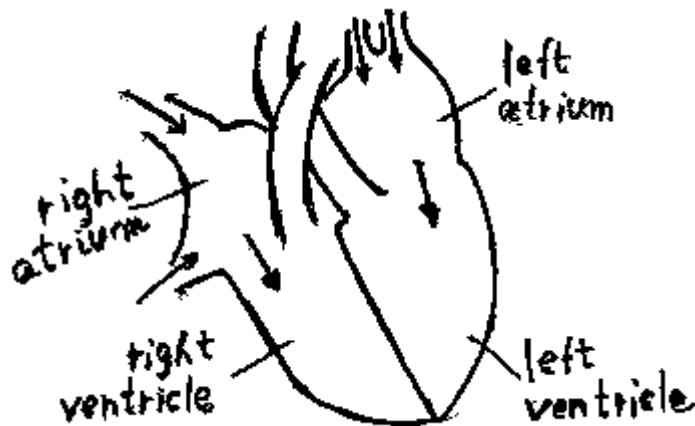


## HEART MUSCLE

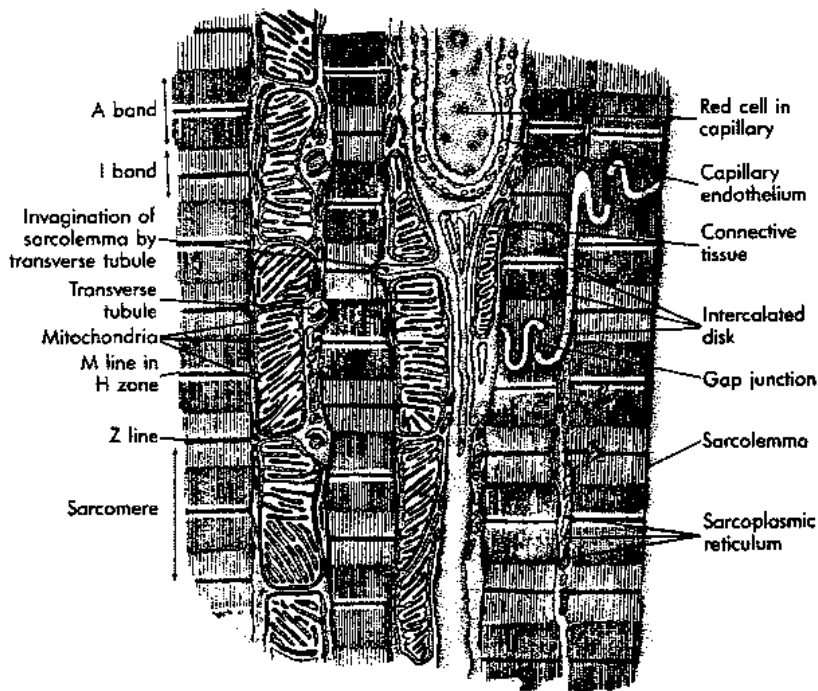
Basic physiology: William Harvey recognized at the beginning of the 17<sup>th</sup> century that the heart pumps the blood through the whole body. The heart consists of four chambers, two atria that receive the blood and the two ventricles that pump the blood. The venous blood enters the heart via the right atrium, flows into the right ventricle and is pumped by the right ventricle through the lungs to pick up O<sub>2</sub> and lose CO<sub>2</sub>. The rejuvenated blood enters the left atrium and delivers O<sub>2</sub> to the tissues by the pumping action of the left ventricle (Fig. H1).



**Fig. H1.** Scheme of the heart compartments and the direction of the blood flow during diastole. Note the pulmonary artery on the top of the right ventricle and the aorta on the top of the left ventricle. The valves separating the atria from the ventricles are not shown.

Alternating contractions and relaxations of the heart muscle, called myocardium, causes the pumping of the heart. There is a pacemaker in the right atrium that generates electrical impulses causing the atria to contract and thereby forcing blood into the ventricles. Following contraction (systole) the ventricles relax (diastole); the entire process is called the cardiac cycle. Normal human heart beats about 70-times per minute at rest. The rate of heart beat increases during exercise, emotional excitement and fever, and decreases during sleep.

Ultrastructure: Cardiac muscle is composed of interconnected mono-nucleated cells. The cells are imbedded in a weave of collagen. The ultrastructure of the heart contains a large number of myofibrils, striated like in skeletal muscle. A large fraction of the cell volume is occupied by mitochondria, which synthesize ATP to supply energy for the constantly working heart muscle. Myofibrils and mitochondria occupy about 85% of the heart cell volume, the rest contains the sarcolemma, T-tubules, sarcoplasmic reticulum, and specialized structures such as the intercalated disk, which connects adjacent heart cells (cardiomyocytes), and gap junction or nexus which makes contact between the plasma membranes of adjacent heart cells (Fig. H2).



**Fig. H2.** Electron micrograph of cardiac muscle (Courtesy of Dr. Helen Rarick).

## Contractile Proteins

**Myosin.** Cardiac myosin is composed of two heavy chains (HCs) and four light chains (LCs). The ventricles contain only two types of light chains (skeletal muscle myosin possess three) with an estimated molecular mass of 19,000 dalton (regulatory light chain, RLC) and 27,000 dalton (essential light chain, ELC). The mass of entire cardiac myosin is about 480,000 and its length is approximately 1,700 Å (Katz 1992), similar to these parameters in skeletal muscle myosin (For structural organization see [Figure M1](#)).

The multigene families of HCs and LCs generate different isoforms that occur at various stages of heart development. Two HC isoforms are known as  $\alpha$ - and  $\beta$ -isoforms. First it was thought that the functional properties of heart muscle are determined by their LC composition, but it turned out that HCs determine the myosin ATPase activity *in vitro* and the shortening velocity in the intact heart (Katz, 1992). In experimental diabetes the HC isoform expression was greatly altered (Rundell et al., 2004). After 6 weeks in diabetes the  $\alpha$ -myosin isoform decreased to 41% and after 12 weeks to 33% as compared to the 78% and 74% control values, respectively. Cross-bridge cycling rate was significantly decreased in diabetes and the tension cost decreased linearly with the decreased  $\alpha$ -myosin content.

In the atria there are two myosin isoforms, A<sub>1</sub> and A<sub>2</sub>, with high and low ATPase activity, respectively, whereas in the ventricles there are three myosin isoforms, V<sub>1</sub> ( $\alpha$ - $\alpha$ ), V<sub>2</sub> ( $\alpha$ - $\beta$ ), and V<sub>3</sub> ( $\beta$ - $\beta$ ), with high, intermediate, and low ATPase, respectively. A comparative study on the various ATPase activities of rabbit heart and skeletal muscle myosins

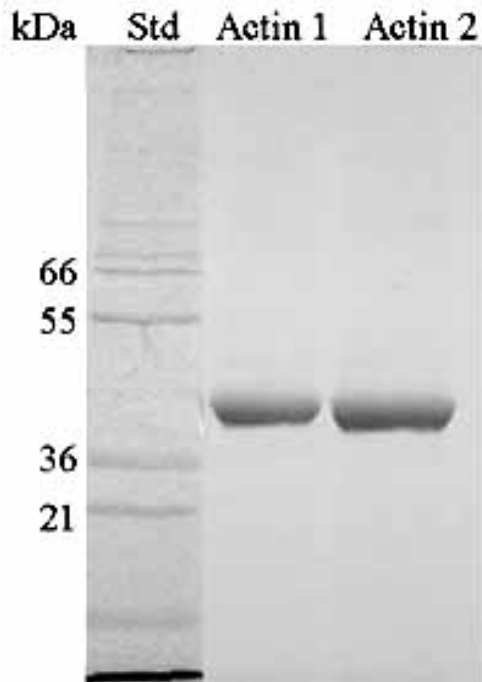
showed that under all conditions the activities of the skeletal source are higher (Bárány et al., 1964).

