Understanding Cardiomyopathy Phenotypes Based on the Functional Impact of Mutations in the Myosin Motor

Jeffrey R. Moore, Leslie Leinwand, David M. Warshaw

Abstract: Hypertrophic (HCM) and dilated (DCM) cardiomyopathies are inherited diseases with a high incidence of death due to electric abnormalities or outflow tract obstruction. In many of the families afflicted with either disease, causative mutations have been identified in various sarcomeric proteins. In this review, we focus on mutations in the cardiac muscle molecular motor, myosin, and its associated light chains. Despite the >300 identified mutations, there is still no clear understanding of how these mutations within the same myosin molecule can lead to the dramatically different clinical phenotypes associated with HCM and DCM. Localizing mutations within myosin’s molecular structure provides insight into the potential consequence of these perturbations to key functional domains of the motor. Review of biochemical and biophysical data that characterize the functional capacities of these mutant myosins suggests that mutant myosins with enhanced contractility lead to HCM, whereas those displaying reduced contractility lead to DCM. With gain and loss of function potentially being the primary consequence of a specific mutation, how these functional changes trigger the hypertrophic response and lead to the distinct HCM and DCM phenotypes will be the future investigative challenge. (Circ Res. 2012;111:375-385.)

Key Words: familial hypertrophic cardiomyopathy ■ dilated cardiomyopathy ■ myosin ■ super-relaxed state ■ cardiac energetics ■ strain-dependent kinetics ■ mutation

Dilated and hypertrophic cardiomyopathies (DCM and HCM, respectively) are the 2 most common forms of genetic heart muscle disease. Although the mechanisms underlying HCM and DCM phenotypes are complex and incompletely understood, the principle pathology is manifest at the level of the ventricle in both diseases.¹ HCM affects nearly 0.5% of the general population,² contributes to a significant percentage of sudden unexpected

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cardiac death in any age group, and is a cause of debilitating cardiac symptoms. This disease is characterized by hypertrophy, especially of the ventricles and interventricular septum, due to aberrant sarcomere replication and excessive connective tissue deposition. Hundreds of mutations, affecting at least 20 different genes have been identified, all of which affect proteins in the cardiac muscle sarcomere.\(^5,6\) The sarcomeric proteins associated with HCM perform different functions, including enzymatic and force-generating roles (β-cardiac myosin, myosin light chains, actin), structural scaffolds (myosin binding protein C, actin, titin, desmin), and regulatory proteins (tropomyosin, troponin, and myosin binding protein C).

In contrast to HCM, DCM is characterized by a thinner than normal ventricular wall and can culminate in heart failure. Although the genetics of DCM mutations are more complex than those of HCM, DCM is also due to aberrant sarcomere replication and excessive connective tissue deposition. Hundreds of mutations, affecting at least 20 different genes, have been identified, all of which affect proteins in the cardiac muscle sarcomere.\(^5,6\) The sarcomeric proteins associated with DCM perform different functions, including enzymatic and force-generating roles (β-cardiac myosin, myosin light chains, actin), structural scaffolds (myosin binding protein C, actin, titin, desmin), and regulatory proteins (tropomyosin, troponin, and myosin binding protein C).

Although many mutations have been associated specifically with either HCM or DCM, mutations in a single protein can lead to either disease. The underlying molecular basis for the differing HCM and DCM phenotypes is unknown, and there is no clear mechanistic hypothesis for how individual mutations could lead to such divergent phenotypes. Several mechanistic pathways have been identified including alterations in acto-myosin force generation,\(^7\) transmission of the generated forces\(^8,9\) disruptions in calcium homeostasis,\(^10–12\) and myocardial energetics.\(^13\) We present an overview of the HCM and DCM mutations in myosin, the force producing molecular motor of cardiac muscle, and provide evidence that altered contractile properties of mutant myosin lead to altered myocardial contraction and ATP consumption, suggesting that altered myocardial energy usage ultimately leads to the disease phenotype.\(^13,14\)

