

Troponin Structure and Function in Health and Disease

LEARNING OBJECTIVES

1. Explain structure and function of the components in striated muscle contraction.
2. Describe the isoforms of troponin and explain the functional role of each isoform in striated muscle contraction.
3. Explain how mutations in the amino acid sequence or protein structure of troponin could cause changes to muscle contraction.
4. Describe the use of troponin as a clinical biomarker for cardiac diseases.

ABBREVIATIONS:

ADP – adenosine diphosphate, ATP – adenosine triphosphate, Ca^{2+} – calcium, cTn – cardiac troponin, fsTn – fast skeletal troponin, MI – myocardial infarction, ssTn – slow skeletal troponin, Tm – tropomyosin, Tn – troponin, TnC – calcium-binding subunit of troponin, TnI – inhibitory subunit of troponin, TnT – tropomyosin binding subunit of troponin

INDEX TERMS: Troponin; High Sensitivity Troponin Assays; Myocardial Infarction

ABSTRACT

Troponin (Tn) is a heterotrimeric protein containing three subunits (C, T, and I) with different molecular weights and distinctive functions. The subunits of Tn work cohesively to regulate the contraction and relaxation activities of striated muscles: troponin C (TnC) binds calcium (Ca^{2+}), troponin T (TnT) interacts with tropomyosin (Tm) and anchors Tn to actin, and troponin I (TnI) inhibits the ATPase activity of the actomyosin cross-bridge and effectively blocks the myosin binding site on actin subunits. At a genetic level, there are 8 distinctive Tn genes (isoforms) which code for tissue specific, heart and skeletal muscle protein subunits: TNNI1, TNNI2, TNNI3, TNNT1, TNNT2, TNNT3, TNNC1, and TNNC2. The gene isoforms are regulated throughout development via post-transcriptional and post-translational modifications. Genetic mutations in any of the three protein subunits could be linked to hypertrophic, dilated and restrictive cardiomyopathies. Tn release by damaged cardiomyocytes is clinically utilized as a biomarker for myocardial infarction, and its release into the serum is measured at specific times post injury for diagnostic or prognostic purposes. Current tests that measure serum Tn are fifth generation assays which have improved sensitivity and specificity compared to previous assays. However, increased serum Tn levels have been seen in chronic diseases such as Fabry disease and chronic renal disease. Therefore, it is important to remember that when clinically evaluating a patient, Tn levels are only one piece of the puzzle as patients' history, symptoms, etc. are essential for making an accurate diagnosis.

Introduction

Troponin (Tn) is an intracellular protein which plays an important role in regulating striated muscle contraction.¹ First discovered in 1965, it was originally named ‘tropomyosin-like protein’² until 1973 when it was purified and characterized from the skeletal muscles of a rabbit as a separate complex consisting of three different protein subunits. Tn is a heterotrimeric protein containing three subunits (C, T, and I) with different molecular weights and distinctive functions.³ The subunits of Tn work cohesively to regulate the contraction and relaxation activities of striated muscles: troponin C (TnC) binds calcium (Ca^{2+}), troponin T (TnT) interacts with tropomyosin (Tm) and anchors Tn to actin, and troponin I (TnI) inhibits the ATPase activity of the actomyosin cross-bridge and effectively blocks the myosin binding site on actin subunits. Based on the muscle type and stage of development, three subunits (from eight different isoforms) come together to form various triplets in either heart or skeletal muscle (Table 1).

At a genetic level, there are 8 distinctive Tn genes (isoforms) which code for tissue specific, heart and skeletal muscle protein subunits. TnI has three isoforms encoded by three homologous genes: TNNI1 for slow skeletal muscle TnI, TNNI2 for fast skeletal muscle TnI, and TNNI3 for cardiac TnI. Similarly, TnT has three isoforms encoded by three homologous genes: TNNT1 for slow skeletal muscle TnT, TNNT3 for fast skeletal muscle TnT, and TNNT2 for cardiac TnT. TnC has two isoforms encoded by 2 homologous genes: TNNC1 for slow skeletal and cardiac muscle TnC and TNNC2 for fast skeletal muscle TnC. Unlike TnI and TnT, TnC does not have a unique cardiac isoform (Table 1).⁵

The different isoforms of Tn encode for proteins with similar, but not identical function which allows the body to fine tune muscle contraction in the cardiac and slow and fast twitch skeletal muscle. The 8 subunit isoforms undergo further fine tuning at the transcriptional level

via alternative splicing of the mRNA. The tight regulation of Tn, specifically cardiac Tn (cTn), at both the transcriptional and the translational level, play an important role in cardiac development, health and muscle pathology.

Striated Muscle Structure

Striated muscle, which includes human skeletal and cardiac muscle tissues, is composed of individual muscle cells (myofibers) that consist of bundles of thin filamentous structures called myofibrils. The myofibrils are composed of individual functional units called sarcomeres, arranged end to end throughout the length of the myofibrils. Skeletal muscle cells are long, unbranched, and multinucleated, whereas cardiac muscle cells are relatively short, branched, and usually contain a single nucleus. Despite these differences, both skeletal and cardiac muscles share a striated appearance that is due to the arrangement of proteins within the sarcomeres.

