Physiol. Res. 48: 235-247, 1999

MINIREVIEW

Isoforms of Troponin in Normal and Diseased Myocardium

M. ADAMCOVÁ,V. PELOUCH[1]

Department of Physiology, Faculty of Medicine, Charles University, Hradec Králové and [1]Department of Medical Chemistry and Biochemistry, Second Medical School, Charles University, Prague, Czech Republic

Received November 4, 1998
Accepted March 26, 1999

Summary

The regulatory protein troponin (Tn) located on actin filament consists of three subunits: TnT – binds troponin to tropomyosin, TnC – binds divalent calcium ions, and TnI – affects myosin-actin interactions. Tn subunits display several molecular and calcium binding variations. During ontogenetic development of cardiac and skeletal muscles the synthesis of multiple isoforms of Tn subunits was detected. Expression of Tn isoforms and the extent of phosphorylation of both TnT and TnI *via* protein kinase C or protein kinase A under different pathological situations (e.g. ischemia, congenital heart disease, heart failure) can affect the Ca^{2+}-stimulated contraction function and the myofibrillar ATPase activity of the heart.

Key words

Troponin • Isoforms of troponin • Myocardium • Heart failure • Congenital heart disease • Ontogeny

Introduction

The contractility of skeletal and cardiac muscles is determined by the interaction of *contractile proteins* (actin and myosin) and *regulatory proteins* (troponin and tropomyosin) in the presence of calcium ions and ATP; binding of Ca-ions to regulatory proteins (tropomyosin-troponin complex) enhances the ATPase-activity of the actomyosin complex (AM). Furthermore, the structure of individual proteins of AM is affected by the *modulatory proteins* of myofibrils (C-, F-, M-proteins, myomesin, actinins, titin, CapZ protein, nebulette) and by the extracellular matrix proteins (different types of interstitial components – collagens, glycoproteins, glycosaminoglycans, elastins) (Pollack 1990, Pelouch *et al.* 1993, Malhotra 1994, Pelouch 1995, Schaub *et al.* 1997, 1998). Extracellular proteins affect the deposition of individual contractile proteins during perinatal development of myofibrils. Moreover, these collagenous proteins play a role during remodeling of muscle fibers under different pathological conditions. It has been demonstrated that treatment of embryonic cardiomyocytes with either cis-hydroxy-L-proline or ethyl-3,4-dihydroxybenzoate disrupt myofibrillogenesis (Fisher and Periasamy 1994). Furthermore, these inhibitors of collagen synthesis alter the expression of muscle specific genes. Therefore, collagen synthesis plays an essential role in maintaining the differentiated phenotype of

Fax +420 2 293 894
E-mail physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

cardiomyocytes not only during normal development but also during protein remodeling under different pathological conditions.

Regulatory proteins (RP) are responsible for both transducing the effect of calcium ions on the contractile machinery and modulation of molecular interactions between myosin and actin filaments. They serve a dual role: in the presence of calcium ions, RP transform the effect of these divalent ions on the activation of the contractile machinery, while in the absence of calcium ions, RP inhibit myosin-actin interaction by blocking the attachment of myosin cross-bridges to actin. RP are composed of troponin (Tn) and tropomyosin (Tm). Both Tn and Tm display multiple isoforms which are encoded by distinct genes. They are tissue-specific and developmentally regulated (Brenner 1991). Whereas the function and structure of both myosin and actin have been the subject of numerous reviews (Swynghedauw 1986, Schaub *et al.* 1998), the structure and different isoforms of Tm-Tn proteins have yet been less studied. There are two isotypes of Tm α and β which were found in both skeletal and cardiac muscles. Their proportion and phosphorylation concern an important molecular mechanism regulating the contractility during the development of muscles and under different pathological conditions (Humphreys and Cummins 1984, Perry 1985). However, this short review analyzes the existing literature on Tn isoforms. Special attention was given to cardiac muscle under physiological condition and in different cardiac diseases.

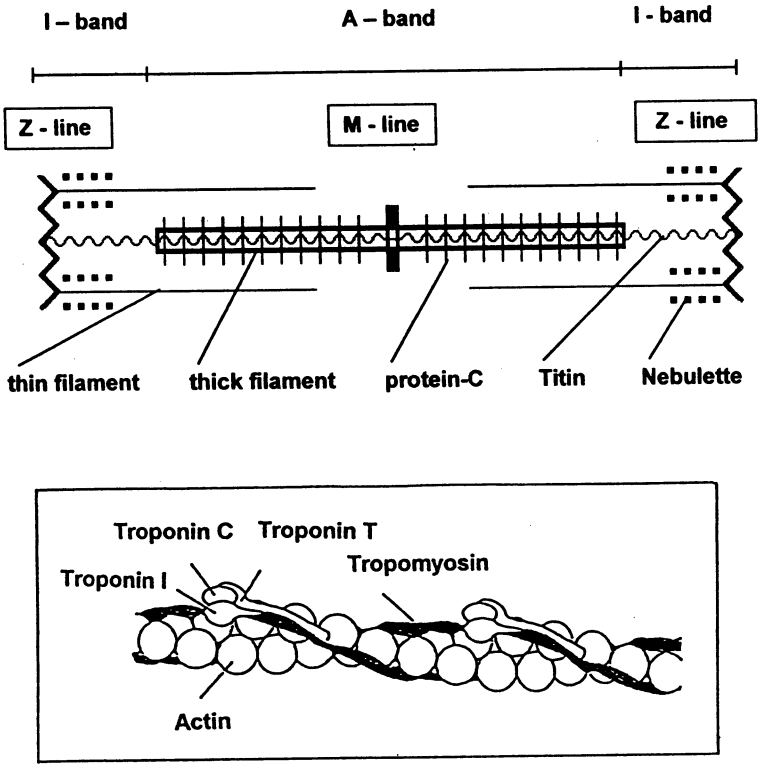

Fig. 1. *Upper part: Molecular mechanisms of contraction. Localization of protein in thick (A-band) and thin (I-band) filament: The myosin molecules in the two halves of each thick filament are oriented in opposite direction; C protein, myosin binding proteins, is located at most of transverse stripes; M line is formed from M protein, creatine kinase isoforms and myomesin; Z lines contains many different proteins: such α-, β-, and γ-actinins, titin is bound by N-terminus to Z line and by C terminus to M line. Nebullete (or nebulin) is part of actin structure in the thin filament. Lower part: Two helical chains of actin molecules form the primary structure of thin filaments; two chains of tropomyosin in a thin filament block cross-bridge binding sites on actin. Molecule of troponin bound to tropomyosin chains has three subunits: troponin C, troponin I and troponin T. For details see text. (Data derived from Pelouch 1995, Schaub et al. 1997, Schaub et al. 1998).*

The first report on troponin appeared in 1969 (Ebashi *et al.* 1969). Later, it was discovered that this regulatory protein located on the actin filament (Fig. 1) has three subunits and the nomenclature proposed by Greaser *et al.* (1972) was adopted: troponin T (TnT) – binds Tn subunits to tropomyosin, troponin C (TnC) – binds divalent covalent ions and troponin I (TnI) – affects myosin-actin interactions. It has recently been shown that troponins display several molecular and calcium binding variations described on the basis of antibody studies and molecular cloning (Campbell *et al.* 1992, Schiaffino *et al.* 1993, Farah and Reinach 1995, Townsend *et al.* 1995, Mair 1997).

