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Multiscale biophysical models of cardiomyopathies reveal complexities challenging existing dogmas

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ABSTRACT Mutations in sarcomeric proteins, including myosin, cause a variety of cardiomyopathies. A prominent hypothesis has been that myosin mutations causing hypercontractility of the motor lead to hypertrophic cardiomyopathy, while those causing hypocontractility lead to dilated cardiomyopathy; however, recent biophysical studies using multiscale computational and experimental models have revealed complexities not captured by this hypothesis. We summarize recent publications in *Biophysical Journal* challenging this dogma and highlighting the need for multiscale modeling of these complex diseases.

Cardiomyopathies (CM), the leading causes of heart failure and sudden death, can be caused by mutations in sarcomeric proteins, including cardiac myosin. Recent studies in *Biophysical Journal* have revealed new insights into the molecular basis of these diseases and described new tools for studying these mutations across increasing scales of organization.

Familial cardiomyopathies are relatively common forms of heart disease affecting >1:250 individuals (1). They are divided into categories based on their differential effects on ventricular remodeling including hypertrophic (HCM), dilated (DCM), restrictive, and left ventricular noncompaction cardiomyopathies. Clinical studies have defined the genetic landscape of these diseases and shown that point mutations in cardiac myosin (*MYH7*) are prominent causes of all four of these diseases (2); however, it is not well understood how genotype relates to phenotype. As a consequence, genetic testing is generally not used to inform patient care. Improving our understanding of the connections between genotype and phenotype could help 1) better classify variants of unknown significance, 2) improve monitoring and/or treatment of genotype-positive patients before the onset of ventricular remodeling, and 3) set the stage for the development of novel therapeutics that improve outcomes for patients based on genotype.

The two most common forms of CM are DCM, clinically characterized by dilation of the left ventricle and reduced cardiac contractility, and HCM, characterized by hypertrophy of the ventricular wall and preserved or enhanced cardiac contractility. Excellent in vitro biophysical and biochemical reconstitution studies have shown that CM mutations in sarcomeric proteins, including cardiac myosin, can affect molecular-based contractility (2,3). Based on these studies and the contractile phenotype seen in patients, a prominent hypothesis has emerged that HCM is caused by molecular hypercontractility while DCM is caused by molecular hypocontractility. In the case of mutations in cardiac myosin, it has been assumed that HCM mutations increase motor function while DCM mutations decrease it (Fig. 1 *A*).

While this hypothesis has been widely cited to explain the effects of myosin mutations, recent studies employing multiscale experimental and computational models have demonstrated complexities not captured by this hypothesis (Fig. 1 *B*). First, mutations in myosin can cause all four CM types, and this cannot be explained by a hypothesis with only two outcomes. Moreover, the field has now developed more sophisticated experimental models, and these models have shown that different phenotypes can be observed at the level of single molecules, macromolecular assemblies, cells, tissues, and organs. Here, we describe several recent publications in *Biophysical Journal* describing experimental and computational approaches that challenge existing dogma and reveal new insights into these complex diseases.

Tang et al. combine several in vitro techniques including steady state and stopped flow kinetics, fluorescence resonance energy transfer, and in vitro motility assays to characterize the properties of cardiac myosin motors with mutations in their converter domains, R723G associated with HCM and F764L associated with DCM (4). At first glance, these mutations appear to follow the standard model, with the HCM mutant showing an increased ADP release