**Myosin: The Cardiac Muscle Force-Generating Molecular Motor**

Cardiac muscle contraction is powered by the ATP-dependent interaction of myosin with actin. Myosin, the major component of the thick filament, consists of 2 heavy chains (MyHCs) and 4 light chains (2 regulatory light chains [RLCs] and 2 essential light chains [ELCs]). Each MyHC associates with 1 of each light chain and 2 MyHC-LC complexes self-associate via an α-helical coil-coil domain to form a dimeric myosin molecule (Figure 1A). The myosin molecule can be divided into the head, neck, and tail domains. An alternating 28 residue pseudo repeat of the α-helical coiled-coil tail domain drives myosin dimers to assemble in a head-to-tail fashion, forming the core of the sarcomere thick filaments. The head domain is more globular in shape and can be further separated into the motor domain and the neck domain. The motor domain contains the ATPase and actin binding regions of MyHC and has been shown to be the minimal subdomain sufficient for, albeit compromised, movement and force production.\(^15\) To achieve full motion generation, small conformational changes in the myosin head are converted into large movements by the myosin neck region acting as a lever (Figure 1B).\(^16–20\) The ELC and RLC wrap around the lever arm, supporting the neck and imparting stiffness to the lever arm.\(^18,21,22\) The proteolytic subfragment that contains the motor and neck domains has historically been referred to as the subfragment 1 (S1); a domain predicted to be a less stable coiled-coil region that connects S1 to the tail domain is referred to as subfragment 2 (S2).

**Many Disease-Causing Mutations Map to the Myosin Gene**

Numerous biophysical and biochemical studies have revealed important information about the function of myosin molecular motors.\(^23,24\) However, being such a large molecule, systematic site-by-site genetic mutagenesis and characterization of each of myosin’s 2002 residues would be an impractical task. With HCM and DCM, Mother Nature has already done this series of experiments for us, presenting scientists with a set of mutations that alter protein function in a way that ultimately leads to disease. It is important to note that the mutations probably highlight residues that are critical for modulating function, whereas mutation of the residues essential for function probably results in embryonic lethality.
HCM Mutations: Gain of Function Versus Loss of Function?

Velocity and ATPase

An attractive early hypothesis for HCM was that reduced enzymatic/contractile activity of mutant myosin molecules leads to compensatory hypertrophy to counteract the contractile deficit. Consistent with this notion, several early studies of R403Q mutant β-myosin isolated from either human leg and ventricular muscles (ie, the same isoform of cardiac β-myosin is expressed in the slow soleus leg muscle) revealed a severe decrease in the velocity of actin filaments over a coverslip surface coated with mutant myosin, which serves as a model system for muscle contraction under unloaded conditions. However, these initial attempts at determining the molecular defects in myosin that give rise to HCM were confounded by the difficulties in studying this large complex molecule in the small biopsies available from patients. In the intervening years, several studies using transgenic mice and myosin expressed in vitro have led to a different picture where instead of reducing contractility many of the HCM mutations enhance activity as measured by increased hydrolytic or force and motion producing capabilities either in the active or relaxed states (Table).