Subunits of troponin regulatory complex

Troponin T (m.w. 37-39 kDa) is an asymmetric protein with globular C-terminal domain. It has a binding site for Tm and is thought to be responsible for the binding of Tn molecules to Tm, thereby positioning the complex on the thin filament. It has been demonstrated that the binding site for both TnI and TnC is at its C-terminus. On the other hand, the binding site for Tm is on its N-terminus (Chen *et al.* 1997). A new study of the properties of the isolated proteins suggests that an important role of TnT is to change the TnC/TnI complex from a calcium insensitive to a calcium sensitive form in the Mg-ATPase system. It can do this by reducing the binding constant for the TnI/TnC complex in the absence of calcium (Perry 1999).

Troponin C (m.w. 18 kDa) is a dumbbell-shaped protein with two globular heads (N-terminal and C-terminal domains) connected by a long central helix; it belongs to the helix-loop-helix calcium binding protein family. It binds calcium ions which subsequently relieve the inhibition of actin-myosin interaction by inducing a steric shift and reversing the inhibitory activity of TnI. On the basis of recent findings (Solaro 1995, Solaro and Rarick 1998), the control of the actin-myosin formation has been shown to be more complex: interference of all myofibrillar proteins and different covalent and non-covalent mechanisms have to be taken into consideration.

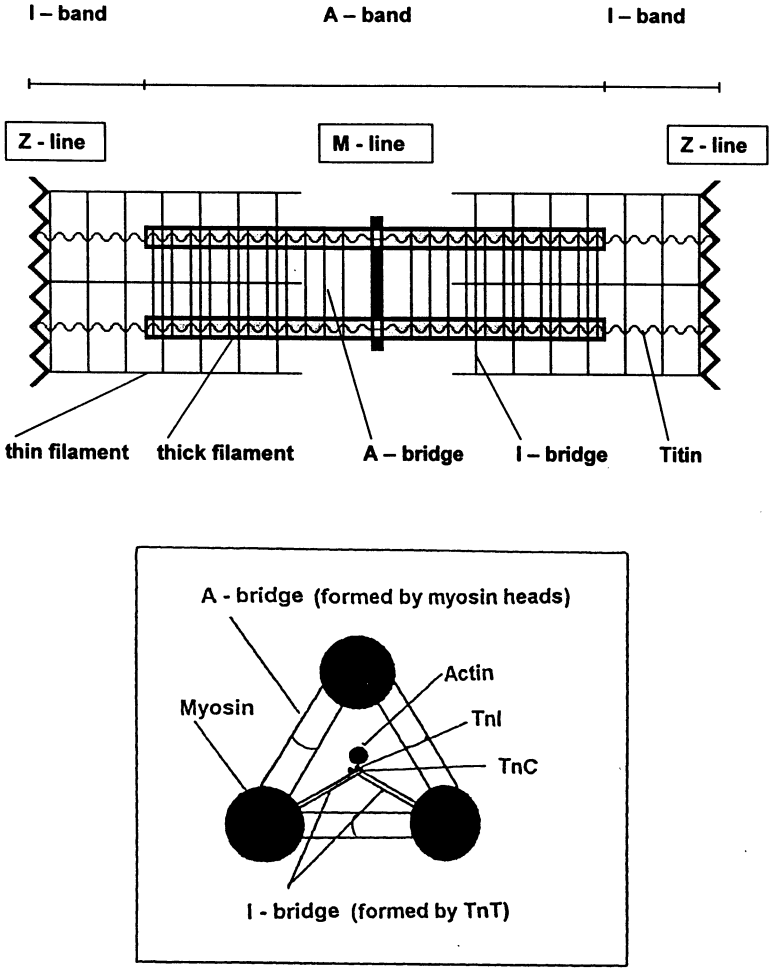

Fig. 2. *Structure of both I-band and A-band (for protein composition see Fig. 1 and text). Formation of A-bridges (HMM - myosin heavy chains: head of myosin molecule) and I-bridges (formed by troponin T). For details see text. (Data derived from Pollack 1990 and Wünsch 1992).*

Troponin I (TnI, m.w. 24-26.5 kDa) is a key component of the actin-filament regulatory complex. In the absence of calcium ions, TnI interacts with actin and inhibits Mg-ATPase activity of AM. This clearly demonstrates that in some way TnI blocks the interaction of action and myosin that is responsible for activation of Mg-ATPase. It has yet to be determined whether TnI acts directly as inhibitor or indirectly by interacting with associated proteins to facilitate their role in the regulatory system. According to the steric hypothesis, tropomyosin has a direct inhibitory role. Perhaps the most significant property of TnI is its ability to induce different conformational changes in each of the proteins with which it interacts (i.e. actin, tropomyosin, TnC and TnT) in order to facilitate their function. With TnC it increases the affinity for calcium so that this cation effectively triggers contraction (Perry 1999).

Localization of troponins in sarcomeres

Tn underlies intracellular compartmentation (Katus *et al.* 1991). There are two pools of Tn: *the majority of Tn* is myofibril bound (in the thin filaments), *the minority of Tn* is found as a soluble, cytoplasmic pool, which probably serves as a precursor pool for the synthesis of the Tn complex. The cytoplasmic fraction of cardiac TnT (cTnT) is estimated to be 6 % of total TnT (Katus *et al.* 1991). However, cytosolic pool for cardiac TnI (cTnI) is only 2.8 % (Mair *et al.* 1996). However, based on Pollack's new theory, the localization of troponin is more complex (Pollack 1990). TnT forms I-bridges (Fig. 2) that are responsible for the connection between thin filaments (where actin is dominant) and thick filaments (where myosin is dominant).

Troponin in skeletal and cardiac muscle

The functional differences between various muscle types are based on important structural differences of contractile and regulatory proteins including Tn. However, in smooth muscles, the Tn's are not present, the same functional role being played by calmodulin. As far as striated muscles are concerned, TnT and TnI exist in three different isoforms unique in structure, one for slow-twitch skeletal muscle, one for fast-twitch skeletal muscle and one for the cardiac muscle (Wilkinson and Grand 1978, Pearlstone *et al.* 1986). The three isoforms are encoded by three different genes. TnC has only two isoforms – one specific for fast-twitch skeletal muscles and one that is coexpressed in the myocardium and slow-twitch skeletal muscles (Pharmacek and Leiden 1991). Recently, the determination of both cardiac TnT and cardiac TnI have been considered to be one of the most specific and sensitive methods for diagnosis of myocardial damage (Mair 1997, Keffer 1996, Adamcová *et al.* 1995, 1996, 1997). However, TnC can not be used as a marker of cardiac muscle damage.

