Myosin-binding protein C (MyBP-C) is a multi-domain protein that interacts with myosin and actin filaments in cardiac and skeletal muscle. Dynamic interactions of MyBP-C with the myofilaments help to coordinate proper muscle contraction and relaxation by providing additional layers of regulation for the ATPase cycle of actomyosin. The C terminus of MyBP-C is anchored to the myosin filament along titin. However, the disposition(s) of the N terminus of MyBP-C has remained elusive, in particular whether binding occurs with myosin or actin. Several models have been proposed where N-terminal MyBP-C effects on function are explained by interactions with regions of myosin or actin filaments. N-terminal MyBP-C binds to myosin and actin in solution, but this had yet to be seen in intact myofilaments of muscle.

It is important to know where the N terminus of MyBP-C is in the intact myofilament lattice because it is this region of the molecule that is responsible for influencing actomyosin structure and contractile function. Others have attempted to solve this, providing clues to the location of the MyBP-C N terminus relative to myosin and actin filaments. For instance, in ideally preserved relaxed skeletal muscle, electron tomography showed that MyBP-C binds to actin (Luther et al., 2011). While the tomograms resolve extension of MyBP-C from myosin filaments to areas near actin filaments, they did not reveal molecular details on the actin interaction, and MyBP-C’s positioning during contraction was not studied. The uncertainty of MyBP-C’s binding is likely due to inherent dynamics (flexibility/disorder) at this end of the molecule. In an electron microscopy study, antibodies specific for N- and C-terminal and central domain regions of MyBP-C were used to define its relative orientation in the myofilament lattice (Lee et al., 2015). These findings confirmed an organization where C-terminal domains lay along myosin filaments and suggested that most of MyBP-C and its N terminus reach out perpendicularly from myosin toward nearby actin filaments. Given this general arrangement, researchers must now use techniques with the spatial and temporal resolution to capture the location(s) and dynamics of the MyBP-C N terminus bound to actin or myosin or positioned in the interfilament space. For more detailed molecular interactions with actin filaments, cryo-electron microscopy has been used recently to uncover specific amino acid contacts between N-terminal domains of MyBP-C and actin (Harris et al., 2016) and its regulatory tropomyosin strand (Risi et al., 2018) in solution. A major remaining question is how MyBP-C interacts with actin (or myosin) in intact muscle, including its tendency to be bound to actomyosin or free in the interfilament space. This gap in knowledge creates a challenge for deciphering the mechanisms by which MyBP-C modulates muscle contraction and relaxation. Understanding these mechanisms is needed to understand MyBP-C function in normal physiology and dysfunction in muscle disorders, and in development of MyBP-C-targeted therapies for treating heart and skeletal muscle disease.

Super-resolution microscopy captures MyBP-C in muscle

Muscle is highly organized in its lattice arrangement of myosin and actin filaments (Fig. 1). This allows for investigations of isolated muscle tissue to resolve the myofilament architecture at the molecular level using high-resolution structural biology approaches. These techniques include cryo-electron microscopy, electron tomography, x-ray diffraction, and electron paramagnetic resonance (EPR) spectroscopy. To resolve MyBP-C images of disordered N-terminal domain positions relative to the myofilament lattice (Fig. 1), in this issue of the Journal of General Physiology, Rahmanseresht et al. (2021) made technological advancements in studying muscle structure using super-resolution microscopy, particle averaging, and computer modeling. STORM (stochastic optical reconstruction microscopy) is a type of single-molecule super-resolution microscopy that uses stochastic excitation of only a few fluorophores at a time by a very-low-intensity light source as opposed to conventional fluorescence microscopy, where all fluorophores in a region of interest are excited. Separating excitation of neighboring fluorophores in time permits
fitting of each fluorophore’s position. Thus, STORM detects the individual center location of fluorophores, typically in a two-dimensional plane, and combined with multiple fluorophore colors, is used for reconstructing images. While conventional fluorescence microscopy is limited by diffraction (180 nm), STORM allows for 20–40-nm resolution, which is well suited for surveying the myofilament lattice.

Rahmanseresht et al. (2021) characterize cardiac muscle isolated from transgenic mouse hearts carrying a Myc-tag at the end of MyBP-C protein (Sadayappan et al., 2005). The Myc-tag allows for specific immunostaining of MyBP-C with a fluorescent antibody, and actin filaments were labeled with dye-conjugated phalloidin, a peptide that tightly binds actin. The fixed muscle preparations were imaged using two-color STORM, and the resolution of the images was improved by particle averaging. The authors take great care in validating that the reconstructed images arise from actin filaments arranged in hexagon shapes and not random signal. Further, Rahmanseresht et al. (2021) compare the experimental images with images generated in silico to help with defining the disposition of MyBP-C N-terminal domains within the actin hexagon (Fig. 1) by considering scenarios of binding to actin or myosin under relaxed and activated conditions.

Dynamic equilibrium of MyBP-C binding
Following from the study by Rahmanseresht et al. (2021) resolving structures down to tens of nanometers, it will now be necessary to define the molecular level interactions (<1 nm) between N-terminal MyBP-C and actin and myosin filaments. This includes the amino acid contacts and domain regions involved in binding, how the structure and dynamics of actin and myosin filaments are impacted by MyBP-C binding, and more information about the dynamic equilibrium of MyBP-C in bound and unbound states. In addition, determining the structure and dynamics of the MyBP-C molecule unbound in muscle, with particular focus surrounding the intrinsically disordered and phosphorylatable M-domain, will also enhance understanding of the molecular mechanism(s) by which MyBP-C modulates contraction and relaxation. The next steps in resolving further details of MyBP-C structure and dynamics in muscle will be aided by approaches under physiological conditions including FRET spectroscopy, single-molecule fluorescence, and cryo-electron spectroscopy, single-molecule fluorescence, and cryo-electron
microscopy. Rahmanseresht et al. (2021) make a major advancement by defining the localization of MyBP-C N-terminal domains in fixed cardiac muscle preparations under relaxed and active states. This sets the stage for future studies of additional regulators of MyBP-C, including phosphorylation, mutations, and small-molecule interventions developed to treat heart failure.

Acknowledgments
Henk L. Granzier served as editor.
This work was supported by National Institutes of Health grant R01 HL141564 (to B.A. Colson).
The author declares no competing financial interests.

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