distinction among models is whether activation depends on both cross-bridge and Ca^{2+} binding or on Ca^{2+} binding alone. In one approach, increases in [Ca^{2+}] are envisioned to increase the rate of cross-bridge binding as a pseudo–second order process (Landesberg and Sideman, 1994). Such a mechanism does not rely upon cooperative spread of activation along the thin filament, although it is conceivable that cooperativity could play a modulatory role. In another approach, the rate of cross-bridge cycling is viewed as invariant with the level of activation, but the extent and rate of cross-bridge binding vary due to cooperative recruitment of additional cross-bridges subsequent to initial cross-bridge binding (Campbell, 1997), as shown in Fig. 1. This mechanism would be most prominent at low levels of activation because entire regions of the thin filament without Ca^{2+} bound to troponin would be inactive and would therefore present a substrate for cross-bridge recruitment, at least within the persistence length of the propagated activating effects of initial cross-bridge binding. In such a mechanism, the slowing of the rate of force development at low levels of activation would be a manifestation of the time required for the progressive recruitment of cross-bridges and not an activation-dependent slowing of the rate of cross-bridge cycling per se. The finding that NEM-S1 accelerates the rate of force development at low [Ca^{2+}] is consistent with this model because NEM-S1 presumably fully activates the thin filament in terms of cross-bridge binding, resulting in a system that is simply switched on and off by Ca^{2+} binding.

Given the differing levels of Ca^{2+} that are typically achieved during twitches of cardiac and skeletal muscles, it seems likely that the two types of muscle rely differently on thin filament cooperativity in the activation of force in vivo. Because the levels of Ca^{2+} are typically

