This Review is part of a thematic series on Regulatory Signaling by Thin Filament Modulation, which includes the following articles:

- Modulation of Thin Filament Activation by Breakdown or Isoform Switching of Thin Filament Proteins: Physiological and Pathological Implications
- Modulation of Thin Filament Activation by Crossbridges
- Covalent and Noncovalent Modification of Thin Filament Action: The Essential Role of Troponin in Cardiac Muscle Regulation
- At the Crossroads of Myocardial Signaling: The Role of Z-Disks in Intracellular Signaling and Cardiac Function

R. John Solaro, Guest Editor

Modulation of Thin Filament Activation by Breakdown or Isoform Switching of Thin Filament Proteins

Physiological and Pathological Implications

Steven B. Marston, Charles S. Redwood

Abstract—In the heart, the contractile apparatus is adapted to the specific demands of the organ for continuous rhythmic contraction. The specialized contractile properties of heart muscle are attributable to the expression of cardiac-specific isoforms of contractile proteins. This review describes the isoforms of the thin filament proteins actin and tropomyosin and the three troponin subunits found in human heart muscle, how the isoform profiles of these proteins change during development and disease, and the possible functional consequences of these changes. During development of the heart, there is a distinctive switch of isoform expression at or shortly after birth; however, during adult life, thin filament protein isoform composition seems to be stable despite protein turnover rates of 3 to 10 days. The pattern of isoforms of actin, tropomyosin, troponin I, troponin C, and troponin T is not affected by aging or heart disease (ischemia and dilated cardiomyopathy). The evidence for proteolysis of thin filament proteins in situ during ischemia and stunning is evaluated, and it is concluded that C-terminal cleavage of troponin I is a feature of irreversibly injured myocardium but may not play a role in reversible stunning. (Circ Res. 2003;93:1170-1178.)

Key Words: actin • troponin • tropomyosin • calpain • stunning

The contractile apparatus of muscle is made up from interdigitating thick and thin filaments arrayed in sarcomeres. Contraction is attributable to the sliding of thick filaments past thin filaments and is powered by the molecular motor of the myosin crossbridge cyclically attaching to actin, changing conformation, and detaching. Contractility is controlled by sarcoplasmic Ca\(^{2+}\), which acts on the receptor molecules troponin and tropomyosin in the thin filaments. Native thin filaments are built up from a double helix of actin monomers, with the elongated tropomyosin molecule forming a continuous strand along each actin helix and the troponin complex bound every 7th actin. Troponin is made up from three subunits. Troponin I is the inhibitory protein that binds to actin and prevents myosin crossbridge cycling. Troponin C is the Ca\(^{2+}\)-binding component; at micromolar Ca\(^{2+}\) concentrations, Ca\(^{2+}\) is bound to troponin C which then binds to troponin I, releasing it from its inhibitory site on actin. Troponin T is an elongated molecule that binds to both troponin C and troponin I and also to tropomyosin, thus anchoring the complex on the thin filament. The interaction...
of the troponin complex with tropomyosin propagates the regulatory signal from 1 troponin to up to 14 adjacent actin molecules; thus, troponin tropomyosin is the cooperative-allosteric Ca\(^{2+}\) switch of the thin filament.\(^2\)

In the heart, the contractile apparatus is adapted to the specific demands of the organ for continuous rhythmic contraction and is one of the features of cardiac muscle that differentiates it from slow and fast skeletal muscles. The specialized contractile properties of heart muscle are attributable to the expression of cardiac-specific isoforms of contractile proteins. This review describes the isoforms of the thin filament proteins actin, tropomyosin, and the three troponin subunits found in human heart muscle, how the isoform profiles of these proteins change during development and disease, and the possible functional consequences of these changes. In addition, the possible role of proteolytic cleavage of thin filament proteins in certain disease states is discussed, along with the likely impact on thin filament regulation.