The atria and the ventricles contain different LC isoforms. The atrial LCs are also found in developing heart and fast skeletal muscle, and in adult slow skeletal muscle. The 19,000-dalton heart LC is phosphorylated and possibly participates in the regulation of heart contraction (Kopp and Bárány, 1979).

Actin: Purification Heart actin has not been studied in detail. Recently we reported a procedure for the preparation of pure rat heart actin (Bárány and de Tombe, 2004). The hearts from freshly killed rats (15-20) were dropped into 1000 volumes of 0.15 M NaCl, 0.01 M Tris-HCl, pH 7.4 (kept in ice). The ventricles were isolated and repeatedly washed with the NaCl-Tris solution to remove blood. The ventricles were minced with a Latapie mincer, then myosin was extracted with 3 volumes of a solution containing 0.3 M KCl, 0.15 M  $KP_i$ , 2.5 mM  $MgSO_4$  and 2.5 mM ATP, pH 6.5, in ice with stirring for 10 min. After centrifugation at 10,000 rpm for 10 min, the residue was washed, throughout in ice, with 5 vol of 0.4%  $NaHCO_3$ , for 10 min. and centrifuged at 5,000 rpm for 10 min. The residue was washed with 10 vol of dist. water for 5 min and centrifuged at 10,000 rpm for 10 min. The residue was treated with acetone three times, 5, 3, and 2 vols, each time for 10 min of acetone, and centrifuged each time at 5,000 rpm for 10 min. The final residue was air dried overnight.

The dried powder was extracted with 30 vols of 0.5 mM ATP in ice for 1 hour; the supernatant was isolated by centrifugation at 15,000 rpm for 15 min. About half of the supernatant protein was actin that could be purified as described ((Bárány and de Tombe, 2004). To obtain the main bulk of actin in the heart powder, we extracted the residue of the first extract with 30 vol of a solution containing 0.25 M KI, 0.02 M Tris-HCl, pH 7.4, and 1 mM ATP, in ice, for 1 hour. After centrifugation at 15,000 rpm for 30 min, the supernatant containing the actin was dialyzed against large volumes of a solution containing 0.1 M NaCl and 2 mM  $MgCl_2$  with frequent changes, at 4°C for 20 h. To the viscous F-actin solution NaCl was added (from a concentrated solution) to 0.6 M, it was stirred in ice for 30 min, then centrifuged at 30,000 rpm, 5°C, for 3 h. The F-actin pellet was rinsed with dist water to remove salts, then dissolved by a Teflon-glass homogenizer in 0.5 mM ATP, pH 7.4, to yield a non-viscous G-actin (5-6 mg/ml). Upon addition of salts to 0.1 M NaCl and 2 mM  $MgCl_2$ , this actin solution became very viscous.

The purified rat heart actin migrated as a single protein on gels (Fig.H3). At low ionic strength, it activated the ATPase activity of rat skeletal myosin, or rat cardiac myosin to the same extent as purified rat skeletal muscle actin did at high ionic strength it formed a very viscous complex with rat skeletal myosin, or rat cardiac myosin to the same extent as purified rat skeletal muscle actin.

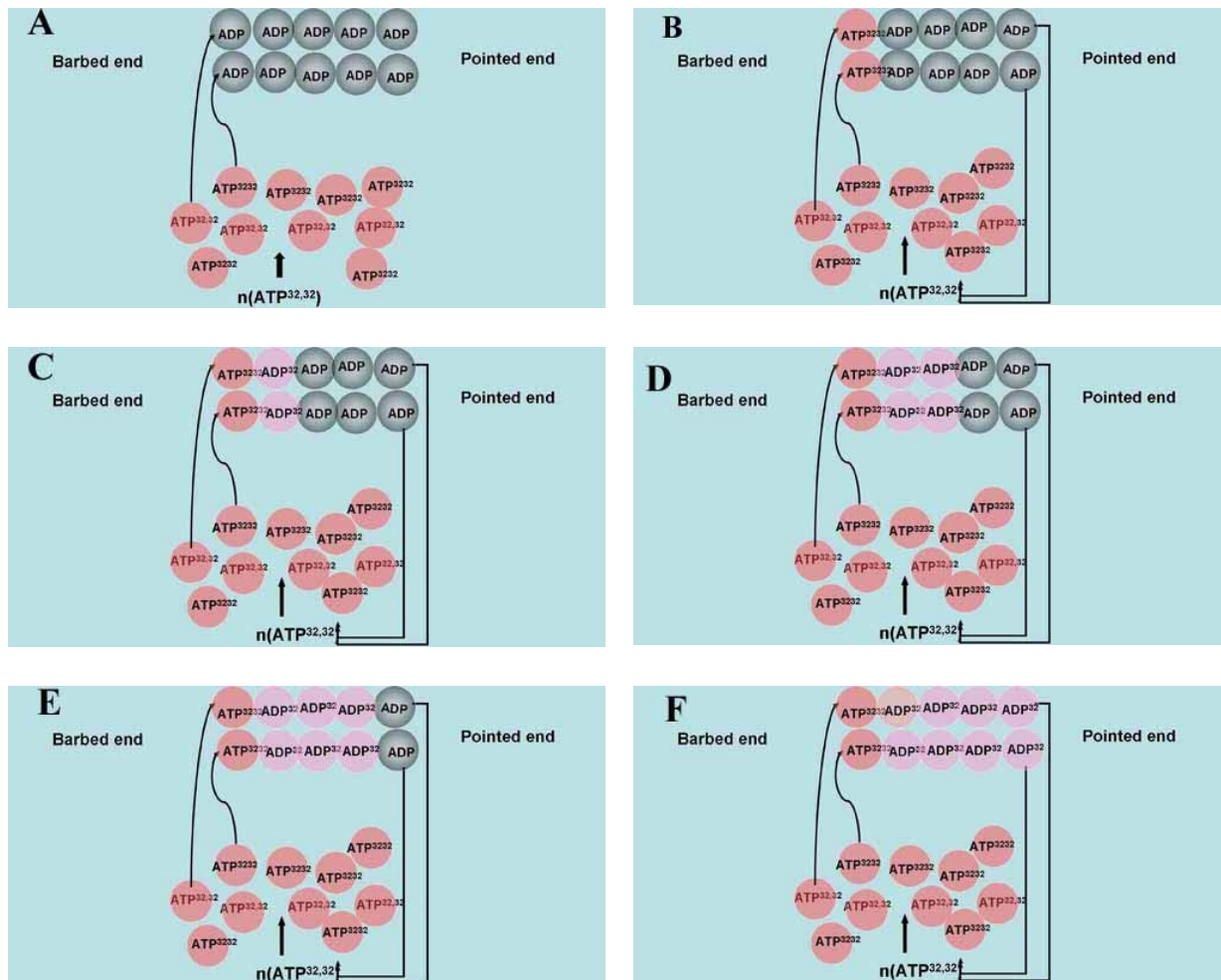


**Fig. H3.** Electrophoresis of purified rat heart actin on 10% Bis-Tris gels. Actin 1 and Actin 2 refer to two different actin preparations. The gels were loaded with 10 and 15  $\mu\text{g}$  actin protein, respectively.