Contraction of striated muscle is a highly regulated and coordinated process, involving interplay among multiple protein components which are arranged in specific patterns. The sarcomere is composed primarily of the proteins actin (thin filaments) and myosin (thick filaments). The actin is associated with Tm, which acts to block the myosin binding sites on actin and, with the troponin complex, in conjunction with Ca^{2+} , works to regulate the formation of cross bridges between the thick and thin filaments. Each sarcomere runs from Z disc to Z disc, which is composed of the protein alpha-actinin and anchors the thin filaments (Figure 1). According to the sliding filament model of muscle contraction proposed in 1954, during contraction, actin and myosin interact with each other in such a way that when activated the thin filaments slide over the thick filaments, drawing the Z discs toward each other, effectively shortening the sarcomere.^{4, 5} As successive sarcomeres shorten collectively, myofibrils shorten, and contraction takes place.

Troponin in Muscle Contraction

Contraction is initiated when muscle fibers are stimulated by a nerve impulse and cytoplasmic Ca^{2+} increases, in skeletal muscle by release from the sarcoplasmic reticulum or in cardiac muscle by the release from the sarcoplasmic reticulum and influx of extracellular Ca^{2+} . Tn, which is associated with actin filaments, binds the Ca^{2+} ions and binding of Ca^{2+} to Tn subsequently displaces Tm, exposing myosin binding sites on actin. The three Tn protein subunits each play a specific role in regulating muscle contraction within the actin-myosin cross bridge. The TnT subunit binds the whole Tn protein to Tm and to the TnC subunit. The TnC subunit acts as a Ca^{2+} sensor in the muscle contraction process; when bound to Ca^{2+} TnC, which is also bound to TnI, causes TnI to shift on actin, exposing the binding sites that allow myosin to bind. Despite the similar organization and function of the Tn complex in regulating skeletal and cardiac muscle contraction, some evidence suggests that there are subtle differences in the molecular interaction between Ca^{2+} and specific regions in the Tn isoforms. See Table 2 for key terms.

TnC

There are only two isoforms of TnC, cardiac TnC (cTnC) and fast twitch skeletal TnC (fsTnC). cTnC encodes for a 210 AA molecule with a molecular weight of 18kDa that does not undergo alternative splicing.⁶ Structurally the cTnC and fsTnC protein subunits have similar C-terminal domains but have different N-terminal domains. There are two Ca^{2+} or Mg^{2+} binding sites, Sites III and IV, on the C-terminal domain in both cTnC and fsTnC that play a role in anchoring TnC to other myo-filaments within the cross bridge. These high affinity Ca^{2+} or Mg^{2+} binding sites help maintain structural integrity throughout the protein. It is the N-terminal domain of TnC that differs between the fast skeletal and cardiac isoforms of this protein. fsTnC

contains two low affinity Ca^{2+} binding sites (Sites I and II), which regulate muscle contraction. In cTnC, there is only one Ca^{2+} site (Site II), which acts as the regulatory region of cardiac muscle contraction; in sTnC, Site I does not bind Ca^{2+} .⁷ To determine if the Ca^{2+} binding Site I could regulate cardiac muscle contraction, Sweeney et al. mutated Ca^{2+} binding site I to bind Ca^{2+} , while also mutating Ca^{2+} binding site II to no longer bind Ca^{2+} .⁸ In this mutated muscle, cardiac muscle contraction was no longer triggered by Ca^{2+} confirming that Ca^{2+} binding Site II is the sole regulator of cardiac contraction.⁸ Ca^{2+} binding to TnC can also be regulated by other proteins in the cross bridge including interactions with TnI, TnT, actin, myosin and Tm.¹

TnT

TnT contains three distinct genes which produce three different protein isoforms, 1) TNNT1 for slow twitch skeletal muscle (ssTnT), 2) TNNT2 for cardiac muscle and some neonatal skeletal muscles (cTnT), and 3) TNNT3 for fast twitch skeletal muscle (fsTnT).⁹ The TnT protein isoforms undergo developmental, transcriptional, and translational regulation and are 220-300 amino acids long with a molecular weight of 30-35 kDa. The C-terminal domain and the middle portion of the protein bind to TnC, TnI, Tm, and Ca^{2+} and therefore directly interact with other proteins in the actin-myosin cross bridge. The main difference between the three TnT protein isoforms is the N-terminal region, which plays a role in regulating conformational changes without directly binding to the cross bridge. For example, in patients who undergo ischemia reperfusion injury, such as after a myocardial infarction, there is a truncation of the N-terminal domain of cardiac TnT (cTnT) by μ -calpain that alters cardiac contraction.¹⁰ Muscle fibers from transgenic mice overexpressing the truncated cTnT had an increased affinity for Tm and *ex vivo* isolated working heart perfusions from these mice showed decreased ventricular contractile velocity but a preserved stroke volume.¹⁰ These data suggest that the post-

translational modification of the end of the N-terminal domain of cTnT during ischemia reperfusion is cardio-protective by improving/maintaining stroke volume in damaged hearts.