Changes in Cardiac Thin Filament Expression

During development of the heart there is a distinctive switch of isoform expression at or shortly after birth; however, during adult life, thin filament protein isoform composition seems to be stable despite protein turnover half-lives of 3 to 10 days. The developmental changes in isoform expression are summarized in Figure 1 and described in detail below. The question of whether this pattern changes in adult heart in pathological states has been the subject of dispute, and here we review the existing information, giving precedence to the data collected in humans when available. Comparatively little is known about actin and tropomyosin isoform alterations in humans, and there is general agreement that troponin I and troponin C isoform expression does not change in adult heart. On the other hand, it has been suggested that the isoforms of troponin T are changed in the failing heart.

Actin

Actin is the most highly conserved of contractile proteins. There are six actin genes in the human genome, of which two are commonly expressed in striated muscles. The \(\alpha\) skeletal actin is encoded by the gene \(ACTA1\) on chromosome 1q42, and \(\alpha\) cardiac actin is encoded by \(ACTC\) on chromosome 15q11-q14. The encoded proteins are both composed of 377 residues, but the mature polypeptides are of only 375 amino acids because of successive removal of the initiator methionine and the cysteine at position 2 during posttranslational modification.\(^3\) The only difference between the two isoforms are two amino acid substitutions (Met299Leu and Ser358Thr) and the transposition of the amino acids at positions 2 and 3 in the mature protein. Determination of the amounts of the isoforms expressed in tissue has been hampered by the extreme similarity of the isoforms, which cannot be separated using 2D gel electrophoresis. Present understanding of the ratios is based on the use of N-terminal amino acid sequencing to distinguish between isoforms.\(^3\) The technique is reliable but very tedious; mass spectrometry has been shown to be capable of quantitation of the two isoforms in mixtures.\(^6\) and isoform-specific antibodies have been demonstrated.\(^7\) These techniques may be expected to yield more detailed information in the near future.

Both isoforms have been shown to be expressed in human cardiac muscle. Analysis of the N-termini of actin preparations indicates that the \(\alpha\)-cardiac actin is the predominant isoform in adult hearts, accounting for approximately 80% of total actin in both human ventricular and atrial samples. The ratio of 4:1 with respect to \(\alpha\)-skeletal actin was also observed in pig and beef hearts.\(^5\)

Less \(\alpha\)-skeletal actin seems to be present in the human heart during development; a recent study using isoform-specific antibodies has shown that \(\alpha\)-skeletal actin is localized in a small proportion of subendocardial and papillary muscle myocytes in the 20-week-old fetal heart, becoming more widespread in the neonate. In adult myocardium, the proportion of fibers containing \(\alpha\)-skeletal actin was found to be 28% (compared with 0.9% of fibers in fetal heart), and the skeletal isoform was found to be consistently more abundant in the subendocardial (67%) than in the subepicardial (28%) regions.\(^7\) In addition, in the fetal heart, transient expression of the \(\alpha\)-smooth muscle isoform (\(ACTA2\), chromosome 10q23), localized at foci throughout the entire myocardium, was detected at up to 20 weeks. It is not clear whether this is attributable to staining of myocytes or fibroblast cells.

At the mRNA level, a trend of increased \(\alpha\)-skeletal actin expression in human heart with age has also been shown, although the proportions of \(\alpha\)-skeletal and \(\alpha\)-cardiac transcripts do not match those of the protein. \(\alpha\)-Smooth muscle actin mRNA was detected in fetal heart tissue at up to 20 weeks as well as \(\alpha\)-cardiac and \(\alpha\)-skeletal muscle actin mRNA. In fetal and neonatal hearts, \(\alpha\)-skeletal actin mRNA accounted for up to 20% of all sarcomeric actin mRNA, increasing to 48% during the first decade of life and becoming the predominant actin mRNA (\(\approx\)60% of actin transcripts) in adult hearts.\(^8\) Quantitatively, mRNA and protein levels do not match; however, localization studies of \(\alpha\)-actin mRNA in fetal heart using in situ hybridization are in accord with the