Cardiac troponin T (cTnT) differs only by the 6-11 amino acid residues from its skeletal muscle isoforms. When comparing the cTnT amino acid sequence with the sequences of both skeletal TnT isoforms, only 10-30 % of its amino acid sequences show a homology with skeletal TnT isoforms (Pearlstone *et al.* 1986). The human cTnT gene was located on 1q32 (Mesnard *et al.* 1995).

The human *cardiac troponin C* (cTnC) gene was located on 3p14.3–p21.3 (Barton *et al.* 1998). Although 70 % of the cardiac TnC is identical to that of fast skeletal TnC, there are significant differences in the first 40 residues, the most crucial being the inactivation of Ca^{2+} binding site I due to an insertion of valine (Val^{28}) and substitutions of key ligands relative to skeletal TnC (Leu^{29} and Ala^{31} in cardiac TnC, instead of Asp^{30} and Asp^{32} in skeletal TnC). The regulatory domain exists in a closed conformation even in the Ca^{2+}-bound (the on) state, differing significantly from the calcium-induced structure observed in skeletal TnC (Sia *et al.* 1997). Skeletal TnC binds four calcium ions (two at the high affinity sites III and IV – located at the C-terminal end, two at low affinity sites I and II – located at N-terminal end), while cardiac TnC binds only three calcium ions (two at high affinity sites III and IV and one at the single low affinity site II) . The high affinity sites III and IV are located in the C-terminal end (association constant 2.10^7 and 3.10^8 M for skeletal and cardiac muscle, respectively), sites II and I are located in the N-terminal end (association constant 2.10^5 and 2.10^6 M for skeletal and cardiac muscle, respectively) (Campbell *et al.* 1992).

Cardiac TnI (cTnI) has an extra 31 amino acid residues at the N-terminus and its amino sequence show about 40 % dissimilarity from both skeletal isoforms (Wilkinson and Grand 1978). The gene of cTnI has been assigned to chromosome 19p13.2–19q13.2 (MacGeoch *et al.* 1991).

The inhibitory regions of TnI in both cardiac and skeletal muscles are identical - except for a single amino-acid substitution of proline at position 110 in skeletal sequence and for threonine at position 143 in the cardiac sequence; these regions are extremely basic (Campbell *et al.* 1992). The major structural difference between the

slow skeletal and cardiac isoforms is the absence of cardiac-specific amino terminus in the slow skeletal isoform. The amino terminus has an important functional role: in the presence of protein kinase A it could be phosphorylated. This phosphorylation decreases calcium sensitivity of myofibrils (Holroyde *et al.* 1979, Wattanapermpool *et al.* 1995).

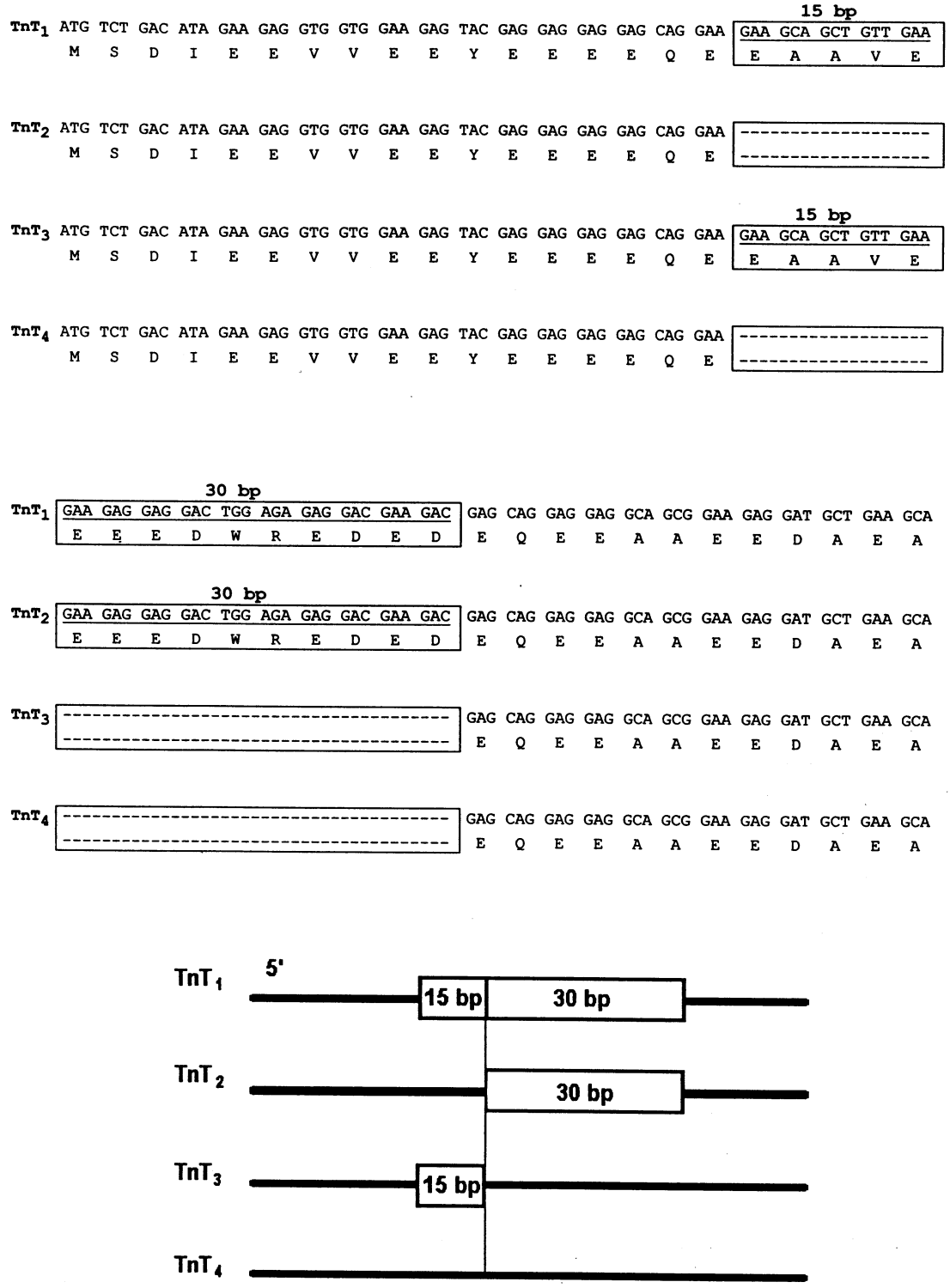

Fig. 3. *Upper part: Human cardiac troponin T (TnT) 5'heterogeneity. Nucleotide and deduced amino acid sequences are shown of the variably included or excluded 15- and 30-nt sequences identified by sequencing reverse transcriptase-polymerase chain reaction products of the 5'end of cardiac TnT cDNA. The sequences were confirmed by sequencing full-length cDNAs. (Data are taken from Anderson et al. 1995). Lower part: Missing 15 base pairs and 30 base pairs sequences of TnT_1 – TnT_4 are schematically shown.*