Exchange of the actin-bound nucleotide in perfused rat heart: In order to study the functional role of the actin bound nucleotide (ATP or ADP) in intact muscle there is need for a method to separate the free nucleotides in the cytoplasm from the actin-bound nucleotides in the myofibril structure. Common organic solvents do this job at  $-15$  to  $-20^{\circ}\text{C}$ . We have used 50% ethanol for arterial smooth muscle (Bárány et al., 2001, see in the [Smooth Muscle Chapter](#)) and 75% methanol for rat heart (Bárány and de Tombe, 2004). If the muscles are incubated in physiological saline containing  $^{32}\text{P}$ -orthophosphate ( $^{32}\text{P}_i$ ), first the  $\gamma$ -P and then the  $\beta$ -P of ATP will be labeled to generate  $[\gamma^{32}\text{P}, \beta^{32}\text{P}]\text{ATP}$  in a short time. The exchange of actin bound nucleotide under various physiological conditions can be followed by comparing the specific radioactivity of the  $^{32}\text{P}$ -phosphate in the actin bound nucleotides with that of the  $^{32}\text{P}$ -phosphate in the cytoplasmic nucleotides. The exchange of the actin-bound nucleotides is complete when its specific radioactivity equals that of the cytoplasmic nucleotides. In rat hearts perfused with  $^{32}\text{P}_i$  the actin-bound nucleotide rapidly exchanged with the cytoplasmic ATP. Furthermore, the rate of exchange of the bound-nucleotide was comparable to the rate of the heart beat (Bárány and de Tombe, 2004).

Our work has also revealed the presence of monomeric-actin in the cytoplasm of rat heart (about 30% of the total actin). The mechanism of exchange of actin-bound nucleotide is visualized in Fig. H4.

**Fig. H4** Mechanism of exchange of actin-bound nucleotides in perfused rat heart.



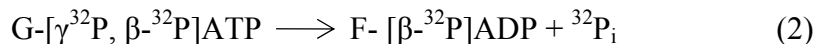
Part A of the Figure shows that the double labeled  $\text{ATP}^{32,32}$  in the cytoplasm (black) exchanges with the bound-ATP of free monomeric-actin (G-actin) in the cytoplasm to yield G-  $\text{ATP}^{32,32}$ -actin (red). The polymeric-actin (F-actin) filament in the upper part of Part A contains non-radioactive ADP (gray).

Part B of the Figure shows the attachment of G- $\text{ATP}^{32,32}$ -actin molecules to the barbed end of the polymeric-actin double filaments, while the terminal ADP-actin monomers are leaving the filaments at the pointed end.

In Part C, we see the attachment of new G- $\text{ATP}^{32,32}$ -actin molecules, while the previously attached G- $\text{ATP}^{32,32}$ -actins are hydrolyzed to actin- $\text{ADP}^{32}$  monomers (pink) in the filaments. At the same time new terminal ADP-actin monomers are released from the pointed ends.

This process is repeated in Parts D, E, and F; all nucleotides in the filaments are radioactive at the end.

The chemical interpretation of Figure H4 is shown by the following two equations:



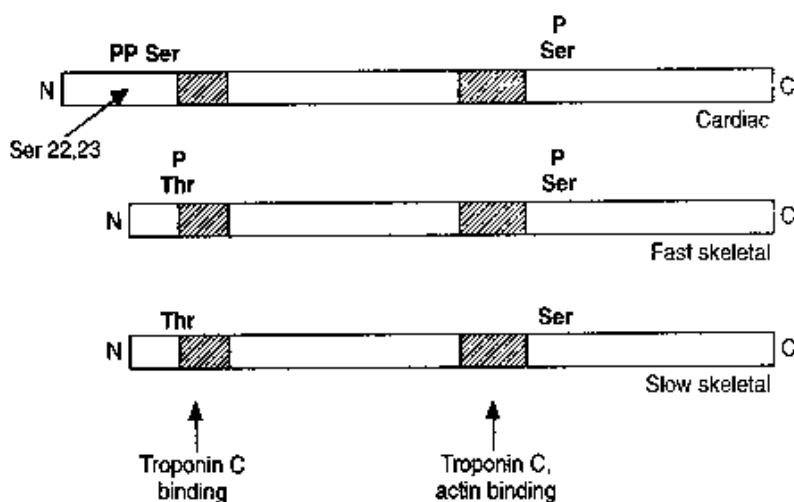
The double labeled ATP in the cytoplasm exchanges with the ATP bound to G-actin (equation 1). Subsequently, the double labeled G-actin polymerizes to F-actin containing single labeled ADP while  ${}^{32}\text{P}_i$  is liberated (equation 2).

The data presented in Figure H4 and equations 1 and 2 suggest that actin polymerization takes place in the perfused rat heart.

## Regulatory Proteins

**TN-C.** The cardiac muscle TN-C (cTN-C) differs from fast skeletal muscle TN-C in that it contains only one  $\text{Ca}^{2+}$ -binding site in the N-terminal domain of the protein. The neighboring site at the N-terminal does not contain aspartic acid, a prerequisite for coordination of  $\text{Ca}^{2+}$ . This site is called site 1, whereas the  $\text{Ca}^{2+}$ -binding site is called site 2 of cTN-C, or the regulatory N-terminal lobe of cTN-C. The binding of  $\text{Ca}^{2+}$ -ions has been shown to open a hydrophobic cleft in the regulatory N-terminal lobe of skeletal TN-C, in cTN-C  $\text{Ca}^{2+}$  alone is not sufficient to stabilize the open conformation so that a target peptide is necessary. The orientation of the regulatory N-terminal lobe of cTN-C with respect to its structural C-terminal lobe varies, with the central linker that connects them adopting a partially disordered or fully  $\alpha$ -helical conformation (Brown and Cohen, 2005).

**TN-I:** The cardiac TN-I (cTN-I) differs from the fast skeletal muscle TN-I by containing an N-terminal extension of 33 amino acid residues (Fig. H5).



