

## FUTURE LEADER REVIEW

# Cardiac myosin super relaxation (SRX): a perspective on fundamental biology, human disease and therapeutics

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## ABSTRACT

The fundamental basis of muscle contraction ‘the sliding filament model’ (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954) and the ‘swinging, tilting crossbridge-sliding filament mechanism’ (Huxley, 1969; Huxley and Brown, 1967) nucleated a field of research that has unearthed the complex and fascinating role of myosin structure in the regulation of contraction. A recently discovered energy conserving state of myosin termed the super relaxed state (SRX) has been observed in filamentous myosins and is central to modulating force production and energy use within the sarcomere. Modulation of myosin function through SRX is a rapidly developing theme in therapeutic development for both cardiovascular disease and infectious disease. Some 70 years after the first discoveries concerning muscular function, modulation of myosin SRX may bring the first myosin targeted small molecule to the clinic, for treating hypertrophic cardiomyopathy (Olivetto et al., 2020). An often monogenic disease HCM afflicts 1 in 500 individuals, and can cause heart failure and sudden cardiac death. Even as we near therapeutic translation, there remain many questions about the governance of muscle function in human health and disease. With this review, we provide a broad overview of contemporary understanding of myosin SRX, and explore the complexities of targeting this myosin state in human disease.

This article has an associated Future Leaders to Watch interview with the authors of the paper.

**KEY WORDS:** Myosin, Super relaxed state (SRX), Interacting heads motif (IHM), Myosin mesa, Hypertrophic cardiomyopathy, Targeted therapy

## Introduction

Fundamentally, myosins are able to produce force when they are available to bind actin and hydrolyse ATP. These interactions drive the sliding of thick and thin filaments past one another, contracting sarcomeres, and creating the force and muscular shortening that allows the heart to circulate blood. If myosins are not in close proximity to actin they cannot use ATP to generate force and drive muscular contraction. Filamentous myosins that are available to bind actin exist in a wide range of structural states and proximities to the thin filament, these conformations have more recently been defined as the disordered relaxed state (DRX) (Cooke, 2011; Fusi et al., 2015;

Stewart et al., 2010). When viewed by electron microscopy or indirectly by X-ray diffraction, these active states occupy a wide variety of structures and proximities to the thin filament, and look ‘disordered’ (Cooke et al., 1982; Thomas and Cooke, 1980). DRX myosin is in balance with a well-ordered state of myosin termed the super relaxed state (SRX). Myosin SRX is a biochemical and likely structural state, where myosin heads interact with one another and the thick filament backbone (Alamo et al., 2016; Hooijman et al., 2011). These interactions form the basis of structural sequestration of myosin heads away from the thin filament, typified by deposition of these catalytic domains onto the thick filament backbone, providing the more ordered structure, myosin SRX (Alamo et al., 2008; Huxley and Brown, 1967; Woodhead et al., 2005). SRX myosin heads are unavailable for binding actin to produce force. This is a curious characteristic of myosin regulation within a filament; why would a cell produce a protein for it to be dormant? What is becoming clearer is that myosin SRX is one of the key determinants of the constant  $N_a$  (cross bridge number) in Huxley’s model of force production, and that it is incredibly important to regulate the activity of these motors to ensure mechanical and energetic homeostasis within the cell. Especially pertinent in cardiac myocytes, which do not readily divide, cannot be replaced, and must contract and relax on average 60 times a minute throughout an individual’s entire life.

Myosin SRX was first defined with its discovery in rabbit skeletal muscle (Stewart et al., 2010; Myburgh et al., 1995). SRX was found to account for a significant proportion of myosin in cardiac thick filaments (Hooijman et al., 2011). Rather than switching the motor unit ‘on’ and ‘off’ to allow contraction like in smooth muscle myosin (Cross et al., 1988), myosin SRX in cardiac muscle is more modulatory and provides more graded recruitment of myosin heads and force production (Hooijman et al., 2011). This difference goes hand in hand with the functional demands of both cardiac and skeletal muscle. Skeletal muscle remains in a relaxed state as long as no force is needed, and is able to rapidly adapt cross-bridge recruitment to satisfy demand. In contrast, cardiac muscle must contract and relax on average 60 beats a minute over the lifetime of an individual, while contracting in a graded and tightly moderated manner, which is adapted gradually not beat to beat, to raise or lower cardiac output (Kobirumaki-Shimozawa et al., 2014; McNamara et al., 2015). There are many properties of the sarcomere that influence recruitment of myosin cross-bridges from the SRX state. These include pre-load of the ventricle and post-translational modification. It has become clear that SRX recruitment contributes to the Frank-Starling law and is important in adapting cardiac output (Marcucci et al., 2019; Reconditi et al., 2017). Myosin SRX has its fundamental roots in earlier evolutionary phylogenies of myosins, and is intricately controlled by the interactions that form the basis of the myosin interacting heads motif (IHM) (Alamo et al., 2016). Myosin SRX dysregulation has been observed as a driving mechanism in HCM. Modulation of myosin SRX has become a central theme for the first targeted therapeutics for genetic cardiomyopathies (Repetti et al., 2019b). The

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utility of targeting myosin SRX therapeutically may extend beyond inherited cardiac conditions to acquired cardiovascular disease and even infectious disease (Trivedi et al., 2020).

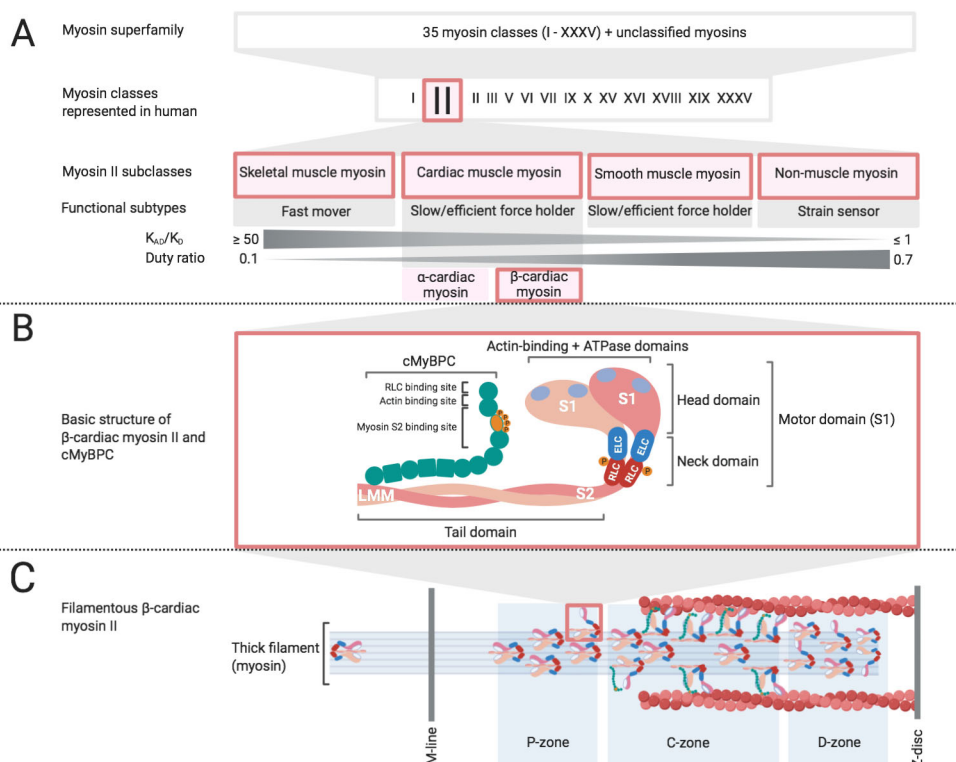
### The evolutionary origin of the myosin interacting heads motif (IHM) and the myosin super relaxed state (SRX)

Myosin is an actin-based motor protein that hydrolyzes ATP driving the motile properties of cells, tissues, and organs. A wide variety of myosin motors exist in nature and they share an evolutionary ancestor with kinesins, the microtubule-based molecular motor. The diverse set of myosin molecular motors have been classified into subgroups by several criteria (Fig. 1A) (Bloemink and Geeves, 2011; Odrionitz and Kollmar, 2007). All myosins have an amino-terminal catalytic head domain, which contain actin- and ATP-binding sites. The major structural differences are found in the carboxyl-terminal, also known as the tail region, which varies widely in length and sequence, and allows the diverse cellular functions that myosins play within cells (Sellers, 2000). Among all these subgroups, Myosin IIs, also referred to as conventional myosins, were the first to be discovered (Kühne, 1864), and the only myosin subgroup that is known to assemble filaments (Sellers, 2000).

Myosin II molecules are made up of two identical heavy chains, each of these possess a globular head, a coiled-coiled tail domain, and a neck region that links the head and tail (Fig. 1B). Each neck domain of a heavy chain possess two IQ motifs that act as binding sites for two light chains, known as the essential (ELC) and the regulatory light chain (RLC) (Bähler and Rhoads, 2002; Heissler and Manstein, 2013). The Myosin II phylogeny possess a wide range of functional subtypes, which differ from one another by their respective duty ratio and thermodynamic coupling (Fig. 1A). The duty ratio defines the fraction of the ATPase cycle that the myosin spends in a strongly bound state to actin, while thermodynamic coupling ( $K_{AD}/K_D$ ) describes the ratio of ADP affinity for actomyosin, versus the ADP

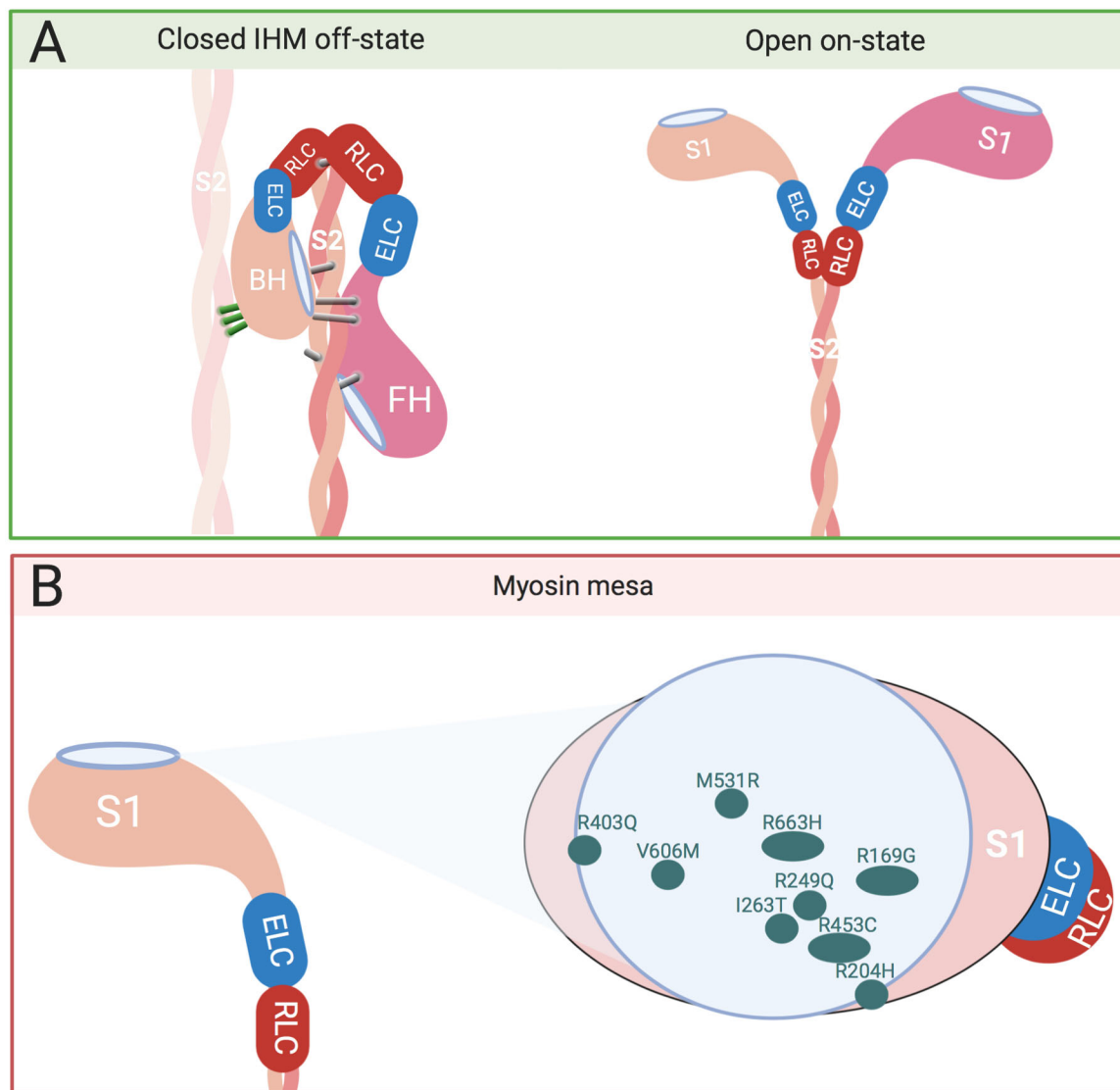
affinity for myosin (Bloemink and Geeves, 2011). This sub-classification highlights the functional diversity of Myosin IIs, which encompass non-muscle myosins that are integral to cytokinesis (Pollard, 2020) as well as cell adhesion, cell migration and tissue architecture (Vicente-Manzanares et al., 2009), smooth muscle myosins that line many of the visceral organs and the circulatory system, and skeletal and cardiac muscle myosins that are responsible for providing force and power to move and pump blood in the heart.