![Figure 1. Diagram summarizing the likely changes in composition of actin, tropomyosin, and troponin isoforms during development from fetal to adult in human myocardium. The width of each line indicates the approximate proportion of each isoform with respect to other isoforms of the same protein.](https://example.com/figure1.png)
protein data.9 In sharp contrast, in the rat, α-skeletal actin has been found to be present in embryonic heart (28% of total actin mRNA 17 to 19 days in utero) and neonatal heart (40% 3 weeks after birth) but barely detectable in the adult (<5% of total actin mRNA).

The early expression of the α-smooth isoform in the fetal human heart and its replacement by α-skeletal actin suggests that these proteins may play distinct roles in myocyte growth and development. The isoform changes may be causing subtle but important differences in contractility; for example, the overexpression of smooth muscle actin in the heart in a mouse model has been shown to decrease Ca²⁺ sensitivity and tension cost,10 and α-skeletal actin has been associated with increased contractility in the mouse heart.11 On the other hand, direct comparisons of purified cardiac and skeletal muscle actin failed to detect any differences in the kinetics of actomyosin ATPase.12 It is possible that the temporal shifts in the actin isoform composition may be influencing steps in differentiation or myofibrillogenesis, independent of the changes they bring about to contractility. Low-level expression of human α-skeletal actin, but not the cardiac isoform, has been reported to cause a profound decrease in cell spreading and the induction of a specific subset of genes in a mouse fibroblast system, suggesting that the order of actin isoform expression may be important during myogenesis.13,14

There have been several reports of increases in α-skeletal actin, at least at the mRNA level, in animal models of hypertrophy and in diseased human myocardium. In a rat model of pressure overload, α-skeletal actin expression in the heart increased profoundly from barely detectable levels within 2 days of aortic coarctation.15 Analysis of actin mRNA levels in human dilated cardiomyopathy hearts has been reported to show significant increases in α-skeletal (and α-smooth) actin mRNA compared with normal control hearts; however, these data also showed similar large increases in cardiac α-actin transcript levels as well.16 Because total actin content does not increase in diseased muscle, there is a suggestion that either translation becomes less efficient or actin turnover is greatly increased in hypertrophic and failing heart.17 In patients with dilated cardiomyopathy or ischemic heart disease, most mRNA studies have found either no significant difference in the relative transcript abundance for the two isoforms8 or have concluded that there is variability in both diseased and nondiseased myocardium and hence the variations are not linked to the disease state.17

Quantification of the actin isoform proteins in the heart from a hypertrophic cardiomyopathy patient showed a 79% to 21% percentage ratio of the α-cardiac and α-skeletal forms, identical to that found in normal myocardium.5 A recent morphometric analysis using isoform-specific antibodies comparing normal and diseased heart suggested there was a significant increase in the proportion of fibers expressing α-skeletal actin in hearts from patients with marked left ventricular hypertrophy or eccentric hypertrophic cardiomyopathy (but not those with dilated cardiomyopathy), especially in the epicardial regions.7 It is suggested that discrepancies with reports showing no increase in α-skeletal actin may be attributable to the use of endocardial biopsies that are not adequately representative of the entire ventricular wall.