Despite the fact that modifying genes may alter the ability of transgenic mouse models to develop a phenotype typical of the human disease, the first mouse model for HCM showed that mice heterozygous for the R403Q mutation developed a hypertrophic phenotype with myocyte disarray, resembling the human disease. Subsequent structural studies found that R403Q mutant smooth muscle myosin S1 was attached to actin at highly variable angles compared with wild-type, suggesting that the histopathologic myocyte disarray may be linked to an underlying disarray at the acto-myosin level. Functional studies showed that the R403Q mutation increased actin filament velocity ($V_{\text{actin}}$) using $\alpha$-cardiac or smooth muscle myosin heavy-chain backbones, but caution is warranted given the observation that expression of the R403Q mutation in the mouse $\alpha$-cardiac myosin showed increased $V_{\text{actin}}$, whereas in the mouse $\beta$-cardiac myosin there was no significant effect. This result in and of itself suggests that mutations would best be studied in the context of the human $\beta$-myosin backbone. However, the enhanced contractile properties observed in vitro were consistent with the observation that hearts from the mutant R403Q mice exhibited an accelerated systolic pressure rise and increased contractile dynamics. Interestingly, the enhancement in enzymatic activity observed with the R403Q mutation was subsequently observed for several other HCM-associated mutations. Myosin purified from hearts of patients heterozygous for the D906G and L908V mutations had a 34% and 24% increase, respectively, in $V_{\text{actin}}$ when compared with normal controls. Similarly, in vitro–expressed myosin containing the D778G mutation or myosin isolated from hearts harboring the R403W, R403Q, and R453C mutations exhibit an increase in contractility as measured by $V_{\text{actin}}$, ATPase and/or power output. Skinned fiber studies have also shown an increased ATPase and unloaded shortening velocity...
for the R719W and R723G mutations, respectively, and faster tension development and relaxation in human cardiac myofibrils with the R403Q mutation. Similarly, cultured mouse myoblasts expressing mutant HCM myosin also revealed that unloaded shortening velocity was increased for the D778G mutant, whereas velocity was unchanged for the G741R mutant. Thus, several mutant myosins either expressed in vitro, purified from mouse cardiac tissue, or studied in the myofibrillar environment display enhanced ATPase activity, tension development and relaxation kinetics, and/or unloaded velocity.

### Isometric Force and Calcium Sensitivity

HCM mutations in myosin have also been shown to have effects on the sensitivity of the contractile apparatus to calcium, isometric force generation, and myosin stiffness (Table). Similar to what was observed for unloaded velocity and ATPase, the majority of the myosin mutations studied resulted in increases in force (Table). For example, the R403Q and R453C mutations result in an increased force and power production. Similarly, 2 mutations in the converter region displayed an increase in isometric force, which resulted from an increase in myosin stiffness.

#### Table. HCM- and DCM-Associated Mutations

<table>
<thead>
<tr>
<th>HCM mutation</th>
<th>Myosin</th>
<th>Experiment</th>
<th>Velocity</th>
<th>ATPase</th>
<th>Contractile Kinetics</th>
<th>Duty Ratio</th>
<th>Force</th>
<th>( \mu \text{Ca}_{50} )</th>
<th>Stiffness, ( K_{myo} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys207Asn</td>
<td>Human β-cardiac</td>
<td>Motility</td>
<td>No change</td>
<td></td>
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<td></td>
<td></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Gly256Glu</td>
<td>Human slow skeletal (β-MHC)</td>
<td>Fiber</td>
<td>No change</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>No change</td>
<td></td>
</tr>
<tr>
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<td>Human β-cardiac</td>
<td>Myofibril</td>
<td>Increase</td>
<td>Decrease</td>
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<td>No change</td>
<td></td>
</tr>
<tr>
<td>Arg403Gln</td>
<td>Chicken embryonic</td>
<td>Motility</td>
<td>Increase</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>No change</td>
<td></td>
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<tr>
<td>Gly584Arg</td>
<td>Chicken embryonic</td>
<td>Motility</td>
<td>Decrease</td>
<td>Increase</td>
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<td></td>
<td></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Arg719Trp</td>
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<td>Fiber</td>
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<td>Increase</td>
<td></td>
<td></td>
<td></td>
<td>Increase</td>
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<tr>
<td>Arg719Trp</td>
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<td>Decrease</td>
<td>Increase</td>
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<td></td>
<td></td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
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<td>Fiber</td>
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<td>Increase</td>
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<td></td>
<td>Increase</td>
<td>Increase</td>
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<tr>
<td>Gly412Arg</td>
<td>Human slow skeletal (β-MHC)</td>
<td>Fiber</td>
<td>Decrease</td>
<td>Increase</td>
<td></td>
<td></td>
<td></td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Gly412Arg</td>
<td>Mouse β-cardiac</td>
<td>Fiber</td>
<td>No change</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>Increase</td>
<td>Increase</td>
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<tr>
<td>Asp778Gly</td>
<td>Chicken smooth muscle myosin</td>
<td>Fiber</td>
<td>Increase</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>Increase</td>
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</tr>
<tr>
<td>Asp778Gly</td>
<td>Mouse β-cardiac</td>
<td>Fiber</td>
<td>Increase</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Asp906Gly</td>
<td>Human β-cardiac</td>
<td>Motility</td>
<td>Increase</td>
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<td></td>
<td></td>
<td>No change</td>
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<tr>
<td>Leu908Val</td>
<td>Human β-cardiac</td>
<td>Motility</td>
<td>Increase</td>
<td>No change</td>
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<td></td>
<td></td>
<td>No change</td>
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</tr>
<tr>
<td>Leu908Val</td>
<td>Human β-cardiac</td>
<td>Motility</td>
<td>Increase</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>No change</td>
<td></td>
</tr>
<tr>
<td>Asp906Gly/Leu908Val</td>
<td>Human β-cardiac</td>
<td>Motility</td>
<td>Increase</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
<td>No change</td>
<td></td>
</tr>
</tbody>
</table>