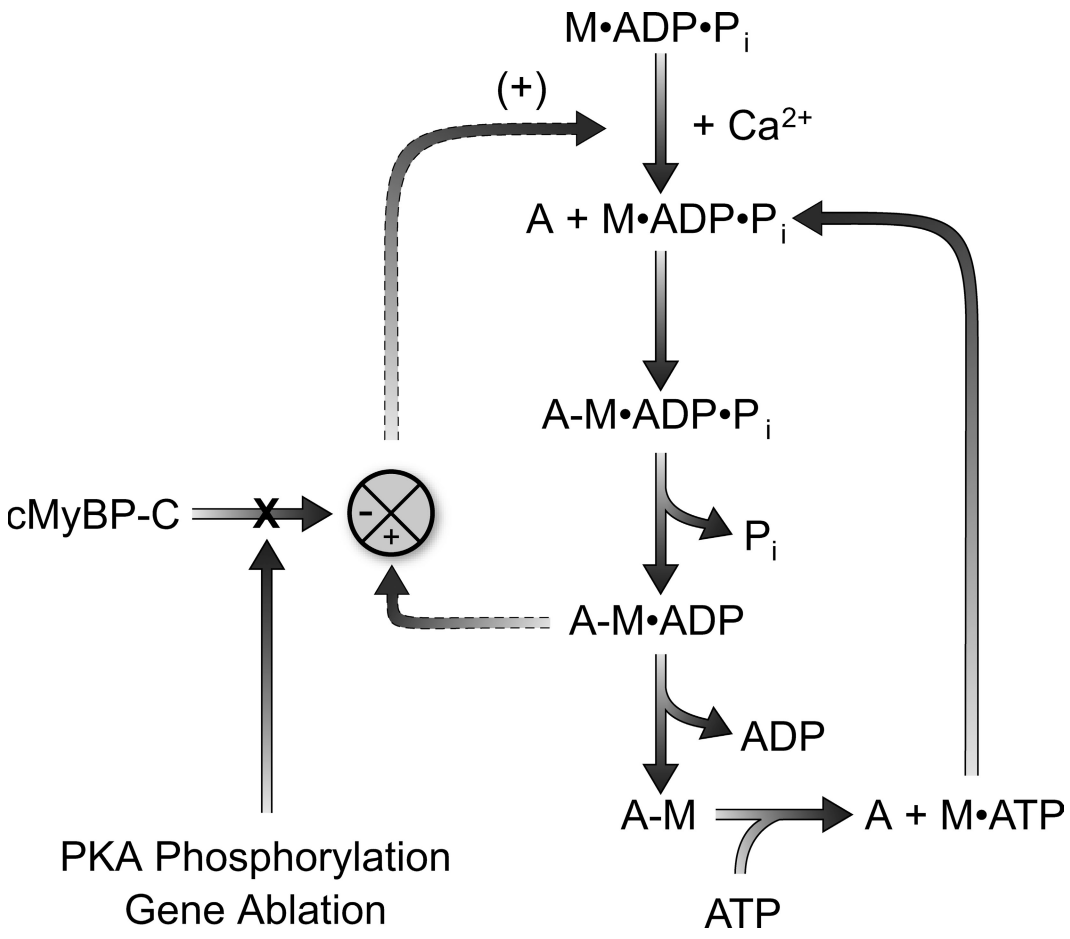

Figure 1. Diagram showing proposed effects of cMyBP-C on the cross-bridge interaction cycle in cardiac muscle. Based on Campbell's (1997) model, strongly bound cross-bridges (predominantly A-M · ADP) cooperatively recruit cross-bridges to bind to the thin filament (represented by A). We propose that cMyBP-C is normally repressive to this mechanism by constraining cross-bridges. However, this constraint is relieved by ablation or PKA phosphorylation of cMyBP-C, resulting in increased cooperative recruitment and rates of recruitment of cross-bridges. As shown in the diagram, Ca^{2+} is required for activation of contraction (+Ca^{2+}), but once initiated, the feedback mechanism shown here cooperatively increases the numbers and rate of cross-bridge binding to actin.

higher in skeletal muscle fibers, especially at tetanic stimulus frequencies that result in fusion of successive twitches, Ca^{2+} activation of the thin filament is high, and the likelihood of cooperative recruitment of cross-bridges into regions of the thin filament without Ca^{2+} bound would be low. Thus, the rate of force development would be near-maximal or maximal in contractions of skeletal muscle. In contrast, the smaller Ca^{2+} transient that is typical of myocardium under resting conditions, e.g., low adrenergic tone, implies that regions of the thin filament without Ca^{2+} bound provide a substrate for cooperative recruitment of cross-bridges, which would slow the rate of rise of force, as predicted by Campbell's (1997) model of regulation. As Ca^{2+} delivery increases during adrenergic stimulation of the heart, greater lengths of the thin filament will have Ca^{2+} bound, thereby reducing the opportunity for cooperative recruitment of cross-bridges and speeding force development. Of course, the kinetics of the Ca^{2+} transient also vary with sympathetic tone, becoming faster during adrenergic stimulation, which contributes to accelerated twitch kinetics. The idea that altered kinetics of cross-bridge cycling or recruitment contribute to accelerated twitch kinetics is evident in a shorter time interval between the peak of the Ca^{2+} transient and peak of twitch during β-adrenergic stimulation of myocardium (Okazaki et al., 1990).

Thick filament cooperativity in the activation of force development

The possibility that there is positive cooperativity in interactions among thick filament proteins, particularly the heads of myosin, has been raised informally by many who are interested in regulatory processes in striated muscles. A straightforward but unsubstantiated possibility is that the two heads of myosin interact in a cooperative manner, such that the binding of one to actin could facilitate or inhibit the binding of the other head to actin. If it is the former, positive cooperativity in binding would be a means for increasing the force developed by a muscle but at the cost of slowing the rate of rise of force as a consequence of the time taken for the second head to seek out an appropriately oriented binding site on actin. Conversely, negative cooperativity would have the effect of limiting force but would increase the rate of force development, which in the extreme would approach the rate of cross-bridge cycling.

On a broader scale, the binding of one or both heads of a myosin molecule might promote the binding of adjacent heads due to localized structural distortion of the thick filament as a consequence of initial binding. Because the heads of a myosin molecule lay on or near subfragment 2 of an adjacent myosin, a conceptually simple (and simplistic) model is one in which the binding of the adjacent myosin head(s) causes distortion of the first myosin head(s), thereby increasing the probability of myosin binding to actin. Such a mechanism has yet to be substantiated by experimental results.