**Tropomyosin**

Tropomyosins are α-helical coiled coil dimers that lie along the actin filament in all muscle fibers and many nonmuscle cells. There are four human tropomyosin genes, TPM1 (located on chromosome 15q22), TPM2 (9p13), TPM3 (1q21.2), and TPM4 (19p13.1), and each gene can produce multiple tissue-specific mRNA transcripts via the use of alternative promoters, alternative splicing, and different 3′ processing.18,19 The principal tropomyosin proteins found in human striated muscles are α-tropomyosin (a product of TPM1), β-tropomyosin (from TPM2), and α-slow tropomyosin (also known as TPM 3 or TM-30, transcribed from TPM3) (Figure 2). Adult hearts of small animals such as rabbit, guinea pig, rat, and dog seem to contain almost exclusively the α isoform, whereas in beef, pig, and sheep myocardium, β-tropomyosin is also present at approximately a 1:4 ratio to the α isoform.20,21 Both α and β isoforms were reported to be present in adult human heart extracts, with the α to β ratio measured at 1:4.8, based on densitometry of stained gels after separating whole tissue by SDS-PAGE; however, purified human ventricular tropomyosin, identified by immunoblotting, has been reported to be exclusively α.22

The significance of the TPM3 gene product to heart muscle is uncertain. At the mRNA level, α-slow tropomyosin has been shown to be expressed in adult human myocardium,23 although the proportion of this isoform present at the protein level remains unclear. The presence of the α-slow tropomyosin protein has not been detected in mouse hearts24 and has not been investigated in human cardiac muscle.
Developmental changes in human cardiac troponyosin content are not well characterized. At the protein level, no data are available. In rodents, α and β troponyosin mRNA are both present in the embryonic heart, and the presence of smaller nonmuscle isoforms has also been detected at both the protein and mRNA levels.\textsuperscript{15,25,26} In the mouse, β-troponyosin mRNA is present at a 1:4.5 ratio to the α isoform mRNA in the 11-day embryonic heart, declining to \(\approx 1:18\) at birth and ultimately 1:60.5 in the adult.\textsuperscript{25} It thus appears that at least in the mouse, the β isoform may be important during cardiogenesis.

Knockout and transgenic mouse studies have been carried out to determine the functional effects of switching the troponyosin isoform composition in the heart from its normal state (almost exclusively α). Lines of mice have been created with the α-troponyosin gene ablated by homologous recombination; in the homozygote, this caused an embryonic lethal phenotype, with death occurring between 8 and 11.5 days postcoitum. Surprisingly, in the heterozygotes, which had a 50% reduction in α-troponyosin mRNA, the level of α-troponyosin protein was unaffected and there were no phenotypic differences compared with control mice, suggesting that the levels of the protein are regulated at the translational level.\textsuperscript{27} Significant effects have been reported in transgenic mice overexpressing the β isoform to 57.8% of total heart troponyosin. As well as altered diastolic function, fibers from these hearts showed an increased Ca\(^{2+}\) sensitivity of force generation, and isolated myocytes displayed reduced rates of contraction and relaxation.\textsuperscript{28–30} Additional overexpression of β-troponyosin to at least 75% of total heart troponyosin has been reported to cause more severe cardiac abnormalities and thrombus formation, resulting in death 10 to 14 days after birth.\textsuperscript{31} In an additional mouse model, overexpression of the α-slow isoform to \(\approx 50%\) of total heart troponyosin was found to result in increased heart rate and reduced Ca\(^{2+}\) sensitivity.\textsuperscript{32} In a pressure-overload rat model, aortic coarctation did result in marked reexpression of both isoforms (TnT1 and TnT2) giving a higher Ca\(^{2+}\) sensitivity.\textsuperscript{33} Interestingly, the greater sensitivity to acidosis of cardiac troponin I has been attributed to a single amino acid difference: histidine 163 in the cardiac isoform is replaced by alanine in the equivalent position (residue 132) in slow skeletal troponin I.\textsuperscript{40} Some or all of these functional differences are likely be of importance during cardiac development and myofibrillogenesis. Cardiac troponin I is distinguished from slow skeletal troponin I by its N-terminal extension containing two serine residues, 23 and 24, that are substrates in vivo for protein kinase A (PKA). Phosphorylation of cardiac troponin I by PKA reduces Ca\(^{2+}\) sensitivity.\textsuperscript{41} Protein kinase C (PKC) can also phosphorylate cardiac troponin I at ser43, ser45, and thr144, and this phosphorylation seems to increase Ca\(^{2+}\) sensitivity.\textsuperscript{42,43} The modulation of cardiac performance by troponin phosphorylation is covered by another review in this series.

