Further progress in understanding of myofibrillar function in health and disease

Christine Cremo1, Richard L. Moss2, and Henk Granzier3

Introduction
This is the second of two special thematic issues focused on regulation of contractile systems in muscle and nonmuscle cells. The first issue, published earlier this year, is JGP volume 153, issue 3 (https://rupress.org/jgp/issue/153/3), and has additional background information with regards to the Myofilament Conference that motivated the thematic issues. As in the earlier issue, the papers here address distinct topics focused on the mechanisms and determinants of contractility studied over a range of spatial organization from molecules to cells, tissues, and organisms.

Papers
Two papers (Hanft et al., 2021; Mamidi et al., 2021) add to the several papers in the first special issue focused on cardiac myosin binding protein-C (cMyBP-C) as a determinant of myocardial function and, particularly, the importance of the phosphorylation state of this protein in regulating the interactions of myosin and actin. The study from the McDonald laboratory (Hanft et al., 2021) convincingly shows that phosphorylation of cMyBP-C modulates the steepness of the Frank-Starling relationship in mouse hearts. As briefly reviewed by the authors, the Frank-Starling relationship describes the increase in stroke volume when end-diastolic volume increases, or, in isolated myocardium, the increase in power output as muscle length is increased. In their experiments, the length dependence of power output was greatest in myocardium expressing cMyBP-C with phosphomimetic substitutions for three PKA-phosphorylatable serines in cMyBP-C and least in myocardium expressing nonphosphorylatable alanines in place of these serines. These results strongly suggest that the phosphorylation state of cMyBP-C is a key determinant of stroke volume in vivo, which the authors confirmed by determining Frank-Starling relationships in working hearts in vivo. Their observation that the steepness of the relationship from WT hearts and myocardium with ~50% phosphorylation of cMyBP-C was between the extremes exhibited by the two cMyBP-C mutants suggests that the relationship in the mouse heart at rest is at a point in a phosphorylation-dependent dynamic range that is modulated in vivo by β-adrenergic tone.

The paper from Stelzer’s group (Mamidi et al., 2021) provides a complementary perspective that reinforces the importance of the phosphorylation state of cMyBP-C as a determinant of myocardial function in response to a candidate heart failure therapeutic omecamtiv mecarbil. The authors show that the effects of the therapy on myocardial force development and on relaxation kinetics differed in myocardium expressing either WT or nonphosphorylatable cMyBP-C. As suggested by the authors, the results may have implications for the clinical effectiveness of the therapy, possibly being more effective at earlier stages of disease progression. This work is the subject of a Research News article in the current special issue (Short, 2021).

The paper by Fazlollahi et al. (2021) adds to the cMyBP-C theme of this special issue. The authors investigated the two main phases of the mammalian cardiac cycle, i.e., contraction and relaxation. How the two are coupled and respond to exercise and injury is not well understood in large mammals, including in humans. Exercise is known to improve cardiac function, and multiple changes occur in the heart in response to injury, disease, and stress. The authors investigated how exercise and myocardial injury affect contraction–relaxation coupling. They show that contraction and relaxation remain tightly coupled in intact canine myocardium after exercise training and/or myocardial infarction. The authors propose that contraction–relaxation coupling is a fundamental myocardial property that resides in the structural arrangement of proteins at the level of the sarcomere, and that a key role might be played by cMyBP-C.

Two papers provide new insights into the consequences of mutations in the lever arm region of cardiac myosin. The lever arm of each myosin head consists of one regulatory light chain
(RLC) and one essential light chain (ELC), each of which wrap around the helical heavy chain. It is not surprising that mutations associated with hypertrophic cardiomyopathy (HCM) exist in this region known to be important to coupling of the actomyosin ATPase activity to the working stroke.

The work of Rasici et al. (2021) is an example of the careful approach that must be taken to assess the impact of HCM mutations on myosin mechanochemistry in general. Here, the RLC charge-switch mutation K104E near the C terminus of the RLC was studied in the background of heavy chains of human β-cardiac myosin subfragment-1 (accessible through an expression system) and the full-length mouse α-cardiac myosin (purified from transgenic mice). Interestingly, mechanochemistry depends on either the length or the isoform of the myosin heavy chain backbone, and the mutation may disrupt RLC interactions with the myosin lever arm domain as well.

Sitbon et al. (2021) provide new insights into the importance and function of the 43-amino-acid-long N-terminal extension of the cardiac ELC previously shown to directly interact with the C-terminal region of actin. Additionally, the study builds upon prior work from this group and co-workers with focus on the A57G and E143K mutations in the ventricular ELC that were shown by population studies to cause human HCM or restrictive cardiomyopathy (RCM), respectively. In a comprehensive approach, new insights into the relationships between the super-relaxed (SRX) to disordered relaxed (DRX) state equilibrium, the degree of myosin RLC phosphorylation, and aspects of ELC mutation-dependent mitochondrial and metabolic remodeling, including changes in oxidative phosphorylation (OXPHOS) and ATP respiration in all three ELC models, have been revealed.

