Troponin I Isoform Expression in Human Heart

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Troponin I is the inhibitory component of troponin, the thin filament regulatory complex in striated muscle. Separate genes encode cardiac-specific fast and slow skeletal-specific isoforms of this protein. We have previously described gene switching from the slow skeletal to the cardiac troponin I mRNA expression in developing rat heart. The purpose of this work was to characterize the expression of the different troponin isoforms in the human heart. Human cardiac and slow skeletal troponin I cDNA probes were obtained by screening an adult cardiac cDNA library and by Taq polymerase amplification of RNA from an infant's heart, respectively. We found that the cardiac troponin I isoform is tissue-specific in its expression in normal adult tissues. RNA blot analysis of cardiac ventricular RNA from infants with congenital heart disease and from an adult with cardiomyopathy revealed expression of human cardiac troponin I in all analyzed specimens. In addition, we found expression of slow skeletal troponin I mRNA and protein in infant hearts but no detectable mRNA expression in the adult heart. We conclude that troponin I isoforms are developmentally regulated in the human heart by a mechanism similar to that in the rat heart. (Circulation Research 1991;69:1409–1414)

Troponin, a contractile protein of the thin filament of striated muscle, consists of three subunits: troponin C (TnC), troponin T (TnT), and troponin I (TnI). Troponin I is the inhibitory subunit of the troponin complex. It has an important function in the regulation of striated muscle contraction.1 At basal levels of calcium, TnI inhibits actin–myosin crossbridges. In systole, as calcium binds to the regulatory site of TnC, the inhibitory action of TnI is released, thereby activating muscle contraction.

Separate isoforms of TnI are present in fast-twitch and slow-twitch skeletal muscle fibers and in cardiac muscle.2 These isoforms may contribute to the functional differences of the various muscle types. Amino acid and cDNA sequences have been reported in several animal species2–4 as well as in humans.5,6 Separate genes encode the three isoforms. This is supported by the degree of primary sequence difference of both the translated and untranslated regions among isoforms of a single species, as well as others3 have previously described in the rat.

TnI is developmentally regulated at the mRNA level in rat heart, with coexpression of the slow skeletal and cardiac mRNA and protein in fetal heart.4,7 With maturation, the quantity of slow skeletal TnI mRNA decreases, and the quantity of the cardiac TnI mRNA increases.4 The slow skeletal mRNA is detectable by RNA blotting in the rat heart as late as 14–21 days after birth.4 The sequential activation of different members of a contractile gene protein family is a well-described mechanism for the developmental regulation of contractile proteins, as reviewed by Wade and Kedes.8 However, developmental gene switching that is present in smaller mammals, such as rats, often does not occur in the human heart. This difference in developmental regulation between smaller and larger mammals has been described for both the α-actin and the myosin heavy chain (MHC) genes.9–12 In rats, the V3 MHC isoform, which is a homodimer or the β-MHC gene product, predominates in the fetal heart and is expressed in conditions of mechanical overload.11,13 The MHC V1 isoform, a homodimer of the α-MHC gene product, is the isoform found in the adult rat heart.11 In the human heart, however, there is no shift from the V1 to the V3 isoform.9 The human V1 isoform remains predominant throughout development in the human heart.9 Similarly, the cardiac and skeletal α-actin genes are coexpressed in fetal rat heart, but the skeletal mRNA is markedly downregulated in the adult rat heart.12 However, in the adult human heart, both isogenes are highly expressed.10

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Thus, if the TnI isoforms were developmentally regulated in human heart, the TnI gene family would provide an interesting model system to examine regulatory mechanisms common among lower and higher mammals.

The purpose of this work was to characterize the expression of the cardiac and slow skeletal TnI isoforms and to search for evidence of developmental regulation in the human heart. In addition to the potential use of the TnI genes as models for contractile protein gene regulation, variations in TnI isoform expression during cardiac maturation are important because they may result in alterations in cardiac function.