What is clear is that all four subtypes of Myosin II have developed regulatory mechanisms to adapt energy consumption, to allow muscle relaxation, or to reduce myosin activity in cells not undergoing cellular division. This is a very important quality of these motors as molecular and cellular motion is energetically costly and needs to be tightly controlled and in balance with energy production within the cell. Even though smooth and nonmuscle Myosin IIs are distantly related to skeletal and cardiac muscle within the Myosin II phylogeny (Golomb et al., 2004), they all possess an IHM (Fig. 2A), a structure that is common to the Myosin II phylogeny. The Myosin IHM is highly conserved across the Myosin II phylogeny (González-Solá et al., 2014; Pinto et al., 2012; Woodhead et al., 2013; Zhao et al., 2009). One would predict that the IHM provided an evolutionary advantage for it to be so highly conserved. In both *Cnidaria* (the most primitive animals with muscles) (Sulbarán et al., 2015) and *Porifera* (considered to be the oldest animal phylum which lack muscle tissue) the IHM is present (Lee et al., 2018a,b). This suggests that the IHM motif emerged up to 700 and 900 million years ago (Lee et al., 2018a,b). Even in the eukaryote *Dictyostelium* modified folding of the Myosin tail similar to what one expects from the IHM has been found (Lee et al., 2018a,b). The formation of myosin molecules by the coiled-coiled domains of two myosin heavy chains brings myosin heads into close proximity at their amino terminus, this forms the basis of the IHM (Alamo et al., 2018). This folded structure of myosin has recently been resolved in spectacular detail in smooth muscle



**Fig. 1. Classification, functional and structural hallmarks of Myosin II.**

(A) 35 groups of myosin (I-XXXV) have been classified based on the phylogenetic relation of their motor domains where unclassifiable myosins are denoted as orphan myosins (Odrionitz and Kollmar, 2007). Subclasses of Myosin II include striated, smooth and non-muscle myosin, functionally differing from one another by duty ratio and thermodynamic coupling (Bloemink and Geeves, 2011).  $\beta$ -cardiac myosin II is primarily expressed in myocardium and encoded by the gene MYH7. (B)  $\beta$ -cardiac myosin II is formed by the characteristic two-headed structure with motor domains, neck regions including ELC and RLC, and a coiled-coiled tail domain. **Inset** cMyBPC exhibits integral binding sites for sarcomeric proteins. (C) Illustration of a half-sarcomere, which shows zone-specific myosin head conformations adapted from Brunello et al., 2020. The majority of actin-attached and force producing myosin heads are located in the C-zone whereas proximal (P) and distal (D) zones primarily comprises disordered and folded myosin heads.



**Fig. 2. The interacting heads motif (IHM) and the Myosin Mesa.** (A) The IHM interactions include the folded back conformation of the myosin dimer for interactions with the core of the thick filament, as well as interactions with adjacent myosin molecules (left) (adapted from Anderson et al., 2018). This is in contrast to the open on-state (right). Characteristic intermolecular (green) and intramolecular (grey) interactions defined by Alamo et al. maintain the IHM off structure, thus the blocked head (BH) is prevented from binding to actin by interacting with the free head (FH) (Alamo et al., 2016). The ATPase activity of the free head is inhibited through the attachment with the blocked head. (B) The side view (left) of the flat surface of a myosin head (left) shows the location of the myosin mesa. The top view (right) illustrates its relevance within the catalytic domain as a crucial location for well described HCM causing mutations displayed as green spots (adapted from Spudich, 2015).

myosin by cryo-EM (Scarff et al., 2020; Yang et al., 2020). The IHM has been conserved and diversified evolutionarily, yet the IHMs importance in regulating cardiac muscle has largely been overlooked, until recently, when it became clear that residues within the IHM defined a significant proportion of cases of the genetic cardiomyopathy HCM (Alamo et al., 2017). SRX may have been adaptive even in earlier mammalian evolution during hibernation and in response to cold (Toepfer et al., 2020). States of myosin that are not fully described as either SRX or DRX have been observed by Caremani et.al. where mouse skeletal muscle cells at sub physiologic temperatures form a refractory state of myosin, that is not consistent with the sequestered structure of SRX nor the high nucleotide utilisation and cross-bridge formation of DRX (Caremani et al., 2019), a similarity with another well-ordered state observed when infusing muscle with 2'-deoxy-ATP (dATP) (Ma et al., 2020). These findings show that there is still much to be understood about the fundamental

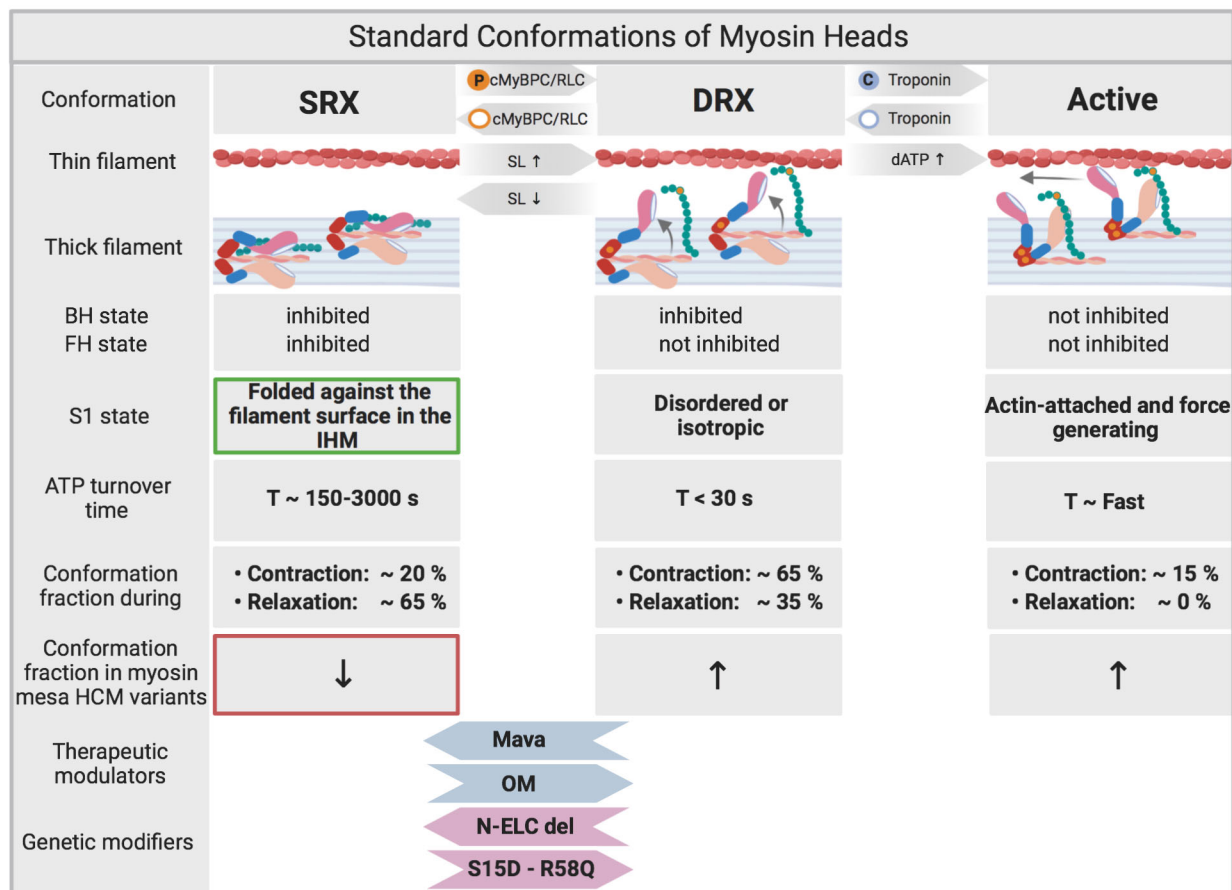
myosin states in relaxed and active muscle. But it seems that myosin SRX is an evolutionarily conserved energy saving mechanism that enables animals to have high-energy efficiency of muscular function, as an adaptation to survive and move in extreme conditions, such as during hibernation (Toepfer et al., 2020), or in the conditions that the wildebeest experiences during periods of nutritional deprivation and high ambient temperature (Curtin et al., 2018). SRX could also be essential in decreasing the metabolic rate of cardiomyocytes during periods of myocardial stress. This is an area of particular interest as myosin SRX regulation has not been studied in a variety of inherited and acquired cardiovascular diseases, including myocardial infarction, atrial fibrillation, heart failure with reduced ejection fraction (HFrEF), heart failure with preserved ejection fraction (HFpEF), amongst other cardiac conditions. One could hypothesise that during myocardial stunning/hibernation post-ischemia that myosin SRX could form as a consequence or in compensation to reduced blood flow. Indeed, if

stunned myocardium does not form SRX one could therapeutically increase myosin SRX acutely to reduce metabolic demand during ischemia.

