JGP Journal of General Physiology

COMMENTARY

Contractile Function

Resolving zone-specific regulation of cardiac myosin

Shane R. Nelson[1]

Cardiac contractility is driven by shortening of ∼2-µm–long, macromolecular assemblies known as sarcomeres. During contraction, the motor protein myosin binds to, and exerts force upon actin filaments, utilizing energy from the hydrolysis of ATP. When not actively contracting, myosin partition into two subpopulations, distinguished by their basal rates of ATP hydrolysis, known as the "Disordered Relaxed" (DRX) and "Super Relaxed" (SRX) states. Additionally, the slower hydrolyzing SRX state has been proposed as a sequestered or "reserve pool" of myosin that do not contribute to contraction but can be recruited for enhanced contractility in response to external stimuli. Thus, the fraction of myosin in the SRX state is thought to reflect the overall regulatory state of the myosin population. In this volume of the *Journal of General Physiology*, a study by Pilagov et al. explores how the SRX state is regulated by phosphorylation or haploinsufficiency of a key regulatory protein, Myosin Binding Protein-C (MyBP-C). Surprisingly, they found that perturbations of MyBP-C led to a negligible change in the overall abundance of SRX. Instead, they found a rearrangement of SRX myosin throughout the sarcomere – specifically a decrease in SRX in regions of the sarcomere that contain MyBP-C and a compensatory increase in SRX in regions lacking MyBP-C. Their findings suggest that the influence of MyBP-C extends beyond its immediate vicinity and can simultaneously exert both positive and negative effects in a location-specific manner.

Introduction

Contraction of both cardiac and skeletal muscle is driven by shortening of ∼2-µm–long, macromolecular assemblies known as sarcomeres. These sarcomeres are arranged into long, tandem arrays known as myofibrils, which are the contractile organelles within muscle cells. Sarcomeres are comprised of interdigitated myosin-containing thick filaments and actin-containing thin filaments (Fig. 1 A), which slide past each other during contraction, resulting in shortening of the sarcomere and consequently, the whole muscle cell. Myosin is the motor protein that converts chemical energy arising from the hydrolysis of ATP into the mechanical work of sarcomere force generation and shortening. However, analogous to an idling car, myosin continues to hydrolyze ATP at a reduced rate when muscle is not actively contracting. This slow, basal rate of ATP hydrolysis is commonly known as the "relaxed" rate of ATP hydrolysis. However, a conundrum was long known in the field as this biochemical rate was far too fast to reconcile with the measured metabolic rate of resting muscle (Kushmerick and Paul, 1976). In 2010, Stewart et al. demonstrated that there are two distinct subpopulations of myosin in relaxed muscle—one exhibiting the conventional relaxed ATPase rate and another population exhibiting a ∼10-fold slower rate, which they termed the "super-relaxed" or SRX population. The SRX state was suggested as a mechanism for energy conservation in resting muscle, perhaps functioning as a "reserve pool" that could be called upon for increased mechanical output. Additionally, based on similarities with smooth and molluscan myosins, the SRX state was also suggested to be correlated with a folded configuration of myosin known as the "interacting heads motif" or IHM (Fig. 1 B; Lee et al., 2018), and the helical ordering of the myosin heads along the thick filament backbone (Dutta et al., 2023; Tamborrini et al., 2023). On this basis, the conventional relaxed rate was rebranded as the disordered relaxed or DRX rate, as it presumably arises from myosin heads that are not in an ordered arrangement and that are competent to interact with the thin filament. The sequestration of myosin into the IHM/SRX/helically ordered state underpins the emerging theory of a thick filament–based mechanism that regulates the number of myosin available to bind to the thin filament and generate force and motion upon muscle activation, thus potentiating the magnitude of each individual contraction. Breakdown of this regulatory mechanism has been proposed as the underlying cause of sarcomeric myopathies, such as hypertrophic cardiac myopathy (Sarkar et al., 2020; Toepfer et al., 2020). In an earlier issue of the *Journal of General Physiology*, a recent study by Pilagov et al.