Materials and Methods

Human Cardiac and Slow Skeletal TnI cDNAs

A near full-length human cardiac cDNA was obtained by screening an adult human heart Agt11 library with an oligonucleotide probe derived from a carboxy terminal region of the rabbit cardiac amino acid sequence. The clone was subjected to sequencing on both strands after subcloning into pGEM-3Z (Promega Corp., Madison, Wisc.), a plasmid vector. A cDNA encoding human slow skeletal TnI was obtained by Taq polymerase amplification. The cDNA template was synthesized from human cardiac total cellular RNA. This RNA was isolated from cardiac ventricular tissue removed from a 7-month-old infant during surgical repair of congenital heart disease. Oligo d(T) was used as the annealing primer for the cDNA synthesis. The primer sequences used for polymeric chain reaction (PCR) amplification were selected from the 5' and 3'-untranslated regions of the published cDNA sequence of human slow skeletal TnI to produce a 685-bp product. Restriction enzymes sites were included in the primer sequences to facilitate subcloning of the PCR product. The following cycles were used in the PCR amplification: for cycle 1, denature at 95°C for 5 minutes, anneal at 55°C for 3 minutes, and extend at 72°C for 6 minutes; for cycles 2–40, denature at 95°C for 2 minutes, anneal at 55°C for 3 minutes, and extend at 72°C for 2 minutes. On analysis of the products, a single product of the predicted length for slow skeletal TnI was evident on gel electrophoresis. It was cloned into pGEM-3Z (Promega) and subjected to sequence analysis to confirm its identity with human slow skeletal TnI. RNA blots were probed with the cDNAs after isolation and purification of the inserts from an agarose gel.

RNA Analysis

Human tissue was obtained at time of open heart surgery, cardiac transplantation, or autopsy and was immediately frozen in liquid nitrogen. Only tissue that would normally be removed during the course of the surgery was used. This protocol was approved by the Human Studies Committee at Washington University School of Medicine. RNA was prepared by lysis in guanidine isothiocyanate buffer and cesium trifluoroacetate (Pharmacia Laboratories, Piscatway, N.J.) gradient centrifugation. RNA was separated on 1.2% agarose/formaldehyde gels and transferred onto Genescreen membranes (New England Nuclear, Boston). Probes were labeled by the random primer technique with [α-32P]. Hybridization was performed at 42°C. Final washing was in 15 mM NaCl, 1.5 mM sodium citrate, 1 mM EDTA, and 0.1% sodium dodecyl sulfate (SDS) at 65°C.

Myofibrillar Protein Preparation and Immunoblotting

Myofibrils were prepared by modification of the method of Solaro, as described by Murphy and Solaro, including extensive use of protease inhibitors. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of the muscle proteins was done according to standard techniques. The protein gel was stained with Coomassie blue. The gel was transferred to an Immobilon membrane (Millipore Corp., Bedford, Mass.). The blot was probed with monoclonal anti-cardiac TnI antibody and an anti-TnI monoclonal antibody that reacts with all three TnI isoforms. These were provided by Drs. Bodor and Ladenson of the Department of Pathology at Washington University. Antibody concentration was 0.2 μg/ml. The Protoblot (Promega) alkaline phosphatase system was used to detect interaction of the primary antibody with myocardial protein. The specificities of these antibodies have been previously reported.

Results

The human cardiac cDNA nucleotide and derived amino acid sequence is illustrated in Figure 1. Although a similar sequence was recently reported, our clone contains 36 nucleotides of additional 5'-untranslated sequence. The derived amino acid sequence of this clone differs from the previously reported clone in the sequence of nucleotides 381–383. In this position, the nucleotides GCC encode an alanine, whereas the nucleotides ACC encode a threonine in the previously reported sequence. The derived amino acid sequence of human cardiac TnI is highly identical to previously reported sequences from other species. However, in comparison with our previously reported rat cardiac cDNA sequence, there is no significant homology in the untranslated regions of these cDNAs.

To characterize the expression of cardiac and slow skeletal TnI isoforms in normal human tissues, we analyzed RNA isolated from adult human tissues. Figure 2 shows a tissue blot probed with human cardiac TnI and slow skeletal TnI cDNAs. The ventricular tissue was obtained at autopsy from an adult who had no cardiac disease. Cardiac TnI was only expressed in cardiac ventricle. There was no evidence of cardiac TnI cDNA hybridization with skeletal muscle. Similarly, the slow skeletal mRNA was only detected in the skeletal muscle. Thus, the isoforms are specific in their expression in these adult tissues.
A developmental blot of human RNA from cardiac ventricles obtained at the time of open heart surgery or cardiac transplantation in children and adults with congenital or acquired heart disease was analyzed (Figure 3). The 2-week-old infant was the recipient of a heart transplant, the 7-month-old infant had Fallot's tetralogy, and the 1- and 2-year-old children had ventricular septal defects and muscle bundles in the right ventricle. The adult patient had cardiomyopathy and underwent cardiac transplantation. Equal amounts of RNA were loaded onto each lane. Cardiac TnI was expressed in cardiac tissue at all ages with some increase in the mRNA content with age. In contrast, the slow skeletal TnI cDNA hybridized with slow skeletal TnI mRNA in young infants, but the expression was not detectable in the adult heart. The lack of mRNA-encoding slow skeletal TnI in adult heart was also confirmed by the inability to obtain a slow skeletal clone from an adult cardiac cDNA library that had yielded several cardiac TnI clones. These data demonstrate both the specificity of TnI expression in adult tissues as well as the striking switch in expression pattern in early life.