### The structure of myosin SRX and its relation to the IHM in cardiac muscle

To better understand the role of myosin SRX in cardiac health and disease it is important to have a clear picture of its role within the sarcomere. Myosin SRX is primarily characterized by a decreased ATPase activity, which is correlated to and likely enabled by a specific orientation of myosin heads in relation to the thick filament, where the blocked and free myosin heads bind each other (Fig. 3) (Naber et al., 2011). This observation has been consistent with previously described switched-off states of myosin heads in non-muscle Myosin II forming a characteristic asymmetric J-like conformation which reduces ATP turnover (Craig and Woodhead, 2006; Jung et al., 2008). In the thick filaments of the sarcomere these SRX heads are ordered as they are conformationally uniform due to the intra- and intermolecular interactions that allow the SRX

to form, as previously observed in tarantula muscle (Alamo et al., 2016; Wendt et al., 1999). Here the actin-binding site of the blocked head attaches to the ATP converter region of the free head (Wendt et al., 2001). While doing so the interacting heads of the myosin dimer bend back and bind to the thick filament helical rod-like core (Fig. 2A) (Woodhead et al., 2005). During this asymmetric conformation the phosphorylatable serine of the RLC on the free head becomes more exposed and freely accessible to MLCK whereas the serine on the blocked head becomes less accessible (Alamo et al., 2008). The head-head binding of the myosin dimer is not the only interaction that stabilizes myosin SRX, anchoring interactions between two adjacent myosin dimers have also been observed. These intermolecular binding events are located between the ELC of the blocked head of one myosin dimer and the free head of an adjacent myosin dimer (Woodhead et al., 2005). These intermolecular interactions have been suggested to contribute to cooperative activation in skeletal muscle (McNamara et al., 2015), and cardiac muscle. Cardiac muscle cooperative activation seems to be modulated by RLC phosphorylation, where phosphorylation



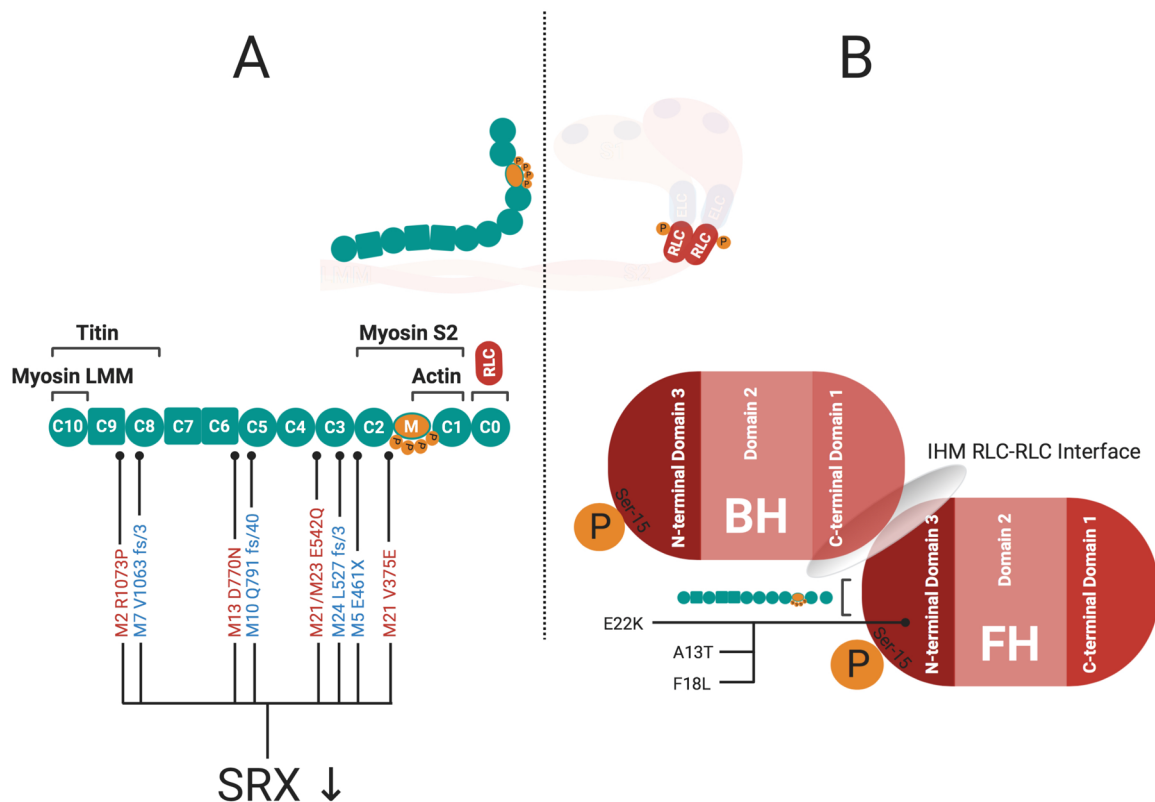
**Fig. 3. Standard conformations of myosin heads.** Physiologically the SRX state and DRX state of myosin is balanced and subject to variation depending on regulating mechanisms such as cRLC and cMyBPC phosphorylation as well as sarcomere length (SL). Calcium binding to Troponin allows myosin heads to bind actin. SRX/DRX/Active states are dynamic over the time course of contraction. The majority of myosin heads remain in the SRX conformation during relaxation (Brunello et al., 2020). Reduced myosin SRX occurs in certain HCM causing mutations including those within the myosin mesa (Fig. 2B). Mavacamten (Alamo et al., 2016) has been shown to increase myosin SRX conformations (Rohde et al., 2018) (Anderson et al., 2018; Green et al., 2016; Olivetto et al., 2020). In contrast, Omecamtiv Mecarbil (OM) activates cardiac muscle contraction (Cleland et al., 2011; Malik et al., 2011; Teerlink et al., 2011, 2016) and decreases the proportion of myosin heads in the SRX conformation. Based on that, a recent study has proven OM to have a positive therapeutic impact in patients with heart failure and reduced ejection fraction (Teerlink et al., 2020). N-terminally truncated ELC has been shown to lead to stabilization of myosin SRX (Sitbon et al., 2020). Constitutive cRLC phosphorylation in the MYL2 R58Q variant has been shown to increase DRX myosin (Yadav et al., 2019a).



prompts alterations in myosin orientation in neighbouring unphosphorylated myosin heads (Kampourakis and Irving, 2015). Five structural intramolecular interactions in total constitute the IHM including the aforementioned head-head interactions (Fig. 2A) (Alamo et al., 2016). The intramolecular interactions are proposed to keep the IHM in a compact off-structure, while the intermolecular interactions are suggested to allow the uniform formation of helical tracks of myosin heads on the surface of the backbone (Alamo et al., 2016; Yang et al., 2020). IHM interactions must be adjustable to regulate and maintain sarcomeric contractility an especially important characteristic to adapt cardiac output in the heart. The IHM regulatory complex includes the ELC, which possess a  $\text{Ca}^{2+}$  binding site, and RLC (Rayment et al., 1993; Scarff et al., 2020), which has a single serine in human myocardium that is phosphorylatable by the cardiac myosin light chain kinase (Chan et al., 2008; Chang et al., 2015; Davis et al., 2001; Ding et al., 2010). In addition to  $\text{Ca}^{2+}$  binding and RLC phosphorylation, stretch activation and mechanosensing have likewise been suggested to trigger the disruption of IHM interactions and thus initiate activation of the thick filament (Padrón et al., 2020). There is still much to be learnt about the IHM and its structural states, interactions, and dynamic physiologically driven regulation as well as its exact role and accountability for the structural and biochemical changes observed in the myosin SRX. Understanding the interplay between its function and structure will allow a deeper insight into disease pathomechanisms.

### Regulation of myosin SRX by protein level phosphorylation, and other key regulators

The RLC and ELC at the neck region of myosin play an essential role in converting chemical energy into mechanical work as they are key players in altering the interactions between actin and myosin. RLC phosphorylation is a requirement for myosin function in smooth (Ikebe et al., 1994) and non-muscle myosin II, but acts as a modulator of contractile force and power in cardiac muscle (Heissler and Manstein, 2013; Toepfer et al., 2013). Phosphorylation of cardiac RLC (cRLC) occurs at serine 15 and is enacted primarily by the cardiac specific myosin light chain kinase (MLCK) (Scruggs et al., 2010). It has been demonstrated by EM and X-ray imaging, that the phosphorylation of RLC by MLCK in the striated muscle of the tarantula results in a loss of the helical ordered assembly of myosin heads, inferring a reduction in the organised myosin SRX (Fig. 3) (Padrón et al., 1991). Colson et al. (2010) have shown similar results by analysing the proximity of myosin cross bridge mass in relation to actin after treatment with cMLCK. cRLC phosphorylation lead to an increased abundance of cross-bridges in proximity to the thin filament reducing myosin SRX in cardiac muscle. The N-terminus of the RLC has shown to be essential to stabilize the IHM off state in smooth and skeletal muscle (Fig. 4) (Ikebe et al., 1994; Nogara et al., 2016). Until recently, it wasn't clear to what extent this N-terminal fragment was needed for regulation of SRX in cardiac muscle. Kampourakis et al. induced a 12 amino acid N-terminal truncation of the cRLC, which increased



**Fig. 4. The location and role of cMyBPC and cRLC mutations in HCM Pathogenicity.** (A) Truncating (blue) and missense (red) mutations in cMyBPC have been linked to C and also shown to reduce SRX proportions in the cardiac muscle (McNamara et al., 2017). It isn't clear how many of the missense variants in MYBPC3 could alter SRX. The cardiac specific CO domain of cMyBPC serves as a binding site for the N-terminal domain 3 of cRLC (Ratti, 2009). (B) The N-terminal domain 3 of cRLC itself contains the regulating phosphorylation site (Toepfer et al., 2013), functions as IHM RLC-RLC interface (Alamo et al., 2016), and has been shown to harbour HCM-related mutations (A13T, F18L, E22K) (Farman et al., 2014; Szczesna-Cordary et al., 2005), where the RLC E22K mutation in particular has been hypothesized to destabilize myosin SRX (Alamo et al., 2017).

contractility much like phosphorylation of WT cRLC, increasing DRX myosin abundance (Kampourakis and Irving, 2015). This indicated that the N-terminus of cRLC in cardiac muscle is required to regulate SRX by stabilizing the parallel state of myosin heads with respect to the thick filament axis. Increasing sarcomere length analogously leads to a greater proportion of DRX myosin heads in the perpendicular state to the thick filament axis (Fig. 3) (Kampourakis et al., 2016). cRLC phosphorylation and sarcomere length act additively to alter DRX abundance and neither can fully activate the thick filament alone (Kampourakis et al., 2016). These findings support the notion that a certain proportion of cross-bridges/myosin heads remain in the SRX conformation (Fig. 1C) (Hooijman et al., 2011).

Structural insights into the IHM and SRX are supported by functional measurements in isolated trabecular muscle, where it has been shown that cRLC phosphorylation increased the rate of force redevelopment after muscle release (Sevrieva et al., 2020 and Toepfer et al., 2016b). This result inferred that the mechanism of action of RLC phosphorylation is the increase in Huxley's  $N_a$  parameter, which is consistent with a reduction of SRX and increase in DRX myosin states (Craig et al., 1987; Nag et al., 2017; Toepfer et al., 2016b). This has recently been validated in a synthetic thick filament system (Gollapudi et al., 2020). In addition phosphorylation of cRLC leads to a higher power production and  $V_{max}$  in trabecular muscle (Toepfer et al., 2013), inferring that cRLC phosphorylation is central to adapting sarcomeric power, which is at least partly mediated by altering IHM interactions. Experiments using the *in-vitro* motility assay found that cRLC phosphorylation increased actin sliding velocities proving that this mechanism is also partially independent of filamentous myosin (Karabina et al., 2015). Taken together, these results indicate the importance of cRLC phosphorylation as a modulator of cardiac contractile performance and suggest that cRLC phosphorylation could be a crucial target in therapeutic interventions. It is becoming clearer that cRLC phosphorylation is responsible for functional and orientational changes to myosin characteristics, but we are yet to establish if cRLC phosphorylation is only responsible for altering myosin states, or if it also alters intrinsic myosin properties such as the power stroke force and kinetics within the filamentous myosin (Greenberg et al., 2014). What complicates matters somewhat with using cRLC phosphorylation as a therapeutic target is the governance of cRLC phosphorylation and de-phosphorylation within the myocardium (Chang et al., 2016), and if the cell will counteract a therapeutic intervention.

