COMMENTARY

Myosin light chain phosphorylation to the rescue

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A common cause of heart failure is hypertrophic cardiomyopathy (HCM) with a prevalence of at least 1 in 500 in adults (1); more recent data suggest that the prevalence of HCM may be as high as 1 in 200 (2). HCM is characterized by disorganized myocyte structure; formation of excess connective tissue (fibrosis); and, importantly, enlargement of cardiac myocytes that increases the ventricular wall thickness and lowers the ventricular chamber volume (1). Clinical manifestations of HCM vary among patients from mild shortness of breath to end-stage heart failure; HCM can also result in sudden cardiac arrest in young adults, especially in vigorously exercising athletes (3). In the majority of patients, HCM is an inherited disease (1). The first identified mutation to cause dilated cardiomyopathy was in myosin heavy chain, the molecular motor that underlies contraction of the heart (4). Since the discovery of this first mutation, more than 1,000 distinct gene mutations have been identified to cause HCM (5). Most of these gene mutations affect proteins that comprise the thin, thick, and titin myofilaments of the sarcomere (the contractile unit of the heart) (Fig. 1). HCM is therefore considered a disease of the sarcomere. It is not possible to revert HCM-causing mutations back to their WT state, and it is therefore important to study the mechanisms of disease that are triggered by the mutations and to identify therapeutic targets that could lessen disease progression and improve quality of life. Such targets have, so far, been elusive. The work of Yuan et al. (6) in PNAS shows that normalizing the phosphorylation status of the myosin regulatory light chain (RLC) rescues the HCM phenotype due to an RLC mutation. This is an important finding with clinical implications.

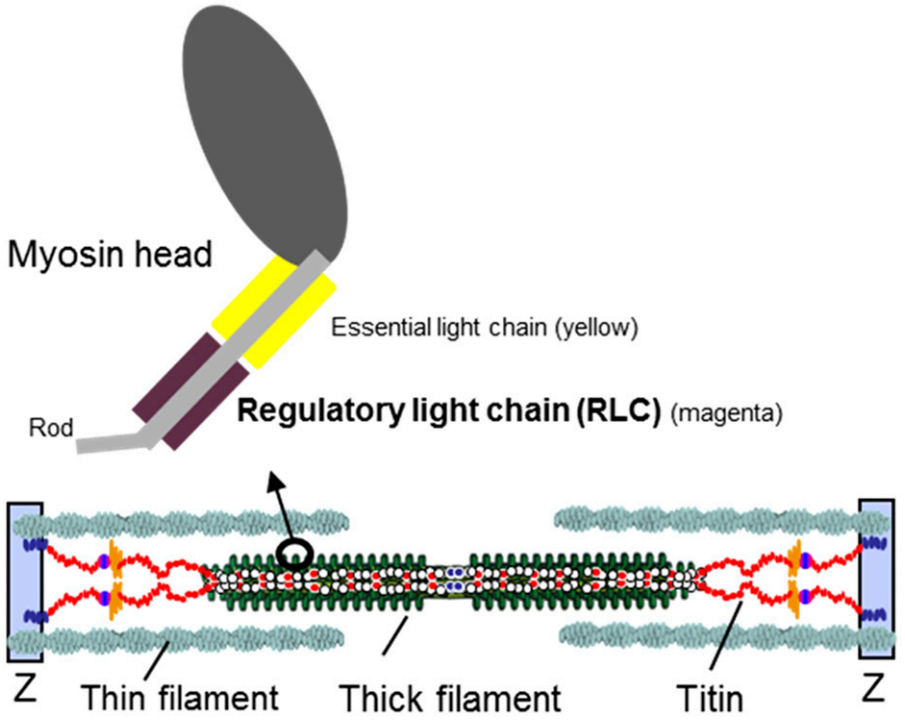

Fig. 1. Schematic of sarcomere. Z-disks (Z) anchor the actin-based thin filaments that penetrate the sarcomere and overlap with the thick filaments. Thick filaments are myosin-based, with the head of the myosin molecule shown at the top. The regulatory light chain is found at the neck region of the myosin head. HCM genes include thick filament proteins [β myosin heavy chain (*MYH7*), myosin regulatory light chain (*MYL2*), myosin essential light chain (*MYL3*), and cardiac myosin binding protein C (*MYPBC3*)] and thin filament proteins [actin (*ACTC*), troponin T (*TNNT2*), α tropomyosin (*TPMI*), and troponin C (*TNNT1*)]. HCM mutations have also been reported in titin (*TNT*), a major disease gene (7). The majority of human mutations occur in *MYH7* and *MYPBC3* (5).