[1]Department of Molecular Physiology and Biophysics, Larner College of Medicine, University of Vermont, Burlington, VT, USA.

Correspondence to Shane R. Nelson: srnelson@med.uvm.edu

This work is part of a special issue on Myofilament Structure and Function.

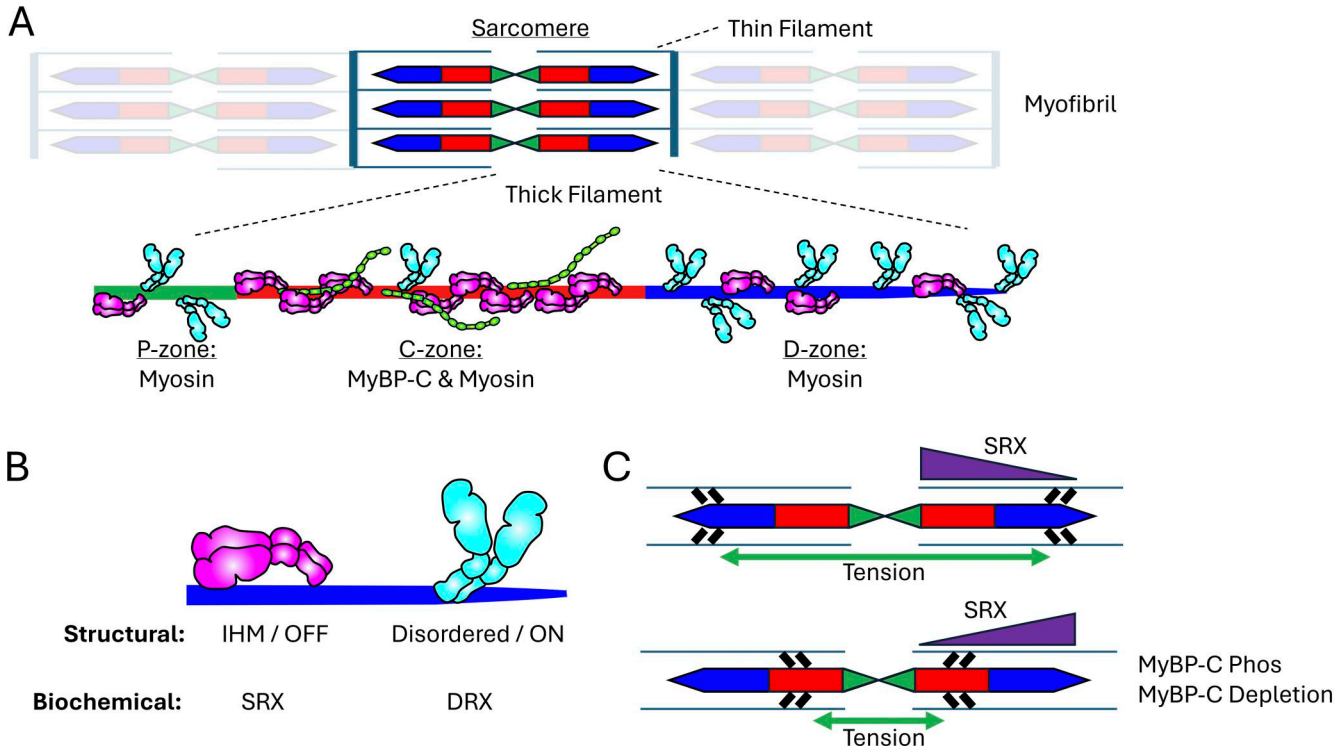

Figure 1. **Myofibrils, sarcomeres, and myosin. (A)** The organization of the thick and thin filaments within a sarcomere. Callout shows one half of a thick filament with the P, C, and D zones. **(B)** Myosin in the SRX and DRX states and the associated structural states. **(C)** The arrangement of SRX myosin may determine the availability of individual myosins for binding to the thin filament. This determines the extent of tension along the thick filament, which in turn may regulate availability of other SRX myosin.

