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EDITORIAL

Toward an understanding of myofibrillar function in health and disease

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Introduction

This special thematic issue of the *Journal of General Physiology* is focused on the function and dynamic regulation of contractile systems in muscle and non-muscle cells. Originally, this issue was to be published following a Myofilament Conference (http://cvrc.wisc.edu/myofilament-conference/#meeting-home) scheduled for June 2020. After the meeting was cancelled due to the COVID-19 pandemic, the editors of the *JGP* generously offered to publish papers in this special issue featuring original research papers and thematic reviews that would be representative of the topical theme of the cancelled meeting, "*Myofilament Form and Function: Determinants of Sarcomeric Contractility.*" Participants in past Myofilament Conferences and others who had already accepted invitations to present their work at the 2020 meeting were invited to submit original manuscripts that would be considered for publication in the special issue, making use of the journal's usual peer review process. The response to the call for papers has been remarkable both in number and in breadth of topics covered, leading to a decision by the editors to authorize a second special issue focused on contractile systems to be published in the summer of 2021.

Papers

The papers in this issue address distinct topics related to the mechanisms and determinants of contractility and its regulation over a range of spatial organization that includes molecular, cellular, and organismal approaches. A rising consensus within the field is that the regulation of contractility involves both switch-like and modulatory processes. In highly organized systems such as the sarcomere in striated muscles or contractile units in smooth muscles, contraction is initiated by Ca^{2+} binding or phosphorylation events involving myofibrillar proteins that activate the thick and thin filaments. But once contraction is activated, the speed and strength of contraction also depends upon modulatory mechanisms such as cooperativity in the binding of myosin to actin and/or phosphorylation of proteins such as myosin regulatory light chain or myosin binding protein-C, both of which appear to increase the activation state of the thick filament and the probability of cross-bridge binding to actin. However, there are critical outstanding questions about the importance of each in various contractile systems, the mechanisms through which these processes have their effects, and the possible involvement of each of these in the physiological manifestations of myopathies and compensatory responses to primary diseases. An important consideration in addressing these questions is that the switch-like and modulatory mechanisms appear to be interactive and synergistic, making it difficult or even undesirable to study a given process in isolation.

Several papers focus on potential mechanisms of the cardiac isoform of myosin binding protein-C (cMyBP-C) in regulating myocardial contractility. These studies follow on nearly two decades of work showing that ablation (Harris et al., 2002) or phosphorylation of cMyBP-C (in the M domain near the N terminus) accelerates contraction kinetics (Stelzer et al., 2006), and that the hypercontractility of myocardium expressing specific hypertrophic cardiomyopathy mutations in cMyBP-C is associated with the absence or near absence of bound cMyBP-C within myofibrils (van Dijk et al., 2009).

In a compelling Viewpoint, Harris (2021) proposes a regulatory function of MyBP-C that could serve to match myocardial contractility to load on the heart and thereby optimize energetic efficiency on a beat-to-beat basis. Using a novel method for modifying myofibrillar proteins in situ, Harris and collaborators recently reported that the spontaneous oscillatory contractions (SPOC) that occur at low levels of Ca^{2+} were reduced or absent in myofibrils in which the endogenous cMyBP-C was modified to eliminate binding of its N-terminal region to myosin (Harris, 2021). Harris proposes that MyBP-C interaction with myosin stabilizes cross-bridges in a super-relaxed inactive configuration

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(SRX). Phosphorylation may lead to accelerated contraction kinetics by two tightly coupled effects, weakening of the SRX state leading to increased likelihood of binding of cMyBP-C to the thin filament, and increased activation of the thin filament by myosin and cMyBP-C binding to actin (Mun et al., 2014). The relative contributions of these mechanisms to increased contractility in vivo are not known, but the possibility that the N-terminal region of cMyBP-C switches its binding from myosin to actin on a beat-to-beat basis is intriguing.

Rahmanseresht et al. (2021) report results that provide novel insights into the position of the N terminus of cMyBP-C with respect to actin and to myosin. Using super-resolution microscopy, the authors found that the position of the N terminus of cMyBP-C in fixed muscle is biased toward the thin filaments in both active and relaxed muscle. In this position, the N terminus could bind to actin or to myosin, at least when the myosin head is near to the thin filament. These results strongly support the idea that the N terminus can bind to either myosin or actin, with the probability of binding to one or another varying with the phosphorylation status of cMyBP-C. As discussed by the authors, binding events could conceivably introduce an internal load to slow muscle shortening and also influence the state of activation of the thick or thin filaments.