Cardiac myosin-binding protein C (cMyBPC) also functions as a modulator of cross-bridge formation and force production (Fig. 3) (McClellan et al., 2001). cMyBPC is structurally integrated in the sarcomeric architecture as it binds to actin and titin in addition to myosin (Fig. 4A) (Lee et al., 2015). The C-terminal part of the protein is bound to the thick filament as observed in intact frog sartorius muscle and rat trabecular preparations (Luther et al., 2008, 2011), while the N-terminus with its cardiac specific C0 domain (Gautel et al., 1995) is attached to both myosin (Gruen and Gautel, 1999) and actin (Barefield and Sadayappan, 2010; Shaffer et al., 2009; van Dijk et al., 2014). Knocking out MYBPC3 in mice showed that sarcomeres without cMyBPC had reduced IHM conformations, which is now rationalised as an inability to form myosin SRX, slowing relaxation (Zoghbi et al., 2008). The structure and function of cMyBPC within the sarcomere is altered by phosphorylation and is likely to be phosphorylation site specific (Previs et al., 2016; Wang et al., 2014). There are several kinases that are able to phosphorylate cMyBPC (Barefield and Sadayappan, 2010). PKA-mediated phosphorylation of cMyBPC has been shown to lead to an increased proximity of cross

bridges to the thin filament, facilitating interactions between myosin and actin (Colson et al., 2008). More recent studies have evaluated the impact of phosphorylation of cMyBPC on the structural state of myosin by specifically substituting the three main phosphorylatable serines of cMyBPC (Ser273, Ser282, Ser302) with either alanine (non-phosphorylatable residue) or aspartic acid (constitutive phosphorylation). These experiments compared the phosphomimetic state of cMyBPC to the nonphosphorylated state. It was found that filaments with the phosphomimetic cMyBPC have myosins that appear more disordered, while dephosphorylated cMyBPC displayed highly ordered cross bridge arrays insinuating an increased proportion of SRX myosins (Kensler et al., 2017). These structural studies have been followed up with single-nucleotide turnover experiments in skinned ventricular preparations where it was found that phosphorylation of cMyBPC reduced SRX myosins, which resulted in increased force production (McNamara et al., 2019). Most notably phosphorylation of Ser282 was sufficient for diminishing SRX (McNamara et al., 2019). Disrupting binding between cMyBPC and myosin subfragment-2 irrespective of cMyBPC phosphorylation results in decreased stabilization of the SRX (McNamara et al., 2019; Toepfer et al., 2019).

During contraction and relaxation the myosin filament backbone itself appears in several regulatory states depending on the different domains of the myosin filament (Fig. 1C) (Brunello et al., 2020). Specific regions of the thick filament have different proportions of myosins in the SRX and DRX states. These regions of the thick filament are regulated and important to its tightly controlled function during contraction and relaxation (Nelson et al., 2020). It is clear that there are many different physiological adapters of myosin SRX and each specific IHM interaction. There is an evolving picture of the importance of these confirmations in terms of contractile output of the sarcomere and energetic demand within the cardiomyocyte. It is not yet clear how these physiologic adapters of SRX and IHM interactions are altered in a variety of human diseases ranging from inherited to acquired conditions such as HCM, DCM, atrial fibrillation, diabetic cardiomyopathy, myocardial infarction, HFpEF and HFrEF.

### The role of myosin SRX in human disease and its utility as a druggable target

The myosin SRX conformation is an intricately controlled state within the myocardium. The interactions that allow the formation of myosin SRX are highly conserved throughout evolution. This has two significances when thinking about human disease (i) dysregulation of the myosin IHM and SRX in either inherited or acquired cardiac conditions would be severely detrimental to maintaining contractile and energetic homeostasis within the cardiomyocyte, and (ii) as a highly conserved set of interactions within the IHM that are central to contractility, relaxation, and energy usage, modulating myosin SRX is an attractive therapeutic target. HCM is estimated to affect at least 1 in 500 individuals (Maron et al., 1995; Semsarian et al., 2015), and is primarily caused by mutations in genes that control and encode sarcomeric proteins (Seidman and Seidman, 2001). The predominance ~50% of HCM mutations are found in the genes encoding human beta cardiac myosin (MYH7) and cMyBPC (MYBPC3). The majority of the remaining HCM variants are found in other sarcomeric proteins such as TNNT2, TNNI3, TPM, MYL2 and MYL3 (Konno et al., 2010). In the case of MYH7 many of the mutations that cause HCM are clustered on a highly conserved surface of the catalytic domain of the myosin head. This region has recently been coined the cardiac myosin mesa and is one of the important IHM interaction sites in the

myosin head domain (Fig. 2B) (Spudich, 2015). The surface of the myosin mesa consists of positively charged domains that could act as binding sites for the negatively charged surfaces on binding partners such as cMyBPC. Based on this it has been hypothesized that HCM causing mutations of the myosin mesa could cause decreased binding affinity for cMyBPC leading to an increased availability of the myosin head for binding to actin and contraction (Spudich, 2015; Trivedi et al., 2018). Pathogenic HCM mutations on the myosin mesa of either the blocked or the free head destabilize major IHM inter- and intramolecular interactions that are also integral for forming SRX (Alamo et al., 2017; Robert-Paganin et al., 2018; Spudich, 2019). Many HCM mutants in the mesa show increased numbers of swaying myosin heads (Adhikari et al., 2019; Sarkar et al., 2020) leading to hypercontractility, impaired relaxation and energy deprivation in cellular models of disease (Toepfer et al., 2020), key clinical phenotypes of HCM (Spudich, 2019; Toepfer et al., 2020). The dysregulation of SRX has been identified as a major determinant of cellular HCM pathophysiology accounting for impaired cellular relaxation, hypercontractility, and metabolic drain within the cell (Toepfer et al., 2020). There are multiple inter- and intramolecular interactions formed by the IHM that lie outside of the mesa that are likely to affect myosin SRX (Alamo et al., 2017; Homburger et al., 2016), which include regions of the myosin tail and S2 that are needed for allowing the sequestration of myosin heads against the thick filament backbone (Fig. 2A) (Adhikari et al., 2019). Outside of the HCM mutations in myosin the predominance of HCM mutations are located in MYBPC3 (Fig. 4A) (van Dijk et al., 2009), another protein that has a central role in the regulation of myosin SRX under physiological conditions. Variants that cause truncations in MYBPC3 predominantly lead to cellular haploinsufficiency (Marston et al., 2009). The lack of cMyBPC in the sarcomere releases the restricting effect of cMyBPC on myosin heads (Moss et al., 2015; Saber et al., 2008; Stelzer et al., 2006) decreasing myosin SRX, increasing contractility and slowing cellular relaxation, both central hallmarks of clinical HCM (McNamara et al., 2016; Toepfer et al., 2019). Certain missense variants found in the MYBPC3 gene cause HCM and have also been identified drivers of SRX depletion (Fig. 4A) (McNamara et al., 2017). But the direct mechanism that causes this is unclear, especially as the missense variants are found throughout the length of cMyBPC, and do not only cluster around the phosphorylation residues or myosin IHM binding regions (Fig. 4A).

As genetic sequencing technologies have evolved there is an ever greater pool of variants discovered in MYH7 and MYBPC3, these more recently identified variants often have no previously identified genotype to phenotype relationship. Identifying a genotype to phenotype relation is important to informing future clinical screening and follow-up. The bottleneck in understanding if a genotype is pathogenic or benign is a new hurdle for the field, where previously genotyping had been key to identifying genetic HCM. It is becoming ever more important to understand pathomechanisms of disease and genotype-phenotype relations when trying to understand HCM, especially as treatments move closer to personalised medicine. Not all variants within a specific gene may cause disease by the same mechanism, and indeed not all HCM variants across the multitude of classical HCM genes are likely to function through the same mechanisms. It is likely that the overarching classification of HCM is in fact a distinct subset of different diseases that manifest as classical HCM phenotypes at the organ level. Many well-studied pathogenic thick filament associated proteins when mutated are likely to interfere with IHM interactions

and destabilise myosin SRX as a driving mechanism. But it is still unclear if IHM destabilization is a driving mechanism in many of the variants of unknown pathogenicity. This includes MYH7 missense variants outside of classical IHM sites, and MYBPC3 missense variants outside of myosin interacting domains (Fig. 4A). This extends to missense variants in MYL2 and MYL3, where the R58Q mutation of cRLC has been shown to increase myosin SRX, whilst structural predictions infer that many cRLC mutations may decrease myosin SRX (Alamo et al., 2017). Deletion of the N-terminus of the ELC has been shown to increase myosin SRX (Fig. 4B) (Sitbon et al., 2020; Yadav et al., 2019a,b). Many of these variants are in close proximity to important IHM forming motifs in the IHM RLC-RLC interface, and may additionally interfere with RLC phosphorylation (Fig. 4B). Further experimental clarification will be needed to determine which of the variants found in these genes alter myosin SRX. In addition to this it is unclear how a significant proportion ~30% of genotype positive HCM cases with variants in TNNT2, TNNI3, TPM and other thin filament associated variants would destabilise the IHM directly, as they do not interfere with residues on myosin. Indeed, it is quite likely that we may see an increase in SRX conformations of myosin in these HCM variants as a compensatory mechanism to reduce inappropriate hypercontractility (Repetti et al., 2019a). There is evidence that this is occurring as cRLC phosphorylation is depleted in thin filament HCM variants (Tucholski et al., 2020). This is an important distinction, both thin and thick filament HCM is typified by a hypercontractile phenotype and poor cellular relaxation, but this simplistically similar phenotype is driven by different mechanisms. These separate mechanisms may explain some of the differences in outcomes between patients that harbour variants in thick versus thin filaments (Coppini et al., 2014). Targeting the myosin ATPase with the allosteric myosin ATPase inhibitor Mavacamten has been shown to increase myosin SRX conformations (Fig. 3) by acting on multiple stages of the myosin chemomechanical cycle (Kawas et al., 2017), and in doing so relieves many of the organ, cellular, energetic, biochemical and also clinically apparent hallmarks of HCM in thick filament models of HCM (Anderson et al., 2018; Green et al., 2016; Jacoby et al., 2018; Olivotto et al., 2020; Rohde et al., 2018; Toepfer et al., 2020, 2019). Mavacamten has also been shown to partially restore  $\text{Ca}^{2+}$ -sensitive molecular and cellular changes of HCM found in the TNNT2 R92Q and the TNNI3 R145G variants (Sparrow et al., 2020). However, a complete resolution of the HCM phenotype was not achieved by Mavacamten in thin filament variants. This may be because the direct mechanism of disease pathogenicity is not addressed by the action of Mavacamten (Repetti et al., 2019a). Thin filament HCM variants may have some benefit from increasing myosin SRX, but a novel therapeutic targeting the thin filament may be needed for complete resolution of sequelae.

In thinking about clinical non-ischemic genetically linked dilated cardiomyopathy (DCM) a disease that afflicts ~1 in 250 people (Hershberger et al., 2013), there is a diverse genetic and environmental architecture that plays a role in disease outcome (McNally and Mestroni, 2017). By definition the disease is diagnosed by a reduction in contractile function characterised by a reduced fractional shortening and ejection fraction (Mestroni et al., 1999). At the cellular level this is often observed as a reduction in contractile function. Targeting myosin SRX in this setting may have a beneficial effect (Mamidi et al., 2017). Reducing myosin SRX would increase the proportion of available heads to form strong cross-bridges, which could salvage systolic insufficiency in DCM. This could be achieved by the positive cardiac inotropic



compound Omeamtiv Mecarbil (OM) (Fig. 3) (Cleland et al., 2011; Kampourakis et al., 2018; Liu et al., 2018; Planelles-Herrero et al., 2017; Swenson et al., 2017; Teerlink et al., 2011; Woody et al., 2018). However, much like HCM, DCM is likely to be comprised of a far more complex sub-set of distinct pathomechanisms, so one drug targeting one specific mechanism may not salvage phenotype in all DCM variants.