(2025) explores the regulation of myosin's SRX state in human and porcine cardiac muscle. Their data suggest the presence of a previously undescribed mechanism of anti-cooperative regulation within this muscle, such that changes favoring activation in one region are offset by a commensurate repression in a neighboring region.

Factors that regulate SRX include thick filament strain, temperature, ionic strength, regulatory light chain phosphorylation, and myosin-binding protein C (MyBP-C). MyBP-C has garnered particular attention, as mutations in this protein are linked to a large proportion of cases of familial hypertrophic cardiomyopathy (Tardiff, 2005). Present in both skeletal and cardiac muscle, MyBP-C has a distinctive distribution within sarcomere, present in 9–11 "stripes", spaced 43-nm apart within a select region of the thick filament of each half of the myosin thick filament (Fig. 1 A). This region, known as the C zone, contains both myosin and MyBP-C in a 3:1 M ratio and is flanked by a smaller P zone on one side and a larger D zone on the other, both of which contain myosin, but not MyBP-C.

The SRX state was first described using a nucleotide chase assay to examine the rate of ATP turnover, specifically the apparent rate of ADP release (Stewart et al., 2010). This assay has since been adapted to myofibrils (Walklate et al., 2022), thick filaments (Gollapudi et al., 2021), and even recombinant myosin (Anderson et al., 2018). An alternative single-molecule approach to measure the SRX state has been pioneered by the Kad group, as well as our own (Pilagov et al., 2023; Nelson et al., 2020). This approach examines the residence time of single molecules of fluorescent ATP as they are taken up and hydrolyzed in isolated myofibrils. These residence times provide a measure of the rates of product release from myosin heads, but with the added advantage of high spatial precision for each individual nucleotide turnover event, allowing the proportions of SRX and DRX to be mapped within a sarcomere.

Previous studies found that the more central region of the thick filament contains a higher proportion of myosin sequestered in the SRX state than the more distal regions, suggesting that the ends of myosin thick filaments are biochemically more active and potentially more available for force-generating interactions with the thin filament upon muscle activation. Additionally, these prior studies have indicated a critical role of MyBP-C in regulation of the SRX state in muscle. In the current study, Pilagov and colleagues have extended their prior studies to examine two key perturbations of MyBP-C in human and porcine cardiac muscle.

Experimental findings

The first perturbation was treatment with PKA, a protein kinase that is activated by β-adrenergic stimulation, leading to enhanced muscle contractility (Stelzer et al., 2007). MyBP-C is a key target of PKA, with 3–4 specific phosphorylation sites near the N-terminus, as well as others in the central domains of the protein (Jia et al., 2010). Upon phosphorylation of MyBP-C by PKA, the authors unexpectedly saw no net change in the overall

abundance of SRX myosin. However, they did find a redistribution of where SRX myosin was located within the sarcomere. Within the C zone, they saw a decrease in SRX abundance, consistent with repression of MyBP-C activity by phosphorylation. Unexpectedly, this was offset by a commensurate increase in SRX abundance within the D zone, resulting in no net change in overall SRX abundance.

As MyBP-C phosphorylation represses MyBP-C activity, Pilagov et al. then extended the study to samples of human cardiac muscle that were heterozygous for a MyBP-C–truncating mutation, which leads to a ∼30% reduction in total MyBP-C expression (Pioner et al., 2023). Here, they observed much the same result—no change in the overall SRX abundance, but a shift in where SRX myosin are located, from predominantly in the C zone in normal myocardium to predominantly in the D zone of the mutant samples.

These results are surprising because a change in SRX within the C zone upon MyBP-C perturbation is perhaps expected, as this is the region where MyBP-C is found. However, as both of our groups (Nelson et al., 2023; Pilagov et al., 2023) and the current study (Pilagov et al., 2025) have shown, effects of MyBP-C perturbations extend throughout the sarcomere, even to regions that do not contain MyBP-C, such as the D zone. These findings suggest communication along the thick filament, where changes in the regulatory state of one region are conveyed throughout the thick filament. Interestingly, those prior studies found that deletion, truncation, or phosphorylation of MyBP-C led to a reduction in SRX content in both the C and D zones, resulting in the entire thick filament shifting to the more active DRX state. In contrast, this new study shows changes in opposite directions between the C and D zones, suggesting that communication along the thick filament may be more nuanced than previously thought.