**Troponin C**

Troponin C is the Ca\(^{2+}\)-sensing component of the troponin complex and belongs to the EF-hand family of proteins. The cardiac isoform is a product of the TNNT2 gene (1q32) potentially produces multiple transcripts via alternative splicing involving exons 4, 5, 10, and 13.\textsuperscript{44–46} The most common isoforms are attributable to combinatorial alternative splicing of exons 4 and 5 and have been termed TnT1, TnT2, TnT3, and TnT4\textsuperscript{47} (Figure 3). Each is expressed in fetal heart, with TnT1 and TnT3 being the predominant isoforms; during perinatal development, the expression of TnT1 declines and that of TnT3 increases, until, in the adult, TnT3 is the major isoform, TnT4 is a minor isoform, and TnT1 and TnT2 are barely detectable.\textsuperscript{37} Characterization of recombinant cardiac human troponin T isoforms in skinned fibers has shown that both fetal isoforms (TnT1 and TnT2) give a higher Ca\(^{2+}\) sensitivity than either the TnT3 or TnT4 protein (by 0.1 to 0.15 pCa units) and that the fetal proteins gave reduced switch off under relaxing conditions.\textsuperscript{48} TnT isoform expression is very variable between species, and this makes the application of results from animal models problematic. Biesiedacki et al\textsuperscript{49} recently demonstrated the presence of up to six protein species being simultaneously
expressed in adult heart muscles of dog and guinea pig. In addition to the exon 4 and 5 splice variants, species lacking exon 6 (guinea pig) or exon 7 (dog) made up as much as 25% of total adult troponin T.

It has been suggested that troponin T isoform expression is changed in failing heart. Although initial observations were made in animal models of failing heart (rabbit and guinea pig), it was later shown that in failing human heart tissue, up to 20% of troponin T detected by Western blotting ran with a faster mobility than the single band observed in nonfailing heart tissue. Fetal heart muscle showed both bands plus a major band with a lower mobility than the main band (the fetal-specific T1 isoform); therefore, it was suggested that “a partial recapitulation of the fetal expression of T isoforms” occurred in failing human heart. Investigations of the molecular basis for the isoform variation established that troponin T predominantly expressed in the adult human heart was the T3 protein isoform, whereas the T4 isoform was identified as the higher mobility band. The comprehensive study of Mesnard-Rouiller et al examined both mRNA and protein in normal and failing human heart tissue. The troponin T isoform composition found in the diseased samples was shown to be similar to that found in the control hearts, with cTnT3 being the major mRNA and protein variant. Protein isoform quantities followed the same pattern as mRNA; thus, the TnT4 mRNA represented 9.4 ± 1.3% of normal and 14.7 ± 1.9% of failing heart troponin T mRNA, and the protein was 11.7 ± 1.3% of total TnT for normal and 15.3 ± 2.3% for failing heart. The difference between normal and failing hearts was not significant for either measurement. The TnT1 protein was detected at very low levels in both normal adult and failing heart tissue (7.0 ± 0.1% and 3.5 ± 0.3%, respectively; a statistically significant difference).

Thus, there is no direct evidence for significant reexpression of the fetal TnT isoforms in failing heart. A higher mobility TnT variant, presumed to be the T4 isoform, has been reported in failing heart tissue by some, but it was not observed by others. Solaro et al observed the higher mobility band in only 10 failing hearts examined, whereas Knott et al observed only a single TnT band in six failing hearts and van der Velden et al recently reported a single troponin T band in 26 failing hearts. All of these studies reported decreased maximally activated myofibrillar ATPase activity, unloaded shortening or sliding speed, and increased Ca2+ sensitivity in failing heart muscle independent of whether the putative T4 band was present in the sample. It is possible that a particular subset of patients with heart failure reexpress TnT4 isoform; however, the possibility that the higher mobility band, when observed, could be a proteolytic fragment of TnT3 was suggested by the work of Knott and colleagues and has not been ruled out in other studies.