Two papers focus on titin and in particular on titin’s N2A element that is located in the molecular spring region of skeletal muscle titin, fetal cardiac titin, and adult cardiac N2BA titin (Granzier and Labeit, 2005). This N2A element contains four Ig-like domains and several unique sequences, of which the 104-residue unique sequence (N2A-Us) with flanking Ig domains Ig80 and Ig81 is a major part. The N2A element is considered a signaling hub that assembles a signalosome with muscle ankyrin repeat protein 1 (MARPI) as an important component. Stronczek et al. (2021) analyze the structure of the N2A components and their association with F-actin. They report that the N2A Ig domains contain unique loop structures, consistent with their selective recruitment of binding partners. The N2A element has previously been proposed to play a role in force production through its Ca2+-regulated association with actin. However, the authors were unable to identify specific Ca2+-binding sites, and F-actin co-sedimentation assays failed to reveal binding to N2A.

van der Pijl et al. (2021) focus on the N2A element and MARPI. MARPI is of particular interest as in skeletal muscle it is normally present at very low levels, but its level increases markedly under conditions of mechanical stress. MARPI is known to interact with several sarcomere proteins and primarily with the N2A element of titin, but its effect on skeletal muscle function is poorly understood. The authors find that MARPI binds to F-actin, and that this interaction is stronger when MARPI forms a complex with titin’s N2A element. Mechanical and structural studies showed that MARPI “locks” titin-N2A to the sarcomeric thin filament, causing increased extension of titin’s elastic PEVK element and, importantly, causing an increase in passive force. Thus, MARPI regulates passive force by locking titin to the thin filament. The details of this novel mechanism of regulating passive force and its clinical significance in critically ill patients is further discussed by van der Pijl et al. (2021).

Scellini et al. (2021) study mavacamten (MYK-461), a small-molecule allosteric inhibitor of sarcomeric myosins that is being used in clinical trials for hypertrophic cardiomyopathy treatment. An in-depth understanding of mavacamten’s impact on force generation has been limited by diffusional barriers that exist in intact or skinned striated muscle preparations. These limitations were overcome in this study by using myofibrils and rapid solution changes. Scellini et al. (2021) characterize the action of mavacamten in human ventricular myofibrils and compared the results with those of fast skeletal myofibrils from rabbit psoas muscle. The authors report that mavacamten has a fast and reversible mechanical action on cardiac muscle that is mediated by a shift of motor heads out of the force-generating cycle, with no effect on the kinetics of force development. The authors propose that mavacamten may alter the interplay between thick and thin filament regulatory mechanisms of contraction, and that this includes a stabilization of myosin motor heads in autoinhibited states.

Solis and Solaro (2021) review the sarcomere regulatory mechanisms and focus on cardiac-specific modifications to the three-state model of thin filament activation. The authors discuss modulation by Ca2+, cross-bridges, cMyBP-C, cardiac RLC (cRLC), and titin. Included are a discussion of long- and short-range interactions with the regulatory units of thin filaments, including proteins at the barbed end (in and near the Z-disc) and the pointed end (near the M-band). The authors also discuss mechanisms that sustain the physiological cardiac state with varying hemodynamic loads and focus on genetic and acquired cardiac disorders.

Coleman et al. (2021) study microtubules, dynamic polymers of α-β protein dimers, with their filament longevity and binding to other cytoskeletal filaments regulated by posttranslational modifications (PTMs) such as deacetylation or acetylation. Microtubules tune cytoskeletal stiffness, which affects cytoskeletal mechanics and mechanotransduction of striated muscle. While recent evidence suggests that microtubules enriched in deacetylated α-tubulin regulate these processes in healthy muscle and increase them in disease, the authors here focus on other α-tubulin modifications. Using various strategies, the authors show that microtubules enriched in acetylated α-tubulin increase cytoskeletal stiffness and viscoelastic resistance. These findings further support the concept that tubulin PTMs regulate cytoskeletal mechanics and tune the magnitude of striated muscle mechanotransduction.

Pütz et al. (2021) focus on smooth muscle and studied caldesmon (CaD), an actin-, myosin-, and calmodulin-binding protein. CaD is expressed in two splice isoforms: h-CaD, which is an integral part of the actomyosin domain of smooth muscle cells, and l-CaD, which is widely expressed and is involved in many cellular functions. To study the role of CaD in smooth muscle contraction in vivo, the authors generate a mouse that is deficient in both isoforms. Homozygous mutants die perinatally,
likely because CaD is indispensable for abdominal wall closure. Based on results from mechanical assays as well as protein analyses on urinary bladder and abdominal aorta, the authors conclude that, in smooth muscle, CaD acts as a molecular brake on contraction, and that it maintains the structural integrity of the contractile machinery.