Many forms of acquired cardiovascular disease manifest with changes in sarcomeric function. Some of these may be driven by changes in myosin SRX created by compensating levels of cMyBPC and cRLC phosphorylation among other protein modifications within the myocardium. Surviving myocardium within the chronically infarcted heart has been shown to initially compensate and increase power production, which is concomitant with increasing cRLC phosphorylation (Toepfer et al., 2016a). But the time-course of this compensation is not fully understood, with multiple models of myocardial infarction (MI) showing varying degrees of protein phosphorylation level change over time (Avner et al., 2012; van der Velden et al., 2011; Walker et al., 2010). One could hypothesise that post-MI in the failing myocardium there may be some benefit to increasing contractile function of the myocardium by employing OM, but it is not clear if this would eventually have a deleterious effect on cellular function as increasing DRX myosins would increase cellular energy demand on a cell likely to be exhibiting mitochondrial dysfunction (Zhou and Tian, 2018). As we do not yet know the effect of MI on SRX regulation this would be a good place to begin investigating the possible utility of therapeutically altering SRX to compensate heart failure. There is a wide variety of cardiac conditions that are diseases of muscular contractile insufficiency that may be partially mediated by myosin states, these include myocardial stunning (Bolli, 1990), heart failure with preserved ejection fraction (HFpEF) (Hamdani et al., 2013), and heart failure with reduced ejection fraction (HFrEF). MYH7 variants in the mesa have also been shown to have a higher incidence of atrial fibrillation compared to variants outside of the mesa region (Lee et al., 2018a,b). Atrial fibrillation is also a condition that often manifests with changes in cardiac contractile function, and has yet to be explored for changes in myosin SRX. Atrial fibrillation is an important atrial disorder, and we also have little understanding of SRX regulation in the atria as all investigations have used ventricular tissue. Changes in SRX abundance may even be a process that occurs during aging in skeletal muscle (Phung et al., 2018), but this is yet to have been investigated in the heart.

Great strides have been made towards understanding the regulation of muscle contraction in the heart in recent years. The discovery of SRX has provided important insight into the complex regulatory mechanisms of cardiac muscle activity in health and disease, but there is yet much to be understood.

#### Competing interests

C.N.T. has previously consulted for Myokardia Inc. M.S. has no competing interests to disclose.

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#### References

Adhikari, A. S., Trivedi, D. V., Sarkar, S. S., Song, D., Kooiker, K. B., Bernstein, D., Spudich, J. A. and Ruppel, K. M. (2019).  $\beta$ -Cardiac myosin hypertrophic cardiomyopathy mutations release sequestered heads and increase enzymatic activity. *Nat. Commun.* **10**, 2685. doi:10.1038/s41467-019-10555-9

- Alamo, L., Wriggers, W., Pinto, A., Bártoli, F., Salazar, L., Zhao, F.-Q., Craig, R. and Padrón, R. (2008). Three-dimensional reconstruction of tarantula myosin filaments suggests how phosphorylation may regulate myosin activity. *J. Mol. Biol.* **384**, 780-797. doi:10.1016/j.jmb.2008.10.013
- Alamo, L., Qi, D., Wriggers, W., Pinto, A., Zhu, J., Bilbao, A., Gillilan, R. E., Hu, S. and Padrón, R. (2016). Conserved intramolecular interactions maintain myosin interacting-heads motifs explaining tarantula muscle super-relaxed state structural basis. *J. Mol. Biol.* **428**, 1142-1164. doi:10.1016/j.jmb.2016.01.027
- Alamo, L., Ware, J. S., Pinto, A., Gillilan, R. E., Seidman, J. G., Seidman, C. E. and Padrón, R. (2017). Effects of myosin variants on interacting-heads motif explain distinct hypertrophic and dilated cardiomyopathy phenotypes. *Elife* **6**, e24634. doi:10.7554/eLife.24634
- Alamo, L., Pinto, A., Sulbaran, G., Mavarez, J. and Padrón, R. (2018). Lessons from a tarantula: new insights into myosin interacting-heads motif evolution and its implications on disease. *Biophys. Rev.* **10**, 1465-1477. doi:10.1007/s12551-017-0292-4
- Anderson, R. L., Trivedi, D. V., Sarkar, S. S., Henze, M., Ma, W., Gong, H., Rogers, C. S., Gorham, J. M., Wong, F. L., Morck, M. M. et al. (2018). Deciphering the super relaxed state of human  $\beta$ -cardiac myosin and the mode of action of mavacamten from myosin molecules to muscle fibers. *Proc. Natl. Acad. Sci. USA* **115**, E8143-E8152. doi:10.1073/pnas.1809540115
- Avner, B. S., Shioura, K. M., Scruggs, S. B., Grachoff, M., Geenen, D. L., Helseth, D. L., Jr., Farjah, M., Goldspink, P. H. and Solaro, R. J. (2012). Myocardial infarction in mice alters sarcomeric function via post-translational protein modification. *Mol. Cell. Biochem.* **363**, 203-215. doi:10.1007/s11010-011-1172-z
- Bähler, M. and Rhoads, A. (2002). Calmodulin signaling via the IQ motif. *FEBS Lett.* **513**, 107-113. doi:10.1016/S0014-5793(01)03239-2
- Barefield, D. and Sadayappan, S. (2010). Phosphorylation and function of cardiac myosin binding protein-C in health and disease. *J. Mol. Cell. Cardiol.* **48**, 866-875. doi:10.1016/j.jmcc.2009.11.014
- Bloemink, M. J. and Geeves, M. A. (2011). Shaking the myosin family tree: biochemical kinetics defines four types of myosin motor. *Semin. Cell Dev. Biol.* **22**, 961-967. doi:10.1016/j.semcdb.2011.09.015
- Bolli, R. (1990). Mechanism of myocardial "stunning". *Circulation* **82**, 723-738. doi:10.1161/01.CIR.82.3.723
- Brunello, E., Fusi, L., Ghisleni, A., Park-Holohan, S. J., Ovejero, J. G., Narayanan, T. and Irving, M. (2020). Myosin filament-based regulation of the dynamics of contraction in heart muscle. *Proc. Natl. Acad. Sci. USA* **117**, 8177-8186. doi:10.1073/pnas.1920632117
- Caremani, M., Brunello, E., Linari, M., Fusi, L., Irving, T. C., Gore, D., Piazzesi, G., Irving, M., Lombardi, V. and Reconditi, M. (2019). Low temperature traps myosin motors of mammalian muscle in a refractory state that prevents activation. *J. Gen. Physiol.* **151**, 1272-1286. doi:10.1085/jgp.201912424
- Chan, J. Y., Takeda, M., Briggs, L. E., Graham, M. L., Lu, J. T., Horikoshi, N., Weinberg, E. O., Aoki, H., Sato, N., Chien, K. R. et al. (2008). Identification of cardiac-specific myosin light chain kinase. *Circ. Res.* **102**, 571-580. doi:10.1161/CIRCRESAHA.107.161687
- Chang, A. N., Battiprolu, P. K., Cowley, P. M., Chen, G., Gerard, R. D., Pinto, J. R., Hill, J. A., Baker, A. J., Kamm, K. E. and Stull, J. T. (2015). Constitutive phosphorylation of cardiac myosin regulatory light chain in vivo. *J. Biol. Chem.* **290**, 10703-10716. doi:10.1074/jbc.M115.642165
- Chang, A. N., Mahajan, P., Knapp, S., Barton, H., Sweeney, H. L., Kamm, K. E. and Stull, J. T. (2016). Cardiac myosin light chain is phosphorylated by Ca<sup>2+</sup>/calmodulin-dependent and -independent kinase activities. *Proc. Natl. Acad. Sci. USA* **113**, E3824-E3833. doi:10.1073/pnas.1600633113
- Cleland, J. G., Teerlink, J. R., Senior, R., Nifontov, E. M., Mc Murray, J. J., Lang, C. C., Tsyrlin, V. A., Greenberg, B. H., Mayet, J., Francis, D. P. et al. (2011). The effects of the cardiac myosin activator, omeamtiv mecarbil, on cardiac function in systolic heart failure: a double-blind, placebo-controlled, crossover, dose-ranging phase 2 trial. *Lancet* **378**, 676-683. doi:10.1016/S0140-6736(11)61126-4
- Colson, B. A., Bekyarova, T., Locher, M. R., Fitzsimons, D. P., Irving, T. C. and Moss, R. L. (2008). Protein kinase A-mediated phosphorylation of cMyBP-C increases proximity of myosin heads to actin in resting myocardium. *Circ. Res.* **103**, 244-251. doi:10.1161/CIRCRESAHA.108.178996
- Colson, B. A., Locher, M. R., Bekyarova, T., Patel, J. R., Fitzsimons, D. P., Irving, T. C. and Moss, R. L. (2010). Differential roles of regulatory light chain and myosin binding protein-C phosphorylations in the modulation of cardiac force development. *J. Physiol.* **588**, 981-993. doi:10.1113/jphysiol.2009.183897
- Cooke, R. (2011). The role of the myosin ATPase activity in adaptive thermogenesis by skeletal muscle. *Biophys. Rev.* **3**, 33-45. doi:10.1007/s12551-011-0044-9
- Cooke, R., Crowder, M. S. and Thomas, D. D. (1982). Orientation of spin labels attached to cross-bridges in contracting muscle fibres. *Nature* **300**, 776-778. doi:10.1038/300776a0
- Coppini, R., Ho, C. Y., Ashley, E., Day, S., Ferrantini, C., Girolami, F., Tomberli, B., Bardi, S., Torricelli, F., Cecchi, F. et al. (2014). Clinical phenotype and outcome of hypertrophic cardiomyopathy associated with thin-filament gene mutations. *J. Am. Coll. Cardiol.* **64**, 2589-2600. doi:10.1016/j.jacc.2014.09.059
- Craig, R. and Woodhead, J. L. (2006). Structure and function of myosin filaments. *Curr. Opin. Struct. Biol.* **16**, 204-212. doi:10.1016/j.sbi.2006.03.006



