Myocardium exhibits a remarkably broad dynamic range of function that is normally well matched to the circulatory load on the heart. Regulation of cardiac contraction is a multifaceted process that is well understood in terms of the activating role of Ca\textsuperscript{2+} but much less well in terms of modulation by thick filament accessory proteins and by post-translational modification of thick and thin filament proteins.\textsuperscript{1,2} In this issue of Circulation Research, an elegant study by Fishman and colleagues\textsuperscript{3} dramatically reinforces the idea that the light chain 2 subunit of myosin (MLC-2) is critically important in myocardium by showing that MLC-2 loss because of mutation abolishes myofibrillar assembly in zebrafish, resulting in embryonic lethality. Earlier work had shown that mutations in MLC-2 account for some cases of hypertrophic cardiomyopathy\textsuperscript{4} and that phosphorylation of MLC-2 contributes to cardiac pump function,\textsuperscript{5} but now it is evident that MLC-2 has an obligatory role in development.

Cardiac isoforms of myosin II comprise the motor of myocardial contraction and like all members of this family are composed of 6 subunits\textsuperscript{6}: 2 myosin heavy chains of \approx200 kDa molecular weight, 2 so-called essential light chains (or light chain 1) of \approx17 kDa, and 2 regulatory light chains (or light chain 2) of >20 kDa. Subfragment 1 of the heavy chain (Figure) comprises the business end of the motor, containing both the nucleotide and actin-binding sites, whereas the light chains wrap around the rod-like extension of subfragment 1 and are believed to function (minimally) as mechanical stabilizers of this part of myosin during force generation and mechanical work performance. This motor is impressive for its efficiency and its reliability and just as much for the dramatic phenotypic consequences of mutations in its various domains, as in human hypertrophic cardiomyopathies.\textsuperscript{7}

**MLC-2 Tunes Myocardial Contraction**

Across species, the enzymatic activities of myosin are well matched to heart rate (see Epstein and Davis\textsuperscript{8}), which is achieved through variations in the ratios of \( \alpha \) (faster) and \( \beta \) (slower) myosin heavy chain isoforms expressed in the heart and also species-specific differences in turnover kinetics of these isoforms.\textsuperscript{6} But tuning of the motor also takes place on a beat-to-beat basis, principally through phosphorylation of MLC-2 or of thick and thin filament accessory proteins as a means to optimize work capacity and energetic efficiency. Work capacity can be varied by regulating the number of cross-bridges binding to actin by controlling the amount of Ca\textsuperscript{2+} delivered to myoplasm or by controlling myosin turnover kinetics. The rate of myocardial force development increases when [Ca\textsuperscript{2+}] is increased,\textsuperscript{9} but this does not appear to be caused by acceleration of cross-bridge cycling kinetics, which are invariant with level of activation. Rather, activation dependence of contraction kinetics appears to be a consequence of cooperative binding of cross-bridges to the thin filament.\textsuperscript{10} At low [Ca\textsuperscript{2+}], thin filaments are partially activated by the binding of Ca\textsuperscript{2+} to troponin, but once cross-bridges bind, the activation state is enhanced and additional cross-bridges bind. This positive cooperation in cross-bridge binding increases the force at a given [Ca\textsuperscript{2+}] but also slows the rate of force development because of the time taken to recruit additional cross-bridges.

Phosphorylation of MLC-2 by myosin light chain kinase\textsuperscript{11} increases force and accelerates the rate of force development in myocardium\textsuperscript{12} but slows relaxation\textsuperscript{13}; however, in this case also, the effects on mechanical properties appear to be mediated by changes in the cooperation of cross-bridge binding to actin. Electron microscopy studies have shown that phosphorylation of MLC-2 in isolated thin filaments results in displacement of cross-bridges away from the thick filament, presumably because of electrostatic repulsion between the phosphate group and fixed charge on the surface of the filament.\textsuperscript{14} In the intact myofilament, MLC-2 phosphorylation would therefore displace cross-bridges to positions closer to actin and thereby increase the likelihood of binding. Such effects are limited to submaximal Ca\textsuperscript{2+} concentrations,\textsuperscript{13} suggesting the net effect of the displacement is to speed the cooperative recruitment of cross-bridges to force generating states, which would increase force and the rate of force development. Likewise, slowed rates of relaxation would be a consequence of increased likelihood of cross-bridge rebinding to actin once detached and not a slower rate of detachment.