| DCM mutation | Motility | Decrease | Decrease | Decrease |                      |            |       | No change |                   |
| Ser523Pro    | Motility | Decrease | Decrease | Increase |                      |            |       | Increase |                   |
| Phe764Leu    | Motility | Decrease | Decrease | Increase |                      |            |       | Increase |                   |
In vitro studies have also revealed that many HCM mutations result in an increased calcium sensitivity of the contractile apparatus. Several myosin mutations are among them. The R403Q mutation and the R453C mutation increase calcium sensitivity of isometric force production. Similarly, a mutation in the converter region, I736T, increased calcium sensitivity and demonstrated significant active force even under relaxing conditions. It is important to note, however, that increased calcium sensitivity is not the universal cause of HCM, as there are reports of several mutations having no effect or even decreased calcium sensitivity.

With HCM mutations affecting velocity, ATPase, force, and calcium sensitivity of activation, a major question remains as to how these phenotypes could lead to HCM. One unifying hypothesis consistent with most of the data is that the mutations (Table) result in increased contractile activity.

**DCM Mutations**

Although it has been established for the past 2 decades that HCM and DCM are “diseases of the sarcomere,” the pathway that leads from the individual mutant gene to the distinct cardiac phenotypes is largely unknown. Unlike the numerous studies of the HCM causing mutations described above and elsewhere, information about the contractile alterations in DCM mutant myosins is only recently emerging (Table). Mouse models of the DCM-causing mutations S532P and F764L both faithfully recapitulate the human disease phenotype. Interestingly, unlike the myosin mutations associated with HCM, myosin purified from hearts expressing the S532P and F764L mutations showed a contractile deficit. Mutant S532P and F764L myosin propelled actin filaments at 57% and 80%, respectively, the velocity of wild-type myosin. AT-

Pase and power output were also similarly reduced. Cellular contractile measurements revealed similar deficits in performance that preceded the onset of cardiac dilation, indicating that the contractile deficit is the primary insult and not a consequence of the dilated phenotype.

Thus, these data support the idea that the primary insult for the HCM and DCM mutations in the myosin motor domain is an alteration in myosin motor activity and that the resulting effects on contractility at the cellular and organ levels precede the disease phenotype. Mutations that enhance motor activity lead to HCM while mutations that diminish motor function lead to DCM.