The physiological significance of fetal troponin T isoform reexpression in failing heart now seems doubtful. At most, 10% to 20% of cardiac troponin T is altered in vivo in some failing hearts, and recent studies indicate that the functional differences between TnT3 and TnT4 are minimal and could not solely account for the observed increased Ca2+ sensitivity found in failing heart muscle. Present thought favors alterations in troponin phosphorylation as the cause of the defective contractility of failing heart muscle.

**Degradation of Thin Filament Proteins**

Despite the absence of evidence for isoform changes in any thin filament proteins, differences in thin filament function in failing human heart have been consistently observed. These effects could be explained by posttranslational modifications such as phosphorylation by PKC and PKA or by proteolytic degradation.

Degradation of thin filament proteins can occur as part of the natural turnover of protein or as a result of injury. In rat heart muscle, troponin I and troponin T have half-lives of 3.2 and 3.5 days, respectively. Troponymosin has a half-life of 5.5 days, and actin has a half-life of 10.3 days. It has been shown that the new proteins exchange into preexisting sarcomeres at random locations; this implies that there must be some mechanism for degrading old proteins in situ to allow for them to be released from the thin filament. Nothing is known about the mechanisms, although it is possible that in some cases the appearance of degraded thin filament proteins could be the consequence of an imbalance in the dynamic process of thin filament maintenance.

Degraded thin filament proteins have generally been observed as a result of injury to the heart; the release of degraded as well as intact cardiac troponin I and troponin T...
into the bloodstream is a well established test for the presence of infarcted tissue.\textsuperscript{64} The enzyme most frequently considered to be involved is \(\mu\)-calpain (also known as calpain I), because it is activated by micromolar Ca\(^{2+}\) concentrations that are likely to be found in the cytoplasm of myocytes under Ca\(^{2+}\) overload conditions. In the isolated troponin complex, troponin I and troponin T are rapidly degraded by \(\mu\)-calpain but not by m-calpain; interestingly, the rate of degradation of troponin I is phosphorylation dependent, being substantially reduced by PKA phosphorylation but enhanced by PKC phosphorylation in vitro.\textsuperscript{65} It is important to note that in intact myofibrils, degradation of troponin I and troponin T by \(\mu\)-calpain is minimal and the most sensitive proteins are the cytoskeletal and Z-line proteins nebulin, desmin, caldesmin, and \(\alpha\)-actinin, with titin and filamin being degraded at an intermediate rate.\textsuperscript{66–68}

The primary cleavage site associated with calpain digestion of cardiac troponin I (210 amino acids) is at the C-terminus, producing a peptide containing residues 1 to 193.\textsuperscript{69} However, most studies of protein degradation have not identified the cleavage point, and one cannot assume that troponin I is always split near the C-terminus. In necrotic human heart tissue,\textsuperscript{64} troponin isolated from human heart,\textsuperscript{56} and in a tail suspension rat model,\textsuperscript{70} proteolytic cleavage at the N terminus between amino acids 25 and 28 has been positively identified using sequencing, antibody epitope analysis, and mass spectrometry. N-terminally cleaved troponin I was also identified in normal mouse, rabbit, cat, and human heart muscle homogenates.\textsuperscript{70} The enzyme responsible for this cleavage is unknown but may be related to the normal protein turnover process.