**Fig. H5.** Comparison of the overall structure of cardiac muscle TN-I with that of skeletal muscle TN-I. The shaded areas correspond to homologous regions in the structures (From Perry 1996).

This extension has two adjacent serine residues, No. 23 and 24 (at the time when Fig. H5 was prepared these residues were believed No. 22 and 23, respectively) in the sequence (Kobayashi and Solaro, 2005). Solaro et al. (1976) discovered that TN-I is phosphorylated in perfused rabbit heart stimulated by adrenaline. It was shown later (Mittmann et al., 1992) that both serines at positions 23 and 24 of rabbit cardiac TN-I can be phosphorylated by cyclic AMP-dependent protein kinase (PKA). Ser-24 is phosphorylated first and, subsequently, much slower phosphorylation occurs at Ser-23. Since PKA is activated by adrenaline in the beating heart it appears that PKA is responsible for the diphosphorylation of cTN-I in the heart. Phosphorylation of Ser-23 and Ser-24 appears specialized for regulation of sensitivity of the myofilaments to  $\text{Ca}^{2+}$  and for enhancing crossbridge cycling rate (Kobayashi and Solaro, 2005).

Protein kinase C (PKC) phosphorylates Ser-42, Ser-44 and Thr-106 residues in cTN-I. This phosphorylation appears specialized for depressing crossbridge cycling rate (Kobayashi and Solaro, 2005).

NMR, fluorescence resonance energy transfer, and mutation studies indicate that the N-terminal part of cTN-I combines with cTN-C. Upon phosphorylation of cTN-I, the site 1 of cTN-C undergoes a conformational exchange consistent with an equilibrium between closed and opened forms of cTN-C (Gaponenko et al., 1999). In addition, cTN-I phosphorylation changes the binding of  $\text{Ca}^{2+}$  to cTN-C, the structure of cTN-I, and the cooperative binding of cTN-I to actin-TM (Solaro and Van Eyk, 1996). Previously, phosphorylation has been shown to modulate cardiac function by reducing the  $\text{Ca}^{2+}$  affinity for the N-terminal regulatory site of cTN-C (Solaro, 1986). Thus, cTN-I phosphorylation is a unique property of the myocardium that plays a key role in cardiac function.

Fluorescence resonance energy transfer was used (Dong et al., 2001) to investigate the global conformation of the inhibitory region of a full-length TN-I mutant from cardiac muscle in the unbound state and in reconstituted complexes with the other cardiac TN subunits. The mutant contained a single tryptophan residue at the position 129 which was used as an energy transfer donor, and a single cysteine residue at the position 152 labeled with IAEDANS as an energy acceptor. The distance between the donor and acceptor sites was found to be 19.4 Å and it was insensitive to reconstitution of cTN-I with cTN-T, cTN-C, or cTN-C plus cTN-T, in the absence of bound regulatory  $\text{Ca}^{2+}$  in cTN-C. A large increase in the Trp129-Cys152 distance was observed upon saturation of the  $\text{Ca}^{2+}$  regulatory site of cTN-C in the complexes. This increase suggests an extended conformation of the inhibitory region in the interface between cTN-C and cTN-I in the holo cardiac troponin, which may pull away the inhibitory region of cTN-I from actin upon  $\text{Ca}^{2+}$  activation in cardiac muscle.

The review of Layland et al., (2005) on the regulation of cardiac function by cTN-I phosphorylation highlights the physiological and pathophysiological aspects of this reaction.

Many mutations of cTN-I have been described that are related to cardiomyopathy. The cause of the myopathy is the mechanical defect in the cardiac muscle fibers. For instance,

inserting mutated human cTN-I from inherited restrictive cardiomyopathy, with a small structural change, into skinned cardiac muscle fibers greatly increased the  $\text{Ca}^{2+}$  sensitizing effect on force generation (Yumoto et al., 2005). Furthermore, increased  $\text{Ca}^{2+}$  affinity of cardiac thin filaments reconstituted with cardiomyopathy-related mutant cTN-I was reported (Kobayashi and Solaro, 2006). This finding may be an important factor in triggering arrhythmias in cardiomyopathy.

In general, analyses of the mechanism by which a single mutation selectively perturbs one component of cardiac physiology one may get an insight into the complex process of heart failure (Morita et al., 2005)

TN-T: As skeletal TN-T, cardiac TN-T (cTN-T) also has several isoforms (McAuliffe et al., 1990). Two TN-T isoforms have been identified in adult beef heart, which show differences in their sequence and activation of the actinS1 ATPase. Five isoforms were found in rabbit heart and two in rat heart. During development of rabbit heart, there are shifts in the isotype population of cTN-T and these are related to the differences in the  $\text{Ca}^{2+}$  regulation between neonate and adult hearts.

Mutations between residues 92 and 110 of cTN-T impair its TM dependent function (Palm et al., 2001). Recombinant human cardiac TN, with wild type TN-T and  $\Delta\text{Lys-210}$  TN-T mutant, altered thin filament regulation by increasing  $\text{Ca}^{2+}$  sensitivity ( $\Delta p\text{Ca}_{50} = +0.2$  pCa units), while virtually abolishing the cooperativity of  $\text{Ca}^{2+}$  activation (Robinson et al., 2002).

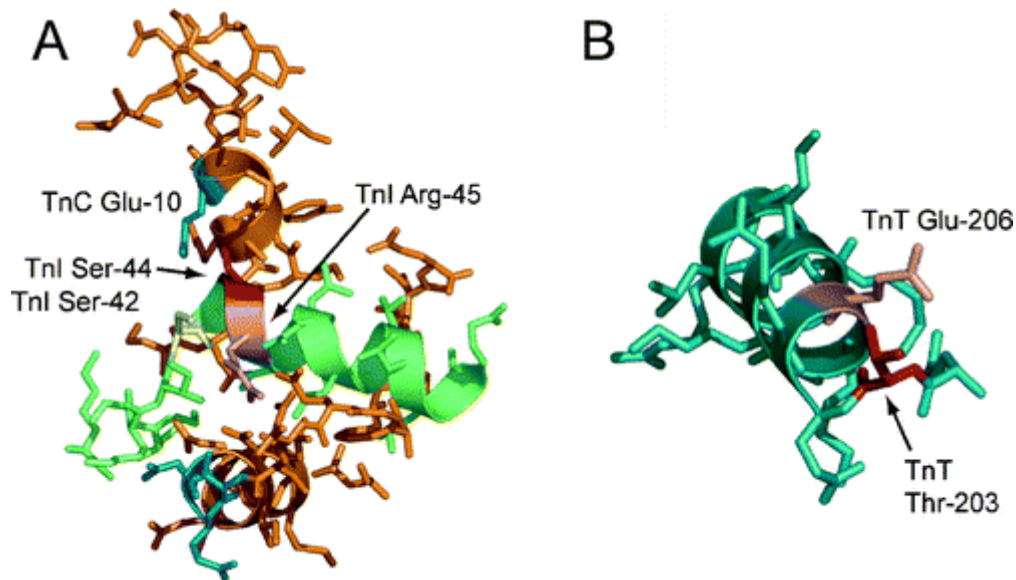
Residues Thr-194, Ser-198, Thr-203, and Thr-284 are phosphorylated by PKC in cTN-T. Among these four sites, Thr-203 is the most significant: it significantly inhibits tension and  $\text{Ca}^{2+}$  sensitivity of skinned heart fiber bundles (Kobayashi and Solaro, 2005).