**Mutations in the Light Chains: A Common Theme for HCM?**

The myosin light chains function to support the elongated α-helical domain that extends from the motor domain. Given the importance of the myosin neck/lever in force and motion generation, it is not surprising that several mutations in the myosin light chains and the myosin neck region were found to be associated with HCM. Similarly, analogous to the light chains, numerous MyHC mutations probably function to destabilize the proximal portion of the helix.

Myosin light chains belong to the EF-hand family of calcium-binding proteins. With the exception of the N-terminal EF-hand of the RLC, the EF-hands of the myosin light chains have lost their ability to bind calcium or magnesium. Mutation of the divalent metal binding site of the RLC has been shown to alter myosin cross-bridge attachment and detachment kinetics, indicating that an intact N-terminal EF-hand is critical to proper function of myosin.

Thirteen mutations have been identified in the myosin light chains: 8 mutations in the RLC and 5 mutations in the ELC. The ELC mutations are located primarily in exons 3 and 4 (Figure 3) indicating that while incapable of binding calcium, the EF hand motifs of the ELC play an important structural role. Data on the effects of ELC mutations on myosin enzymology are limited but, consistent with the increased contractility hypothesis, one ELC mutation, M149V, has been shown to have a 40% increase in Vactin compared with control myosin.

Mutations identified in the RLC are located throughout the molecule (Figure 3). The positions of the known RLC mutations are mapped onto the crystal structure of the chicken skeletal regulatory domain shown in Figure 3. The mutations appear to be clustered in 3 regions: near the phosphorylatable serine (A13T, F18L, and E22K), the calcium/magnesium binding site (E22K, N47K, and K58Q), and the linker/C-terminal regions of the RLC (P95A, K104E, and D166V). Although not studied as thoroughly as the MyHC mutations, there is strong evidence that LC mutations affect several aspects of myosin structure and function. We will discuss N47K and R58Q, 2 of the most malignant mutations in the RLC, focusing on how they alter the sensitivity of myosin biochemistry to strain and ultimately support the gain of function hypothesis for HCM.

When the N47K and R58Q RLC mutations are exchanged onto a β-MyHC backbone, there is no effect on Vactin or ATPase activity, seemingly at conflict with the gain of function hypothesis. It is important to point out, however, that these experiments were performed under unloaded conditions.

Because the heart must work to pump blood during systole, myosin will always contract against a load and thus the loaded kinetics reflect the ability of myosin to produce work and power under more physiological conditions. Myosin’s ability to perform mechanical work and power is driven by...
ATP hydrolysis and requires the coupling of its multistep enzymatic and mechanical cycles. In 1923, Fenn showed that the kinetics of the myosin molecule are modulated by strain. ADP release from myosin’s catalytic site has been shown to be the rate limiting step for detachment kinetics in myosin and thus a principal determinant of Vactin. Thus, when exogenously added ADP competes for ATP binding, velocity is slowed (K_i = 160 ± 10 μmol/L; Figure 4A). On the other hand, in vitro motility assays examining Vactin as a function of ADP concentration in the presence of load show that load causes a significant 3-fold increase in the inhibition constant (K_i = 400 ± 60 μmol/L; Figure 4A). To explain this result, it was proposed that straining the myosin traps ADP in the myosin catalytic site preventing its release and rendering it relatively insensitive to exogenously added ADP. This result is consistent with a strain-dependent isomerization in the ADP bound state. Interestingly the N47K and R58Q mutations lack the load dependent increase in the K_i (Figure 4B and 4C), consistent with the idea that the RLC mutations reduce myosin stiffness and disrupt transfer of load to the active site.

In the healthy heart, straining the myosin molecule causes the myosin cross-bridges to cycle more slowly, thus hydrolyzing less ATP. It has been suggested that this physiologically important strain dependence underlies the hyperbolic shape of the force velocity curve and also serves to increase the economy of maintaining and generating tension in loaded muscle. The increase in the economy of tension development is critical for proper functioning of the heart. The lack of strain dependence in the mutant myosins harboring the N47K and R58Q mutant RLCs would result in an unusually high ATP consumption (like that described above for the MyHC mutations) but only clearly observed under more physiological loaded conditions.