Regulation of the availability of cross-bridges to actin

A growing body of evidence suggests that myosin heads are dynamically recruited to the thin filaments via mechanisms that displace myosin from the thick filament backbone and toward actin. The earliest evidence of such a process was the inference from x-ray diffraction patterns that there is a transfer of molecular mass from thick to thin filaments during the activation of muscle contraction (Haselgrove and Huxley, 1973). Although such a phenomenon could, in part, be a simple manifestation of myosin head binding to actin, the transfer of mass is observed even at long sarcomere lengths at which there is little overlap of thick and thin filaments and therefore little binding of myosin heads to actin. This apparent activation of head displacement is a puzzling but potentially important aspect of muscle contraction because movement of myosin heads closer to actin would presumably increase the probability of binding to actin and increase the rate and amplitude of force development. Recent work (Brunello et al., 2009) focusing on myosin layer lines in x-ray patterns from frog skeletal muscles suggests that the transition of myosin heads to an activated orientation proceeds with a time course that precedes active force development, an orientation that is maintained during active force development. Then, when muscle relaxes due to cessation of electrical stimulation and the sequestration of Ca^{2+}, the activation-related changes in the myosin reflections persist during the isometric phase of relaxation but return toward the resting pattern during the subsequent chaotic phase of relaxation. It is conceivable that these dynamic changes in cross-bridge head disposition are related to activity-dependent phosphorylation of myosin regulatory light chains, which could be determined from measurements of the time course of phosphorylation during contraction and relaxation. However, at this point it seems unlikely that light chain phosphorylation is the basis for the observed changes in the x-ray pattern during the onset of force development, as this occurs early in contraction before significant changes in light chain phosphorylation would be expected to occur. Thus, the basis for these activation-related changes in cross-bridge disposition is not known.

Recent results in heart muscle pose the interesting possibility that mechanisms have evolved in at least some striated muscles to regulate the availability of myosin to actin as a means to vary contractility. In mammalian cardiac muscles, a thick filament accessory protein, myosin-binding protein C (cMyBP-C), appears to constrain myosin heads to take up positions close to the thick filament backbone and away from myosin-binding sites on actin, which we predict would have the effect of reducing the rate and amplitude of force development. Consistent with this idea, findings by Stelzer et al. (2006) have shown that

ablation or phosphorylation of cMyBP-C increases the rate of force development in permeabilized myocardium. The idea that this acceleration is due to relief of a cMyBP-C–mediated physical constraint on the myosin head is supported by x-ray studies (Colson et al., 2007, 2008) of myocardium showing that ablation or phosphorylation of cMyBP-C results in the transfer of molecular mass from the lattice plane comprised principally of myosin to the plane that also includes actin. Movement of cross-bridges toward actin would increase the probability of weak or strong binding to the thin filament and thereby increase the rate of force development. It is important to consider that the structural changes resulting from relief of a cMyBP-C constraint, which we infer from x-ray results, need not be a gross radial movement of the cross-bridge head, but instead could be a relatively subtle azimuthal movement that better aligns the head with potential binding sites on actin. Nonetheless, electron microscopy studies by Weisberg and Winegrad (1996) strongly suggest that phosphorylation of cMyBP-C causes myosin heads to extend radially from the backbone of thick filaments in solution.