Further, cTnT is essential for development; studies conducted in rodents show that a complete loss of cTnT is embryonic lethal.¹¹ There are 17 exons that are encoded by TNNT2, cTnT; and exons 4, 5 and 13 can undergo alternative splicing. Although the cause of exon 13 splicing is unknown, exon 13 plays a role in linking two functional domains of the TnT protein together.¹² Alternative splicing of exon 5 is developmentally regulated in the heart; it is only expressed in the embryonic heart.¹³ This exon contains 10 amino acids that cause the protein to become more negatively charged, therefore it binds Ca^{2+} more tightly, which decreases cardiac muscle contraction.¹³ Both embryonic and developing skeletal muscles also express cTnT that is alternatively spliced like that in the heart.^{14, 15} This suggests that the alternative splicing of cTnT is regulated systemically and not in response to organ demands.

Exons 4 and 5 are alternatively spliced in the N-terminal variable region and give rise to four distinctive protein products: 1) cTnT1: no splicing; 2) cTnT2: splicing of exon 4; 3) cTnT3: splicing of exon 5; 4) cTnT4: splicing of exons 4 and 5, that have different molecular weights, Ca^{2+} sensitivity and inhibition of force development.¹⁶ Both cTnT1 and cTnT2 have higher levels of Ca^{2+} sensitivity and are more likely to inhibit the ATPase activity of the actin-tropomyosin-activated myosin ATPase. The presence of exon 5 in the final cTnT protein product decreases the contractility of the actin-myosin cross bridge. Further, in various animal models of dilated cardiomyopathies, there are other alternatively spliced out exons in the cTnT protein which exacerbate disease. These models include exon 4 splicing in heart failure patients and familial hypertrophic cardiomyopathy, and diabetic rat hearts, exon 5 splicing in canine dilated cardiomyopathy, exon 6 splicing in abnormal cardiac structural changes in guinea pigs, exon 7

splicing in canine cardiomyopathy, and exon 8 splicing in dilated cardiomyopathy in turkeys.⁹ Further research is still needed to determine the functional significance of these alternatively spliced isoforms in human health and disease.

TnI

TnI has three genes coding for three protein isoforms 1) TNNI1 in slow twitch skeletal muscle (ssTnI), 2) TNNI2 in fast skeletal muscle (fsTnI), 3) TNNI3 in cardiac muscle (cTnI). TnI interacts with both TnC and TnT and is able to inhibit muscle contraction by binding to actin with or without Ca^{2+} ; this region is termed the inhibitory peptide region.^{3, 17} Ca^{2+} regulation of TnI is through TnI's interaction with TnT and TnC but not direct binding of Ca^{2+} itself.⁹ TnI undergoes developmental and translational regulation and is 182-210 amino acids long with a molecular weight of 21-24kDA.¹ There are eight exons which code for the various TnI genes; exons 4-8 are conserved among the three isoforms.^{18, 19} cTnI is the largest of the three isoforms and contains a unique 30 amino acid N-terminal sequence coded by exons 1-3.^{19, 20} There is no known alternative splicing that occurs in cTnI but there are posttranslational modifications which affect muscle contraction.^{1, 21} In cardiac myocytes, activation of protein kinase A (PKA) in response to beta-adrenergic stimulation of the heart leads to phosphorylation of two adjacent serine residues, Ser-23 and Ser-24, in cTnI. These residues are part of a 32-residue extension of the N-terminal region of the protein that is unique to the cardiac isoform of TnI. Phosphorylation of these two serine residues leads to a decrease in affinity between cTnI and cTnC, a decrease in the sensitivity of cTnC to Ca^{2+} , and an increase in the rate of relaxation.²² Interestingly, PKA-dependent phosphorylation of these specific serine residues in cTnI has decreased in the hearts of patients with heart failure.^{23, 24}

cTnI expression is developmentally regulated in the heart and is only expressed in the adult heart.²⁵ This is important for clinical application of diagnostic tests, which utilize various Tn isoforms to determine cardiac damage. Protein and mRNA expression levels of the TnI isoforms were assessed during pre and post-natal cardiac development and showed that slow twitch skeletal TnI is expressed in the fetal and newborn heart, but by 9 months post-natal, cTnI is the sole TnI fiber in the heart.²⁶ The main difference between these two proteins is the cTnI contains a unique 27-33 amino acid N-terminal sequence. This sequence contains two phosphorylation sites that can further regulate TnI interactions with TnC and TnT through cAMP protein kinase. Studies conducted in adult transgenic mice, containing slow twitch muscle TnI instead of wild type cTnI in the heart, show no developmental dysfunction and no changes to mortality. The transgenic mice did exhibit diastolic dysfunction, which was exacerbated by treatment with the β -adrenoreceptor agonist isoprenaline. The slow twitch skeletal TnI isoform has a lower affinity for TnC compared to TnI. The altered affinity altered the Ca^{2+} regulation in the transgenic mice and therefore caused cardiac dysfunction. Further, recent studies have also shown that the C-terminal mobile domain of cTnI also plays a role in modifying cardiac contraction.¹⁷ Together, these data suggest that any mutations of cTnI can cause cardiac myopathies.