In another study, Giles et al. (2021) show that the slowing of unloaded shortening velocity in skinned myocardium as shortening proceeds is apparent only at intermediate levels of Ca^{2+} activation *and* when cMyBP-C is less than fully phosphorylated at the regulatory PKA sites in the M domain. Previously, a similar slowing of shortening velocity was observed in skeletal muscle fibers (Hofmann et al., 1991) and was thought to be due to an internal load that arises due to strain of an elastic structure that was slack during the initial phase of shortening. Here, because the unphosphorylated N-terminal region of cMyBP-C is most likely bound to myosin, which induces the SRX state and reduces the number of available cross-bridges, the authors attributed the slow phase to a shortening-induced decrease in the activation of the thin filament as a consequence of a reduced number of cross-bridges bound to the thin filament during shortening. In cMyBP-C knockout myocardium, the slow phase of shortening is much less prominent or completely absent. PKA phosphorylation of the N terminus of cMyBP-C, which disrupts its binding to myosin, also eliminates the slow phase of shortening. Both results are consistent with the proposed mechanism for the slow velocity phase, but this does not eliminate other possibilities, including an internal load. As suggested by Rahmanseresht et al. (2021), the binding of the N terminus of cMyBP-C to the myosin head could introduce an internal load that slows the power stroke and thus the sliding velocity of thin filaments. If such is the case, phosphorylation of the N terminus would disrupt its binding to myosin, reduce or eliminate the internal load, and increase the speed and power of myocardial contraction.

To an increasing degree, it has become evident that both spontaneous and heritable mutations in cardiac MyBP-C can cause cardiac myopathies which in their most virulent forms can cause sudden cardiac death (Greenberg and Tardiff, 2021). Also, in end-stage heart disease, such as congestive heart failure, the myocardium exhibits diminished or absent responsiveness to physiological processes such as increased β-adrenergic tone or the Frank–Frank–Starling mechanism (increased stroke volume due to increased end-diastolic volume) that are normally activated when cardiac output and systolic blood pressure are reduced. To address these issues, efforts to develop therapeutic interventions that mimic or inhibit the effects of cMyBP-C phosphorylation or its binding to specific partners in the thick or thin filaments have increased in recent years. The paper by Bunch et al. (2021) in this issue reports the development of an innovative method to assess protein binding to actin, with emphasis on the binding of the C0–C2 domain of cMyBP-C. Their method involves the use of a novel time-resolved fluorescence assay to assess binding, with results that compare favorably to the more laborious cosedimentation assays that are typically used. The new method has potential value in studies to identify cMyBP-C binding partners and to also screen candidate therapeutics designed to enhance or repress specific binding events.

Two additional articles in this issue focus on mutations that cause myopathies. Greenberg and Tardiff (2021) review the biophysical basis of cardiomyopathies that are due to mutations in myofilament proteins. Based on their analyses, they propose that developing effective treatments for these disorders will greatly benefit from binning patient subpopulations based on common underlying biophysical mechanisms that drive the molecular disease pathogenesis. Tobacman and Cammarato (2021) hone in on the locations of pathogenic and non-pathogenic troponin mutations in a thin filament atomic model. They show that the large majority of pathogenic sites are in troponin regions that inhibit contraction via direct contacts with actin or tropomyosin. These are significant findings that warrant follow up experimental work.

Four papers add to recent advances in the understanding of the structure of myosin filaments in smooth and skeletal muscle and how structural features relate to the performance requirements of the muscles. Wang et al. (2021) provide a timely review of myosin filaments in smooth muscles. Many types of smooth muscles must maintain contractility over a large range of cell lengths. The authors discuss how the unique properties of smooth muscle myosin together with the side-polar filament structure may explain adaptation to changing muscle lengths. Evidence that smooth muscle myosin filaments can assemble and disassemble in response to changing cellular conditions and forces is discussed and mechanistic hypotheses are proposed.