- Craig, R., Padrón, R. and Kendrick-Jones, J. (1987). Structural changes accompanying phosphorylation of tarantula muscle myosin filaments. *J. Cell Biol.* **105**, 1319–1327. doi:10.1083/jcb.105.3.1319
- Cross, R. A., Jackson, A. P., Citi, S., Kendrick-Jones, J. and Bagshaw, C. R. (1988). Active site trapping of nucleotide by smooth and non-muscle myosins. *J. Mol. Biol.* **203**, 173–181. doi:10.1016/0022-2836(88)90100-3
- Curtin, N. A., Bartlam-Brooks, H. L. A., Hubel, T. Y., Lowe, J. C., Gardner-Medwin, A. R., Bennitt, E., Amos, S. J., Lorenc, M., West, T. G. and Wilson, A. M. (2018). Remarkable muscles, remarkable locomotion in desert-dwelling wildebeest. *Nature* **563**, 393–396. doi:10.1038/s41586-018-0602-4
- Davis, J. S., Hassanzadeh, S., Winitzky, S., Lin, H., Satorius, C., Vemuri, R., Aletras, A. H., Wen, H. and Epstein, N. D. (2001). The overall pattern of cardiac contraction depends on a spatial gradient of myosin regulatory light chain phosphorylation. *Cell* **107**, 631–641. doi:10.1016/S0092-8674(01)00586-4
- Ding, P., Huang, J., Battiprolu, P. K., Hill, J. A., Kamm, K. E. and Stull, J. T. (2010). Cardiac myosin light chain kinase is necessary for myosin regulatory light chain phosphorylation and cardiac performance in vivo. *J. Biol. Chem.* **285**, 40819–40829. doi:10.1074/jbc.M110.160499
- Farman, G. P., Muthu, P., Kazmierczak, K., Szczesna-Cordary, D. and Moore, J. R. (2014). Impact of familial hypertrophic cardiomyopathy-linked mutations in the NH2 terminus of the RLC on  $\beta$ -myosin cross-bridge mechanics. *J. Appl. Physiol.* (1985) **117**, 1471–1477. doi:10.1152/jappphysiol.00798.2014
- Fusi, L., Huang, Z. and Irving, M. (2015). The conformation of myosin heads in relaxed skeletal muscle: implications for myosin-based regulation. *Biophys. J.* **109**, 783–792. doi:10.1016/j.bpj.2015.06.038
- Gautel, M., Zuffardi, O., Freiburg, A. and Labeit, S. (1995). Phosphorylation switches specific for the cardiac isoform of myosin binding protein-C: a modulator of cardiac contraction? *EMBO J.* **14**, 1952–1960. doi:10.1002/j.1460-2075.1995.tb07187.x
- Gollapudi, S. K., Yu, M., Gan, Q. F. and Nag, S. (2020). Synthetic thick filaments: A new avenue for better understanding the myosin super-relaxed state in healthy, disease, and mavacamten-treated cardiac systems. *J. Biol. Chem.* (in press).
- Golomb, E., Ma, X., Jana, S. S., Preston, Y. A., Kawamoto, S., Shoham, N. G., Goldin, E., Conti, M. A., Sellers, J. R. and Adelstein, R. S. (2004). Identification and characterization of nonmuscle myosin II-C, a new member of the myosin II family. *J. Biol. Chem.* **279**, 2800–2808. doi:10.1074/jbc.M309981200
- González-Solá, M., Al-Khayat, H. A., Behra, M. and Kensler, R. W. (2014). Zebrafish cardiac muscle thick filaments: isolation technique and three-dimensional structure. *Biophys. J.* **106**, 1671–1680. doi:10.1016/j.bpj.2014.01.050
- Green, E. M., Wakimoto, H., Anderson, R. L., Evanchik, M. J., Gorham, J. M., Harrison, B. C., Henze, M., Kawa, R., Oslob, J. D., Rodriguez, H. M. et al. (2016). A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. *Science* **351**, 617–621. doi:10.1126/science.aad3456
- Greenberg, M. J., Shuman, H. and Ostap, E. M. (2014). Inherent force-dependent properties of  $\beta$ -cardiac myosin contribute to the force-velocity relationship of cardiac muscle. *Biophys. J.* **107**, L41–L44. doi:10.1016/j.bpj.2014.11.005
- Gruen, M. and Gautel, M. (1999). Mutations in  $\beta$ -myosin S2 that cause familial hypertrophic cardiomyopathy (FHC) abolish the interaction with the regulatory domain of myosin-binding protein-C. *J. Mol. Biol.* **286**, 933–949. doi:10.1006/jmbi.1998.2522
- Hamdani, N., Bishu, K. G., von Frieling-Salewsky, M., Redfield, M. M. and Linke, W. A. (2013). Deranged myofilament phosphorylation and function in experimental heart failure with preserved ejection fraction. *Cardiovasc. Res.* **97**, 464–471. doi:10.1093/cvr/cvs353
- Heissler, S. M. and Manstein, D. J. (2013). Nonmuscle myosin-2: mix and match. *Cell. Mol. Life Sci.* **70**, 1–21. doi:10.1007/s00018-012-1002-9
- Hershberger, R. E., Hedges, D. J. and Morales, A. (2013). Dilated cardiomyopathy: the complexity of a diverse genetic architecture. *Nat. Rev. Cardiol.* **10**, 531–547. doi:10.1038/nrcardio.2013.105
- Homburger, J. R., Green, E. M., Caleshu, C., Sunitha, M. S., Taylor, R. E., Ruppel, K. M., Metpally, R. P., Colan, S. D., Michels, M., Day, S. M. et al. (2016). Multidimensional structure-function relationships in human  $\beta$ -cardiac myosin from population-scale genetic variation. *Proc. Natl. Acad. Sci. USA* **113**, 6701–6706. doi:10.1073/pnas.1606950113
- Hooijman, P., Stewart, M. A. and Cooke, R. (2011). A new state of cardiac myosin with very slow ATP turnover: a potential cardioprotective mechanism in the heart. *Biophys. J.* **100**, 1969–1976. doi:10.1016/j.bpj.2011.02.061
- Huxley, H. E. (1969). The mechanism of muscular contraction. *Science* **164**, 1356–1365. doi:10.1126/science.164.3886.1356
- Huxley, H. E. and Brown, W. (1967). The low-angle x-ray diagram of vertebrate striated muscle and its behaviour during contraction and rigor. *J. Mol. Biol.* **30**, 383–434. doi:10.1016/S0022-2836(67)80046-9
- Huxley, H. and Hanson, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973–976. doi:10.1038/173973a0
- Huxley, A. F. and Niedergerke, R. (1954). Structural changes in muscle during contraction; interference microscopy of living muscle fibres. *Nature* **173**, 971–973. doi:10.1038/173971a0
- Ikebe, M., Ikebe, R., Kamisoyama, H., Reardon, S., Schwonek, J. P., Sanders, C. R., II and Matsuura, M. (1994). Function of the NH2-terminal domain of the regulatory light chain on the regulation of smooth muscle myosin. *J. Biol. Chem.* **269**, 28173–28180. doi:10.1016/S0021-9258(18)46910-4
- Jacoby DLS, O. A., Wang, A., Young, D., Tripuraneni, R. and Semigran, M. H. S. (2018). Reduction in left ventricular outflow tract gradient with mavacamten (MYK-461) in symptomatic obstructive hypertrophic cardiomyopathy patients (PIONEERHCM). Paper presented at the ACC.18, Orlando, Florida.
- Jung, H. S., Komatsu, S., Ikebe, M. and Craig, R. (2008). Head-head and head-tail interaction: a general mechanism for switching off myosin II activity in cells. *Mol. Biol. Cell* **19**, 3234–3242. doi:10.1091/mbc.e08-02-0206
- Kampourakis, T. and Irving, M. (2015). Phosphorylation of myosin regulatory light chain controls myosin head conformation in cardiac muscle. *J. Mol. Cell. Cardiol.* **85**, 199–206. doi:10.1016/j.yjmcc.2015.06.002
- Kampourakis, T., Sun, Y.-B. and Irving, M. (2016). Myosin light chain phosphorylation enhances contraction of heart muscle via structural changes in both thick and thin filaments. *Proc. Natl. Acad. Sci. USA* **113**, E3039–E3047. doi:10.1073/pnas.1602776113
- Kampourakis, T., Zhang, X., Sun, Y.-B. and Irving, M. (2018). Omecamtiv mercabil and blebbistatin modulate cardiac contractility by perturbing the regulatory state of the myosin filament. *J. Physiol.* **596**, 31–46. doi:10.1113/JP275050
- Karabina, A., Kazmierczak, K., Szczesna-Cordary, D. and Moore, J. R. (2015). Myosin regulatory light chain phosphorylation enhances cardiac  $\beta$ -myosin in vitro motility under load. *Arch. Biochem. Biophys.* **580**, 14–21. doi:10.1016/j.abb.2015.06.014
- Kawas, R. F., Anderson, R. L., Ingle, S. R. B., Song, Y., Sran, A. S. and Rodriguez, H. M. (2017). A small-molecule modulator of cardiac myosin acts on multiple stages of the myosin chemomechanical cycle. *J. Biol. Chem.* **292**, 16571–16577. doi:10.1074/jbc.M117.776815
- Kensler, R. W., Craig, R. and Moss, R. L. (2017). Phosphorylation of cardiac myosin binding protein C releases myosin heads from the surface of cardiac thick filaments. *Proc. Natl. Acad. Sci. USA* **114**, E1355–E1364. doi:10.1073/pnas.1614020114
- Kobirumaki-Shimozawa, F., Inoue, T., Shintani, S. A., Oyama, K., Terui, T., Minamisawa, S., Ishiwata, S. and Fukuda, N. (2014). Cardiac thin filament regulation and the Frank-Starling mechanism. *J. Physiol. Sci.* **64**, 221–232. doi:10.1007/s12576-014-0314-y
- Konno, T., Chang, S., Seidman, J. G. and Seidman, C. E. (2010). Genetics of hypertrophic cardiomyopathy. *Curr. Opin. Cardiol.* **25**, 205–209. doi:10.1097/HCO.0b013e3283375698
- Kühne, W. (1864). *Untersuchungen über das Protoplasma und die Contractilität*. Leipzig: W. Engelmann.
- Lee, K., Harris, S. P., Sadayappan, S. and Craig, R. (2015). Orientation of myosin binding protein C in the cardiac muscle sarcomere determined by domain-specific immuno-EM. *J. Mol. Biol.* **427**, 274–286. doi:10.1016/j.jmb.2014.10.023
- Lee, K. H., Sulbaran, G., Yang, S., Mun, J. Y., Alamo, L., Pinto, A., Sato, O., Ikebe, M., Liu, X., Korn, E. D. et al. (2018a). 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. doi:10.1073/pnas.1715247115
- Lee, S.-P., Ashley, E. A., Homburger, J., Caleshu, C., Green, E. M., Jacoby, D., Colan, S. D., Arteaga-Fernandez, E., Day, S. M., Girolami, F. et al. (2018b). Incident atrial fibrillation is associated With MYH7 sarcomeric gene variation in hypertrophic cardiomyopathy. *Circ. Heart Fail.* **11**, e005191. doi:10.1161/CIRCHEARTFAILURE.118.005191
- Liu, C., Kawana, M., Song, D., Ruppel, K. M. and Spudich, J. A. (2018). Controlling load-dependent kinetics of  $\beta$ -cardiac myosin at the single-molecule level. *Nat. Struct. Mol. Biol.* **25**, 505–514. doi:10.1038/s41594-018-0069-x
- Luther, P. K., Bennett, P. M., Knupp, C., Craig, R., Padrón, R., Harris, S. P., Patel, J. and Moss, R. L. (2008). Understanding the organisation and role of myosin binding protein C in normal striated muscle by comparison with MyBP-C knockout cardiac muscle. *J. Mol. Biol.* **384**, 60–72. doi:10.1016/j.jmb.2008.09.013
- Luther, P. K., Winkler, H., Taylor, K., Zoghbi, M. E., Craig, R., Padrón, R., Squire, J. M. and Liu, J. (2011). Direct visualization of myosin-binding protein C bridging myosin and actin filaments in intact muscle. *Proc. Natl. Acad. Sci. USA* **108**, 11423–11428. doi:10.1073/pnas.1103216108
- Ma, W., Childers, M., Murray, J., Moussavi-Harami, F., Gong, H., Weiss, R., Daggett, V., Irving, T. and Regnier, M. (2020). Myosin dynamics during relaxation in mouse soleus muscle and modulation by 2'-deoxy-ATP. *J. Physiol.* **598**, S165–S182. doi:10.1113/JP280402
- Malik, F. I., Hartman, J. J., Elias, K. A., Morgan, B. P., Rodriguez, H., Brejc, K., Anderson, R. L., Sueoka, S. H., Lee, K. H., Finer, J. T. et al. (2011). Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science* **331**, 1439–1443. doi:10.1126/science.1200113
- Mamidi, R., Li, J., Gresham, K. S., Verma, S., Doh, C. Y., Li, A., Lal, S., Dos Remedios, C. G. and Stelzer, J. E. (2017). Dose-dependent effects of the myosin activator omecamtiv mercabil on cross-bridge behavior and force generation in failing human myocardium. *Circ. Heart Fail.* **10**, e004257. doi:10.1161/CIRCHEARTFAILURE.117.004257