The authors suggest that a key difference in this study may lie in the isoform of myosin that is expressed. There are two genetically and biochemically distinct myosin isoforms present in the hearts of adult mammals. The α-myosin heavy chain is predominant in most rodent species, including mice—the most used model system for these studies. However, humans and pigs predominantly express β-myosin heavy chain in their hearts. The findings here may suggest that cardiac β-myosin behave very differently in this regard than skeletal or cardiac α-myosin.

Knowledge gaps in the field

In the model of thick filament regulation described by Linari et al. (2015), myosin near the tip of the thick filament (i.e., the D zone) are the most available and first to bind the thin filament upon muscle activation. Forces generated by these first myosins create tension along the thick filament (Fig. 1 C), proportionally freeing more centrally located heads from their sequestered state and making them available to bind the thin filament and contribute more force. Thus, the number of myosin available to contribute to a contraction can be modulated in response to mechanical demands (Wang et al., 2024). In agreement with this model, we (Nelson et al., 2020; Nelson et al., 2023) and Pilagov et al. (2023) show that myosin nearest the thick filament tip, i.e., those in the D zone, show the lowest fraction of SRX of the sarcomere, suggesting that they are the most available for binding to the thin filament to generate force and motion. However, the new findings of Pilagov et al. present a conundrum, as PKA treatment leads to enhanced contractility (Stelzer et al., 2007), which would suggest an overall reduction in SRX content. However, this is not what they found. Perhaps the key lies in the arrangement of the SRX myosin, as they found that upon phosphorylation or a reduction in the total amount of MyBP-C, those "sentinel" myosin near the thick filament tip become more repressed (i.e., increased SRX content), while more centrally located myosin become more activated (i.e., reduced SRX content). This may serve to preserve sarcomere energetics while blunting the force sensitivity of the thick filament as D zone heads would not be expected to sense tension on the thick filament if they are located distally to the force-generating crossbridges (Fig. 1 C).

The relationship between the helically ordered/structural IHM state and the biochemical SRX state remains unclear. Circumstantial evidence suggests that they are highly correlated if not equivalent—the sarcomeric C zone has a high degree of helical order with myosin heads in IHM (Dutta et al., 2023; Tamborrini et al., 2023). This region also demonstrates the highest proportion of the slower, SRX rate of product release (Nelson et al., 2020; Nelson et al., 2023; Pilagov et al., 2023). Many authors use the terms IHM and SRX interchangeably. However, there are many reports of SRX content in preparations of single-headed myosin S1 (Anderson et al., 2018; Gollapudi et al., 2021), which cannot adopt the IHM, as it has a single myosin head with no coiled-coil domain. More recent studies have called these findings into question (Cail et al., 2025; Mohran et al., 2024). In a study combining both biochemical and structural measures, Chu et al. (2021) concluded using soluble myosin that IHM-like folded structures lead to but are not required for SRX-like biochemical rates.

Finally, are SRX and DRX myosin in an equilibrium? Various perturbations have been shown to rapidly change the number of myosin in the SRX state, suggesting that individual myosins must be able to exist in either state. However, absent such a perturbation, do myosin exchanges between SRX and DRX states? Such an exchange must be slower than the apparent SRX rate for that rate to be observed at all (Walklate et al., 2022; Cail et al., 2025). As distinct subpopulations have not been described for any other step in the myosin ATPase cycle, perhaps myosin must exit the SRX state to bind a subsequent ATP, effectively "exiting" the SRX state with each complete hydrolytic cycle. Alternately, perhaps myosin remains in an SRX state for multiple ATPase cycles? That is to say that when Pilagov et al. report that 46% of myosin are in the SRX state, does that mean that 46% of myosin are locked into this state under the given conditions, or are myosin freely exchanging between SRX and DRX states, such that the 46% estimate is more of a steady-state average?