Troponin T modulates sarcomere length-dependent recruitment of crossbridges in cardiac muscle (Chandra et al., 2006)

The troponin complex: A major advance in the understanding of the active and inhibited state of cardiac thin filaments came from the determination of the crystal structure of a ternary cardiac troponin complex (Takeda et al, 2003). These authors described that the core domain of human cardiac TN in the  $\text{Ca}^{2+}$  saturated form is divided into structurally distinct subdomains that are connected by flexible linkers, making the entire molecule highly flexible. The  $\alpha$ -helical coiled-coil formed between TN-T and TN-I is integrated in a rigid and asymmetric structure (about 80 Å long), the IT arm, which bridges putative tropomyosin-anchoring regions. The structures of the TN ternary complex imply that  $\text{Ca}^{2+}$  binding to the regulatory site of TN-C removes the carboxy-terminal portion of TN-I from actin, thereby allowing tropomyosin to move on the actin filament.

Structural model of phosphorylation sites in cardiac troponin. Most of the phosphorylation sites of cTN-I and cTN-T were not resolved in the crystal structure. Some information may be obtained about the surrounding areas of Ser-42 and Ser-44 in cTN-I and Thr-203 of cTN-T which were visible in the crystal structure (Fig. H6).

Ser-42 forms a side chain backbone hydrogen-bond network with Arg-45, which represents the most common pattern of  $\alpha$ -helix capping (Part A of Fig.H6). Phosphorylation of Ser-42 may affect the capping and thus alter cTN-I local structure. Ser-44 of cTN-I interacts with Glu-10 of the N-lobe of cTN-C, indicating that phosphorylation of Ser-42/Ser44 might affect the interaction between cTN-I and cTN-C and thus  $\text{Ca}^{2+}$  activation. Another potential phosphorylation site visible in the crystal structure is Thr-203 of cTNT (Part B of Fig. H6). This residue also acts as an N-cap of a helix. Modeling of the structural change induced by phosphorylation of Thr-203 predicts an extension of the helix. Compared with controls, skinned fiber bundles containing cTN-T-Thr-203-Glu demonstrate a significantly reduced tension and ATPase rate, as well a reduced  $\text{Ca}^{2+}$  sensitivity (Kobayashi and Solaro, 2005).



**Fig. H6.** Structural model of phosphorylation sites in cTN. (A) cTN-I (yellow)/ Ser-44 (red) and nearby residues. Ser-42 forms a capping box with Arg-45 (magenta). Whereas Ser-44 interacts with Glu-10 (blue) from the N-terminal domain of cTN-C in the crystal structure, pseudophosphorylation (Asp substitution) of Ser-42/ Ser-44 perturbs the N-terminal structure of the G-helix (also shown in blue) from the C-terminal domain of cTN-C. (B) cTN-T Thr-203 (red) and nearby residues. Thr-203 forms a capping box with Glu-206 (pale magenta) of cTN-T. (From Tomoyoshi Kobayashi and R. John Solaro, *CALCIUM, THIN FILAMENTS, AND THE INTEGRATIVE BIOLOGY OF CARDIAC CONTRACTILITY*. *Annu. Rev. Physiol.* 2005, 67: 39-67. Reproduced with permission. Copyright 2005 by Annual Reviews.

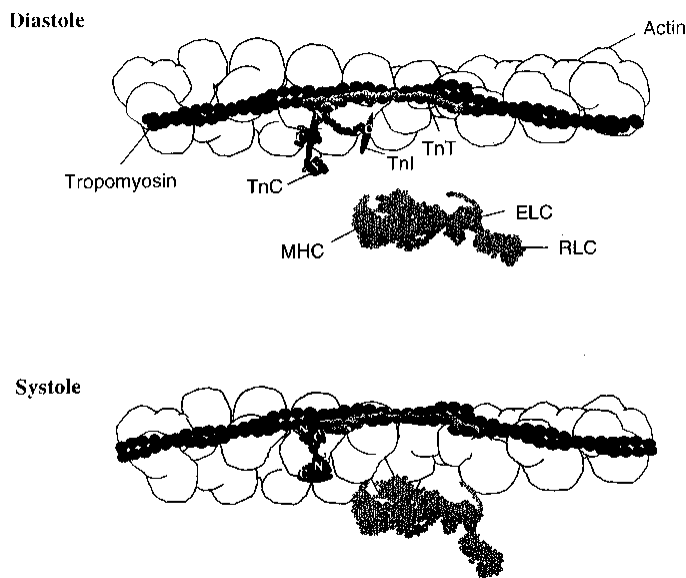
Structural and functional differences between cardiac and skeletal isoforms of troponin:

Comparison of the structures and functions of cardiac and skeletal troponins shows several differences (Vinogradova et al., 2005). The  $\text{Ca}^{2+}$  binding site 1 in cTN-C is inactive because amino acid replacements. The N-terminal lobe of cTN-C does not open as much as in skeletal TN-C in the presence of  $\text{Ca}^{2+}$ . At the N-terminal cTN-I has an extension of 33 amino acid residues.  $\text{Ca}^{2+}$  has less effect on cTN than on skeletal TN.

On the other hand, the overall organization of the subunits in cTN and TN is similar. However, the following features do not match: The structure of the TN-I inhibitory segment is ordered and visible only in the skeletal muscle isoform. The TN-C  $\text{Ca}^{2+}$  regulatory domain which is positioned in space by the central helix of TN-C is rigid in skeletal muscle TN-C, but it is melted in cardiac TN-C

**TM:** In skeletal muscle two isoforms of TM,  $\alpha$  and  $\beta$  (each under different genetic control) are expressed. In contrast, in the heart only the  $\alpha$ -form of TM is expressed. However, using transgenic approaches mice could be produced with overexpressed  $\beta$ -TM in the heart. Novel functions of this TM isoform were detected (Palmiter et al., 1996). Thus, the cardiac myofilaments, containing  $\beta$ -TM demonstrated an increase in the activation of the thin filament by strongly bound cross-bridges, an increase in  $\text{Ca}^{2+}$  sensitivity of steady state force, and a decrease in the rightward shift of the  $\text{Ca}^{2+}$ -force relation induced by cAMP-dependent phosphorylation. These data indicate that switching of TM isoform has a major effect on heart myofilament activity.

**Movement of tropomyosin during the heart cycle:** Fig. H7 shows that in diastole TM is fixed in the groove of the actin double helix by TN-T and TN-I. The position of the TM is such that the myosin cross-bridges (MHC, ELC, RLC) can not react with actin. In systole,  $\text{Ca}^{2+}$ -TN-C interacts with TN-I and TN-T. This allows TM to move on the thin filament removing the steric hindrance of the actin-crossbridge combination and systole ensues.