- Marcucci, L., Washio, T. and Yanagida, T. (2019). Proposed mechanism for the length dependence of the force developed in maximally activated muscles. *Sci. Rep.* **9**, 1317. doi:10.1038/s41598-018-36706-4
- Maron, B. J., Gardin, J. M., Flack, J. M., Gidding, S. S., Kurosaki, T. T. and Bild, D. E. (1995). Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary artery risk development in (Young) adults. *Circulation* **92**, 785-789. doi:10.1161/01.cir.92.4.785
- Marston, S., Copeland, O., Jacques, A., Livesey, K., Tsang, V., McKenna, W. J., Jalilzadeh, S., Carballo, S., Redwood, C. and Watkins, H. (2009). Evidence from human myectomy samples that MYBPC3 mutations cause hypertrophic cardiomyopathy through haploinsufficiency. *Circ. Res.* **105**, 219-222. doi:10.1161/CIRCRESAHA.109.202440
- McClellan, G., Kulikovskaya, I. and Winegrad, S. (2001). Changes in cardiac contractility related to calcium-mediated changes in phosphorylation of myosin-binding protein C. *Biophys. J.* **81**, 1083-1092. doi:10.1016/S0006-3495(01)75765-7
- McNally, E. M. and Mestroni, L. (2017). Dilated cardiomyopathy: genetic determinants and mechanisms. *Circ. Res.* **121**, 731-748. doi:10.1161/CIRCRESAHA.116.309396
- McNamara, J. W., Li, A., Dos Remedios, C. G. and Cooke, R. (2015). The role of super-relaxed myosin in skeletal and cardiac muscle. *Biophys. Rev.* **7**, 5-14. doi:10.1007/s12551-014-0151-5
- McNamara, J. W., Li, A., Smith, N. J., Lal, S., Graham, R. M., Kooiker, K. B., van Dijk, S. J., Remedios, C. G. D., Harris, S. P. and Cooke, R. (2016). Ablation of cardiac myosin binding protein-C disrupts the super-relaxed state of myosin in murine cardiomyocytes. *J. Mol. Cell. Cardiol.* **94**, 65-71. doi:10.1016/j.jmcc.2016.03.009
- McNamara, J. W., Li, A., Lal, S., Bos, J. M., Harris, S. P., van der Velden, J., Ackerman, M. J., Cooke, R. and Dos Remedios, C. G. (2017). MYBPC3 mutations are associated with a reduced super-relaxed state in patients with hypertrophic cardiomyopathy. *PLoS ONE* **12**, e0180064. doi:10.1371/journal.pone.0180064
- McNamara, J. W., Singh, R. R. and Sadayappan, S. (2019). Cardiac myosin binding protein-C phosphorylation regulates the super-relaxed state of myosin. *Proc. Natl. Acad. Sci. USA* **116**, 11731-11736. doi:10.1073/pnas.1821660116
- Mestroni, L., Maisch, B., McKenna, W. J., Schwartz, K., Charron, P., Rocco, C., Tesson, F., Richter, A., Wilke, A. and Komajda, M. (1999). Guidelines for the study of familial dilated cardiomyopathies. Collaborative research group of the European human and capital mobility project on familial dilated cardiomyopathy. *Eur. Heart J.* **20**, 93-102. doi:10.1053/ehj.1998.1145
- Moss, R. L., Fitzsimons, D. P. and Ralphe, J. C. (2015). Cardiac MyBP-C regulates the rate and force of contraction in mammalian myocardium. *Circ. Res.* **116**, 183-192. doi:10.1161/CIRCRESAHA.116.300561
- Myburgh, K. H., Franks-Skiba, K. and Cooke, R. (1995). Nucleotide turnover rate measured in fully relaxed rabbit skeletal muscle myofibrils. *J. Gen. Physiol.* **106**, 957-973. doi:10.1085/jgp.106.5.957
- Naber, N., Cooke, R. and Pate, E. (2011). Slow myosin ATP turnover in the super-relaxed state in tarantula muscle. *J. Mol. Biol.* **411**, 943-950. doi:10.1016/j.jmb.2011.06.051
- Nag, S., Trivedi, D. V., Sarkar, S. S., Adhikari, A. S., Sunitha, M. S., Sutton, S., Ruppel, K. M. and Spudich, J. A. (2017). The myosin mesa and the basis of hypercontractility caused by hypertrophic cardiomyopathy mutations. *Nat. Struct. Mol. Biol.* **24**, 525-533. doi:10.1038/nsmb.3408
- Nelson, S. R., Li, A., Beck-Previs, S., Kennedy, G. G. and Warshaw, D. M. (2020). Imaging ATP consumption in resting skeletal muscle: one molecule at a time. *Biophys. J.* **119**, 1050-1055. doi:10.1016/j.bpj.2020.07.036
- Nogara, L., Naber, N., Pate, E., Canton, M., Reggiani, C. and Cooke, R. (2016). Spectroscopic studies of the super relaxed state of skeletal muscle. *PLoS ONE* **11**, e0160100. doi:10.1371/journal.pone.0160100
- Odonitz, F. and Kollmar, M. (2007). Drawing the tree of eukaryotic life based on the analysis of 2,269 manually annotated myosins from 328 species. *Genome Biol.* **8**, R196. doi:10.1186/gb-2007-8-9-r196
- Olivotto, I., Oreziak, A., Barriaes-Villa, R., Abraham, T. P., Masri, A., Garcia-Pavia, P., Saberi, S., Lakdawala, N. K., Wheeler, M. T., Owens, A. et al. (2020). Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **396**, 759-769. doi:10.1016/S0140-6736(20)31792-X
- Padrón, R., Pante, N., Sosa, H. and Kendrick-Jones, J. (1991). X-ray diffraction study of the structural changes accompanying phosphorylation of tarantula muscle. *J. Muscle Res. Cell Motil.* **12**, 235-241. doi:10.1007/BF01745112
- Padrón, R., Ma, W., Duno-Miranda, S., Koubassova, N., Lee, K. H., Pinto, A., Alamo, L., Bolanos, P., Tsaturyan, A., Irving, T. et al. (2020). The myosin interacting-heads motif present in live tarantula muscle explains tetanic and posttetanic phosphorylation mechanisms. *Proc. Natl. Acad. Sci. USA* **117**, 11865-11874. doi:10.1073/pnas.1921312117
- Phung, L. A., Karvinen, S. M., Colson, B. A., Thomas, D. D. and Lowe, D. A. (2018). Age affects myosin relaxation states in skeletal muscle fibers of female but not male mice. *PLoS ONE* **13**, e0199062. doi:10.1371/journal.pone.0199062
- Pinto, A., Sanchez, F., Alamo, L. and Padrón, R. (2012). The myosin interacting-heads motif is present in the relaxed thick filament of the striated muscle of scorpion. *J. Struct. Biol.* **180**, 469-478. doi:10.1016/j.jsb.2012.08.010
- Planelles-Herrero, V. J., Hartman, J. J., Robert-Paganin, J., Malik, F. I. and Houdusse, A. (2017). Mechanistic and structural basis for activation of cardiac myosin force production by omecamtiv mecarbil. *Nat. Commun.* **8**, 190. doi:10.1038/s41467-017-00176-5
- Pollard, T. D. (2020). Myosins in Cytokinesis. *Adv. Exp. Med. Biol.* **1239**, 233-244. doi:10.1007/978-3-030-38062-5\_11
- Previs, M. J., Mun, J. Y., Michalek, A. J., Previs, S. B., Gulick, J., Robbins, J., Warshaw, D. M. and Craig, R. (2016). Phosphorylation and calcium antagonistically tune myosin-binding protein C's structure and function. *Proc. Natl. Acad. Sci. USA* **113**, 3239-3244. doi:10.1073/pnas.1522236113
- Ratti, J. (2009). *Investigation of Cardiac Myosin Binding protein C (cMyBPC) Domains and their Interactions. (Doctor of Philosophy)*, University of Leicester.
- Rayment, I., Rypniewski, W. R., Schmidt-Base, K., Smith, R., Tomchick, D. R., Benning, M. M., Winkelmann, D. A., Wesenberg, G. and Holden, H. M. (1993). Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* **261**, 50-58. doi:10.1126/science.8316857
- Reconditi, M., Caremani, M., Pinzauti, F., Powers, J. D., Narayanan, T., Stienen, G. J., Linari, M., Lombardi, V. and Piazzi, G. (2017). Myosin filament activation in the heart is tuned to the mechanical task. *Proc. Natl. Acad. Sci. USA* **114**, 3240-3245. doi:10.1073/pnas.1619484114
- Repetti, G., Toepfer, C. N., Garfinkel, A. C., Bellini, V., Padhila, K., Wilcox, J., Venturini, G., Seidman, J. and Seidman, C. (2019a). Abstract 10964: the biophysical consequences of thin filament hypertrophic cardiomyopathy mutations. *Circulation* **140**. doi:10.1161/res.125.suppl\_1.339
- Repetti, G., Toepfer, C. N., Seidman, J. G. and Seidman, C. E. (2019b). Novel Therapies for Prevention and Early Treatment of Cardiomyopathies. *Circ. Res.* **124**, 1536-1550. doi:10.1161/CIRCRESAHA.119.313569
- Robert-Paganin, J., Auguin, D. and Houdusse, A. (2018). Hypertrophic cardiomyopathy disease results from disparate impairments of cardiac myosin function and auto-inhibition. *Nat. Commun.* **9**, 4019. doi:10.1038/s41467-018-06191-4
- Rohde, J. A., Roopnarine, O., Thomas, D. D. and Muretta, J. M. (2018). Mavacamten stabilizes an autoinhibited state of two-headed cardiac myosin. *Proc. Natl. Acad. Sci. USA* **115**, E7486-E7494. doi:10.1073/pnas.1720342115
- Saber, W., Begin, K. J., Warshaw, D. M. and VanBuren, P. (2008). Cardiac myosin binding protein-C modulates actomyosin binding and kinetics in the in vitro motility assay. *J. Mol. Cell. Cardiol.* **44**, 1053-1061. doi:10.1016/j.jmcc.2008.03.012
- Sarkar, S. S., Trivedi, D. V., Morck, M. M., Adhikari, A. S., Pasha, S. N., Ruppel, K. M. and Spudich, J. A. (2020). The hypertrophic cardiomyopathy mutations R403Q and R663H increase the number of myosin heads available to interact with actin. *Sci. Adv.* **6**, eaax0069. doi:10.1126/sciadv.aax0069
- Scarff, C. A., Carrington, G., Casas-Mao, D., Chalovich, J. M., Knight, P. J., Ranson, N. A. and Peckham, M. (2020). Structure of the shutdown state of myosin-2. *Nature* **588**, 515-520. doi:10.1038/s41586-020-2990-5
- Scruggs, S. B., Reisdorph, R., Armstrong, M. L., Warren, C. M., Reisdorph, N., Solaro, R. J. and Buttrick, P. M. (2010). A novel, in-solution separation of endogenous cardiac sarcomeric proteins and identification of distinct charged variants of regulatory light chain. *Mol. Cell. Proteomics* **9**, 1804-1818. doi:10.1074/mcp.M110.000075
- Seidman, J. G. and Seidman, C. (2001). The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell* **104**, 557-567. doi:10.1016/S0092-8674(01)00242-2
- Sellers, J. R. (2000). Myosins: a diverse superfamily. *Biochim. Biophys. Acta* **1496**, 3-22. doi:10.1016/S0167-4889(00)00005-7
- Semsarian, C., Ingles, J., Maron, M. S. and Maron, B. J. (2015). New perspectives on the prevalence of hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* **65**, 1249-1254. doi:10.1016/j.jacc.2015.01.019
- Sevrieva, I. R., Brandmeier, B., Ponnamp, S., Gautel, M., Irving, M., Campbell, K. S., Sun, Y. B. and Kampourakis, T. (2020). Cardiac myosin regulatory light chain kinase modulates cardiac contractility by phosphorylating both myosin regulatory light chain and troponin I. *J. Biol. Chem.* **295**, 4398-4410. doi:10.1074/jbc.RA119.011945
- Shaffer, J. F., Kensler, R. W. and Harris, S. P. (2009). The myosin-binding protein C motif binds to F-actin in a phosphorylation-sensitive manner. *J. Biol. Chem.* **284**, 12318-12327. doi:10.1074/jbc.M808850200
- Sitbon, Y. H., Kazmierczak, K., Liang, J., Yadav, S., Veerasammy, M., Kanashiro-Takeuchi, R. M. and Szczesna-Cordary, D. (2020). Ablation of the N terminus of cardiac essential light chain promotes the super-relaxed state of myosin and counteracts hypercontractility in hypertrophic cardiomyopathy mutant mice. *FEBS J.* **287**, 3989-4004. doi:10.1111/febs.15243
- Sparrow, A. J., Watkins, H., Daniels, M. J., Redwood, C. and Robinson, P. (2020). Mavacamten rescues increased myofilament calcium sensitivity and dysregulation of Ca(2+) flux caused by thin filament hypertrophic cardiomyopathy mutations. *Am. J. Physiol. Heart Circ. Physiol.* **318**, H715-H722. doi:10.1152/ajpheart.00023.2020