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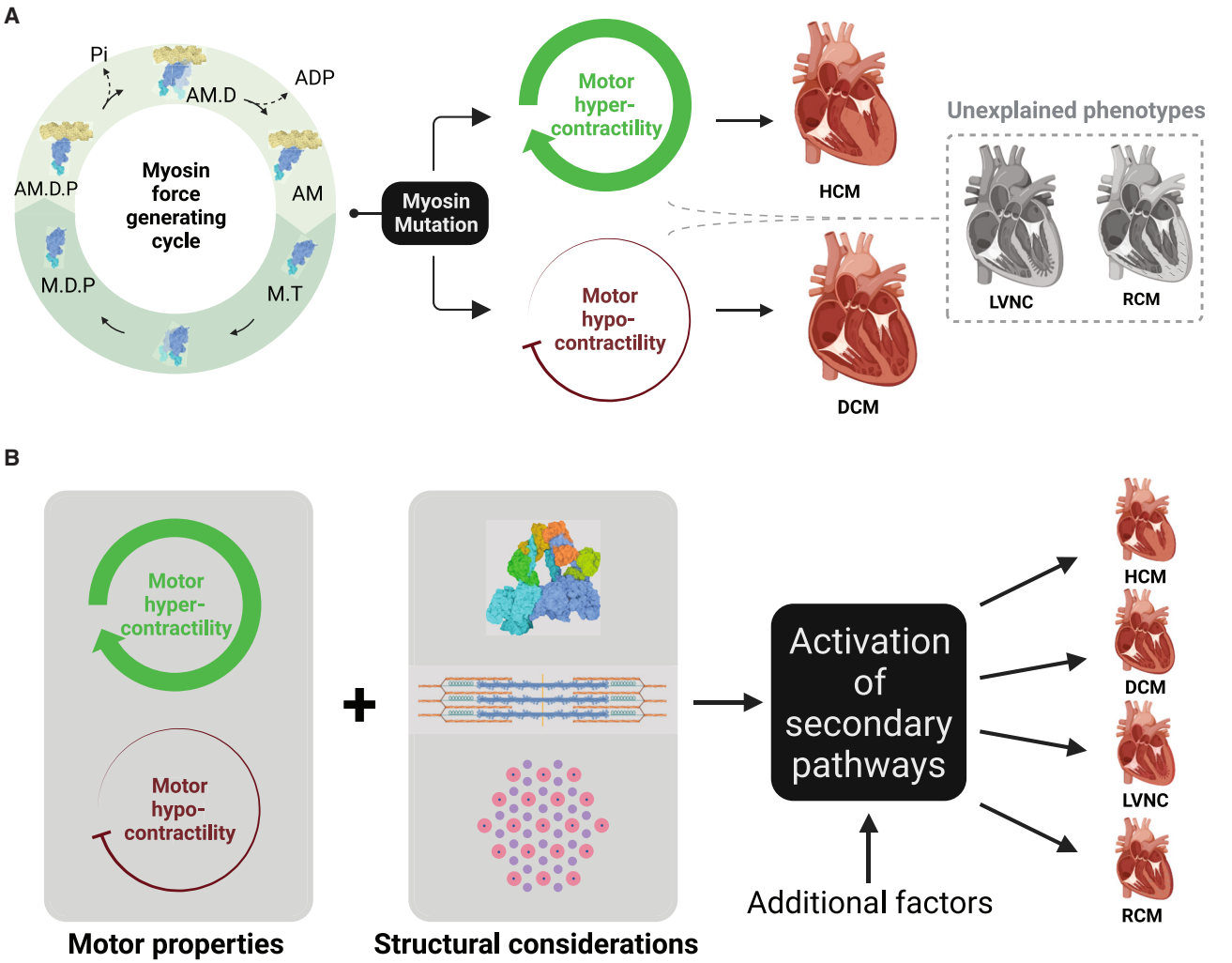

FIGURE 1 Conceptual models of how myosin mutations could cause CMs. (*A*) The frequently invoked model that mutations in myosin affect its motor properties and that motor hypercontractility is associated with HCM and motor hypocontractility is associated with DCM. This model does not explain how mutations in the myosin motor lead to left ventricular noncompaction CM or restrictive CM, and recent studies have shown that some mutations do not follow this model. (*B*) Cartoon illustrating an alternative approach to understanding CMs caused by myosin mutations. Both the biochemical properties of motors and their structural organization within the sarcomere influence contractile phenotypes. Mutation-induced changes in contractility lead to the activation of secondary pathways that can be modulated by additional factors (e.g., hormones, calcium homeostasis). These combined factors then give rise to different CMs. Linking genotype and phenotype will require new tools to model and dissect these factors and pathways. Image was created with BioRender.com including protein structures from the PDB (PDB: 8EFD, 8ACT, and 8D17). To see this figure in color, go online.

rate and in vitro motility speed, while the DCM mutant showed reductions in these rates; however, closer examination of the data reveals critical deviations from this model. Both the HCM and DCM mutants show reduced force production, lower duty ratios, and slower power strokes, consistent with loss of motor contractile function. Therefore, changes in motor contractility alone cannot distinguish the effects of HCM and DCM mutations, and a different model and/or additional factors are necessary to understand their differential effects (Fig. 1 *B*).