In conclusion, this recent study from the Kad group reinforces the role of MyBP-C in regulation of the myosin thick filament. They found that perturbations to MyBP-C led to changes in SRX abundance throughout the sarcomere, extending even to regions that are devoid of MyBP-C. Additionally, they describe a redistribution of SRX, with sarcomeric C and D zones

changing in opposite directions, instead of a global change. This suggests more nuanced role for thick filament regulation by MyBP-C than previously appreciated.

Acknowledgments

Henk L. Granzier served as editor.

The author would like to thank Dave Warshaw and Brandon Bensel (University of Vermont) for critical reading of this commentary and Guy Kennedy (Instrumentation and Model Facility, University of Vermont) for myosin illustrations.

This work was supported by funds from the National Institutes of Health (HL150953 and HL163585).

Author contributions: Shane R. Nelson: visualization and writing—original draft, review, and editing.

Disclosures: The author declares no competing interests exist.

References

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

Cail, R.C., F.A. Báez-Cruz, D.A. Winkelmann, Y.E. Goldman, and E.M. Ostap. 2025. Dynamics of β-cardiac myosin between the super-relaxed and disordered-relaxed states. *J. Biol. Chem.* 301:108412. https://doi.org/10.1016/j.jbc.2025.108412

Chu, S., J.M. Muretta, and D.D. Thomas. 2021. Direct detection of the myosin super-relaxed state and interacting-heads motif in solution. *J. Biol. Chem.* 297:101157. https://doi.org/10.1016/j.jbc.2021.101157

Dutta, D., V. Nguyen, K.S. Campbell, R. Padrón, and R. Craig. 2023. Cryo-EM structure of the human cardiac myosin filament. *Nature*. 623:853–862. https://doi.org/10.1038/s41586-023-06691-4

Gollapudi, S.K., M. Yu, Q.-F. Gan, and S. Nag. 2021. Synthetic thick filaments: A new avenue for better understanding the myosin super-relaxed state in healthy, diseased, and mavacamten-treated cardiac systems. *J. Biol. Chem.* 296:100114. https://doi.org/10.1074/jbc.RA120.016506

Jia, W., J.F. Shaffer, S.P. Harris, and J.A. Leary. 2010. Identification of novel protein kinase A phosphorylation sites in the M-domain of human and murine cardiac myosin binding protein-C using mass spectrometry analysis. *J. Proteome Res.* 9:1843–1853. https://doi.org/10.1021/pr901006h

Linari, M., E. Brunello, M. Reconditi, L. Fusi, M. Caremani, T. Narayanan, G. Piazzesi, V. Lombardi, and M. Irving. 2015. Force generation by skeletal muscle is controlled by mechanosensing in myosin filaments. *Nature*. 528:276–279. https://doi.org/10.1038/nature15727

Kushmerick, M.J., and R.J. Paul. 1976. Relationship between initial chemical reactions and oxidative recovery metabolism for single isometric contractions of frog sartorius at 0 degrees C. *J. Physiol.* 254:711–727. https://doi.org/10.1113/jphysiol.1976.sp011254

Lee, K.H., G. Sulbarán, S. Yang, J.Y. Mun, L. Alamo, A. Pinto, O. Sato, M. Ikebe, X. Liu, E.D. Korn, et al. 2018. Interacting-heads motif has been conserved as a mechanism of myosin II inhibition since before the origin of animals. *Proc. Natl. Acad. Sci. USA*. 115:E1991–E2000. https://doi.org/10.1073/pnas.1715247115

Mohran, S., K. Kooiker, M. Mahoney-Schaefer, C. Mandrycky, K. Kao, A.-Y. Tu, J. Freeman, F. Moussavi-Harami, M. Geeves, and M. Regnier. 2024. The biochemically defined super relaxed state of myosin-A paradox. *J. Biol. Chem.* 300:105565. https://doi.org/10.1016/j.jbc.2023.105565

Nelson, S.R., A. Li, S. Beck-Previs, G.G. Kennedy, and D.M. Warshaw. 2020. Imaging ATP consumption in resting skeletal muscle: One molecule at a time. *Biophys. J.* 119:1050–1055. https://doi.org/10.1016/j.bpj.2020.07.036