Therefore, defects in strain-dependent mechanochemistry can contribute to disease by causing the mutant myosins to hydrolyze more ATP at physiological loads. Interestingly, the sites of HCM mutations in the head domain, as discussed above, map out the critical mechanical communication pathways between the catalytic site and more distant domains that are involved with force transmission and sensing. Furthermore, the data also suggest that future in vitro and in vivo studies examining mutation-based defects in contractility must not only be characterized under unloaded conditions, but also load-dependent changes in myosin mechanochemistry should be considered.

**Mutations in the Myosin Rod Affect Assembly and Stability**

Whereas the mechanisms by which mutations in the myosin motor domain are readily interpreted based on predicted effects on motor function, the pathogenic mechanisms of mutations in the rod and S2 portions of the myosin molecule are less clear. It has been proposed that mutations in the myosin head/motor domain lead to a distinct phenotype compared with those in the rod. The myosin rod is an α-helical coiled-coil protein that self-associates to form the bipolar thick filament. α-Helical coiled-coil proteins are characterized by a common heptad amino acid repeat sequence (abcdefg)n. The a and d positions of the coiled-coil are typically hydrophobic residues that stabilize the interaction interface between the coiled-coil helices, whereas oppositely charged residues in the e and g positions form salt bridges that stabilize the hydrophobic core (Figure 5).

Dimeric myosin molecules assemble into helical-bipolar thick filaments with a helical repeat of 43 nm and an axial stagger of 14.3 nm between myosin molecules. Assembly is a property of the isolated light meromyosin portion of the
myosin rod and is thought to be driven by favorable electrostatic interactions along repeating 28-residue charge zones and the presence of the so-called assembly competent domain. To date, 112 mutations have been identified in the rod region. Overall, the mutations are distributed throughout the heptad positions although mutations in the d positions are more rare and appear to be clustered in the N-terminal rod segments. Interestingly, with the exception of G1057S, mutations in the d and f positions are absent between residues 931 and 1414 (Figure 5). A1379T and S1776G were among the first mutations to be identified in the myosin rod region and were proposed to disrupt thick filament assembly properties. Consistent with this notion, the c position mutation, E1356K, was shown to destabilize the protein and reduce filament formation without any detectable changes in secondary structure. Molecular dynamics simulations of myosin rod mutations have also been used to assay the nanomechanical properties of the human cardiac S2 domain. These models predicted that the R1193S mutant S2 domain was considerably stiffer than wild-type. Proper myosin rod domain structure, mechanics, and stability are probably critical for both proper thick filament assembly and interaction with accessory proteins such as MyBP-C, MyBP-H, myomesin, titin, and myosin head domains (see super-relaxed state below). In addition, forces either generated by the myosin itself or imposed externally as a load to the muscle are transmitted through the myosin filament backbone. Therefore, thick filament stability is critical to the stiffness of the thick filament and its ability to transmit force, providing another potential means of altering the kinetics of the myosin motor itself, which are load sensitive (see above). Biophysical and biochemical studies of myosin rod domain mutations are emerging and likely to provide critical understandings of thick filament assembly and the molecular underpinnings of the mutation phenotype.