The expression of slow skeletal TnI in neonatal heart was also confirmed at the protein level, as illustrated in Figure 4. Myofibrillar protein gel and immunoblot of cardiac ventricle of a 20-day-old and a 4-month-old infant was probed with monoclonal cardiac TnI antibody and with antibody recognizing all three TnI isoforms. A band migrating just above the 26-kd marker is identified by probing with the cardiac-specific antibody and with antibody recognizing all three TnI isoforms. A band representing slow skeletal TnI, which migrates faster in SDS-PAGE than the cardiac isoform.

**Discussion**

We have shown an important developmental regulation of TnI with coexpression of the slow skeletal and cardiac isoforms in immature heart and down-regulation of slow skeletal TnI mRNA with maturation. Our findings in hypertrophied human cardiac specimens are very similar to those we previously described in normal rat cardiac tissue, where slow skeletal and cardiac TnI are coexpressed in fetal heart and regulated in opposite directions with maturation. The slow skeletal mRNA is present for up to 3 weeks after birth in rat hearts. Therefore, it is likely that normal human heart has a similar postnatal pattern of regulation. The time course of the isoform switch, however, might progress at a faster rate in nonhypertrophied human hearts. The down-regulation of the slow skeletal TnI mRNA despite hypertrophy in these human specimens indicates that hypertrophy alone is not a sufficient stimulus to maintain a high level of expression of the TnI "fetal" isoform. In the adult rat cardiac ventricle, the expression of a number of fetal isoform genes can be induced by pressure-overload hypertrophy. These genes include β-MHC, skeletal α-actin, β-tropomyosin, and atrial natriuretic factor. This model may not be valid for slow skeletal TnI, as indicated by the downregulation of this isoform in hypertrophied human hearts. In addition, we have not been able to produce slow skeletal TnI reexpression in adult rat hearts with hypertrophy induced by aortic banding (A. Murphy and R. Payne, unpublished data). These data imply that negative regulatory elements in the slow skeletal TnI gene may have an important role in controlling TnI isoform expression in the heart.

The consistency in the developmental regulation of TnI isoforms in the human and rat hearts contrasts
gene family may be an important model system for determination of developmental regulatory mechanisms in the human heart. The similarities in regulation of TnI in lower and higher mammals and the downregulation of slow skeletal TnI mRNA even in hypertrophied human heart imply tight genetic control of the expression of TnI genes in heart.

Our results contrast with those of Humphreys and Cummins, who did not find any evidence of polymorphic forms of TnI when fetal and adult bovine and human protein preparations were compared. However, fetal cardiac TnI displayed very low levels of charge heterogeneity compared with adult myocardium, suggesting lower phosphorylation. This would be consistent with the presence of the slow skeletal TnI isoform. The protein gels and immunoblots in this report provide strong evidence for the presence of slow skeletal TnI antigen, which is incorporated into the cardiac myofibrils of these infants.
The degree of primary sequence differences between the cardiac and slow skeletal isoforms is likely to result in functional differences in the immature heart. The major structural difference between these two isoforms is the absence of the cardiac-specific amino terminus in the slow skeletal isoform. The amino terminus has an important functional role, because it contains the phosphorylation sites by protein kinase A. Phosphorylation at these sites decreases calcium sensitivity of the contractile apparatus. It has been postulated that TnI phosphorylation may be responsible for the increased rate of relaxation seen in adrenergically stimulated hearts. The presence of slow skeletal TnI, which lacks these phosphorylation sites, might explain the absence of augmentation of relaxation with β-adrenergic stimulation in neonatal hearts. Neonatal rat hearts are also less sensitive than adult hearts to the reduction of tension development by acidosis. This has been attributed to TnI isoform switching in these hearts; thus, this functional difference may be operable in the neonatal human heart as well. Because of the functional consequences of persistence of slow skeletal TnI, it is interesting to note the significant content of slow skeletal TnI in a 20-day-old infant with congenital heart disease (Figure 4). Infants with symptomatic congenital heart disease often undergo complete repair of their defects within the first days or weeks of life. These infants sustain myocardial hypoxia, ischemia, and acidosis during their surgical repair and often require β-adrenergic inotropic support in the postoperative period. Thus, they are likely to be exposed to events in which the content of TnI isoforms present in cardiac myofibrils may be functionally important.

In summary, this work demonstrates an important developmental regulation of TnI, one of the regulatory contractile proteins in human heart. Further investigation should allow us to elucidate factors that regulate the developmental expression of the TnI genes and, thus, to understand signals that mediate the maturation of the human heart.

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References


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