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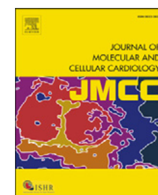

Role of myosin light chain phosphatase in cardiac physiology and pathophysiology

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ABSTRACT

Maintenance of contractile performance of the heart is achieved in part by the constitutive 40% phosphorylation of myosin regulatory light chain (RLC) in sarcomeres. The importance of this extent of RLC phosphorylation for optimal cardiac performance becomes apparent when various mouse models and resultant phenotypes are compared. The absence or attenuation of RLC phosphorylation results in poor performance leading to heart failure, whereas increased RLC phosphorylation is associated with cardiac protection from stresses. Although information is limited, RLC phosphorylation appears compromised in human heart failure which is consistent with data from mouse studies. The extent of cardiac RLC phosphorylation is determined by the balanced activities of cardiac myosin light chain kinases and phosphatases, the regulatory mechanisms of which are now emerging. This review thusly focuses on kinases that may participate in phosphorylating RLC to make the substrate for cardiac myosin light chain phosphatases, in addition to providing perspectives on the family of myosin light chain phosphatases and involved signaling mechanisms. Because biochemical and physiological information about cardiac myosin light chain phosphatase is sparse, such studies represent an emerging area of investigation in health and disease.

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1. Cardiac contractile protein system overview

The heart is an involuntary, striated muscle made up of cardiomyocytes that coordinate the continuous cycles of contraction and relaxation to pump blood out of atria and ventricles to the blood vessels of the pulmonary and systemic circulatory systems. Cardiomyocytes have a specialized membrane system to deliver and remove Ca^{2+} from the highly organized contractile protein system that initiates contraction and relaxation as part of the rhythmic pumping action of the heart [1].

1.1. Sarcomeres, myosin cross-bridge cycling, and Ca^{2+} regulation

Cardiomyocytes contain bundles of myofibrils with sarcomeres that represent the basic contractile units of the myocyte [2]. The sarcomere is composed primarily of thick filaments containing the molecular motor myosin, and thin filaments containing polymerized actin with its associated regulatory proteins tropomyosin and troponin, respectively (Fig. 1). Ca^{2+} binds to a specific troponin subunit resulting in a series of protein conformational changes in troponin and tropomyosin to remove them from an inhibitory state. This allows myosin cross bridges to bind cyclically to actin with the hydrolysis of ATP to generate contractile force [2–4].

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1.2. Cardiac myosin

1.2.1. Function relative to structure

Dimerized heavy chains of myosin contain multiple subdomains including (1) a coiled coil of the two heavy chain subunits embedded in the thick filament, (2) an extended helix lever arm containing sites for the binding of two kinds of light chain subunits (essential and regulatory light chains (RLC), respectively), and (3) the cross-bridge head with its actin-activated ATPase motor domain, (Fig. 1) [5]. The coordinated movement of myosin cross bridges from rest (diastole) to contraction (systole) involves complex interactions with the backbone of the thick filament and myosin-binding protein C as well as with thin filaments [2,6–9]. Cited recent reviews deal with these important topics in detail.

Fine-tuning of contractile performance is partly achieved by post-translational modifications of sarcomeric proteins occurring through signaling modules to regulate the pumping action of the heart to meet circulatory demands of the body [10–14]. RLCs in different kinds of muscles are phosphorylated by myosin light chain kinases (MLCKs) to activate or modulate the myosin, and dephosphorylated by myosin light chain phosphatases (MLCPs) [13,15–20]. A serine in the N-terminus of different RLCs is phosphorylated by tissue-specific, dedicated MLCKs (Fig. 1) [13,21]. In normal beating hearts, Serine 15 is 40% phosphorylated (0.40 mol phosphate/mol RLC) which is greater than the 10% or lower phosphorylation normally observed in resting skeletal and smooth muscles (Fig. 1) [13,15–17,21–23].