Isoforms of cardiac troponins

Multiple cTnT isoforms whose expression correlated with ATPase activity of myofibrils, the sensitivity of myofilaments to calcium and also the binding capacity of myofilaments for calcium ions (Saba *et al.* 1996) are expressed in the mammalian heart (Anderson *et al.* 1988, Sabry and Dhoot 1989), whereas cTnC (Toyota *et al.* 1989) and cTnI (Murphy *et al.* 1991, Hunkeler *et al.* 1991) are expressed as single isoforms in the adult heart. Based on the existence of a single cTnT gene, the various isoforms with individual functional significance are the products of alternative splicing of a primary RNA transcript. Four variable regions in human cTnT have been detected: the first variable domain at the 5′ end of cDNA is composed of two joined cassettes of 15 and 30 base pairs (bp), the latter being potentially excised at the first codon, the second is a single codon at amino-acid 45, and the third is a box of 9 bp (Mesnard-Rouiller *et al.* 1997). The fourth variable domain is in the central region of cDNA. Although the actual number of protein isoforms is not yet known, Anderson *et al.* (1991, 1995) have shown four human cTnT isoforms at the protein level. It was determined that these four isoforms ($cTnT_1$ through $cTnT_4$ numbered in the order of decreasing molecular size) are generated by combinatorial alternative splicing of two 15 and 30 base pairs (Anderson *et al.* 1995). Both exons encode highly acidic peptides, the inclusion of either peptide would add an overall negative charge to the cTnT protein. The isoform $cTnT_1$ and $cTnT_2$, are expressed in the fetal heart, with $cTnT_2$ being expressed at a very low level. Furthermore, TnT_4 is expressed in the fetal heart and re-expressed in the failing adult heart, whereas $cTnT_3$ is the dominant isoform in the adult heart (Fig. 3). It was demonstrated that the 5-residue peptide altered the sensitivity of ATPase activity to calcium (Tobacman and Lee 1987). Studies of Nassar *et al.* (1991) and McAuliffe *et al.* (1990) suggest that the presence of isoforms containing the 10-residue peptide increases myofilament sensitivity to calcium, which may be functionally important in the immature myocardium, where the peak cytosolic calcium concentration transient is significantly less than that of the adult (Anderson *et al.* 1993). Interestingly, it was found that in human left ventricular myocardium, peak myofibrillar ATPase activity correlated with $cTnT_4$ expression (Anderson *et al.* 1991).

Ontogenetic development of troponin isoforms

It seems that Tn's are distributed uniformly throughout atrial and ventricular chambers (Cummins *et al.* 1987, Voss *et al.* 1995). Both cardiac and skeletal TnT are coexpressed in the fetal heart (Anderson *et al.* 1991), but the skeletal isotype is subsequently suppressed during the perinatal period. However, cTnT is downregulated during the prenatal development of skeletal muscle: the expression of cTnT in normal adult skeletal muscles varied widely, ranging from no expression (quadriceps femoris) to expression of up to 20 % of the muscle fibers (diaphragm) (Bodor *et al.* 1997a). Recent studies suggest that cTnT could also be expressed in regenerating human skeletal muscles (Bodor *et al.* 1997a).

The fetal human myocardium contains predominantly slow-twitch troponin I. The presence of slow skeletal TnI (where phosphorylation sites are lacking) even persisted in the first months after birth. Later, from the ninth postnatal month, cTnI is expressed exclusively in both healthy and diseased human myocardium (Bhavsar *et al.* 1991, Hunkeler *et al.* 1991). It could be speculated that this might explain the absence of relaxation after β-adrenergic stimulation in neonatal hearts and the relative insensitivity of neonatal myofilament activity to acidic pH (Park *et al.* 1980, Martin *et al.* 1991). In the rat heart, slow-skeletal and cTnI isoforms are also coexpressed and regulated in opposite directions during fetal and neonatal development and cTnI becomes specific after the neonatal period. (Murphy *et al.* 1991). cTnI is neither expressed in adult skeletal muscle, nor in other tissues (Bodor *et al.* 1995).

However, the relative amount of the adult cTnT isoform increased significantly around the time of birth, this increase being significantly more prominent in left than in right ventricles (Gao *et al.* 1995). On the other hand, cTnI isoform transition was significantly more rapid in right than in left fetal rabbit ventricles (Gao *et al.* 1995). An injection of phenylephrine to pregnant animals facilitated TnT but not TnI isoform transition in the fetal heart. This result indicates that the developmental transition of rabbit cTnI and cTnT isoforms is not coordinated and might be regulated by different mechanisms. This study also provided evidence that the cTnT isoform population is influenced by adrenergic stimulation and stress on the cardiovascular system during development (Gao *et al.* 1995).

Less information is available about the ontogenetic development of cTnC, but similarly to cTnT, TnC is expressed predominantly as a skeletal muscle isoform during fetal life (Toyota and Shimada 1981).

Phosphorylation states of troponin isoforms

The phosphorylation state of Tn *via* protein kinase C (PKC) or protein kinase A (PKA) affected the Ca^{2+}-stimulated contraction function and the myofibrillar ATPase activity of the heart. Two phoshorylated sites were detected in bovine cardiac TnT: the first site is on the N-terminal serine, the second is on the C-terminal in CN 4 (cyanogen bromide fragment) (Swiderek *et al.* 1990). Phoshorylation of TnT in the presence or absence of TnC and TnI led to a lower affinity for binding of tropomyosin to F actin (Noland and Kuo 1992).

The interaction between myosin and actin seems to be more likely when TnI is in its dephosphorylated form (Malhotra *et al.* 1997). Phosphorylation of cTnI at two adjacent N-terminal residues has been shown to reduce the Ca^{2+} affinity of the single Ca^{2+}-specific regulatory site of cTnC and to increase the rate of Ca^{2+} dissociation from this site. β-adrenergic stimulation of intact heart elevated phosphorylation of TnI on serine in positions 23 and 24 (Swiderek *et al.* 1990). The study of Zhang *et al.* (1995) suggest that one of Ser^{23} may be constitutively phosphorylated and that Ser^{24} may be functionally more important. It is possible that the increased availability of PKA phosphorylation sites on TnI could affect TnT isoform switching (McConnel *et al.* 1997). However this hypothesis remains to be tested.