- Spudich, J. A.** (2015). The myosin mesa and a possible unifying hypothesis for the molecular basis of human hypertrophic cardiomyopathy. *Biochem. Soc. Trans.* **43**, 64-72. doi:10.1042/BST20140324
- Spudich, J. A.** (2019). Three perspectives on the molecular basis of hypercontractility caused by hypertrophic cardiomyopathy mutations. *Pflügers Archiv*. **471**, 701-717. doi:10.1007/s00424-019-02259-2
- Stelzer, J. E., Fitzsimons, D. P. and Moss, R. L.** (2006). Ablation of myosin-binding protein-C accelerates force development in mouse myocardium. *Biophys. J.* **90**, 4119-4127. doi:10.1529/biophysj.105.078147
- Stewart, M. A., Franks-Skiba, K., Chen, S. and Cooke, R.** (2010). Myosin ATP turnover rate is a mechanism involved in thermogenesis in resting skeletal muscle fibers. *Proc. Natl. Acad. Sci. USA* **107**, 430-435. doi:10.1073/pnas.0909468107
- Sulbarán, G., Mun, J. Y., Lee, K. H., Alamo, L., Pinto, A., Sato, O., Ikebe, M., Liu, X., Korn ED, P. R. and Craig, R.** (2015). The inhibited, interactingheads motif characterizes myosin II from the earliest animals with muscle. *Biophys. J.* **108**, 301a. doi:10.1016/j.bpj.2014.11.1639
- Swenson, A. M., Tang, W., Blair, C. A., Fetrow, C. M., Unrath, W. C., Previs, M. J., Campbell, K. S. and Yengo, C. M.** (2017). Omecamtiv mecarbil enhances the duty ratio of human beta-cardiac myosin resulting in increased calcium sensitivity and slowed force development in cardiac muscle. *J. Biol. Chem.* **292**, 3768-3778. doi:10.1074/jbc.M116.748780
- Szczesna-Cordary, D., Guzman, G., Zhao, J., Hernandez, O., Wei, J. and Diaz-Perez, Z.** (2005). The E22K mutation of myosin RLC that causes familial hypertrophic cardiomyopathy increases calcium sensitivity of force and ATPase in transgenic mice. *J. Cell Sci.* **118**, 3675-3683. doi:10.1242/jcs.02492
- Teerlink, J. R., Clarke, C. P., Saikali, K. G., Lee, J. H., Chen, M. M., Escandon, R. D., Elliott, L., Bee, R., Habibzadeh, M. R., Goldman, J. H. et al.** (2011). Dose-dependent augmentation of cardiac systolic function with the selective cardiac myosin activator, omecamtiv mecarbil: a first-in-man study. *Lancet* **378**, 667-675. doi:10.1016/S0140-6736(11)61219-1
- Teerlink, J. R., Felker, G. M., McMurray, J. J., Solomon, S. D., Adams, K. F., Jr, Cleland, J. G., Ezekowitz, J. A., Goudev, A., Macdonald, P., Metra, M. et al.** (2016). Chronic Oral Study of Myosin Activation to Increase Contractility in Heart Failure (COSMIC-HF): a phase 2, pharmacokinetic, randomised, placebo-controlled trial. *Lancet* **388**, 2895-2903. doi:10.1016/S0140-6736(16)32049-9
- Teerlink, J. R., Diaz, R., Felker, G. M., McMurray, J. J. V., Metra, M., Solomon, S. D., Adams, K. F., Anand, I., Arias-Mendoza, A., Biering-Sorensen, T. et al.** (2020). Cardiac myosin activation with omecamtiv mecarbil in systolic heart failure. *N. Engl. J. Med.* **384**, 105-116. doi:10.1056/NEJMoa2025797
- Thomas, D. D. and Cooke, R.** (1980). Orientation of spin-labeled myosin heads in glycerinated muscle fibers. *Biophys. J.* **32**, 891-906. doi:10.1016/S0006-3495(80)85024-7
- Toepfer, C., Caorsi, V., Kampourakis, T., Sikkil, M. B., West, T. G., Leung, M. C., Al-Saud, S. A., MacLeod, K. T., Lyon, A. R., Marston, S. B. et al.** (2013). Myosin regulatory light chain (RLC) phosphorylation change as a modulator of cardiac muscle contraction in disease. *J. Biol. Chem.* **288**, 13446-13454. doi:10.1074/jbc.M113.455444
- Toepfer, C. N., Sikkil, M. B., Caorsi, V., Vydyanath, A., Torre, I., Copeland, O., Lyon, A. R., Marston, S. B., Luther, P. K., Macleod, K. T. et al.** (2016a). A post-MI power struggle: adaptations in cardiac power occur at the sarcomere level alongside MyBP-C and RLC phosphorylation. *Am. J. Physiol. Heart Circ. Physiol.* **311**, H465-H475. doi:10.1152/ajpheart.00899.2015
- Toepfer, C. N., West, T. G. and Ferenczi, M. A.** (2016b). Revisiting frank-starling: regulatory light chain phosphorylation alters the rate of force redevelopment (ktr) in a length-dependent fashion. *J. Physiol.* **594**, 5237-5254. doi:10.1113/JP272441
- Toepfer, C. N., Wakimoto, H., Garfinkel, A. C., McDonough, B., Liao, D., Jiang, J., Tai, A. C., Gorham, J. M., Lunde, I. G., Lun, M. et al.** (2019). Hypertrophic cardiomyopathy mutations in MYBPC3 dysregulate myosin. *Sci. Transl. Med.* **11**, eaat1199. doi:10.1126/scitranslmed.aat1199
- Toepfer, C. N., Garfinkel, A. C., Venturini, G., Wakimoto, H., Repetti, G., Alamo, L., Sharma, A., Agarwal, R., Ewoldt, J. F., Cloonan, P. et al.** (2020). Myosin sequestration regulates sarcomere function, cardiomyocyte energetics, and metabolism, informing the pathogenesis of hypertrophic cardiomyopathy. *Circulation* **141**, 828-842. doi:10.1161/CIRCULATIONAHA.119.042339
- Trivedi, D. V., Adhikari, A. S., Sarkar, S. S., Ruppel, K. M. and Spudich, J. A.** (2018). Hypertrophic cardiomyopathy and the myosin mesa: viewing an old disease in a new light. *Biophys. Rev.* **10**, 27-48. doi:10.1007/s12551-017-0274-6
- Trivedi, D. V., Nag, S., Spudich, A., Ruppel, K. M. and Spudich, J. A.** (2020). The myosin family of mechanoenzymes: from mechanisms to therapeutic approaches. *Annu. Rev. Biochem.* **89**, 667-693. doi:10.1146/annurev-biochem-011520-105234
- Tucholski, T., Cai, W., Gregorich, Z. R., Bayne, E. F., Mitchell, S. D., McIlwain, S. J., de Lange, W. J., Wrobbel, M., Karp, H., Hite, Z. et al.** (2020). Distinct hypertrophic cardiomyopathy genotypes result in convergent sarcomeric proteoform profiles revealed by top-down proteomics. *Proc. Natl. Acad. Sci. USA*. **117**, 24691-24700. doi:10.1073/pnas.2006764117
- van der Velden, J., Merkus, D., de Beer, V., Hamdani, N., Linke, W. A., Boontje, N. M., Stienen, G. J. and Duncker, D. J.** (2011). Transmural heterogeneity of myofibrillar function and sarcomeric protein phosphorylation in remodeled myocardium of pigs with a recent myocardial infarction. *Front. Physiol.* **2**, 83. doi:10.3389/fphys.2011.00083
- van Dijk, S. J., Bezold, K. L. and Harris, S. P.** (2014). Earning stripes: myosin binding protein-C interactions with actin. *Pflügers Archiv*. **466**, 445-450. doi:10.1007/s00424-013-1432-8
- van Dijk, S. J., Dooijes, D., dos Remedios, C., Michels, M., Lamers, J. M., Winegrad, S., Schlossarek, S., Carrier, L., ten Cate, F. J., Stienen, G. J. et al.** (2009). Cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy: haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. *Circulation* **119**, 1473-1483. doi:10.1161/CIRCULATIONAHA.108.838672
- Vicente-Manzanares, M., Ma, X., Adelstein, R. S. and Horwitz, A. R.** (2009). Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat. Rev. Mol. Cell Biol.* **10**, 778-790. doi:10.1038/nrm2786
- Walker, L. A., Walker, J. S., Ambler, S. K. and Buttrick, P. M.** (2010). Stage-specific changes in myofilament protein phosphorylation following myocardial infarction in mice. *J. Mol. Cell. Cardiol.* **48**, 1180-1186. doi:10.1016/j.yjmcc.2009.09.010
- Wang, L., Ji, X., Barefield, D., Sadayappan, S. and Kawai, M.** (2014). Phosphorylation of cMyBP-C affects contractile mechanisms in a site-specific manner. *Biophys. J.* **106**, 1112-1122. doi:10.1016/j.bpj.2014.01.029
- Wendt, T., Taylor, D., Messier, T., Trybus, K. M. and Taylor, K. A.** (1999). Visualization of head-head interactions in the inhibited state of smooth muscle myosin. *J. Cell Biol.* **147**, 1385-1390. doi:10.1083/jcb.147.7.1385
- Wendt, T., Taylor, D., Trybus, K. M. and Taylor, K.** (2001). Three-dimensional image reconstruction of dephosphorylated smooth muscle heavy meromyosin reveals asymmetry in the interaction between myosin heads and placement of subfragment 2. *Proc. Natl. Acad. Sci. USA* **98**, 4361-4366. doi:10.1073/pnas.071051098
- Woodhead, J. L., Zhao, F. Q. and Craig, R.** (2013). Structural basis of the relaxed state of a Ca<sup>2+</sup>-regulated myosin filament and its evolutionary implications. *Proc. Natl. Acad. Sci. USA* **110**, 8561-8566. doi:10.1073/pnas.1218462110
- Woodhead, J. L., Zhao, F.-Q., Craig, R., Egelman, E. H., Alamo, L. and Padrón, R.** (2005). Atomic model of a myosin filament in the relaxed state. *Nature* **436**, 1195-1199. doi:10.1038/nature03920
- Woody, M. S., Greenberg, M. J., Barua, B., Winkelmann, D. A., Goldman, Y. E. and Ostap, E. M.** (2018). Positive cardiac inotrope omecamtiv mecarbil activates muscle despite suppressing the myosin working stroke. *Nat. Commun.* **9**, 3838. doi:10.1038/s41467-018-06193-2
- Yadav, S., Kazmierczak, K., Liang, J., Sitbon, Y. H. and Szczesna-Cordary, D.** (2019a). Phosphomimetic-mediated in vitro rescue of hypertrophic cardiomyopathy linked to R58Q mutation in myosin regulatory light chain. *FEBS J.* **286**, 151-168. doi:10.1111/febs.14702
- Yadav, S., Yuan, C.-C., Kazmierczak, K., Liang, J., Huang, W., Takeuchi, L. M., Kanashiro-Takeuchi, R. M. and Szczesna-Cordary, D.** (2019b). Therapeutic potential of AAV9-S15D-RLC gene delivery in humanized MYL2 mouse model of HCM. *J. Mol. Med.* **97**, 1033-1047. doi:10.1007/s00109-019-01791-z
- Yang, S., Tiwari, P., Lee, K. H., Sato, O., Ikebe, M., Padrón, R. and Craig, R.** (2020). Cryo-EM structure of the inhibited (10S) form of myosin II. *Nature*. **588**, 521-525. doi:10.1038/s41586-020-3007-0
- Zhao, F.-Q., Craig, R. and Woodhead, J. L.** (2009). Head-head interaction characterizes the relaxed state of Limulus muscle myosin filaments. *J. Mol. Biol.* **385**, 423-431. doi:10.1016/j.jmb.2008.10.038
- Zhou, B. and Tian, R.** (2018). Mitochondrial dysfunction in pathophysiology of heart failure. *J. Clin. Investig.* **128**, 3716-3726. doi:10.1172/JCI120849
- Zoghbi, M. E., Woodhead, J. L., Moss, R. L. and Craig, R.** (2008). Three-dimensional structure of vertebrate cardiac muscle myosin filaments. *Proc. Natl. Acad. Sci. USA* **105**, 2386-2390. doi:10.1073/pnas.0708912105