The RLC is localized at the neck of the myosin head (Fig. 1), at the head-rod junction, a location well suited to influence the behavior of the myosin head, and thereby affect contraction (7). The RLC contains a cardiac myosin light chain kinase (cMLCK) phosphorylatable Ser (S15) that is dephosphorylated by cardiac myosin light chain phosphatase (cMLCP) (8). Previous work has shown that phosphorylation of the RLC increases the calcium sensitivity of force generation and accelerates both the speed of force development (9) and stretch activation (10, 11). A gradient in the extent of RLC phosphorylation has been demonstrated across the heart that was suggested to aid in the systolic torsion that accompanies cardiac contraction (12). The importance of RLC phosphorylation for normal cardiac function is highlighted by the findings that both KO of cMLCK and the introduction of a nonphosphorylatable form of RLC (13–15) result in the development of cardiomyopathy, whereas overexpression of cMLCK is cardioprotective (16). The phosphorylation level of S15 appears to be critically important, because dephosphorylation leads to diminished calcium sensitivity of force development and a reduction in maximum tension (15). An HCM mutation has been reported in the RLC (Asp replaced by Val at residue 166; D166V) that results in a reduced phosphorylation level of the RLC (17). Using a

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novel mouse model, Yuan et al. (6) tested the hypothesis that restoring the RLC phosphorylation level counteracts the contractile deficiency that is caused by the D166V mutation.

Transgenic S15D-D166V mice were generated that express the pseudophosphorylated S15 (S15D) in the background of the disease causing D166V mutation. In a series of elegant studies Szczesna-Cordary and coworkers (6) found that pseudophosphorylation of S15D-RLC prevented abnormal hypertrophic cardiac growth in D166V mice. Similarly, myofilament disarray and fibrosis present in the D166V mice were absent in the S15D-D166V mice, and systolic and diastolic function were close to normal. Myofilament function was assessed in skinned papillary muscle strips by measuring the calcium sensitivity of tension and the maximal level of tension. Results show increased calcium sensitivity and reduced maximal tension in D166V mice (confirming earlier findings), as well as, importantly, close to WT values in S15D-D166V mice. Thus, pseudophosphorylation of S15 is sufficient to prevent the development of adverse morphological and functional defects observed in D166V mice. Because the mouse model expresses RLCs that contain both the HCM and S15D mutations, it remains to be established in future work whether phosphorylating the RLC can diminish the adverse effects of the mutation after they have existed for some time. This is an important question, because restoring RLC phosphorylation in patients (see below) is likely to take place only after disease manifestation.

The mechanism underlying RLC hypophosphorylation in D166V mice was also addressed by Yuan et al. (6). Minimal changes were found in the expression level of cMLCK, suggesting that the availability of cMLCK was not limiting. However, the activity level of cMLCK remains to be investigated, as do the expression and activity levels of MLCP; thus, presently, it cannot be excluded that reduced cMLCK and/or increased MLCP activity does play a role. Yuan et al. (6) propose that the diminished phosphorylation of D166V mice results from steric constraints caused by the Val-to-Asp substitution, and their results, based on structural modeling studies, provide support for intramolecular changes triggered by the mutation that indeed might make the mutated RLC a less effective substrate for cMLCK. Finally, low-angle X-ray diffraction experiments on skinned papillary muscle strips performed at the BioCAT facility at the Advanced Photon Source (Argonne) revealed increased myofilament spacing and repositioning of cross-bridges closer to actin in D166V mice compared with WT mice, changes that might underlie their contractile abnormalities. Normalization of these structural changes was observed in muscles from pseudophosphorylated S15D-D166V mice.

Yuan et al. convincingly show that a multitude of adverse effects due to the D166V RLC mutation can be avoided by constitutively phosphorylating the RLC.

The benefits of pseudophosphorylation appear to extend beyond improvement in systolic function because hemodynamic studies revealed an enhanced speed of relaxation, which can be explained by the reported reduction in calcium sensitivity. Improved diastolic function is also suggested by the experiments that revealed in D166V mice an increased passive tension upon stretch and its normalization in S15D-D166V mice. Whether the latter is due to alterations in collagen and/or titin [the two main determinants of passive stiffness (18)] remains to be established. Intriguingly, a relationship between passive muscle stretch and RLC phosphorylation has also been observed in previous work on isolated rat heart preparations in which high passive pressure was found to cause an RLC phosphorylation gradient, from epicardium (high) to endocardium (low) (19). Future follow-up study is warranted on the mechanistic link between the intracellular passive force system (titin) and RLC.

In summary, Yuan et al. (6) convincingly show that a multitude of adverse effects due to the D166V RLC mutation can be avoided by constitutively phosphorylating the RLC. This finding is clinically important not only for patients with HCM who have the D166V mutation but also for other patients with mutations elsewhere in the RLC and for patients with end-stage heart failure, in whom significantly reduced RLC phosphorylation has also been reported (20). Although constitutive RLC phosphorylation by introducing an S15D mutation (as done in the mouse) will not be easily feasible in patients, a possible alternative is the manipulation of the activities of either cMLCK (increasing it) or cMLCP (lowering it). Because these two enzymes show high specificity toward the cardiac RLC (21), this might be feasible without adversely affecting other proteins. The work by Yuan et al. (6) therefore takes an important step toward the ultimate goal of restoring normal cardiac structure and function in patients with heart disease.

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