Troponin in various pathological situations

Although regulatory proteins may play a major role in the altered function of diseased myocardium, very few direct links of molecular alterations in troponin to pathological function have so far been demonstrated. Molecular mechanisms that may influence the expression of troponin genes also remain to be determined.

Myocardial ischemia

Ischemia induces many cellular disturbances. The extent of damage of cardiac cells depends on the presence of preformed collateral anastomoses. Whereas brief periods of ischemia (range of minutes) are tolerated, longer periods of ischemia induce leakage of metabolites and macromolecules due to elevated intracellular calcium, activation of phospholipases, endonucleases and the soluble protease – calpain. Furthermore, cardiac ischemia induces an increase in the blood levels of TnI, TnT, creatine kinase, transaminases, lactate dehydrogenase, glycogen phosphorylase BB (Keffer 1996, Adamcová *et al.* 1997, Mair 1997). Irreversible phase is characterized by very low concentrations of both cardiac creatine phosphate and ATP, cessation of glycolysis and by depletion of glycogen stores. Creatine kinase, a key enzyme that transfers energy from mitochodria to the vicinity of myofibrils, and myoglobin are upregulated following cellular damage (Voss *et al.* 1995); the structural and regulatory protein cTnT does not govern a mechanism for replacement of lost protein following cell injury and necrosis.

Table 1. Comparison of selected biochemical markers of myocardial damage

Marker	Molecular weight (D)	Range of times to initial elevation (h)	Mean time to peak elevations (non-thrombolysis)	Time to return to normal range
cTnT	37 000	3 - 12	24 h	5 – 14 d
cTnI	24 000	3 - 12	24 h	5 – 10 d
CK-MB	86 000	3 - 12	24 h	48 – 72 h
Myoglobin	17 800	1 - 4	6 – 7 h	24 h
LD	135 000	10	24 – 48 h	10 – 14 d

CK-MB -MB isoenzyme of creatine kinase LD – lactate dehydrogenase (Data derived from Adams et al. 1993)

Cardiac troponin T concentrations in patients with confirmed acute myocardial ischemia show roughly biphasic release kinetics, with the initial peak closely paralleling that of the mass creatine kinase MB concentration during the first 24 h after the onset of symptoms. The second peak occurs on about the fourth

day after admission. Despite the short biological half-life (2 h), the diagnostic window of cTnT is unusually wide, ranging from a few hours to several weeks after the acute episode. The biphasic release profile of cTnT is probably the result of intracellular compartmentation. The appearance of cTnT within the first few hours after myocardial infarction is the consequence of a rapid loss of the cytoplasmic pool superimposed on prolonged myofibrillar degradation that results in a long plateau effect several days after the onset of pain (Adamcová *et al.* 1997, Mair 1997). The time course of cTnI release is very similar. A recent study gave evidence that the predominant form of cTnI in the circulation is associated with troponin subunits cTnC and cTnT (Morjana 1998). The presence of the troponin complex in the serum suggests that assays that measure TnI or TnT are in fact measuring the same circulating complex with different levels of detection. A comparison of selected biochemical markers of myocardial damage is shown in Table 1.

Heart failure

In failing human myocardium, a significant amount of TnT4 is present; it recapitulates the fetal pattern (Anderson *et al.* 1991). The proportion of TnT_4 in the failing and non-failing human myocardium is inversely correlated with myofibrillar ATPase activity. It has been suggested that alterations in TnT isoforms may underlie the depressed myofibrillar ATPase activity which is characteristic for the failing myocardium and is thought to constitute one of the major pathophysiological aberrations in this condition. The results of Chen *et al.* (1997) supported the idea that the shift in TnT isoforms towards a more "fetal" pattern occurs during mild left ventricular hypertrophy of rabbit myocardium and is therefore, likely to be a general feature of the response to hemodynamic stress rather than a phenomenon confined to the final stage of the disease.

In contrast, there is no qualitative change in TnI isoforms expression associated with end-stages of heart failure (Hunkeler *et al.* 1991). Low skeletal muscle mRNA TnI was not detectable in any sample of right and left ventricular muscle in end-stage heart failure either from pulmonary hypertension, ischemic heart disease, or dilated cardiomyopathy. This means that alterations in the TnI isoform content can not be considered as an underlying mechanism for the altered characteristics of contractility associated with the failing ventricle. Cumulative evidence suggests that the same is true for TnC expression, which remains constant in the diseased myocardium.

Bodor *et al.* (1997b) demonstrated that the failing myocardium contains significantly larger amounts of dephosphorylated cTnI than the control tissue. The extent of TnI phosphorylation can be responsible for enhanced myofibrillar calcium sensitivity of the failing myocardium. The functional consequence of this difference may be an adaptive or maladaptive response to the lower and longer calcium concentration transient of the failing heart (enhancing force development or producing ventricular diastolic dysfunction).

Congenital heart diseases

In recent years, there has been a trend towards earlier repair of congenital cardiac defects in neonates and infants. The data obtained from studies in animals imply that the juvenile myocardium is more resistant to the effect of ischemia and reperfusion than the adult myocardium (Taggart *et al.* 1996). We have shown that both hypoxemic and normoxemic congenital heart diseases affect the pattern of contractile proteins: the changes were detected predominantly on the light chains of myosin (Pelouch *et al.* 1995). Taggart *et al.* (1996) concluded that cardiac troponins are more specific markers of myocardial injury in pediatric cardiac surgery for all cardiac operations. However, they did not support the opinion that myocardium of children is more resistant to ischemic injury. The percentage of TnT_4 isoform expression was significantly higher in hearts with congenital defects that caused congestive failure than in hearts with Fallot's tetralogy (Saba *et al.* 1996). In congenital heart malformations the skeletal isoform of TnI was detectable up to 2 years after birth (Hunkeler *et al.* 1991). Therefore, it is possible to conclude that congenital malformations result in a retarded postnatal TnI transition. The different troponin isoform expression may be functionally important after surgical repair. It can affect the response of the child myocardium to ischemia, acidosis and β-adrenergic inotropic support.

Hypertrophic cardiomyopathy

Recent findings (Moolman *et al.* 1997) have focused attention on the role of cTnT gene mutations, which may account for at least 15 % of cases of familial hypertrophic cardiomyopathy. This represents the most common cause of sudden cardiac death in the young. The gene mutation is facilitated by the relatively small coding

sequence of the cardiac troponin T gene and the presence of the mutation hotspot (cytosine:guanine doublet within codon 92).

Diabetes mellitus

No significant changes in troponin I content have been observed in right and left ventricles from diabetic rats. However, the phosphorylation of TnI was higher (approximately 40 %) in the diabetic hearts. This change was reversible with insulin treatment. Increased phosphorylation of TnI and also the changes of troponin T (Malhotra *et al.* 1995, Malhotra and Sanghi 1997) contribute toward the depression in cardiac myofibrillar ATPase activity in chronic diabetes (Liu *et al.* 1996) due to glycation of different myofibrillar proteins (Syrový and Hodný 1993).

