Perspectives on: SGP Symposium on Muscle in Health and Disease

Emerging questions about the macromolecular machines of muscle

Robert T. Dirksen,[1] Guest Editor, and Edward N. Pugh Jr.,[2,3] Editor

[1]Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY 14642
[2]Center for Neuroscience, University of California, Davis, Davis, CA 95616
[3]*The Journal of General Physiology*

Introduction

The purpose of the Perspectives in General Physiology is to provide a forum where scientific uncertainties or controversies are discussed in an authoritative, yet open, manner. Perspectives are solicited by the editors—often based on recommendations by members of the editorial advisory board. The comments and opinions expressed in the Perspectives are those of the authors and not necessarily those of the editors or the editorial advisory board. The Perspectives are accompanied by a few editorial paragraphs that introduce the problem and followed by an invitation to readers to submit comments.

This Perspectives series was inspired by the 63rd Symposium of the Society of General Physiologists on "Muscle in Health and Disease," which was co-organized by Drs. H. Lee Sweeney (University of Pennsylvania) and David A. Eisner (University of Manchester) and held in Woods Hole, Massachusetts, in September 2009. The contributions cover key novel findings, unresolved questions, and clinical implications of three essential muscle processes (excitation–contraction [EC] coupling, thin/thick filament regulation, and membrane stabilization during contraction) that are each controlled by distinct multiprotein machines.

Historical perspective

The mechanisms that control striated muscle contraction have been the focus of intense research for centuries. The classical studies of Sydney Ringer, conducted while at the University College London and published in a series of four publications in 1882 and 1883, were the first to demonstrate that extracellular Ca^{2+} ions are required to maintain the normal rate and contractile strength of the isolated frog heart. Ringer's work, while both remarkably methodical and meticulous, was also surprisingly serendipitous. As the often recounted legend goes, Ringer's landmark discovery resulted only after realizing that his research assistant had prepared the heart perfusate using hard London tap water, complete with high levels of inorganic constituents including ~1 mM Ca^{2+}! Subsequent experiments using distilled water blocked contractions, and the re-addition of appropriate levels of NaCl, $NaHCO_3$, KCl, and, of course, $CaCl_2$ to the distilled water were required to enable the heart to "continue beating perfectly." Thus was launched the field of ion homeostasis and its role in the control of normal muscle physiology and disease.

Subsequent seminal work in muscle physiology over the last 100 years includes, among others, the characterization of heat production and metabolism in working muscle by A.V. Hill in the early 1900s; the pioneering studies of the sliding filament theory of A.F. Huxley and H.E. Huxley in the 1950s; elegant early electron microscopy ultrastructural studies of transverse tubules and triads in skeletal muscle fibers by C. Franzini-Armstrong in the 1960s; the discovery of intramembrane charge movement within the transverse tubule membrane and its association with the control of intracellular Ca^{2+} release by W.K. Chandler and colleagues in the 1970s; and linkage of the dystrophin gene to Duchenne muscular dystrophy by L.M. Kunkel in 1986 and subsequent characterization of the molecular players in the dystrophin–glycoprotein complex by K.P. Campbell and others.

Fast forward more than 125 years from Sydney Ringer's landmark studies. Leading muscle researchers across the world make a "pilgrimage" of sorts to the Marine Biological Laboratory to participate in the 63rd Symposium of the Society of General Physiologists on "Muscle in Health and Disease." Presentations and discussions at the meeting, both formal and informal, focus around new findings, insights, and how to address current unanswered questions and challenges in the field. As both active participants and interested observers, we were struck by a common recurring theme throughout the conference that involved the use of multidisciplinary cutting-edge approaches to investigate the molecular mechanisms by which complex protein–protein interactions dictate the

Correspondence to Robert T. Dirksen:
Robert_Dirksen@URMC.Rochester.edu

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function (and dysfunction) of several highly evolved "macromolecular machines" in striated muscle. Emphasis was placed on how these macromolecular machines are assembled and operate to control such diverse muscle functions as excitability (EC coupling mechanism), sarcomere shortening (thin-thick filament regulation), as well as lateral force transmission and sarcolemmal integrity (dystrophin–glycoprotein complex). Not surprisingly, current work involving each of these muscle macromolecular machines is strongly linked to one or more of the seminal studies of muscle physiology discussed above. The Perspectives in this series were selected to highlight progress and enduring unresolved issues involving these three areas of emphasis.

Muscle macromolecular machines

The EC coupling mechanism. Beam and Bannister (University of Colorado) provide a comprehensive discussion of the current molecular understanding of the conformational coupling interaction between the dihydropyridine receptor (DHPR) L-type Ca^{2+} channel or voltage sensor in the transverse tubule membrane and the type 1 RYR (RYR1) Ca^{2+} release channel located in the terminal cisternae of the sarcoplasmic reticulum. A particularly intriguing aspect of the DHPR–RYR1 macromolecular machine is that it mediates a bidirectional signaling interaction between two large ion channel complexes located in adjacent membrane compartments, with each channel being an assembly of multiple subunits and associated regulatory proteins. Although the original identification, cloning, and validation of the DHPR and RYR1 proteins as essential players in the EC coupling process were demonstrated by Beam and several others in the late 1980s and early 1990s, the precise molecular nature of this intermembrane macromolecular interaction remains exasperatingly enigmatic. In their Perspective, Beam and Bannister candidly outline the central questions regarding the DHPR–RYR1 interaction that remain elusive, including: How is the ECC signal transmitted from the voltage sensor to the release channel? What are the respective roles of the DHPR α_{1S} and β_{1a} subunits? How is EC coupling modulated by other nonessential proteins (e.g., FKBP12, calmodulin, triadin, junctin, calsequestrin, etc.) associated with the DHPR–RYR1 machine? How does altered function of the EC coupling machine contribute to muscle disease? Like that of their predecessors, current and future EC coupling researchers will likely be driven, and even haunted, by the quest to provide definitive answers to these questions.