Looking ahead
A common goal of JGP and the Myofilament Conference series is to elucidate mechanisms that underlie physiological processes, which, in the case of the conference, is the generation and regulation of contractile force and movement. The conference has evolved to emphasize the structure and function of vertebrate skeletal, cardiac, and smooth muscles, and invertebrate body wall and flight muscles, as well as the basis for altered muscle function in human disease. An important mission for both JGP and the Myofilament Conference is providing support to those who represent future generations of independent researchers.

We are looking forward to the 2022 Myofilament Conference (the seventh in the series) with a heightened sense of enthusiasm and certainty. The meeting will take place May 21–24, 2022 at the Monona Terrace in Madison (http://cvrc.wisc.edu/myofilament-conference#meeting-home).

Acknowledgments
David A. Eisner served as editor.

References
Coleman, A.K., H.C. Joca, G. Shi, W.J. Lederer, and C.W. Ward. 2021. Tubulin acetylation increases cytoskeletal stiffness to regulate mechano-transduction in striated muscle. J. Gen. Physiol. 153:e202012743. https://doi.org/10.1085/jgp.202012743
Fazlollahi, F., J.J. Santini Gonzalez, S.J. Repas, B.D. Canan, G.E. Billman, and P.M.L. Janssen. 2021. Contraction-relaxation coupling is unaltered by exercise training and infarction in isolated canine myocardium. J. Gen. Physiol. 153:e202128289. https://doi.org/10.1085/jgp.202128289
Granzier, H.L., and S. Labeit. 2005. Titin and its associated proteins: the third myofilament system of the sarcomere. Adv. Protein Chem. 71:89–119. https://doi.org/10.1016/S0065-3233(04)70003-7
Hanft, L.M., D.P. Fitzsimons, T.A. Hacker, R.L. Moss, and K.S. McDonald. 2021. Cardiac MyBP-C phosphorylation regulates the Frank-Starling relationship in murine hearts. J. Gen. Physiol. 153:e20212770. https://doi.org/10.1085/jgp.20212770
Mamidi, R., J.B. Holmes, C.Y. Doh, K.L. Dominic, N. Madugula, and J.E. Stelzer. 2021. cMyBPC phosphorylation modulates the effect of ome- camtiv mecarbil on myocardial force generation. J. Gen. Physiol. 153:e20212816. https://doi.org/10.1085/jgp.20212816
van der Pijl, R.J., M. van den Berg, M. van de Locht, S. Shen, S.J.P. Bogards, S. Conijn, P. Langlais, P. Hooijman, S. Labeit, L.M.A. Heunks, et al. 2021. Muscle ankyrin repeat protein 1 (MAR1) locks titin to the sarcomeric thin filament and is a passive force regulator. J. Gen. Physiol. 153:e202112925. https://doi.org/10.1085/jgp.202112925
Pütz, S., L.S. Barthel, M. Frohn, D. Metzler, M. Barham, G. Prymchuk, O. Trunschke, L.T. Lubomirov, J. Hescheler, J.M. Chalovich, et al. 2021. Caldesmon ablation in mice causes umbilical herniation and alters contractility of fetal urinary bladder smooth muscle. J. Gen. Physiol. 153:e20212776. https://doi.org/10.1085/jgp.20212776
Rasicci, D.V., O. Kirkland, F.H. Moonschi, N.B. Wood, D. Szczesna-Cordary, M.J. Previs, J.F. Wenk, K.S. Campbell, and C.M. Yengo. 2021. Impact of regulatory light chain mutation K104E on the ATPase and motor properties of cardiac myosin. J. Gen. Physiol. 153:e20212811. https://doi.org/10.1085/jgp.20212811
Scellini, B., N. Piroddi, M. Dente, G. Vitale, J.M. Pioner, R. Coppini, C. Ferrantini, C. Poggesi, and C. Tesi. 2021. Mavacamten has a differential impact on force generation in myofibrils from rabbit psoas and human cardiac muscle. J. Gen. Physiol. 153:e20212789. https://doi.org/10.1085/jgp.20212789
Short, R. B. 2021. cMyBPC phosphorylation alters response to heart failure drug. J. Gen. Physiol. 153:e202112973. https://doi.org/10.1085/jgp.202112973
Sitbon, Y.H., F. Diaz, K. Kazmierczak, J. Liang, M. Wangpaichitr, and D. Szczesna-Cordary. 2021. Cardiomyopathic mutations in essential light chain reveal mechanisms regulating the super relaxed state of myosin. J. Gen. Physiol. 153:e20212801. https://doi.org/10.1085/jgp.20212801
Solís, C., and R.J. Solaro. 2021. Novel insights into sarcomere regulatory systems control of cardiac thin filament activation. J. Gen. Physiol. 153:e20212777. https://doi.org/10.1085/jgp.20212777
Stronczek, C., S. Lange, B. Bullard, S. Wolniak, E. Börgesen, O. Mayans, and J.R. Fleming. 2021. The N2A region of titin has a unique structural configuration. J. Gen. Physiol. 153:e20212766. https://doi.org/10.1085/jgp.20212766