A possible role in protein degradation has also been proposed for the enzyme caspase 3, which is involved in apoptosis. Actin and \(\alpha\)-actinin were cleaved by this enzyme, but cleavage of tropomyosin, troponin I, troponin T, and troponin C was barely detectable, although troponin T was degraded in the intact troponin complex. It was found that ventricular myocytes undergoing apoptosis contained detectable amounts of cleaved actin and troponin T.\textsuperscript{71}

Investigation of the presence of degradation of thin filament proteins in diseased heart muscle is fraught with difficulty even in well-controlled animal models. The target proteins, the extent of proteolysis, and the site of cleavage depend on the nature of the injury and the species that is investigated. For example, ischemic rat heart is associated with C-terminal cleavage of troponin I, but increased preload is associated with N-terminal cleavage\textsuperscript{59,70}; heart damage induced by reactive oxygen species has been shown to cause troponin I degradation in rat heart, but the same treatment causes troponin T degradation in rabbit heart.\textsuperscript{72}

Troponin I degradation in stunned myocardium has been intensively studied and provides a good example of the difficulties of such investigations. Initially it was observed that after 60 minutes of global ischemia, up to 50% of troponin I and troponin T was degraded, whereas actin, troponin C, and tropomyosin were not significantly altered.\textsuperscript{73} Gao and colleagues\textsuperscript{74,75} developed a rat model of stunning using 20 minutes of ischemia followed by 20 minutes of reperfusion; maximum isometric force was decreased by 40% and Ca\(^{2+}\) sensitivity was reduced (EC\textsubscript{50} doubled). Under these conditions, \(\approx 50\%\) of troponin I (31 kDa) was degraded to a 26-kDa band, whereas troponin T, tropomyosin, and actin were untouched. The troponin I degradation was shown to be specifically linked to the stunning protocol, because it was not observed in 20 minutes of ischemia without reperfusion or when hearts were reperfused under conditions that diminished stunning (low Ca\(^{2+}\), low pH). Furthermore, calpain was implicated in both stunning and troponin I degradation, because added calpain I produced both effects in normally perfused heart and calpastatin blocked the effect.\textsuperscript{74,75}

The same degradation of troponin I without degradation of troponin T or tropomyosin was found in rat heart by Van Eyk et al\textsuperscript{76} after 15 minutes of ischemia and 15 minutes of reperfusion. In this study, although maximum force was reduced, Ca\(^{2+}\) sensitivity of contractile activation was slightly increased. The degraded troponin I fragment was identified as the 1-193 peptide.\textsuperscript{69} The authors also observed substantial degradation of the Z-line protein \(\alpha\)-actinin and the formation of covalent complexes ThnI(1-193)/ThnT(191-298) and ThnI(1-193)/ThnC(1-94) making up 17% of total troponin I. This confirmed an earlier histological study that had suggested the formation of cross-linked complexes.\textsuperscript{77} A more prolonged 60-minute ischemia led to a substantial release of protein on reperfusion, indicating necrosis, and was accompanied by additional degradation of troponin I to 16- and 15-kDa bands attributable to N-terminal cleavage of 1-193 at amino acids 63 and 73. The physiological importance of the loss of 17 amino acids from the C-terminus of troponin I was confirmed in a transgenic mouse model expressing the truncated troponin I, which exhibited the reduced isometric force and lower Ca\(^{2+}\) sensitivity characteristic of stunning.\textsuperscript{78}

Initially, a self-consistent case could be made for a role of troponin I degradation in myocardial stunning. Troponin I degradation occurs in stunned myocardium attributable to \(\mu\)-calpain activation during Ca\(^{2+}\) overload and could cause the defective contractility. Recovery from stunning would occur as new intact troponin I was synthesized and incorporated into the sarcomere. Unfortunately, experiments in models where recovery from stunning was examined showed no correlation between stunning and troponin I degradation. Stunning without degradation and degradation without stunning were observed.\textsuperscript{79}

