Another New Kinase Targets Troponin I

Anne M. Murphy

In this issue of Circulation Research, Haworth et al report that protein kinase D (PKD), a serine kinase, phosphorylates the cardiac thin filament protein, troponin I (TnI). These investigators performed a detailed and complete set of experiments, initially noting the association of TnI and PKD, and then mapping the sites that PKD phosphorylates, Ser22 and Ser23. PKD treatment of myocytes was further demonstrated to result in desensitization of the myofilaments to calcium and accelerated isometric crossbridge cycling.1

Why is finding an additional kinase for TnI important? TnI is a critical component of the calcium switch of striated muscle, and the cardiac specific isoform is further specialized because of the presence of an amino-terminal region containing protein kinase A (PKA) sites. Troponin (Tn) consists of three subunits including TnI, the inhibitory component, which inhibits actin-myosin interactions at diastolic calcium levels, TnC, the calcium binding subunit, and TnT, which anchors Tn to tropomyosin (Tm) but also contributes to regulation of muscle activation. During systole, a regulatory Ca2+-binding site on TnC is occupied, increasing the affinity of TnC for TnI and lessening the interaction between TnI and actin-Tm. This results in movement of Tm-Tn on the thin filament, releasing the inhibition of actin-myosin interactions and increasing the probability of cycling of the crossbridge and muscle shortening (reviewed by Tobacman2).

Studies conducted in the 1970s demonstrated TnI to be a target of PKA phosphorylation, and Solaro et al demonstrated the phosphorylation of TnI on administration of adrenaline to perfused hearts. Since that time, the specific sites of phosphorylation of PKA, PKG (also at PKA sites), PKC, p21-activated kinase (PAK), and now PKD have been elucidated.4–6 More importantly, the functional effects of site-specific phosphorylations have been discovered by using a variety of biochemical, structural, and physiological studies from molecular studies in vitro to whole animal physiology (reviewed by Metzger and Westfall7). As illustrated in the schematic of cardiac TnI (Figure), TnI is a small molecule that has multiple binding partners. Just as binding of regulatory Ca2+ to TnC alters the affinity of binding between TnI and TnC, phosphorylation at the various sites influences interactions between the troponin complex and actin-Tm to regulate the contractile process.

To understand the context of the article by Haworth et al,1 it is important to note that PKD is downstream from PKC, yet it phosphorylates TnI at the “PKA” sites. Numerous studies have indicated that phosphorylation of TnI at PKA and PKC sites have somewhat opposing effects. Phosphorylation at the PKA sites is associated with desensitization of the myofibrillar ATPase to Ca2+, augmenting the rate of relaxation enhancing crossbridge cycling and accelerating unloaded shortening velocity.8–11 In terms of structural studies, unphosphorylated TnI complexed with TnC has an extended conformation and thus has extensive contact throughout the molecule.15–17 Phosphorylation of TnI by PKA shrinks or collapses TnI structure and brings the N terminus in close proximity to the C terminus.18 All of these findings indicate a role for TnI in diastolic relaxation, a key effect of β-adrenergic stimulation. More recent data however suggest TnI phosphorylation at PKA sites also has effects on myocyte power output,19 as well as systolic performance, force frequency relationship, and the response to afterload in vivo.20,21

Although phosphorylation by PKC has been less intensively studied, data indicate phosphorylation of TnI by PKC is associated with a decreased maximal myofibrillar MgATPase.22 The primary PKC phosphorylation sites of mouse TnI are Ser42, Ser44, and Thr143; however, in vitro studies with site-specific mutants indicated that the Ser42 and Ser44 sites (referred to as Ser43 and Ser45 because of including the initiating methionine) are primarily involved in the functional effect.23 A recent in vitro study by Burkart et al24 suggests desensitization of sliding velocity is produced by PKC phosphorylation at the Thr143 site; however, the proximal PKC sites of TnI had the dominant effect on tension-calcium relationships.

Importantly, there is evidence that altered phosphorylation at specific TnI sites may be present in failing hearts. Zakhariev et al25 demonstrated a >25% decrease in PKA phosphorylation of TnI in myocardium from patients with end-stage heart failure. This finding was supported by other studies.26–28 PKC phosphorylation of TnI may be increased in heart failure. Takeishi et al29 reported that transgenic mice overexpressing PKC isoform β2 have diminished cardiac function associated with increased TnI phosphorylation. This PKC isoform is also increased in expression in human heart failure. There is also indirect evidence that failing human heart myofilaments may have increased phosphorylation by PKC.30 Thus, in heart failure, the relative activities and localizations of PKA, PKC isoforms, and PKD, as well as the relevant phosphatases, may alter the balance of phosphorylation of specific sites on TnI that are functionally important.

It should not be a surprise that dynamic regulation of the thin filament by phosphatases and kinases is more complex

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A simplified depiction of cardiac TnI based on the crystal structure of the core troponin domain. The four α-helical regions of TnI are indicated as tubular structures. Major phosphorylation sites for PKA/PKD (Ser22, 23), PKC (Ser-42, Ser-44, and Thr-143), and PAK (Ser-150) are depicted. The TnI minimal-inhibitory domain (137 to 148) falls between helices 2 and 3. This region binds either to actin-Tm or TnC in a calcium-dependent fashion, forming a key part of the molecular switch on the thin filament. Additional TnI functional domains include the near NH2-terminal domain (33 to 136), which anchors TnI to the thin filament and contributes to modulation of the myosin ATPase rate and has PKC sites. Immediately past the inhibitory region is a second TnC-binding region (cardiac TnI residues 149 to 165), followed by the second actin-TM-binding site (cardiac TnI residues 165 to 198). Together, these two sites modulate the calcium sensitivity of the thin filament. Note that this numbering sequence refers to the rat cardiac sequence and omits the initiating methionine, thus differing in the numbering found in some publications.

The article by Haworth et al. has taken the first step of defining PKD phosphorylation of TnI and its functional effects in cardiac muscle. Many questions remain to be answered, such as which TnI kinases and phosphatases contribute at rest or with agonist stimulation? What alterations occur in heart failure or with altered afterload? What is the crosstalk between various kinase pathways? How can these regulated pathways be modulated in targeted therapeutics? Answering these questions will further elucidate the intricate network of posttranslational modifications that regulate contraction on a beat to beat basis.

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