The data from Tang et al. are consistent with a previous study of the R403Q HCM mutation in cardiac myosin which also causes reduced motor function at the level of single motors, despite causing hypercontractility in patients (5,6). This led the field to consider other potential regulatory mechanisms that could be affected by myosin mutations extending beyond the level of single motors. Recently, it has become appreciated that myosin motors can dynamically switch between an inhibited super relaxed state where the motor cannot interact with actin and an activated state that can interact with actin (7). It has been suggested that a subset of mutations that cause HCM or DCM could affect the ability of myosin to form these inhibited states. While myosin can adopt an inhibited biochemical state by itself, potentially stabilized through intramolecular interactions with the other myosin head and the S2 region, this inhibition can be modulated by external mechanisms within the sarcomere, including light-chain phosphorylation, myosin binding protein C

(MyBPC) binding and phosphorylation, mechanical stretch, and cation binding (8). It has been hypothesized that some CM mutations, such as those studied by Tang et al., could affect the ability of myosin to adopt this inhibited state. In such a model, it is possible that HCM mutations could show reduced contractility at the level of single motors, but in the context of a full sarcomere, where there are many motors available to interact with actin, increased recruitment of motors due to destabilization of the inhibited state would result in net hypercontractility, consistent with the clinical HCM phenotype (9,10). It is likely that different mechanisms may be at play for different mutations due to myosin's complex intramolecular interactions (11) and intermolecular interactions with binding partners in the sarcomere (12).

Modeling these new regulatory mechanisms will require the development of new experimental and computational platforms recapitulating key aspects of the sarcomere. One such experimental platform is the "*nanosurfer*" assay developed by Touma et al. (13). In the *nanosurfer* assay, synthetic thick filaments are generated using DNA nanotubes, where the organization and spacing of myosins is set by a DNA linker to match the sarcomere. Moreover, nanotubes allow for the inclusion of MyBPC, which is mutated in ∼30% of HCM cases, at specific locations along the nanotube. Using this assay, Touma et al. were able to investigate the effects of MyBPC phosphorylation and organization on myosin-based motility. We envision that this assay could be a useful tool for future studies of CM mutations in both myosin and MyBPC.

As new regulatory mechanisms are uncovered, new computational models are needed to capture salient features of this regulation. Kosta et al. recently developed a new computational tool, *FiberSim*, to model some of these new mechanisms within a spatially explicit model of the sarcomere (14). A spatially explicit model has the advantage that it can capture the complex architecture of the sarcomere including the arrangement of MyBPC and filament lattice spacing. In *FiberSim*, the user can control multiple parameters that define both thick- and thin-filament-based regulation, enabling the authors to recapitulate key features of muscle physiology, including the force-pCa curve, tension recovery after a length change, and the effects of small molecules on muscle function. This model will likely be useful for modeling of myofibril mechanics in the context of CM-related mutations.

While these recent publications have advanced our understanding of CMs and muscle physiology, they also raise important questions (Fig. 1 *B*). If mutations have complex effects on contractility, which biophysical parameters are essential for connecting genotype and phenotype? What determines whether a given mutation in cardiac myosin leads to HCM, DCM, restrictive CM, or left ventricular noncompaction CM? What are the mechanobiological mechanisms by which molecular-based changes in contractility lead to downstream changes in cellular function including altered electrophysiology and ventricular remodeling? Is it possible to use biophysical mechanisms to better characterize variants of unknown significance? Can we harness our understanding of contractile mechanism to design new therapeutics (15)? Answering these questions will require the development of new experimental and computational approaches, and our biophysical community is uniquely poised to address these questions.

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