The observed structural effects of PKA within the thick and thin filament lattice provide a mechanism that can account for the acceleration of contraction kinetics as a consequence of β-adrenergic stimulation of myocardium. Of course, some of the inotropy due to β stimulation is due to enhanced Ca^{2+} delivery during excitation–contraction coupling, but accelerated rates of rise of twitch force as a consequence of accelerated cross-bridge cycling are also a feature of adrenergic agonist infusion. The troponin I (TnI) subunit of cardiac troponin is also phosphorylated during β-adrenergic stimulation (Solaro et al., 2008), giving rise to questions about the potential role of this protein in β agonist–induced inotropy. PKA phosphorylation of TnI has been shown to reduce the Ca^{2+} sensitivity of isometric force in myocardium, a phenomenon that is thought to contribute to earlier and faster relaxation of twitch force upon infusion of a β agonist. Although this effect of PKA phosphorylation of cTnI is well established, it is controversial whether cTnI phosphorylation contributes to the β agonist–induced acceleration of cross-bridge cycling. For example, Kentish et al. (2001) and Hünlich et al. (2005) reported that cross-bridge cycling is accelerated after PKA treatment, presumably due to phosphorylation of cTnI, whereas work from de Tombe and colleagues (for review see de Tombe, 2003) suggested that PKA-mediated phosphorylation of cTnI does not modulate the rate of cross-bridge cycling. More recent work is consistent with the de Tombe conclusion, in that PKA treatment of skinned myocardium expressing nonphosphorylatable cTnI accelerated force development similar to PKA treatment of native tissue (Stelzer et al., 2007), whereas myocardium expressing a nonphosphorylatable cMyBP-C exhibited no acceleration of force development in response to PKA, even though cTnI in these preparations was robustly phosphorylated (Tong et al., 2008). Such results are consistent with the idea that phosphorylation of cMyBP-C mediates the acceleration of myofibrillar contraction kinetics upon treatment with PKA.

A model in which cMyBP-C functions to constrain myosin heads is consistent with evidence from physiological and structural experiments and is also derivative of a long-standing belief that MyBP-C forms rings around thick filaments at intervals corresponding to the axial repeat of myosin. Such a model has yet to be substantiated definitively. In this regard, three-dimensional reconstructions of the cardiac thick filament suggest that the fixed domains of cMyBP-C are oriented along the long axis of the thick filament (Zoghbi et al., 2008), with no evidence for stable circumferential orientation of the remaining domains of the molecule. There is also growing evidence suggesting the possibility that the N terminus of cMyBP-C binds to actin, for example, as described by Harris and Trewhella (Whitten et al., 2008). Published results by Harris et al. (2004) are consistent with the binding of N-terminal peptides to actin, an interaction that is prevented by PKA phosphorylation of the cMyBP-C motif between domains C1 and C2 of cMyBP-C. Although such interactions have yet to be demonstrated in vivo, their existence could influence the state of activation of the thin filament, e.g., by spatial displacement of the regulatory strand. Also, the binding of cMyBP-C to actin might provide a mechanistic basis for the sudden slowing of velocity after unloaded shortening equivalent to 70–80 nm/half-sarcomere (Moss, 1986). Biochemical extraction of MyBP-C from heart or skeletal muscle fibers reversibly eliminates this slowing, implying that MyBP-C imposes an internal load with continued shortening. The binding of cMyBP-C to actin in cardiac muscle would provide a physical basis for such an internal load, which could be envisioned to arise once shortening takes up slack in cMyBP-C and the protein is strained by further shortening.

Whatever the mechanism of modulation of contraction by cMyBP-C, it seems likely that phosphorylation of cMyBP-C accelerates contraction by allowing or facilitating greater probability of cross-bridge binding to actin, which would also accelerate the rate of propagation of cooperative recruitment of cross-bridge heads into adjacent regions of the thin filament.

Another puzzling feature of the modulatory effects of cMyBP-C on contractile properties is that the protein is localized to every third myosin crown along the thick filament, corresponding to the axial repeat of myosin, and is not found at all in the distal one third or so of each half of the thick filament. Thus, effects due to ablation or phosphorylation of cMyBP-C either involve only a small subset of the population of cross-bridge heads, or the effects on these heads are somehow communicated to adjacent cross-bridges. With regard to the latter possibility, it is

conceivable although not yet shown that local distortions of the thick filament when myosin–cMyBP-C interactions are disrupted are communicated along the thick filament as a result of myosin–myosin interactions. Alternatively, the effects might be communicated along the thin filament due to near-neighbor cooperative recruitment of cross-bridges as a result of activating effects due to the initial binding of the small population of cross-bridges that is released by ablation or phosphorylation of cMyBP-C (Fig. 1).