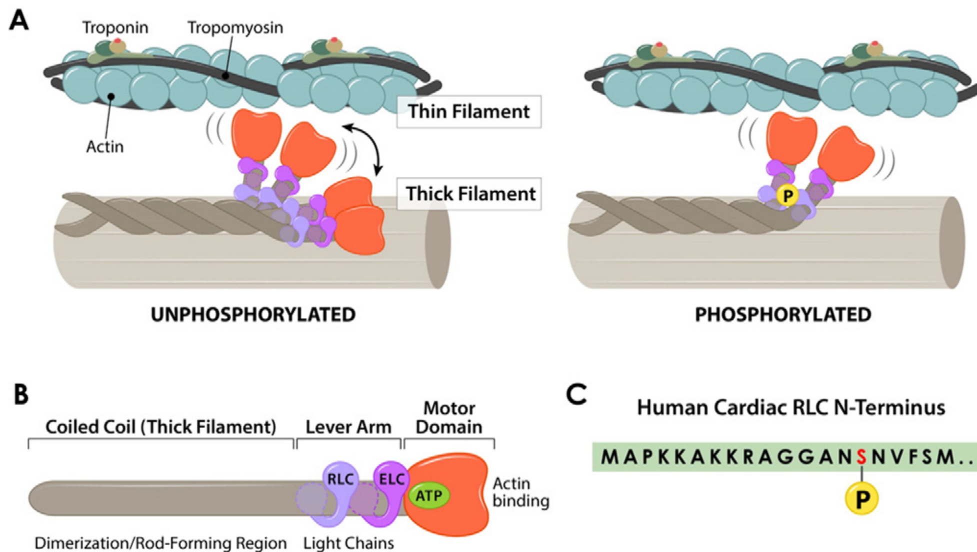

Fig. 1. Cardiac contractile proteins and myosin regulatory light chain phosphorylation. A) Left panel: The cardiac sarcomere is composed primarily of thick filaments containing the molecular motor myosin, and thin filaments containing polymerized actin with its associated regulatory proteins tropomyosin and troponin. The binding of Ca^{2+} to the troponin complex results in movement of troponin/tropomyosin from an inhibitory state exposing myosin binding sites on actin, thus permitting actin-activation of myosin ATPase activity and ensuing contractions to occur. Myosin heads fluctuate between positions against the thick filament or in the inter-filament space, as indicated by the double-headed arrow. Right panel: Phosphorylation (**P**) of RLC favors the inter-filament position and thus modulates the activity of cardiac myosin by promoting its attachment to actin. B) Subdomains of the myosin protomer. The myosin heavy chain folded into a motor domain catalyzes the actin-activated ATPase activity. The lever arm binds an essential light chain (ELC) and a regulatory light chain (RLC). The rod-forming region dimerizes with a second protomer through coiled coil assembly to form the myosin molecule. Thus, myosin II is a hexamer comprised of two each of the heavy chain, ELC, and RLC. Bundles of myosins form the thick filament. C) Amino acid sequence near the human cardiac RLC phosphorylation site, Serine 15 (red).

1.2.2. Biophysical effects of regulatory light chain phosphorylation on myosin cross bridges

In the absence of RLC phosphorylation in striated muscles a portion of the myosin heads are folded back on the myosin tail in a compact *off* conformation in which the long axes of the myosin heads are parallel to the thick filament axis (Fig. 1) [24–27]. However, some of the heads are not bound to the thick filament and are extended to the thin filament in an *on* state in striated muscles. Thus, there is an equilibrium between these two states. RLC phosphorylation in cardiac muscle controls myosin cross-bridge cycling properties on actin (increased myosin cross-bridge repulsion from the thick filament toward actin and the transition of cross-bridge attachment to the strongly bound, force generating state) [28]. RLC phosphorylation also enhances the Ca^{2+}-dependent activation of thin filaments [26,28]. RLC phosphorylation thus potentiates contractility in cardiac muscle by multiple effects on myosin cross bridges, including cooperative effects on adjacent unphosphorylated myosin cross bridges (Fig. 1). This leads to enhanced physiological reserve with contractile forces maintained at a lower Ca^{2+} concentration, and less energy spent pumping Ca^{2+} out of the myoplasm during the contraction cycle. Thus, RLC phosphorylation amplifies the effectiveness of Ca^{2+} release.

Although it is widely understood that the Ca^{2+}-dependence of cardiac contraction is regulated by the troponin complex, and that the phosphorylation of cardiac RLC plays a modulatory role in cardiac function, recent discoveries have advanced the physiological importance of this post-translational modification. Unlike the modulatory phosphorylations of other myofilament proteins, such as troponin I and myosin binding protein C, which are sensitive to acute changes in heart rate and inotropic agents, various investigators have reported baseline RLC phosphorylation in ventricular muscle does not change acutely [21, 29–32]. Lack of dynamic fluctuations in cardiac RLC phosphorylation *in vivo* is supported by the slow turnover rate of the phosphate bound to RLC in the intact rat heart [33]. When procedures are used that preserve phosphorylation, cardiac RLC shows an average 40% phosphorylation in a variety of animals [21,28,31,33–42]. Nearly half-maximal phosphorylation of cardiac RLC from fish to humans suggests this functional reserve for cardiac performance is evolutionarily conserved. The present review will focus on ventricular cardiac RLC although it is appreciated that the distinct atrial RLC is also phosphorylated [30,43].

1.3. Cardiac regulatory light chain phosphorylation and disease

The importance of constitutive RLC phosphorylation for normal cardiac performance is underscored by the reduction of RLC phosphorylation in human heart failure [44–47], and in animal models of myocardial infarction and pressure overload-induced heart failure [42,48–54]. Pressure overload induced by transaortic constriction in wild-type mice reduced the extent of RLC phosphorylation by 40% and cardiac MLCK expression by 85% [54]. The importance of this extent of RLC phosphorylation on cardiac performance becomes apparent when various mouse models and resultant phenotypes are compared (Fig. 2).

Mice that express a non-phosphorylatable cardiac RLC showed RLC phosphorylation was required for optimal cardiac performance and a normal lifespan [28,57]. Unlike human cardiac RLC, mouse cardiac RLC has two serines in tandem, Serines 15/16. Thus, when Serine 15 was mutated to Alanine, Serine 16 was phosphorylated *in vivo*, and a double mutation was necessary to remove the phosphorylation of cardiac RLC *in vivo* [28]. The double phosphorylation site knockin mutant has a shorter lifespan and a more severe phenotype than knockout of cMLCK (Fig. 2). Despite the difference in severity of cardiac phenotype, both mouse models have attenuated hypertrophic response to transaortic constriction, attributed to the lack of RLC phosphorylation [28,54].

There is a discrepancy in the amount of RLC phosphorylation that remains in two models of cMLCK knockout mice described by Warren et al. [54] and Chang et al. [21]. Both cMLCK null lines were generated by crossing cMLCK floxed mice with Cre recombinase transgenic mice with a beta-actin promoter. The cMLCK knockout line described by Chang et al. [21,35] was produced by crossing cMLCK floxed mice with Cre transgenic with cytomegalovirus immediate early enhancer-chicken beta-actin hybrid (CAG) promoter, which deletes floxed genes at two-cell blastomere stage [58]. In both lines, cMLCK protein was not expressed, and mice had heart failure with a normal lifespan. However, Warren et al. reported no RLC phosphorylation was detected by 2D-

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