Both transcriptional and/or translational changes to the Tn subunits and isoforms can cause changes to muscle contraction through their interaction with the actin-myosin cross bridge by altering the exposure of the binding sites on actin. After Ca^{2+} binds to Tn, the T_m shifts, exposing the myosin binding site on actin. The head of each myosin unit is now free to bind to the exposed binding site, forming a cross bridge. Each myosin head has an ADP and phosphate molecule attached to it from the previous cross bridge cycle. Upon formation of the cross bridge,

the ADP and phosphate are released, which triggers a conformational change resulting in the myosin head moving to the un-cocked position. As the myosin head moves, it pulls the thin filament along with it (power stroke). Once the pivot occurs, the affinity for ATP increases and a new ATP attaches to the ATP binding region in the myosin head and the myosin head detaches from actin. ATP is then hydrolyzed to ADP and phosphate, and the cycle repeats itself until Ca^{2+} levels drop below threshold or until maximal shortening of the sarcomere is achieved. Relaxation of the muscle cell occurs due to the action of ATP-dependent Ca^{2+} pumps move Ca^{2+} back into the sarcoplasmic reticulum or out of the cell. As the cytoplasmic concentration of Ca^{2+} drops, the Tn-Tm complex slides back into place, blocking the myosin binding sites on actin. Figure 2.

Stimulation of cardiac muscle cells also leads to increased cytoplasmic concentrations of Ca^{2+} . In these cells, action potentials lead to an influx of extracellular Ca^{2+} in addition to stimulation of Ca^{2+} release from the sarcoplasmic reticulum. Like skeletal muscle, the rise in intracellular Ca^{2+} levels lead to the removal of the Tm block by the interaction of Ca^{2+} and TnC. The role of Tn in muscle contraction is indispensable. The regulation of subunit isoforms is organ-specific during development or for diseases.

Troponin Causing Cardiomyopathies

According to the National Institute of Health National Heart Lung and Blood Institute, cardiac myopathies are a group of diseases of the heart that are either inherited or acquired. A variety of hereditary cardiomyopathies including hypertrophic cardiomyopathy (HCM, thickening of the heart muscle), restrictive cardiomyopathy (RCM, stiffness of the heart muscle) and dilated cardiomyopathy (DCM, thinning of the heart muscle wall), have been associated with mutations in the three subunits of cTn.⁶ A change as small as one amino acid in the Tn sequence, termed a point mutation, can cause changes to Tn subunit interactions in the actin-myosin cross

bridge formation, muscle contraction, and cardiac function. Over 100 mutations in cTn subunits cTnT encoded by TNNT2, cTnI encoded by TNNT1, and cTnC encoded by TNNC1 have been linked to cardiomyopathies.²⁷

In 2001, the first HCM mutation in cTn was discovered in a 60 year old male.²⁸ To test the role of mutations found in cardiomyopathy patients, various *in vivo* and *in vitro* models have been studied. For example the missense mutation, L29Q, in TNNC1 was tested in a variety of *in vivo* and *in vitro* models and results showed inconclusive effects on Ca²⁺ sensitivity.⁶ Other mutations, such as A8V, A31S, C84Y and more, in cTnC cause changes to the Ca²⁺ sensitivity of Tn and therefore affect cardiac muscle contractility.⁶ Mutations in TNNC1 and TNNT2 have also been linked to DCM in sporadic and familial cases.²⁹ These mutations affect the ability of Tn to bind to the actomyosin cross bridge, altering Ca²⁺ sensitivity and muscle contraction. Lastly, mutations in the last 5 amino acids of the C-terminal domain of cTnI have been identified in various patients with HCM, DCM and RCM.³⁰ *In vitro* studies cleaving the last 5 amino acids of cTnI cause an increase in Ca²⁺ sensitivity of the Tn complex and thereby modify muscle contraction. These mutations are only some of the Tn mutations associated with cardiomyopathies, other mutations are reviewed in Chang et al.³¹ Further studies identifying other mutations in cTns in cardiomyopathies and the functional significance of these mutations will play an important role in treating patients in the future.

Degradation of Troponin in Tissue and Release into Serum

The Tn complex is a tightly regulated protein that is essential for striated muscle contraction. However, its importance in detecting ischemia-related cardiac muscle injuries is recognized especially with the appearance of the high sensitivity assays based on cTnI and cTnT. To maintain homeostasis, a normal functional heart is needed to deliver nutrients and oxygen to

different body parts as well as itself. About 4-5% of the cardiac output goes to the heart for supplying its pumping activities.³² When cardiac myocytes die or are injured, Tn is released from these cells and diffuses into the circulation. The release of Tn from cardiac myocytes is mediated through the processes of inflammation, apoptosis, or necrosis. It is worthy to note that very low concentrations of cardiac biomarkers can be detected in people with healthy hearts by the recently developed high sensitivity cTn assay.^{33, 34} Aside from troponins, other biomarkers are released into circulation when myocardial injury occurs including creatine kinase (CK), lactate dehydrogenase, and myoglobin, to name a few.³⁵ The time for these various biomarkers diffusing from myocardial cells into the circulation is determining the earliest time when they could be detected from blood samples after myocardial incidents. Together with the presenting clinical symptoms, measuring these biomarkers help clinicians diagnose cardiac diseases more accurately.