Finally, MyBP-C is also expressed in skeletal muscle in stoichiometric ratios to myosin that are similar to that observed in cardiac muscle. However, the role of MyBP-C in skeletal muscle is not as well understood as in cardiac muscle both because a skeletal isoform knockout mouse has yet to be developed and because the skeletal isoform seems not to be reversibly phosphorylated as a means of modulating muscle function. The latter observation suggests that any modulatory role of MyBP-C in skeletal muscle is static rather than phasic in nature. In this regard, a potentially important clue is that biochemical extraction of MyBP-C from skeletal muscle reversibly increases the velocity of unloaded shortening (Hofmann et al., 1991), suggesting that MyBP-C is repressive to cross-bridge function in skeletal muscle just as it is in cardiac muscle. Paradoxically, such repression in fast-twitch skeletal muscle together with the lesser sensitivity of the skeletal thin filament to the activating effects of strong-binding cross-bridges could contribute to the impulsive, all-or-none nature of the twitch in these muscles, as robust activation would occur only at higher (compared to cardiac muscle) levels of intracellular Ca^{2+}.

Adaptive advantage of multiple regulatory/modulatory processes

The mechanisms of activation and modulation of cross-bridge binding discussed in this Perspective and featured at the Society of General Physiologists meeting at Woods Hole in September 2009 have added significant levels of complexity to previous views that the regulation of muscle contraction could be explained entirely by Ca^{2+} binding to the TnC subunit of troponin. From a design perspective, these levels of complexity enhance the precision of regulation by better matching the functional dynamic range to specific purposes and by introducing the possibility for finer control of muscle force and work rate, which would increase efficiency. Consistent with these statements, cardiac muscle appears to use a greater number of control mechanisms, particularly phosphorylations of cMyBP-C and cTnI, to match contractility to workload on a beat-to-beat basis. In contrast, fast-twitch skeletal muscle has fewer distinct control mechanisms beyond the Ca^{2+} switch but exhibits much greater cooperativity in cross-bridge binding to the thin filament, resulting in much more explosive all-or-none activations of contraction.

Another concept that has emerged in the field of regulation and is emphasized in this Perspective is that steady activation of the thin filament involves the binding of both Ca^{2+} and cross-bridges to the thin filament. Although Ca^{2+} binding initiates activation, the extent and rate of activation depend on the fraction of TnC sites occupied by Ca^{2+} and on cooperativity in Ca^{2+} and cross-bridge binding. Here, again, the more nearly impulsive contractions of fast-twitch skeletal muscles can be viewed as products of a highly cooperative system in terms of cross-bridge binding and the near saturation of TnC by Ca^{2+} as a result of tetanic simulation. Also, any cooperative processes that propagate activation along the skeletal muscle thin filament would presumably occur at a much faster rate than in cardiac muscle due to the faster cycling rates of cross-bridges in fast-twitch muscles. In cardiac muscle, the greater dynamic range in terms of developed force and speed of contraction is a product of finer control of the release of Ca^{2+} from the sarcoplasmic reticulum and the exquisite sensitivity of the cardiac thin filament to the activating effects of even small numbers of bound cross-bridges. Under resting conditions in which sympathetic tone is low, reduced release of Ca^{2+} and low levels of myofibrillar protein phosphorylations in myocardium serve to lower twitch amplitude and slow the rate of force development, the latter being a consequence of the time taken to evoke Ca^{2+} release and to cooperatively recruit cross-bridges to force-generating states. When sympathetic tone is increased, cardiac muscle exhibits contractile characteristics that are more similar to but nonetheless still slower than in skeletal muscles due to slower turnover kinetics of cardiac myosin isoforms and the still-present, even at high Ca^{2+} concentrations, cooperative recruitment of cross-bridges to force-generating states.

Work continues as investigators attempt to understand the mechanisms of primary (Ca^{2+}-mediated) and secondary regulatory processes (cooperation and posttranslational modifications) in myofilaments of vertebrate striated muscles. Important questions drive the field, including: What are the mechanisms of cooperativity in muscle? What are the relative contributions of secondary regulatory processes to muscle function? What is the nature of regulation via thick filament proteins, and to what degree does thick filament regulation contribute to the activation of contraction? How and why does MyBP-C modulate contraction, and how and why does the function of this protein differ in heart and skeletal muscles? The pursuit of these and other questions will shape the direction of inquiry in the field for many years to come, with promise that answers will improve understanding of muscle contraction and its regulation in health and disease, and also inform the development of interventions designed to treat or prevent muscle dysfunction.

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