Myosin Interaction Motifs in the Thick Filament May Affect the ATPase Activity of “Super-Relaxed State”

Cardiac muscle contraction is regulated primarily by calcium binding to the thin filament. However, there is increasing evidence that cardiac muscle force can be modulated at the level of the thick filament. In this regard, recent evidence indicates the presence of at least 2 biochemical states of the relaxed striated muscle thick filament: one state having a rapid ATPase with a time constant similar to the ATP turnover of isolated myosin in the absence of actin and another with a time constant ~5-fold longer, the so-called “super-relaxed state.” The super-relaxed state is not present with isolated myosin but is found when myosin is assembled into thick filaments, suggesting that there is probably a structural component involving a potential interaction between the myosin heads and/or the filament backbone. Head-head interactions have been observed for smooth muscle myosin that appear to be responsible for inhibition of ATPase activity. Specifically, the actin-binding domain of one head (ie, blocked head) binds the converter domain of its dimeric partner head (ie, free head), thus preventing ATPase activity in both heads (see Figure 6). Recent evidence from thick filaments suggests that these interactions represent a general mechanism for regulating myosin activity in striated muscles as well (Figure 6). Therefore, mutation-induced disruption of thick filament regulation could alter energy consumption by the heart.

Interacting Head Motif in Cardiac Muscle Thick Filaments

Electron microscopy has revealed an ordered structural motif in which myosin heads interact helically along the backbone of the mouse cardiac muscle thick filament. Molecular modeling and structural studies have revealed that ionic interactions at head-head and head-tail interfaces are important for the interacting head regulatory motif. Specific interactions were found between D748 in the converter domain of the free head and K368 of the blocked head, and that R406 and/or K416 of the blocked head may interact with E169 of the free head. Interestingly, these residues map in (or very near) some of the regions with increased HCM mutation frequency also shown in Figure 2. Correcting for species differences, mouse R406 corresponds to the well-known human R403Q cardiomyopathy mutation, suggesting that some aspects of the R403Q mutant phenotype could result
from altered head-head interactions. Consistent with this notion, the R719W HCM mutation (near the putative D748/K368 interaction) was not regulated by phosphorylation when incorporated into smooth muscle myosin. 35

A motor domain/S2-rod segment interaction has also been proposed to be an important component of the interacting head motif. 76,80 The primary interaction has been proposed to be between a positive patch on myosin in the actin binding loop and a patch of negative charged residues residing in the N-terminal portion of S2. Again, these regions of the molecule are the ones highlighted in Figure 2. Of these, one of the best-studied mutants is the L908V mutation. Therefore, in addition to the current known effects of the L908V mutation on myosin contractility, consideration of the potential effects of the mutation on relaxed head-head and head-tail interactions are probably important for understanding the mutant phenotype.

In cardiac muscle the ordered array of myosin molecules on the thick filament, and ultimately the relaxed myosin ATPase, is dynamic and can be modulated by several factors including myosin binding protein C 81,82 and myosin RLC phosphorylation. 83 In skeletal muscle, the super-relaxed state has been proposed to play an important role in thermogenesis. 71 In cardiac muscle, transition from the super relaxed state has been proposed to be an important component of the recruitment of cross-bridges out of the relaxed states to states that hydrolyze more ATP. 72,73 Myocardial energy utilization could then be varied by sequestering a population of heads into an inactive state where they have a very low rate of energy utilization. Thus, an HCM mutation– induced disruption of the distribution of heads between relaxed and super-relaxed states could potentially lead to hypertrophy via an altered energetic state of the heart. 13

Summary/Conclusions

In summary, studies of cardiomyopathy-linked mutations in myosin over the past 2 decades have begun to shed light on the underlying molecular cause of cardiomyopathy. As science often does, early attractive hypotheses that hypertrophic cardiomyopathy results from a myocardial compensatory response to compromised myosin molecular motor function have yet to be realized. Instead, a preponderance of data indicate that myosin with HCM mutations has enhanced activity and the relatively small number of DCM mutations studied display reduced contractility. The increased activity in HCM could result from several sources: increased ATPase activity (in both the actin bound and unbound states), velocity, and/or force under loaded and unloaded conditions. However, it seems likely that firm conclusions must await the analysis of mutations in the appropriate human β-myosin backbone. 36