Cardiac troponins are released into the bloodstream 2 to 8 hours after a cardiac injury occurs, peak at 12- 48 hours, and can remain at detectable levels for up to 3 to 5 days for cTnI and 5 to 10 days for cTnT. Therefore, there is a natural rise and fall pattern associated with biomarkers after acute myocardial injury. The concentration of cTnI or cTnT is positively related to the number of injured cardiac myocytes (area of ischemia/necrosis).³⁶ Further, the added value of troponins in the diagnosis of myocardial injury is that the level of Tn remains elevated up to 7-10 days after acute myocardial infarction.³⁶ As what was previously discussed in this article, since there is no cardiac specific TnC, TnC is not used as a clinical diagnostic marker. Unfortunately, in addition to myocardial injuries, cardiac damage from other diseases, such as kidney disease, sepsis, and severe anemia, can also cause an elevation in Tn. For example, patients with end stage renal disease have increased cTnI and cTnT concentrations, and these

biomarkers are prognostic for the adverse events of the end stage renal disease.³⁷ These diagnostic markers are utilized differently for different prognoses and diagnoses. While both cTnT and cTnI levels are usually measured, cTnI was shown to be the preferred biomarker for myocardial damage in patients with chronic renal failure.³⁸

Troponin as a Diagnostic and Prognostic Biomarker

It is a challenge to accurately diagnose patients presenting with symptoms of acute coronary syndrome, especially for those who do not exhibit the classical changes in ST-segment of electrocardiogram recordings. As a result, cardiac markers have played an increasingly important role in aiding in the diagnosis of acute myocardial infarction (MI) especially within the last 2 decades. Troponins, in particular, provide valuable information for helping establish a diagnosis in the case of chest pain presentation. cTnI or cTnT have been recognized as the preferred biomarkers for detecting MI since the publication of the Joint European Society of Cardiology (ESC) and the American College of Cardiology (ACC) definition for myocardial infarction in 2000.³⁹ As myocytes die, they sequentially release intracellular components into the blood that are used as biomarkers that can be detected at specific times after injury, by different types of immunochemical assays. The development of various assays for detecting cTnI and cTnT are utilized worldwide and are key biomarkers of MI in patients.^{36, 40} Although elevated cTnI is an indicator of MI, patients with other chronic diseases also express elevated TnI levels in their blood. For example, patients with Fabry disease have an increased cTnI especially for those with a left ventricular hypertrophy.⁴¹ Therefore, when utilizing blood levels of Tn, it is important to incorporate patient history and symptoms in order to make an accurate diagnosis.

cTn has been shown to have prognostic value as well.^{42, 43} The cTnT can be used for prognostic purpose for non-emergent post percutaneous coronary intervention outcome.⁴⁴ In a

prospective cohort study of 1024 patients with unstable angina/non-ST-segment elevation MI who underwent coronary angiography and subsequent coronary stenting within 24 hours, the risk of in-hospital and long-term mortality was greater with increased levels of baseline cTnT.⁴² The data indicates cTnT is a useful biomarker for post MI prognosis purpose.

Although the current diagnostic systems can reliably diagnose myocardial infarction 3-6 hours after the onset of heart attack, efforts have been made to detect changes in Tn biomarkers earlier with utilization of high sensitivity assays.^{39, 45-47} High sensitivity (hs) assays have been developed and are available from both Abbott (hs-cTnI) and Roche (hs-cTnT) and will be discussed more fully in the second and third articles in this focus series. The use of these high sensitivity assays is going to be evaluated from sensitivity and specificity perspectives for many cardiac diseases including MI. Undoubtedly, a thorough knowledge on Tn biomarkers is critical for healthcare providers to either diagnose or make prognoses in coronary artery or heart diseases.

Table 1. Troponin Isoforms and Chromosomal Location. Adapted from Barton et al 1997⁴⁸.

TnI			
Gene	Protein Isoform	Chromosome	Expression Site
TNNI1	ssTnI	1q32	Slow Twitch Skeletal Muscle
TNNI2	fsTnI	11p15.5	Fast Twitch Skeletal Muscle
TNNI3	cTnI	19q13.4	Cardiac Muscle
TnT			
Gene	Protein Isoform	Chromosome	Expression Site
TNNT1	ssTnT	19q13.4	Slow Twitch Skeletal Muscle
TNNT2	cTnT	1q32	Cardiac Muscle
TNNT3	fsTnT	11p15.5	Fast Twitch Skeletal Muscle
TnC			
Gene	Protein Isoform	Chromosome	Expression Site
TNNC1	cTnC	3p14.3-p21.3	Cardiac or Slow Skeletal Muscle
TNNC2	fsTnC	20q12-q13.11	Fast Twitch Skeletal Muscle

Table 2. Key Biology terms used in this article.

Keyword	Definition
Central Dogma	The two-step process of transcription and translation which takes genetic material in the form of DNA (nucleic acids) and converts it to the final protein product.
Transcription	The process of converting the DNA into RNA
Translation	The process converting RNA into a protein
Alternative Splicing	During transcription, a single gene which codes for multiple proteins by coding for differing exons. This differences can cause changes to protein structure and function.
Post Transcriptional Modification	A modification, such as phosphorylation, to a protein after it has been completely translated. This can cause changes to protein function and activity.