Effect of thyroid hormones

Thyroid hormone levels in the early postnatal period influence the transcriptional activity of a variety of genes including those encoding cardiac myosin (Izumo *et al.* 1986, Černohorský *et al.* 1998) and TnI in the neonatal hearts (Sasse *et al.* 1993). Hypothyroidism in adulthood does not change the TnT isoforms profile of the rat ventricles (Saggin *et al.* 1990). In contrast, hypothyroidism was associated with the delay of the expected isoform switching of TnI in myocardium of rats during the postnatal period (Averyhart-Fullard *et al.* 1994, Dieckman and Solaro 1990).

The changes in the phosphorylation status of TnI or TnC correlated with alterations in the speed of myocardial relaxation and contraction in response to the altered thyroid states (Jakab *et al.* 1994). Left ventricular contraction was significantly higher in hyperthyroid than in euthyroid animals and this was associated with an increase in basal phosphorylation levels of both TnI and TnC in the myofibrils. In hypothyroid rats, the basal phosphorylation levels of cTnI and cTnC were significantly lower than in euthyroid animals.

Pharmacological intervention

It should be noted that therapeutic intervention may itself influence troponin expression, given that cardiac gene expression can be altered by a variety of pharmacological agents (Nadal-Ginard and Mahdavi 1989). It has been shown that cardiac troponin I is phosphorylated in response to adrenergic stimulation of the heart (Solaro *et al.* 1976). This mechanism is involved in phosphorylation-mediated control under β-sympathetic stimulation and it may be responsible for the increased rate of relaxation. The absence of phosphorylation sites in the slow skeletal isoform, present in the myocardium during the first months after birth, might explain the absence of augmented relaxation with β-adrenergic stimulation in neonatal hearts (Park *et al.* 1980).

Cardiac TnC is, probably, a potential target in therapy of patients with acute myocardial infarctions and subsequent congestive heart failure, where the diseased myocardium is "desensitized" to increased cytosolic Ca^{2+} levels. A novel group of positive inotropic agents (known as calcium sensitizers) increases the affinity of cardiac TnC for Ca^{2+}, possibly by binding them to a hydrophobic patch in the N-domain of cardiac TnC (Sia *et al.* 1997)

Acknowledgements

This work was supported by a grant No. 305/98/P261 of the Grant Agency of the Czech Republic.

References

ADAMCOVÁ M, KOKŠTEIN Z, PALIČKA V, PODHOLOVÁ M, KOŠŤÁL M: Troponin T levels in the cord blood of healthy term neonates. *Physiol Res* 44: 99-104, 1995.

ADAMCOVÁ M, KOKŠTEIN Z, PALIČKA V: Troponin T-marker of myocardial injury in asphyxiated neonates. *J Moll Cell Cardiol* **28:** A81, 1996.

ADAMCOVÁ M, KOKŠTEIN Z, VÁVROVÁ J: Clinical utility of cardiac troponin I and cardiac troponin T measurements. *Acta Med* **40:** 83-87, 1997.

ADAMS JE, ABENDSCHEIN DR, JAFFE AS: Biochemical markers of myocardial injury: is MB creatine kinase the choice for the 1990s? *Circulation* **88:** 750-763, 1993.

ANDERSON PAW, MOORE GE, NASSAR RN: Development changes in the expression of rabbit left ventricular troponin T. *Circ Res* **63:** 742-747, 1988.

ANDERSON PAW, MALOUF NN, OAKELEY AE, PAGANI ED, ALLEN PD: Troponin T isoform expression in humans: a comparison among normal and failing adult heart, fetal heart, and adult and fetal skeletal muscle. *Circ Res* **69:** 1226-1233, 1991.

ANDERSON PAW, HENDERSON PM, NASSAR R: The calcium transient in cardiac myocytes increases with development. *Circulation* **88** (Suppl I): I-436, 1993.

ANDERSON PAW, GREIG A, MARK TM, MALOUF NN, OAKELEY AE, UNGERLEIDER RM, ALLEN PD, KAY BK: Molecular basis of human cardiac troponin T isoforms expressed in the developing, adult and failing heart. *Circ Res* **76:** 681-686, 1995.

AVERYHART FULLARD V, FRAKER LD, MURPHY AM, SOLARO RJ: Differential regulation of slow-skeletal and cardiac troponin I mRNA during development and by thyroid hormone in rat heart. *J Mol Cell Cardiol* **26:** 609-616, 1994.

BARTON JRP, BRAND NJ, BHAVSAR PK, MULLEN AJ, YACOUB MH: Physical linkage of human troponin genes: Analysis of loci encoding cardiac and slow-skeletal troponin I and T. *Circulation* **30:** A183, 1998.

BHAVSAR P, DHOOT GK, CUMMING DVE, BUTLER-BROWNE GS, YACUB MH, BARTON PJR: Development expression of troponin I isoforms in fetal human heart. *FEBS Lett* **292:** 5-8, 1991.

BODOR GS, PORTERFIELD D, VOSS EM, SMITH S, APPLE FS: Cardiac troponin I is not expressed in fetal and healthy and diseased adult human skeletal muscle tissue. *Clin Chem* **41**: 1710-1715, 1995.

BODOR GS, SURVANT L, VOSS EM, SMITH S, PORTERFIELD D, APPLE FS: Cardiac troponin T composition in normal and regenerating human skeletal muscle. *Clin Chem* **43:** 476-484, 1997a.

BODOR GS, OAKELEY AE, ALLEN PD, CRIMMINS DL, LADENSON JH, ANDERSON PA: Troponin I phosphorylation in the normal and failing adult human heart. *Circulation* **96:** 1495-1500, 1997b.

BRENNER B: A new concept for the mechanism of Ca^{2+}-regulation of muscle contraction. Implications for physiological and pharmacological approaches to modulate contractile function of myocardium. *Basic Res Cardiol* **86** (Suppl 3)**:** 83-92, 1991.

CAMPBELL AP, VANEYK JE, HODGES RS, SYKES BD: Interaction of troponin I and troponin C: use of the two-dimension transferred nuclear Overhauser effect to determine the structure of a Gly-110 inhibitory troponin I peptide analog when bound to cardiac troponin C. *Biochim Biophys Acta* **1160:** 35-54, 1992.

ČERNOHORSKÝ J, KOLÁŘ F, PELOUCH V, KORECKÝ B, VETTER R: Thyroid control of sarcolemmal Na/Ca-ATPase in developing rat heart. *Am J Physiol* **275:** H264-H273, 1998.

CHEN Z, HIGASHIYAMA A, YAKU H, BELL S, FABIAN J, WATKINS MW, SCHNEIDER DJ, MAUGHAN DW, LEWINTER MM: Altered expression of troponin T isoforms in mild left ventricular hypertrophy in the rabbit. *J Mol Cell Cardiol* **29:** 2345-2354, 1997.