Physiologically, the increased enzymatic activity observed in studies of HCM-mutant myosin and mutant mycardium has been proposed to decrease the concentration of free ATP and result in the accumulation of hydrolysis products, ADP and Pi. 84,85 Consistent with this idea, the resulting decrease in the free energy available from ATP hydrolysis has been correlated with the progression of disease. 86 The free energy from ATP hydrolysis is required for several critical processes in the heart, and disruption of these processes may lead to the development of the disease phenotype. For example, the reduction of sarcoplasmic calcium levels during relaxation requires the activity of the sarcoplasmic reticular ATPase, the function of which can be compromised by reductions in phosphorylation potential. Furthermore, deficient calcium sequestering would lead to improper relaxation of the heart and diastolic dysfunction. This effect of incomplete relaxation would be more pronounced, with the high levels of diastolic calcium resulting from impaired calcium sequestration. Interestingly, in support of the energy depletion hypothesis for HCM, 13 several non-myosin mutations that disrupt energy production in the myocardium have been shown to produce a similar phenotype. 87–90 Future studies are required to determine the mechanistic link that leads to the common consequence of these disparate protein mutations. Studies of Mother Nature’s experiment are beginning to shed light on the underlying molecular cause of cardiomyopathy and in the meantime continue to provide important insight into the molecular mechanism of cardiac myosin function.

Clinical Insights

Individuals with known cardiomyopathy-causing mutations often do not develop overt symptoms until adulthood or are at times asymptomatic, presumably due to epigenetic or environmental influences in the development of the disease phenotype. Clinically, this represents a challenge regarding follow-up strategy for families with HCM. Current guidelines 91,92 recommend genetic screening and recent studies indicate that the addition of molecular genetic screening to the management and diagnosis of HCM is the more likely to be cost effective than clinical tests alone. 93,94 With genotyping feasible for some individuals with HCM and DCM, one question that arises is whether knowing the genetic cause of the disease will lead to better and potentially preventive treatments. Currently, disease management for HCM is primarily focused on alleviating symptoms via β-blockade and/or Ca2+ channel blockers. 95 These treatments as well as reduced exercise and antiarrhythmic drugs have been used to reduce the incidence of sudden cardiac death. For patients judged to be at high risk, an implantable defibrillator can be used. 96 The mechanism for altered electric activity and ultimately ventricular fibrillation is not known but could result from altered calcium buffering by sarcomeric proteins, as was proposed for HCM-linked mutations in the RLC. 97 In cases of HCM where removal of a severe obstruction is required, surgical myectomy or alcohol ablation has been shown to be somewhat effective in terms of symptom relief. 98,99

Newer treatments being proposed and developed include miRNA antagonists, angiotensin II receptor blockade, and small-molecule myosin activators. Recent evidence indicates a role for miRNAs in the development of cardiac hypertrophy and failure. Although MyHC mRNAs themselves have not been shown to be direct targets of any miRNAs, it is clear that regulation of MyHC expression by miRNAs is important. Both α- and β-MyHC encode miRNAs, miR-208a and miR-208b, respectively. 100 Intriguingly, animal studies have shown that inactivation of miR-208a prevents the pathologi-
clical induction of β-MyHC, cardiac hypertrophy and fibrosis without any observable toxicity, suggesting that miRNAs can be good targets for treatment of heart disease. Clinical trials of angiotensin II receptor blockade, based on research using a mouse model of HCM caused by a mutation in α-cardiac MyHC, also show some promise, with one trial showing a reduction in hypertrophy, mostly in patients with myosin mutations. Recently, the small-molecule myosin activator omecamtiv mecarbil has been shown to be effective as a treatment for systolic heart failure. However, use of a myosin activator as a treatment option requires an in-depth understanding of the underlying molecular defect on myosin mecanochemical function. For example, such a treatment probably should only be considered in treating patients with myosin mutations that result in a loss of function to the motor. Thus, we are now at the exciting crossroad where molecular-level information about actomyosin defects are poised to guide the development and administration of appropriate individual therapy.

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