Figure 1. Sarcomere Organization. Image reprinted with permission of John Nagy

A sarcomere is the smallest functional unit of muscle contraction. It is organized from Z disc to Z disc (termed the A Band), with an M line found in the center. The functional unit consists of the actin and myosin filaments, which will slide over one another toward the M line during contraction and shrink the H zone.

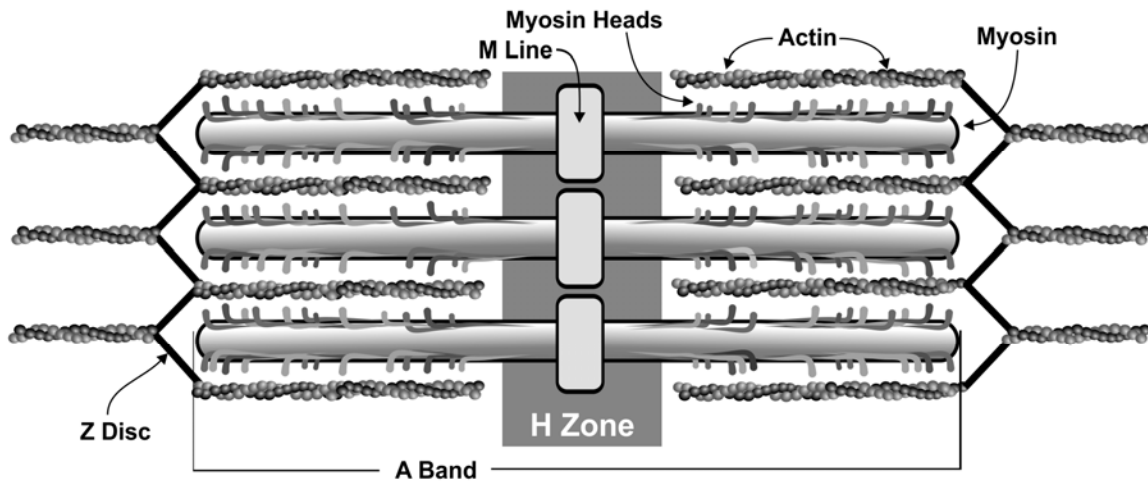
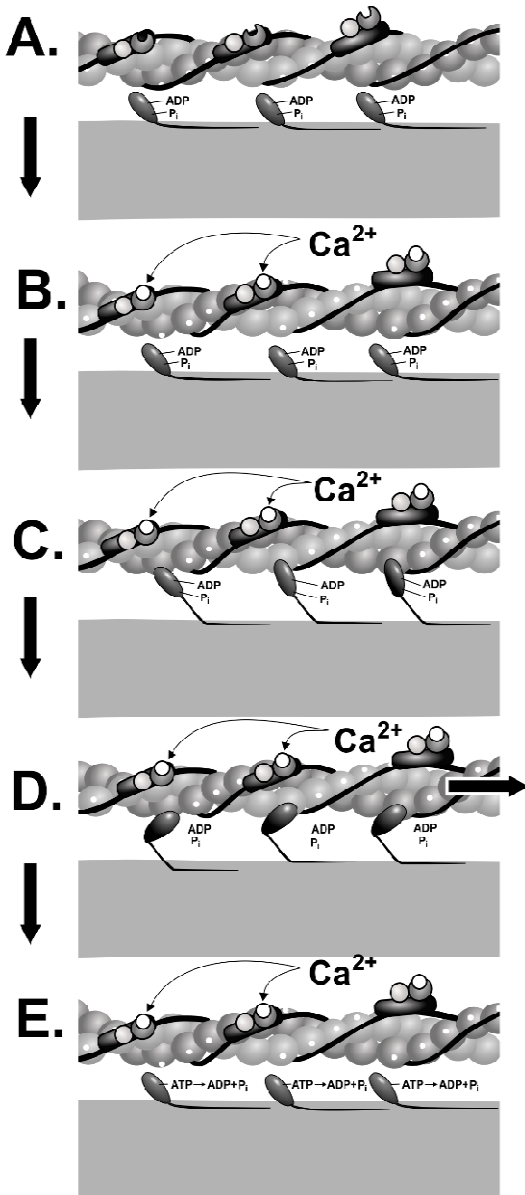


Figure 2. Power Stroke Image of Muscle Contraction. A. resting with myosin binding sites covered, B. calcium binding to troponin, myosin-binding sites exposed, C. Cross bridge formation as myosin binds to the myosin binding sites on actin, D. Power stroke which moves actin toward the M line, E. Dissociation of myosin head from actin. Image reprinted with permission of John Nagy.