CUMMNINS B, AUCKLAND ML, CUMMINS P: Cardiac-specific troponin I radioimmunoassay in the diagnosis of acute myocardial infarction. *Am Heart J* **113:** 1333-1344, 1987.

DIECKMAN LJ, SOLARO RJ: Effect of thyroid status on thin-filament Ca^{2+} regulation and expression of troponin I in perinatal and adult rat hearts. *Circ Res* **67**: 344-351, 1990.

EBASHI S, ENDO M, OHTSUKI I: Control of muscle contraction. *Q. Rev Biophys* **2:** 351-384, 1969.

FARAH CS, REINACH FC: The troponin complex and regulating of muscle contraction. *FASEB J* **9:** 755-767, 1995.

FISHER SA, PERIASAMY M: Collagen synthesis inhibitors disrupt embryonic cardiocyte myofibrillogenesis and alter the expression of cardiac specific genes in vitro. *J Mol Cell Cardiol* **26:** 721-731, 1994.

GAO L, KENNEDY JM, SOLARO RJ: Differential expression of TnI and TnT isoforms rabbit heart during the perinatal period and during cardiovascular stress. *J Mol Cell Cardiol* **27**: 541-550, 1995.

GREASER ML, YAMAGUCHI M, BREKKE C, POTTER J, GERGELY J: Troponin subunits and their interactions. *Cold Spring Harbour Symp Quant Biol* **37:** 235-244, 1972.

HOLROYDE MJ, HOWE E, SOLARO RJ: Modification of calcium requirements for activation of cardiac myofibrillar ATPase by cyclic AMP dependent phosphorylation. *Biochim Biophys Acta* **586**: 63-69, 1979.

HUMPHREYS JE, CUMMINS P: Regulatory proteins of myocardium. *J Mol Cell Cardiol* **16:** 643-657, 1984.

HUNKELER NN, KULLMAN J, MURPHY AM: Troponin I isoform expression in human heart. *Circ Res* **69:** 1409-1414, 1991.

IZUMO S, NADAL-GINARD B, MAHDAVI V: All members of the MHC multigene family respond to thyroid hormone in highly tissue-specific manner. *Science* **231:** 597-600, 1986.

JAKAB G, KISS E, KRANIAS EG, EDES I: Effect of thyroid status on basal phosphorylation of cardiac myofibrillar phosphoproteins in rats. *Cardioscience* **5:** 19-24, 1994.

KATUS HA, REMPPIS A, SCHEFFOLD T, DIEDERICH KW, KUEBLER W: Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. *Am J Cardiol* **67:** 1360-1367, 1991.

KEFFER JH: Myocardial markers of injury. *Am J Clin Pathol* **105:** 305-320, 1996.

LIU X, TAKEDA N, DHALLA NS: Troponin I phosphorylation in heart homogenate from diabetic rat. *Biochim Biophys Acta* **1316**: 78-84, 1996.

MACGEOCH C, BARTON PJR, VALLINS WJ, BHAVSAR P, SPURR NK: The human cardiac troponin I locus: assignment to chromosome 19p13.2-19.q13.2. *Hum Genet* **88:** 101-104, 1991.

MAIR J: Progress in myocardial damage detection: new biochemical markers for clinicians. *Crit Rev Clin Lab Sci* **34:** 1-66, 1997.

MAIR J, GENSER N, MORANDELL D: Cardiac troponin I in the diagnosis of myocardial injury and infarction. *Clin Chim Acta* **245**: 19-38, 1996.

MALHOTRA A: Role of regulatory proteins (troponin - tropomyosin) in pathological states. *Mol Cell Biochem* **135:** 43-50, 1994.

MALHOTRA A, SANGHI V: Regulation of contractile proteins in diabetic heart. *Cardiovasc Res* **34:** 34-40, 1997.

MALHOTRA A, LOPEZ MC, NAKOUZI A: Troponin subunits contribute to altered myosin ATPase activity in diabetic cardiomyopathy. *Mol Cell Biochem* **151:** 165-172, 1995.

MALHOTRA A, NAKOUZI A, BOWMAN J, BUTTRICK P: Expression and regulation of mutant forms of cardiac TnI in reconstituted actomyosin system: role of kinase dependent phosphorylation. *Mol Cell Biochem* **170:** 99-107, 1997.

MARTIN AF, BALL K, GAO L, KUMAR P, SOLARO RJ: Identification and functional significance of troponin I isoforms in neonatal rat heart myofibrils. *Circ Res* **69:** 1244-1252, 1991.

MCAULIFFE JJ, GAO L, SOLARO RJ: Changes in myofibrillar activation and troponin C Ca^{2+} binding associated with troponin T isoform switching in developing rabbit heart. *Circ Res* **66:** 1204-1216, 1990.

MCCONNELL BK, MORAVEC CS, MORANO I, BOND M: Troponin I phosphorylation in spontaneously hypertensive rat heart: effect of β-adrenergic stimulation. *Am J Physiol* **273:** H1440-H1451, 1997.

MESNARD L, LOGEART D, TAVIAUX S, DIORING S, MERCADIER JJ, SAMSON F: Human cardiac troponin T: cloning and expression of new isoforms in the normal and failing heart. *Circ Res* **76 :** 687-692, 1995.

MESNARD-ROUILLER L, MERCADIER JJ, BUTLER-BROWNE G, HEIMBURGER M, LOGEART D, ALLEN PD, SAMSON F: Troponin T mRNA and protein isoforms in the human left ventricle: pattern of expression in failing and control heart. *J Mol Cell Cardiol* **29:** 3043-3055, 1997.

MOOLMAN JC, CORFIELD VA, POSEN B, NGUMBELA K, SEIDMAN CH, BRINK PA, WATKINS H: Sudden death to troponin T mutations. *J Am Coll Cardiol* **29:** 549-555, 1997.

MORJANA NA: Degradation of human cardiac troponin I after myocardial infarction. *Biotechnol Appl Biochem* **28:** 105-111, 1998.

MURPHY AM, JONES L II, SIMS HF, STRAUSS AW: Molecular cloning of rat cardiac troponin I and analysis of troponin I isoform expression in developing rat heart. *Biochemistry* **30:** 707-712, 1991.

NADAL-GINARD B, MAHDAVI V: Molecular basis of cardiac performance. *J Clin Invest* **84:** 1693-1700, 1989.

NASSAR R, MALOUF NN, KELLY MB, OAKELEY AE, ANDERSON PAW: Force-pCa relation and troponin T isoforms of rabbit myocardium. *Circ Res* **69:** 1470-1475, 1991.

NOLAND TA, KUO JF: Protein kinase C phoshorylation of cardiac troponin T decreases Ca^{2+} dependent actomyosin MgATPase activity and troponin T binding to tropomyosin F actin complex. *Biochem J* **288:**123-129, 1992.