**Fig. H7.** Illustration of the movement of tropomyosin in the heart cycle (Courtesy of Dr. Helen Rarick). For details see the text.

In summary, cardiac contraction is a series of interactions between  $\text{Ca}^{2+}$ , the regulatory proteins, and the actomyosin system. In the resting muscle, at low intracellular free  $\text{Ca}^{2+}$  concentration, the TN-TM complex inhibits the actin-myosin combination and with an increase in the myoplasmic  $\text{Ca}^{2+}$  the inhibition is released. The  $\text{Ca}^{2+}$  signaling process starts with the binding of  $\text{Ca}^{2+}$  to a single regulatory site of TN-C and by a tight binding

of TN-C to TN-I. The signal is transmitted by TN and TM to actin in the thin filament. The final step is the combination of actin with myosin.

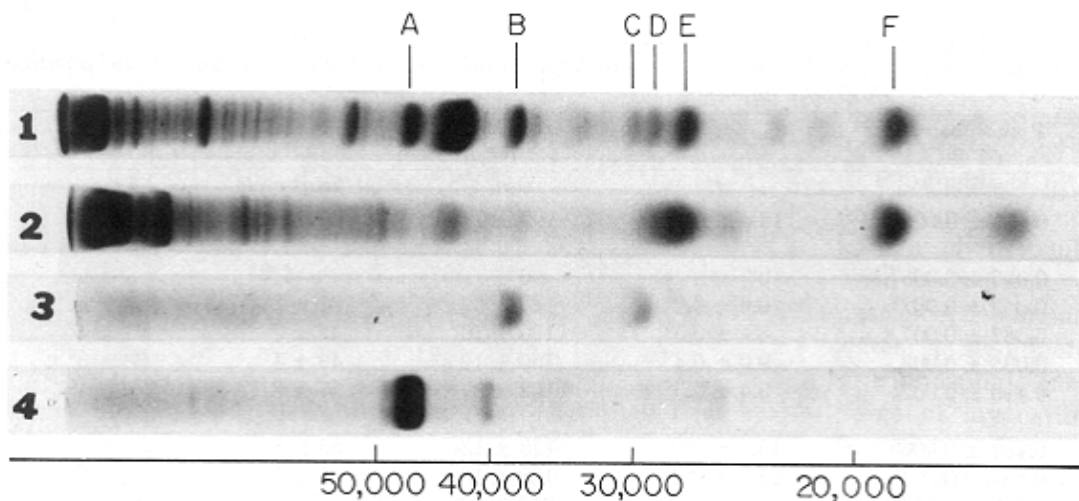
## Methods

Displacement of endogenous TN in skinned fibers with TN mutants: A very sensitive method for mapping the functional domains of TN components is the replacement of native TN in the fibers with mutant or modified TN and then assaying force development or  $\text{Ca}^{2+}$ -sensitivity of the modified fibers. In principle, the fibers are briefly treated with 1% Triton X-100 to remove the sarcolemma and subsequently washed to remove the Triton. The fibers are exposed to 10-20-fold excess of exogenous TN, over the endogenous TN, in a medium containing 250 mM KCl. Finally, unbound TN is removed by washing the fibers. For a long time, the replacement studies were performed with skeletal muscle fibers, but a method for exchanging the TN subunits in cardiac fibers was developed (Chandra et al., 1999). The skinned rat cardiac fiber bundles remained relaxed through much of the extraction/reconstitution procedure. The fibers were treated with a mixture of cTN-T-cTN-I to displace the endogenous TN; 70-80% of the endogenous TN subunits were removed. After reconstitution with cTN-C the  $\text{Ca}^{2+}$ -dependence of the force development by the fibers was restored to the extent of 80-85%.

Reconstitution of a modified TN can also be carried out at the level of myofibrils and the  $\text{Mg}^{2+}$ -ATPase activity of the modified fibrils can be measured as a function of  $p\text{Ca}$  (Rarick et al., 1997).

Purification of cardiac myofibrils is important for studying the interaction of the troponin components with each other, with tropomyosin and actin. Myofibrils purified by conventional methods are contaminated with mitochondrial, sarcolemmal and sarcoplasmic reticulum membranes. Treatment of these myofibrils by 1% Triton X-100 removes the contaminants (Solaro et al., 1971). The MgATPase activity of the purified myofibrils corresponds to that of cardiac actomyosin and they exhibit the normal high  $\text{Ca}^{2+}$ -sensitivity.

Gel electrophoresis of the regulatory proteins: In our laboratory, 10% polyacrylamide gels containing 1% SDS, 0.1 M Na-phosphate, pH 7.0 and 8.0 M urea are used for separation of cTM, cTN-T and cTN-I (Fig. H8). Under these conditions cTM (zone A, 48-kDa) migrates behind actin, cTN-T (zone B, 38-kDa) migrates in front of actin, cTN-I (zones C and D, 30- and 29 kDa) splits into two components. Zones E and F, correspond to the 27- and 19-kDa cardiac myosin LCs. cTN-C migrates in front of the 19-kDa LC, it is not visible because it does not stain with Coomassie blue.



**Fig. H8.** Separation of rat heart myofibril proteins by gel electrophoresis (From Kopp and Bárány, 1979). Gel 1, myofibrils; gel 2, beef heart myosin; gel 3, beef heart troponin; and gel 4, beef heart TM. For other details see the text.

*Literature:* Reviews on the interaction of the TN complex, TM, and actin in cardiac thin filaments are available (Tobacman, 1996; Brown and Cohen, 2005; Kobayashi and Solaro, 2005).

## Regulation of $\text{Ca}^{2+}$ Flow

### Sarcoplasmic Reticulum

$\text{Ca}^{2+}$  transport into the sarcoplasmic reticulum (SR) occurs via the action of the SR  $\text{Ca}^{2+}$  pump. As in skeletal muscle, 1 molecule of ATP has to be hydrolyzed per 2 molecules of  $\text{Ca}^{2+}$  that are pumped against a large concentration gradient into the lumen of the SR. The SR  $\text{Ca}^{2+}$ -ATPase, called sarco(endo)plasmic reticulum Ca-ATPase (SERCA), is regulated by phospholamban (described below).  $\text{Ca}^{2+}$  is released from SR through a  $\text{Ca}^{2+}$  release channel. The channel tightly binds ryanodine and, therefore, it is also referred to as the ryanodine receptor (RyR), or  $\text{Ca}^{2+}$  release units (CRUs). In cardiac muscle, CRUs come in three subtypes that differ in geometry, but have common molecular components (Franzini-Armstrong et al., 2005). Peripheral couplings are formed by a specialized junctional domain of the SR (jSR) with the plasmalemma. Dyads occur where the jSR is associated with transverse (T)-tubules. Corbular SR is a jSR domain that is located within the cells and bears RyRs but does not associate with either plasmalemma or T-tubules.