References

- 1 Sheng JJ, Jin JP. Gene regulation, alternative splicing, and posttranslational modification of troponin subunits in cardiac development and adaptation: a focused review. *Front Physiol.* 2014; 5: 165.
- 2 Ebashi S, Kodama A. A new protein factor promoting aggregation of tropomyosin. *J Biochem.* 1965; 58(1): 107-8.
- 3 Greaser ML, Gergely J. Purification and properties of the components from troponin. *J Biol Chem.* 1973; 248(6): 2125-33.
- 4 Huxley H, Hanson J. Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature.* 1954; 173(4412): 973-6.
- 5 Huxley AF, Niedergerke R. Structural changes in muscle during contraction; interference microscopy of living muscle fibres. *Nature.* 1954; 173(4412): 971-3.
- 6 Li MX, Hwang PM. Structure and function of cardiac troponin C (TNNC1): Implications for heart failure, cardiomyopathies, and troponin modulating drugs. *Gene.* 2015; 571(2): 153-66.
- 7 van Eerd JP, Takahashi K. The amino acid sequence of bovine cardiac tamponin-C. Comparison with rabbit skeletal troponin-C. *Biochem Biophys Res Commun.* 1975; 64(1): 122-7.
- 8 Sweeney HL, Brito RM, Rosevear PR, Putkey JA. The low-affinity Ca²⁺(+)-binding sites in cardiac/slow skeletal muscle troponin C perform distinct functions: site I alone cannot trigger contraction. *Proc Natl Acad Sci U S A.* 1990; 87(24): 9538-42.
- 9 Wei B, Jin JP. TNNT1, TNNT2, and TNNT3: Isoform genes, regulation, and structure-function relationships. *Gene.* 2016; 582(1): 1-13.
- 10 Feng HZ, Biesiadecki BJ, Yu ZB, Hossain MM, Jin JP. Restricted N-terminal truncation of cardiac troponin T: a novel mechanism for functional adaptation to energetic crisis. *J Physiol.* 2008; 586(14): 3537-50.
- 11 Nishii K, Morimoto S, Minakami R, Miyano Y, Hashizume K, Ohta M, *et al.* Targeted disruption of the cardiac troponin T gene causes sarcomere disassembly and defects in heartbeat within the early mouse embryo. *Dev Biol.* 2008; 322(1): 65-73.
- 12 Jin JP, Wang J, Zhang J. Expression of cDNAs encoding mouse cardiac troponin T isoforms: characterization of a large sample of independent clones. *Gene.* 1996; 168(2): 217-21.
- 13 Cooper TA, Ordahl CP. A single cardiac troponin T gene generates embryonic and adult isoforms via developmentally regulated alternate splicing. *J Biol Chem.* 1985; 260(20): 11140-8.
- 14 Kracklauer MP, Feng HZ, Jiang W, Lin JL, Lin JJ, Jin JP. Discontinuous thoracic venous cardiomyocytes and heart exhibit synchronized developmental switch of troponin isoforms. *FEBS J.* 2013; 280(3): 880-91.
- 15 Liu R, Feng HZ, Jin JP. Physiological contractility of cardiomyocytes in the wall of mouse and rat azygos vein. *Am J Physiol Cell Physiol.* 2014; 306(7): C697-704.
- 16 Gomes AV, Guzman G, Zhao J, Potter JD. Cardiac troponin T isoforms affect the Ca²⁺ sensitivity and inhibition of force development. Insights into the role of troponin T isoforms in the heart. *J Biol Chem.* 2002; 277(38): 35341-9.
- 17 Meyer NL, Chase PB. Role of cardiac troponin I carboxy terminal mobile domain and linker sequence in regulating cardiac contraction. *Arch Biochem Biophys.* 2016; 601: 80-7.
- 18 Bhavsar PK, Brand NJ, Yacoub MH, Barton PJ. Isolation and characterization of the human cardiac troponin I gene (TNNI3). *Genomics.* 1996; 35(1): 11-23.
- 19 Jin JP, Zhang Z, Bautista JA. Isoform diversity, regulation, and functional adaptation of troponin and calponin. *Crit Rev Eukaryot Gene Expr.* 2008; 18(2): 93-124.