Brizendine et al. (2021) have focused on the behavior of the fully activated state of smooth muscle myosin filaments while moving on actin in vitro in the absence of load. Using kinetic modeling and direct observations of deflections in single-head domains that differ from the motion of the filament backbone, they provide evidence that the S2 domain of a head at the end of its powerstroke is able to be detached from the filament backbone during motion driven by working heads. This proposed flexibility of S2 independent from the backbone allows for the working heads to experience less compressive strain.

Ma et al. (2021) and Caremani et al. (2021) present advances in understanding the structure of skeletal muscle myosin filaments in the resting or relaxed state using small-angle x-ray

diffraction and interference, but from organisms that are widely separated on the evolutionary tree: tarantula and rabbit. Both studies perturb the muscle by changing the temperature and interpret the observed changes in the context of the interacting heads motif that leads to the helical arrangement of the heads on the filament backbone. By observing responses of tarantula muscle at temperatures lower than the range of optimal temperatures, Ma et al. (2021) propose a structurally explicit thermosensing mechanism that may be similar to a proposed refractory state in mammals. Caremani et al. (2021) present a careful comparison of temperature- and lattice compression–dependent effects on demembranated fibers and intact muscle. Results suggest that at physiological temperature, filament structural changes due to demembranation can be rescued by compressing the filament lattice dimensions. This result is important because thick filament structure is likely directly linked to thick filament–based regulatory mechanisms that relate to performance of the whole muscle in terms of efficiency and metabolic cost.

Kawai et al. (2021) studied single fibers and single myofibrils from skeletal muscle. Using small-amplitude sinusoidal length oscillations on activated fibers/myofibrils, they demonstrate that phosphate (P_i) binding is a key determinant of oscillatory work and suggest that the reverse transition of myosin heads from force- to nonforce-generating states is coupled to P_i binding. Mijailovich et al. (2021) used a multiscale modeling approach that incorporates the spatial and chemomechanical properties of sarcomeres to simulate the mechanical performance of muscle. They demonstrate that when muscle elasticity and thick filament regulation are included in their model, the twitch tension and kinetics and Ca^{2+} sensitivity of force generation by intact cardiac muscle can be accurately simulated.

van der Horst et al. (2021) investigated smooth muscle and focused on the role of the molecular motor dynein in the microtubule-dependent internalization of the voltage-gated Kv7.4 potassium channel. Patch-clamp studies were performed in HEK293B cells in which dynein function was experimentally reduced, and localization and Kv7.4 knockdown studies were performed on mesenteric artery myocytes. These studies revealed that dynein can traffic Kv7.4 channels in vascular smooth muscle in a mechanism dependent on cholesterol-rich caveolae.

Finally, Gohlke et al. (2021) studied the actin filament-associated nebulin. This is a giant protein that consists largely of small modules that are organized into seven-module super-repeats, each containing a tropomyosin binding sequence. Nebulin plays roles in regulating the length of the thin filament in skeletal muscle, and mutations in nebulin are a frequent cause of nemaline myopathy. The authors investigated the nebulin gene in 53 species of birds, fish, amphibians, reptiles, and mammals. They report that the number of super-repeats shows high interspecies variation, and, importantly, scales with body size. The higher number of super-repeats in large animals was shown to increase the thin filament length, which is expected to reduce the number of sarcomeres in series (per unit fiber length) and lower the shortening velocity of muscle. It has been known since the work of A.V. Hill in 1950 (Hill, 1950) that as species increase in size, the shortening velocity of their muscle is reduced, and Gohlke et al. (2021) provide evidence that nebulin contributes to its mechanistic basis.

Looking ahead

We look forward with enthusiasm to the 2022 Myofilament Conference, which will be the seventh conference in the series. 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. Early career investigators and trainees at past conferences have been provided with opportunities to present and discuss their work, to meet established scientists, and to network with colleagues at similar career stages. As in the past, the 2022 conference will begin with research symposia featuring and organized by early career investigators in consultation with faculty advisors. Early career investigators will also be invited to compete for one of two *JGP*-sponsored Early Career Investigator Awards of $250. Six Outstanding Poster Awards of $250 will also be sponsored by the UW–Madison Cardiovascular Research Center. In addition to the cash prize, recipients of an Outstanding Poster Award will be given the opportunity to present an oral summary of their work in the closing session of the conference. A formal announcement of the dates and theme of the 2022 conference will be issued in the early summer of 2021.

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