$\text{Ca}^{2+}$  induced  $\text{Ca}^{2+}$  release (CICR) CICR is specific for the heart. The small amount of  $\text{Ca}^{2+}$  that enters the cell through voltage dependent plasmalemmal  $\text{Ca}^{2+}$  channels (which

open in response to the action potential) causes a much larger amount of  $\text{Ca}^{2+}$  to be released from within the SR.

Calsequestrin is a 44 kDa  $\text{Ca}^{2+}$  binding protein that is located within the lumen of SR and is primarily responsible for  $\text{Ca}^{2+}$  storage within the SR. Both cardiac and skeletal muscle calsequestrins have been crystallized and their structures and  $\text{Ca}^{2+}$  binding capacities compared (Park et al., 2004). The two crystal structures for cardiac and skeletal calsequestrins are nearly superimposable and both proteins can undergo dimerization, tetramerization and oligomerization. The  $\text{Ca}^{2+}$  binding capacities are; 60  $\text{Ca}^{2+}$  ions per cardiac calsequestrin and 80  $\text{Ca}^{2+}$  ions per skeletal calsequestrin, as compared with net charges for these molecules of -60 and -80, respectively. Authors suggest that  $\text{Ca}^{2+}$  binding to calsequestrin is coupled to the polymerization of the protein and the release of large number of  $\text{Ca}^{2+}$  ions during contraction occurs through surface diffusion from calsequestrin to the  $\text{Ca}^{2+}$  release channel.

Phospholamban (PLB): It is a pentamer made up of five identical subunits, each 6,000 dalton (52 amino acid residues). PLB is found in cardiac, slow skeletal and smooth muscle but it is absent in fast skeletal muscle. The protein is associated with the  $\text{Ca}^{2+}$ -pump ATPase in the membrane of the SR. The flexible N-terminus on which the phosphorylation sites are located extends out into the cytoplasm of the cardiac muscle cell. The phosphorylation site at serine 16 is a substrate for PKA, whereas threonine 17 is phosphorylated by a  $\text{Ca}^{2+}$ -calmodulin dependent protein kinase. Phosphorylation of PLB by PKA increases the rate of  $\text{Ca}^{2+}$  transport and the  $\text{Ca}^{2+}$ -sensitivity of the  $\text{Ca}^{2+}$ -pump and thereby facilitates relaxation in heart exposed to  $\beta$ -adrenergic agonists (Katz, 1992; Perry, 1996). Phosphorylation of PLB by a  $\text{Ca}^{2+}$ -calmodulin dependent protein kinase II also stimulates  $\text{Ca}^{2+}$ -uptake *in vitro*, and the physiological significance of this phosphorylation is extensively studied (Maier and Bers, 2002).

Research on the physiological significance of PLB reached an unexpected turn when a PLB deficient mouse was generated, the "PLB knockout (KO) mouse". Surprisingly, there were no detrimental effects in the performance of such animals or in the function of their isolated heart. For instance, the basal contractility and the  $\text{Ca}^{2+}$ -transient of myocytes isolated from PLB deficient hearts were enhanced compared with cells isolated from wild type animals. Furthermore, the contractility of PLB-deficient myocytes could be further enhanced by the  $\beta$ -adrenergic agonist, isoproterenol. These results demonstrate that in the absence of PLB, there are mechanisms available in the heart to adjust its activity, and that phosphorylation of sites other than PLB may play an important role in regulation of contraction-relaxation dynamics of heart responding to  $\beta$ -adrenergic stimulation (Wolska et al., 1996).

#### Factors controlling the $\text{Ca}^{2+}$ release from SR:

Ryanodine (a plant derived alkaloid) is a very effective drug that alters the SR  $\text{Ca}^{2+}$  release channel.

Phospholamban phosphorylation by PKA.

Thapsigargin and cyclopiazonic acid (CPA) that block the  $\text{Ca}^{2+}$  pump.

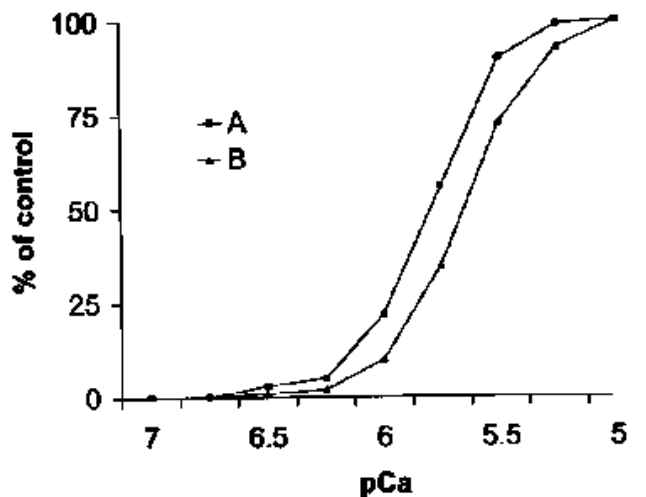
Digitalis, a drug that inhibits the  $\text{Na}^+/\text{K}^+$  pump leading to a small increase in intracellular  $\text{Na}^+$ . This decreases the rate at which the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger extrudes  $\text{Ca}^{2+}$  from the cell and thus leads to enhanced loading of the SR.

## Sarcolemma

$\text{Ca}^{2+}$  enters the cardiac cell during the plateau phase of the action potential via the L-type  $\text{Ca}^{2+}$  channel (The channel tightly binds the  $\text{Ca}^{2+}$  channel blocking agents of the dihydropyridine family). The sarcolemmal  $\text{Na}^+/\text{Ca}^{2+}$  exchanger transports 3  $\text{Na}^+/\text{Ca}^{2+}$ . The energy for this transport is indirectly derived from ATP hydrolysis via the sodium gradient that is established by the  $\text{Na}^+/\text{K}^+$  pump. It is the major  $\text{Ca}^{2+}$  extrusion mechanism of the cardiac myocyte and may contribute significantly to myocardial relaxation. The sarcolemma also houses the  $\text{Na}^+/\text{K}^+$ -ATPase that transports 3  $\text{Na}^+$  out and 2  $\text{K}^+$  into the cell per molecule of ATP, and thus moves out one net charge per cycle. This is the key transporter that sets up the sarcolemmal ionic gradients for Na, K, and Ca, and consequently allows ion channels to function. The  $\text{Na}^+$  and  $\text{K}^+$  channels in the sarcolemma are involved in action potential generation. Fig. H12, illustrates the channels and the ion movements in the sarcolemma.

## $\text{Ca}^{2+}$ Sensitivity

$\text{Ca}^{2+}$  is the central factor in myocardial contraction and the potential of a cardiac system to be activated by  $\text{Ca}^{2+}$  is characterized by its  $\text{Ca}^{2+}$  sensitivity. Originally,  $\text{Ca}^{2+}$  sensitivity was used to define the relationship between  $\text{Ca}^{2+}$  concentration and tension, but later the relationship was extended from tension to other parameters as well, e.g. myofibrillar ATPase activity or fluorescence intensity. Conventionally,  $\text{Ca}^{2+}$  sensitivity is illustrated by plotting the  $p\text{Ca}$  against the selected parameter, expressed as percentage of the control value (Fig. H9).