Nelson, S., S. Beck-Previs, S. Sadayappan, C. Tong, and D.M. Warshaw. 2023. Myosin-binding protein C stabilizes, but is not the sole determinant of SRX myosin in cardiac muscle. *J. Gen. Physiol.* 155:e202213276. https://doi.org/10.1085/jgp.202213276

Pilagov, M., L.W.H.J. Heling, J. Walklate, M.A. Geeves, and N.M. Kad. 2023. Single-molecule imaging reveals how mavacamten and PKA modulate ATP turnover in skeletal muscle myofibrils. *J. Gen. Physiol.* 155:e202213087. https://doi.org/10.1085/jgp.202213087

Pilagov, M., S. Steczina, A. Naim, M. Regnier, M.A. Geeves, and N.M. Kad. 2025. Spatially resolving how cMyBP-C phosphorylation and haploinsufficiency in porcine and human myofibrils affect β-cardiac myosin activity. *J. Gen. Physiol.* 157:e202413628. https://doi.org/10.1085/jgp.202413628

Pioner, J.M., G. Vitale, S. Steczina, M. Langione, F. Margara, L. Santini, F. Giardini, et al. 2023. Slower calcium handling balances faster cross-bridge cycling in human MYBPC3 HCM. *Circ. Res.* 132:628–644. https://doi.org/10.1161/CIRCRESAHA.122.321956

Sarkar, S.S., D.V. Trivedi, M.M. Morck, A.S. Adhikari, S.N. Pasha, K.M. Ruppel, and J.A. Spudich. 2020. The hypertrophic cardiomyopathy mutations R403Q and R663H increase the number of myosin heads available to interact with actin. *Sci. Adv.* 6:eaax0069. https://doi.org/10.1126/sciadv.aax0069

Stewart, M.A., K. Franks-Skiba, S. Chen, and R. Cooke. 2010. Myosin ATP turnover rate is a mechanism involved in thermogenesis in resting skeletal muscle fibers. *Proc. Natl. Acad. Sci. USA*. 107:430–435. https://doi.org/10.1073/pnas.0909468107

Stelzer, J.E., J.R. Patel, J.W. Walker, and R.L. Moss. 2007. Differential roles of cardiac myosin-binding protein C and cardiac troponin I in the myofibrillar force responses to protein kinase A phosphorylation. *Circ. Res.* 101:503–511. https://doi.org/10.1161/CIRCRESAHA.107.153650

Tamborrini, D., Z. Wang, T. Wagner, S. Tacke, M. Stabrin, M. Grange, A.L. Kho, M. Rees, P. Bennett, M. Gautel, and S. Raunser. 2023. Structure of the native myosin filament in the relaxed cardiac sarcomere. *Nature*. 623:863–871. https://doi.org/10.1038/s41586-023-06690-5

Tardiff, J.C. 2005. Sarcomeric proteins and familial hypertrophic cardiomyopathy: Linking mutations in structural proteins to complex cardiovascular phenotypes. *Heart Fail. Rev.* 10:237–248. https://doi.org/10.1007/s10741-005-5253-5

Toepfer, C.N., A.C. Garfinkel, G. Venturini, H. Wakimoto, G. Repetti, L. Alamo, A. Sharma, R. Agarwal, J.K. 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

Walklate, J., K. Kao, M. Regnier, and M.A. Geeves. 2022. Exploring the super-relaxed state of myosin in myofibrils from fast-twitch, slow-twitch, and cardiac muscle. *J. Biol. Chem.* 298:101640. https://doi.org/10.1016/j.jbc.2022.101640

Wang, Y., L. Fusi, J.G. Ovejero, C. Hill, S. Juma, F.P. Cullup, A. Ghisleni, S.-J. Park-Holohan, W. Ma, T. Irving, et al. 2024. Load-dependence of the activation of myosin filaments in heart muscle. *J. Physiol.* 602:6889–6907. https://doi.org/10.1113/JP287434