Prasan et al\textsuperscript{80} investigated stunning in a rabbit model. They found no increase in troponin I fragmentation after reversible stunning, induced by 15 minutes of ischemia and 15 minutes of reperfusion, and they also showed that functional recovery was not related to troponin I breakdown. Oxygen radical scavengers attenuated stunning in viable myocardium, and hypercholesterolemia was associated with poor recovery; however, in neither case did the level of troponin I degradation correlate with the functional recovery of the stunned myocardium. The study of Feng et al\textsuperscript{81} used a rat model in which preload was kept low (10 mm Hg) during reperfusion. Stunning developed, but there was no increase in troponin I degradation over control. In contrast, rat hearts exposed to an elevated preload (25 mm Hg) without ischemia showed that 20% of troponin I degraded, which could be prevented by calpeptin. Because the standard rat Langendorff heart prepa-
ration used in most studies develops at least 30 mm Hg left ventricular end-diastolic pressure during reperfusion, it was concluded that the elevated preload in this preparation was the cause of troponin I breakdown rather than stunning.

Large animal studies (dog, sheep, and pig) have failed to observe any troponin I breakdown associated with reversible stunning. Moreover, there is little evidence for troponin I breakdown in human cardiac muscle. Studies on failing human heart muscle have usually failed to find any degradation of thin filament proteins. A study of biopsy samples from patients undergoing bypass surgery, which involves a period of ischemia followed by reperfusion, identified the 22-kDa fragment of troponin I in the left ventricular muscle of 25 of 68 patients before operation and 26 of 70 patients after. Several of these patients had additional low-molecular-weight degradation products of troponin I, indicative of preexisting necrotic damage to the heart. Up to 15% modification of troponin I was also observed in a study of four hearts explanted from patients with ischemic heart failure, and this was ascribed to increased preload. On average, there was no difference in the level of troponin I degradation before and after coronary artery bypass grafting operation; in a sample of 30 patients, degradation increased for 10 patients, decreased for 10 patients, and stayed the same for 10 patients. We have performed a similar study in heart biopsies from six patients undergoing bypass surgery and have likewise failed to see any change in troponin degradation during cross-clamp (J.R. Pepper, S.H. Harding, and S.B. Marston, unpublished observations, 2002).

The conclusion from all of the work on troponin I degradation would now seem to be that calpain-catalyzed C-terminal cleavage of troponin I is a feature of irreversibly injured myocardium, but it is less clear whether it plays a role in reversible stunning. Although troponin I is not a good substrate of \( \mu \)-calpain until it is released from the myofibrils, the evidence that reversible stunning is related to \( \mu \)-calpain activation is convincing. Proteolysis of other proteins might be involved. Reduced phosphorylation of contractile or Ca\(^{2+}\) handling proteins has been suggested as the cause of stunning, although the observations were not experimentally linked to proteolysis. There is evidence linking breakdown of Z-line proteins to stunning. It is established that proteins of the Z line and cytoskeleton (\( \alpha \)-actinin, desmin spectrin, and titin) are more than 100 times more susceptible to \( \mu \)-calpain proteolysis in the intact myofibril than troponin I, and Papp et al. have recently demonstrated that rat myofibrils treated with calpain primarily produced desmin degradation. When fibers were treated in relaxing conditions, their structure and function were preserved, but after an initial contraction, sarcomere disorder appeared and force-generating capacity was diminished, indicating that the effect of stress on a weakened Z-line structure was responsible for the contractile defect.

Cleavage of \( \alpha \)-actinin, spectrin, and desmin has been reported in ischemic heart and stunned myocardium in animal models and in infarcted human heart tissue. Thus, it is possible that damage to Z-lines could be behind the diminished contractility of stunned myocardium.

Acknowledgments
This research was funded by grants from the British Heart Foundation. We thank Hend Farza for helpful discussions on this manuscript.

References


Modulation of Thin Filament Activation by Breakdown or Isoform Switching of Thin Filament Proteins: Physiological and Pathological Implications
Steven B. Marston and Charles S. Redwood

Circ Res. 2003;93:1170-1178
doi: 10.1161/01.RES.0000105088.06696.17
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/93/12/1170

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/