PARK MK, SHERIDAN PH, MORGAN WW, BECK N: Comparative inotropic response of newborn and adult rabbit papillary muscles to isoproterenol and calcium. *Dev Pharmacol Ther* **1:** 70-82, 1980.

PEARLSTONE J, CARPENTER M, SMILLIE L: Amino acid sequences of rabbit cardiac troponin T. *J Biol Chem* 261: 16795-16810, 1986.

PELOUCH V, DIXON IMC, GOLFMAN L, BEAMISH RE, DHALLA NS: Role of extracellular matrix proteins in heart function. *Mol Cell Biochem* **129:** 101-120, 1993.

PELOUCH V: Molecular aspects of regulation of cardiac contraction. *Physiol Res* **44:** 53-60, 1995.

PELOUCH V., MILEROVÁ M., OŠŤÁDAL B., HUČÍN B., ŠAMÁNEK M: Differences between atrial and ventricular protein profiling in children with congenital heart disease. *Moll Cell Biochem* **147:** 43-49, 1995.

PERRY SV: Properties of the muscle proteins: a comparative approach. *J Exp Biol* **115:** 31-42, 1985.

PERRY SV: Troponin I: inhibitor or facilitator. *Mol Cell Biochem* **190:** 9-32, 1999.

PHARMACEK MS, LEIDEN JM: Structure, function and regulation of troponin C. *Circulation* **84:** 991-1003, 1991.

POLLACK GH: *Muscles and Molecules - Uncovering Principles of Biological Motion.* Ebner and Sons Publishers, Seattle, 1990.

SABA Z, NASSAR R, UNGERLEIDER RM, OAKELEY AE, ANDERSON PAW: Cardiac troponin T isoform expression correlates with pathophysiological descriptors in patients who underwent corrective surgery for congenital heart disease. *Circulation* **94:** 472-476, 1996.

SABRY MA, DHOOT GK: Identification of and changes in the expression of troponin T isoforms in developing avian and mammalian heart. *J Mol Cell Cardiol* **21:** 85-91, 1989.

SAGGIN L, GORZA L, AUSONI S: Cardiac troponin T in developing, regenerating and denerved rat skeletal muscle. *Development* **110:** 547-554, 1990.

SASSE S, BRAND NJ, KIPRIANOU P, DHOOT GK, WADE R, ARAI M, PERIASAMY M, YACOUB MH, BARTON PJR: Troponin I gene expression during human cardiac development and in end-stage heart failure. *Circ Res* **72:** 932-938, 1993.

SCHAUB MC, HEFTI MA, HARDER BA, EPPENBERGER HM: Various hypertrophic stimuli induce distinct phenotypes in cardiomyocytes. *J Mol Med* **75:** 901-920, 1997.

SCHAUB MC, HEFTI MA, ZUELLING RA, MORANO I: Modulation of contractility in human cardiac hypertrophy by myosin essential light chain isoforms. *Cardiovasc Res* **37:** 381-404, 1998.

SCHIAFFINO S, GORZA L, AUSONI S: Troponin isoform switching in the developing heart and its functional consequences. *Trends Cardiovasc Med* **3:** 12-17, 1993.

SIA KS, LI MX, SPYRACOPOULOS L, GAGNÉ SM, LIU W, PUTKEY JA, SYKES BD: Structure of cardiac muscle troponin C unexpectedly reveals a closed regulatory domain. *J Biol Chem* **29:** 18216-18221, 1997.

SOLARO RJ: Troponin C – troponin I interactions and molecular signalling in cardiac myofilaments. *Adv Exp Med Biol* **382:** 109-115, 1995.

SOLARO RJ, RARICK HM: Troponin and tropomyosin: proteins that switch on and tune in the activity of cardiac myofilaments. *Circ Res* **83:** 471-480, 1998.

SOLARO RJ, MOIR AJG, PERRY SV: Phosphorylation of troponin I and the inotropic effect of adrenaline in the perfused rabbit heart. *Nature* **262:** 615-617, 1976.

SWIDEREK K, JAQUET K, MEYER HF, SCHACHTELE C, HOFMANN F, HEILMEYER GH: Sites phoshorylated in bovine cardiac troponin T and I. Characterization by P-NMR spectroscopy and phoshorylation by protein kinases. *Eur J Biochem* **190:** 575-582, 1990.

SWYNGHEDAUW B: Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. *Physiol. Rev* **66:** 710-771, 1986.

SYROVÝ I, HODNÝ Z: In vitro non-enzymatic glycosylation of myofibrillar proteins. *Int J Biochem* 25: 941-946,1993.

TAGGART DP, HADJINIKOLAS L, WONG K, YAP J, HOOPER J, KEMP M, HUE D, YACOUB M, LINCOLN JC: Vulnerability of paediatric myocardium to cardiac surgery. *Heart* **76:** 214-217, 1996.

TOBACMAN L, LEE R: Isolation and functional comparison of bovine cardiac troponin T isoforms. *J Biol Chem* **262:** 4059-4064, 1987.

TOWNSEND PJ, BARTON PJR, YACOUB MH, FARZA H: Molecular cloning of human cardiac troponin T isoforms: expression in developing and failing heart. *J Mol Cell Cardiol* **27:** 2223-2236, 1995.

TOYOTA N, SHIMADA Y: Differentiation of troponin in cardiac and skeletal muscles in chicken embryos as studied by immunofluorescence microscopy. *J Cell Biol* **91:** 497-504, 1981.

TOYOTA N, SHIMADA Y, BADER D: Molecular cloning and expression of chicken cardiac troponin C. *Circ Res* **65:** 1241-1246, 1989.

VOSS EM, SHARKEY WS, GERNERT AE, MURAKAMI MM, JOHNSTON RB, HSIEH CC, APPLE FS: Human and canine cardiac troponin T and creatine kinase-MB distribution in normal and diseased myocardium. *Arch Pathol Lab Med* **119**: 799-806, 1995.

WATTANAPERMPOOL J, GUO X, SOLARO RJ: The unique amino-terminal peptide of cardiac troponin I regulates myofibrillar activity only when it is phoshorylated. *J Mol Cell Cardiol* **27:** 1383-1391, 1995.

WILKINSON JM, GRAND RJA: Comparison of amino acid sequence of troponin I from different striated muscles. *Nature* **271:** 31-35, 1978.

WÜNSCH Z: Machina carnis. *Čs fyziol* **41:** 5-31, 1992.

ZHANG R, ZHAO J, POTTER JD: Phosphorylation of both serine residues in cardiac troponin I is required to decrease teh Ca^{2+} affinity of cardiac tropinin C. *J Biol Chem* **270:** 30773-30780, 1995.

Reprint requests

M. Adamcová, Department of Physiology, Faculty of Medicine, Charles University, Šimkova 870, 500 01 Hradec Králové, Czech Republic.