Epstein and Davis\textsuperscript{4,5,8} have proposed that transmural variations in regulatory light chain phosphorylation contribute to both diastolic and systolic function in the heart. Their finding in rat heart that the outer (epicardial) layers of myocardium are phosphorylated to a greater degree than the innermost layers suggests that there is a transmural gradient in force production, which is greatest in the epicardium. With respect to ejection, they propose that the rightward torsional twist of the heart (when viewed from the base) during systole would...
result in stretch of endocardium, which in turn would result in a stretch activation of the endocardium. Such a mechanism could be critical to the later stages of ejection, and since the properties of stretch activation vary with \([\text{Ca}^{2+}]_{15}\), this could also be a mechanism for kinetic tuning of the ejection phase.

MLC-2 Required for Cardiac Myofibrillogenesis

The article by Rottbauer et al in this issue exploits the finding that zebrafish heart expresses a single isoform of cardiac MLC-2 at all stages of development, so that disruption of MLC-2 expression did not lead to compensatory upregulation of another isoform. Earlier work in a murine model showed that ablation of the MLC-2v gene resulted in abnormal myofibrillogenesis and lethality at embryonic day 12.5, leading to the important conclusion that MLC-2v is required for normal myofibrillogenesis in mouse ventricle. However, a compensatory increase in the expression of the atrial MLC-2a isoform in the ventricle presumably contributed to the structural and functional phenotypes that were observed in those experiments. In the present study, the absence of compensatory expression of an alternate isoform resulted in failure to form thick filaments, even while thin filament assembly was evident. Painstaking control experiments showed that this effect was caused by absence of MLC-2 expression and occurred in a cell autonomous manner. These results show conclusively that MLC-2 is required for thick filament assembly by an as yet unknown mechanism.

Interesting Possibilities

\(\text{Ca}^{2+}\) Binding by MLC-2

Cardiac MLC-2 has a \([\text{Ca}^{2+}]/\text{Mg}^{2+}\) binding site that could conceivably play a role in myofibrillogenesis. The site has high sequence homology with other high-affinity \([\text{Ca}^{2+}]/\text{Mg}^{2+}\) sites and is located in the amino-terminal region of MLC-2 in close proximity to the serine residue that is phosphorylated by myosin light chain kinase. Because of high levels of \(\text{Mg}^{2+}\) in the myoplasm, this site is most likely occupied by \(\text{Mg}^{2+}\) when the muscle is relaxed and appears to exchange \(\text{Ca}^{2+}\) too slowly to participate in the regulation of contraction. However, under conditions of sustained elevations in intracellular \(\text{Ca}^{2+}\), the site could bind \(\text{Ca}^{2+}\) and in this way buffer \(\text{Ca}^{2+}\) and/or regulate myosin function. Such binding could plausibly be important in myofibrillogenesis, since it is well established that spontaneous \(\text{Ca}^{2+}\) transients occur during embryonic development of striated muscles.

Similar Outcomes Caused by Mutations/Deletions of Other Myofibrillar Proteins

Although the mechanism by which deletion of MLC-2 disrupts thick filament assembly and myofibrillogenesis is not known, important clues could emerge from findings that other myofibrillar proteins are important in the process. As Rottbauer et al point out, zebrafish lacking titin display abnormalities similar to deletion of MLC-2, and mice lacking the atrial isoform of MLC-2 exhibit disruption of myofibrillar organization in the atria.

Previous work has also shown that phosphorylation of MLC-2 is important in development, as inhibition of myosin light-chain kinase disrupts thick filament assembly in embryonic myocytes in culture. Thus, when \(\text{Ca}^{2+}\) increases during development, myosin light-chain kinase is activated and MLC-2 is phosphorylated and contributes critically to thick filament assembly.

Thus, Rottbauer et al have shown that MLC-2 plays an essential role in the assembly of cardiac thick filament and the sarcomere, but whether this is because of a structural or contractile effect of MLC-2 is presently unknown. Either way, MLC-2 is emerging as an important element in the constellation of factors that affect cardiac function in health and disease.

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References


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