Thin-thick filament regulation of the sarcomere. The control of actin–myosin interactions in striated muscle is considered in two Perspectives authored by Solaro et al. (University of Illinois and University of Manchester) and by Moss and Fitzsimons (University of Wisconsin). A classical schematic of muscle force generation simply depicts a myosin with the head anchored to a thick filament and undergoing an ATP-dependent cross-bridge cycle. But as Solaro et al. point out, the contractile unit is the sarcomere, a complex macromolecular machine comprising not only actin, myosin, tropomyosin, and troponins, but also other proteins that stoichiometrically bind to the core components and regulate cycling. Solaro et al. opine that studies of the role of altered interactions between sarcomeric proteins regulating striated muscle contractility have been metaphorically treated as the meddlesome, ugly stepsister of its more winsome membrane ion channel and transporter siblings. This Perspective details a new and evolving appreciation for the molecular mechanisms by which these various sarcomeric proteins dynamically affect the myofilaments' response to the changing calcium landscape during the cardiac contraction cycle, as exemplified by adrenergic modulation of the rise and maintenance of cardiac systolic contraction. The Perspective submitted by Moss and Fitzsimons focuses on both the mechanisms that underlie the thin filament cooperativity of Ca^{2+} activation, and recent evidence indicating a previously unappreciated role of thick filament processes in the regulation of contraction and in β adrenergic stimulation of the myocardium. With respect to the former, there is increasing evidence that cross-bridge heads are "activated" during muscle contraction, although the mechanism(s) underlying this phenomenon is not yet known. With respect to the latter, in concert with phosphorylation of troponin I (TnI), the rate of cardiac force development is increased by PKA-mediated phosphorylation of the thick filament accessory protein myosin-binding protein C (MyBP-C), which enhances the availability of myosin cross-bridges to interact with actin. Together, these two Perspectives highlight several unresolved issues with regard to the sarcomeric macromolecular machine: What are the respective roles of the thick and thin filament proteins in conferring the cooperativity of Ca^{2+} activation of cross-bridge cycling? To what degree, if any, do secondary regulatory mechanisms now well-established in the heart (e.g., constraint of cross-bridges by MyBP-C, phosphorylation of TnI or MyBP-C) impact function of the skeletal muscle molecular motor? How do familial hypertrophic and dilated cardiomyopathic missense mutations in sarcomeric proteins trigger structural, electrical, and metabolic remodeling of the heart? How can new molecular insights into the control and operation of the sarcomeric macromolecular machine be exploited to develop novel therapeutic agents to treat life-threatening and debilitating cardiac and skeletal muscle diseases?

The dystrophin–glycoprotein complex. Goldstein and McNally (University of Chicago) provide a provocative Perspective on the multifunctional role of the dystrophin–glycoprotein complex (DGC) in skeletal muscle and how defects of this macromolecular machine lead

to muscular dystrophy. The DGC, concentrated within costameres, a cytoskeletal lattice along the plasma membrane, is a multiprotein complex consisting of dystrophin, dystroglycan, syntrophins, actin, and numerous other associated proteins, including sarcoglycans, dystrobrevin, and nitric oxide synthase. The DGC structurally couples the intracellular actin cytoskeleton to the extracellular matrix and mediates multiple critical functional roles, including: maintenance of membrane integrity/stiffness, lateral force transmission, and scaffolding of signaling molecules. Loss of this complex in muscular dystrophy results in costamere disorganization, increased membrane fragility and leakiness, muscle weakness, necrosis, as well as altered signaling via Ca^{2+} influx/release, nitric oxide, reactive oxygen species, and the extracellular matrix. Many unresolved issues continue to plague investigators in the field, including: What is the relative importance of muscle fiber loss and muscle fiber dysfunction to muscle weakness that occurs in the muscular dystrophies? What is the relative importance of the different DGC functions (membrane stability, force transmission, and signaling) in muscle fiber loss and dysfunction? What are the most effective ways in restoring dystrophin expression (e.g., viral-mediated gene transfer, oligonucleotide-directed exon skipping, utrophin up-regulation, and stem cell transplantation)? What are the most effective ways to limit damage due to loss of DGC function (e.g., inhibition of stretch-activated Ca^{2+} influx, RYR stabilization, TGF-β inhibition, augmented NO signaling, and enhanced membrane stabilization using synthetic membrane sealing poloxamers)?

Summary and future directions

These Perspectives manifest major shifts underway in understanding of the molecular mechanisms of physiological processes that mediate and regulate muscle contraction. On the one hand, there is a shift toward elucidating dynamic changes in the macromolecular complexes that control muscle excitation, contraction, and membrane stability, and on the other a continuing shift toward deeper characterization of the specific molecular interfaces involved in these protein–protein interactions. It is increasingly clear that a comprehensive understanding of the molecular mechanisms of muscle disease and the future development of rational therapies requires continued advances in both directions.

Letters to the editor related to these Perspectives will be published in the October 2010 issue of the Journal. Letters to the editor should be received no later than Monday, August 16, 2010, to allow for editorial review. The letters may be no longer than two printed pages (approximately six double-spaced pages) and will be subject to editorial review. They may contain no more than one figure, no more than 15 references, and no significant references to unpublished work. Letters should be prepared according to the Journal's instructions and can be submitted electronically, or as an e-mail attachment to jgp@rockefeller.edu.