- 20 Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: from myocardial infarction to chronic disease. *Cardiovasc Res*. 2017; 113(14): 1708-18.
- 21 Jin JP, Yang FW, Yu ZB, Ruse CI, Bond M, Chen A. The highly conserved COOH terminus of troponin I forms a Ca²⁺-modulated allosteric domain in the troponin complex. *Biochemistry*. 2001; 40(8): 2623-31.
- 22 de Tombe PP, Solaro RJ. Integration of Cardiac Myofilament Activity and Regulation with Pathways Signaling Hypertrophy and Failure. *Annals of Biomedical Engineering*. 2000; 28(8): 991-1001.
- 23 Zhang J, Guy MJ, Norman HS, Chen Y-C, Xu Q, Dong X, *et al*. Top-Down Quantitative Proteomics Identified Phosphorylation of Cardiac Troponin I as a Candidate Biomarker for Chronic Heart Failure. *Journal of Proteome Research*. 2011; 10(9): 4054-65.
- 24 Zhang P, Kirk JA, Ji W, dos Remedios CG, Kass DA, Van Eyk JE, *et al*. Multiple Reaction Monitoring to Identify Site-Specific Troponin I Phosphorylated Residues in the Failing Human Heart. *Circulation*. 2012; 126(15):1828-37.
- 25 Rittoo D, Jones A, Lecky B, Neithercut D. Elevation of cardiac troponin T, but not cardiac troponin I, in patients with neuromuscular diseases: implications for the diagnosis of myocardial infarction. *J Am Coll Cardiol*. 2014; 63 (22): 2411-20.
- 26 Sasse S, Brand NJ, Kyprianou P, Dhoot GK, Wade R, Arai M, *et al*. Troponin I gene expression during human cardiac development and in end-stage heart failure. *Circ Res*. 1993; 72(5): 932-8.
- 27 Lu QW, Wu XY, Morimoto S. Inherited cardiomyopathies caused by troponin mutations. *J Geriatr Cardiol*. 2013; 10(1): 91-101.
- 28 Hoffmann B, Schmidt-Traub H, Perrot A, Osterziel KJ, Gessner R. First mutation in cardiac troponin C, L29Q, in a patient with hypertrophic cardiomyopathy. *Hum Mutat*. 2001; 17(6): 524.
- 29 Mogensen J, Murphy RT, Shaw T, Bahl A, Redwood C, Watkins H, *et al*. Severe disease expression of cardiac troponin C and T mutations in patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*. 2004; 44(10): 2033-40.
- 30 Gilda JE, Xu Q, Martinez ME, Nguyen ST, Chase PB, Gomes AV. The functional significance of the last 5 residues of the C-terminus of cardiac troponin I. *Arch Biochem Biophys*. 2016; 601: 88-96.
- 31 Chang AN, Parvatiyar MS, Potter JD. Troponin and cardiomyopathy. *Biochem Biophys Res Commun*. 2008; 369(1): 74-81.
- 32 Smith J, Kampine J. *Circulatory Physiology-the essentials* Baltimore: Lippincott, Williams & Wilkins 1984.
- 33 Sou SM, Puelacher C, Twerenbold R, Wagener M, Honegger U, Reichlin T, *et al*. Direct comparison of cardiac troponin I and cardiac troponin T in the detection of exercise-induced myocardial ischemia. *Clin Biochem*. 2016; 49(6): 421-32.
- 34 Rubini Gimenez M, Twerenbold R, Reichlin T, Wildi K, Haaf P, Schaefer M, *et al*. Direct comparison of high-sensitivity-cardiac troponin I vs. T for the early diagnosis of acute myocardial infarction. *Eur Heart J*. 2014; 35(34): 2303-11.
- 35 Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol*. 2000; 36(3): 959-69.

- 36 Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. *Am Heart J.* 1987; 113(6): 1333-44.
- 37 Jacobs LH, van de Kerkhof J, Mingels AM, Kleijnen VW, van der Sande FM, Wodzig WK, *et al.* Haemodialysis patients longitudinally assessed by highly sensitive cardiac troponin T and commercial cardiac troponin T and cardiac troponin I assays. *Ann Clin Biochem.* 2009; 46(1): 283-90.
- 38 Flores LM, Hernández Dominguez JL, Otero A, González Juanatey JR. [Cardiac troponin I determination in patients with chronic renal failure]. *Nefrologia.* 2006; 26: 107-12.
- 39 Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, *et al.* Third universal definition of myocardial infarction. *Circulation.* 2012; 126(16): 2020-35.
- 40 Katus HA, Looser S, Hallermayer K, Remppis A, Scheffold T, Borgya A, *et al.* Development and in vitro characterization of a new immunoassay of cardiac troponin T. *Clin Chem.* 1992; 38(3): 386-93.
- 41 Tanislav C, Guenduez D, Liebetrau C, Giese AK, Eichler S, Sieweke N, *et al.* Cardiac Troponin I: A Valuable Biomarker Indicating the Cardiac Involvement in Fabry Disease. *PLoS One.* 2016; 11(6): e0157640.
- 42 Gerhardt W, Nordin G, Herbert AK, Burzell BL, Isaksson A, Gustavsson E, *et al.* Troponin T and I assays show decreased concentrations in heparin plasma compared with serum: lower recoveries in early than in late phases of myocardial injury. *Clin Chem.* 2000; 46(6Pt 1): 817-21.
- 43 Apple FS, Murakami M, Panteghini M, Christenson RH, Dati F, Mair J, *et al.* International survey on the use of cardiac markers. *Clin Chem.* 2001; 47(3): 587-8.
- 44 Herrmann J, Lennon RJ, Jaffe AS, Holmes DR, Rihal CS, Prasad A. Defining the optimal cardiac troponin T threshold for predicting death caused by periprocedural myocardial infarction after percutaneous coronary intervention. *Circ Cardiovasc Interv.* 2014; 7(4): 533-42.
- 45 Kavsak PA, MacRae AR, Yerna MJ, Jaffe AS. Analytic and clinical utility of a next-generation, highly sensitive cardiac troponin I assay for early detection of myocardial injury. *Clin Chem.* 2009; 55(3): 573-7.
- 46 Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, *et al.* Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med.* 2009; 361(9): 858-67.
- 47 Diercks DB, Peacock WF, Hollander JE, Singer AJ, Birkhahn R, Shapiro N, *et al.* Diagnostic accuracy of a point-of-care troponin I assay for acute myocardial infarction within 3 hours after presentation in early presenters to the emergency department with chest pain. *Am Heart J.* 2012; 163(1): 74-80.e4.
- 48 Barton PJ, Townsend PJ, Brand NJ, Yacoub MH. Localization of the fast skeletal muscle troponin I gene (TNNI2) to 11p15.5: genes for troponin I and T are organized in pairs. *Ann Hum Genet.* 1997; 61(Pt 6):519-23.