**Fig. H9.** Graph for a  $p\text{Ca}$  - activity relationship in a biological system. "A" and "B" represent the variations within the same system, e.g. animals of different ages.

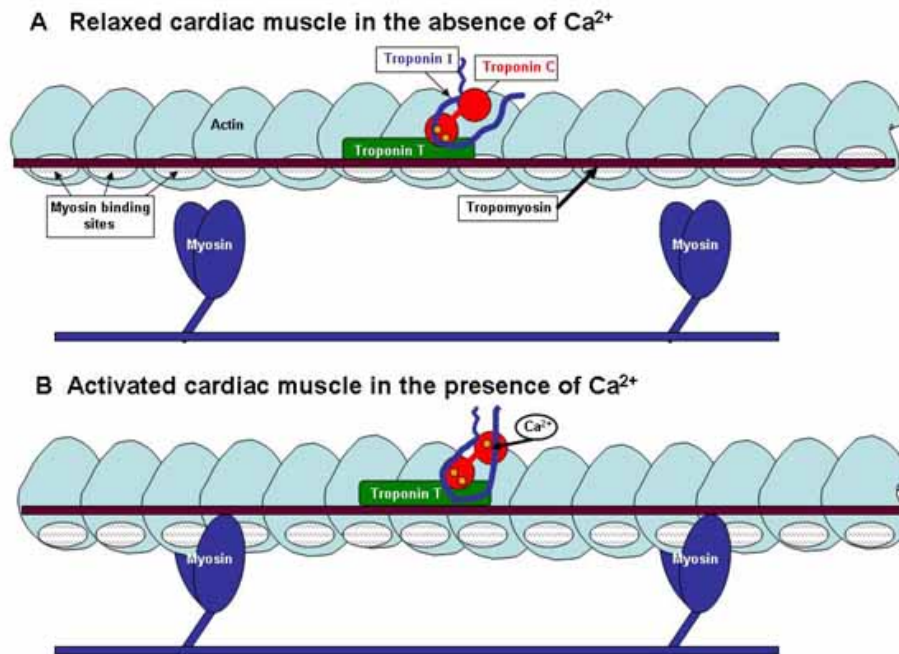
The  $pCa$  curve can shift to the left "increased  $Ca^{2+}$  sensitivity" or to the right "decreased  $Ca^{2+}$  sensitivity". The extent of shift can be estimated from the difference of the  $pCa$  values at 50% activity. The shift may be small, 0.05  $pCa$ , or large  $>1.0 pCa$ .

There are several factors, which influence the  $Ca^{2+}$  sensitivity of the myocardium. In case of the Force -  $pCa$  relationship the factors are: TN-I phosphorylation,  $\beta$ -tropomyosin, aging, acidosis, sarcomere length, temperature, ionic strength, caffeine, or other agents (Bers, 2001). It should be mentioned that most Force -  $pCa$  relationships were measured on cardiac myofilaments with their sarcolemma removed by Triton treatment, "skinned fibres"; the  $Ca^{2+}$  sensitivity of the skinned preparations may differ from those of intact heart ventricles.

Some authors attribute the differences in  $Ca^{2+}$  sensitivity among hearts of various animals (frog, guinea pig, rat, rabbit, and cow) to differences in their TN-C content. On the other hand, others feel that the  $Ca^{2+}$  sensitivity is not based on the single reaction between  $Ca^{2+}$  and TN-C but it rather reflects a series of reactions involved in the signal transduction initiated by  $Ca^{2+}$ .

## Excitation-Contraction Coupling

$Ca^{2+}$  is an absolute requirement for excitation-contraction coupling (E-C) in the heart (Fig. H10). In the absence of  $Ca^{2+}$  the conformation of the troponin complex keeps tropomyosin in a blocking position along the actin filament. This effectively inhibits the myosin crossbridges to combine with actin and results in cardiac muscle relaxation. Upon release of  $Ca^{2+}$ , from the SR and through channels and transport system in the sarcolemma, the regulatory site in the N-terminal lobe of troponin-C binds  $Ca^{2+}$  thereby inducing a conformational change in the troponin complex. This causes tropomyosin to travel away from and expose binding sites on actin. Myosin crossbridges are now able to interact with actin, resulting in cardiac muscle contraction.



**Fig. H10.** (A) Cardiac muscle relaxed in the absence of  $\text{Ca}^{2+}$ . (B) Cardiac muscle activated in the presence of  $\text{Ca}^{2+}$  (Courtesy of Dr. Patti Engel).

There is a recurring theme that skeletal muscle contraction depends almost exclusively on  $\text{Ca}^{2+}$  released from SR with insignificant  $\text{Ca}^{2+}$  entry across the sarcolemma during a normal twitch. Cardiac muscle contraction, on the other hand, depends on both  $\text{Ca}^{2+}$  entry across the sarcolemma and  $\text{Ca}^{2+}$  release from the SR. There are notable differences in the ultrastructure: Skeletal muscle has an extensive and well organized SR network, abutting the narrow T-tubules. In contrast, the SR of cardiac muscle is rather sparse and less organized, and surrounded with T-tubules of much larger diameter. In addition, cardiac myocytes are only 0.02 nm thick, whereas the diameter of the skeletal muscle fibers goes up to 0.2 nm. Thus, a substance from the extracellular space reaches the center of the heart cell much faster than the center of the skeletal muscle cell. In general the structure of the cardiac cell is consistent with a larger role of the transsarcolemmal  $\text{Ca}^{2+}$  fluxes.  $\text{Ca}^{2+}$ -calmodulin dependent protein kinase II regulates  $\text{Ca}^{2+}$ -transporters in the heart (Maier and Bers, 2002).

Major events in cardiac E-C coupling (courtesy of Dr. Pieter de Tombe):

Excitation

$\text{Ca}^{2+}$  induced  $\text{Ca}^{2+}$  release

Activation of contractile proteins

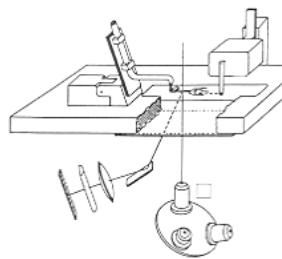
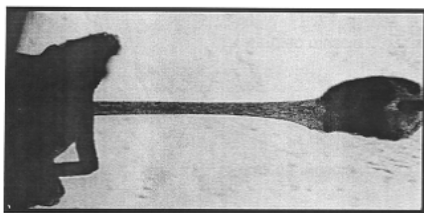
$\text{Ca}^{2+}$  reuptake into the SR and  $\text{Ca}^{2+}$  extrusion leading to relaxation

## Biochemistry of Starling's Law

The Starling law describes an enhancement of contractile function of the heart resulting from the increased end-diastolic ventricular volume and consequently from an increase in muscle length. The Starling law also applies to skeletal muscle but in cardiac muscle it is much more pronounced. The simplest explanation of the phenomenon is based on the constant volume principle, *i.e.* the increased muscle length requires a decrease in the interfilament space in order to keep the volume of the muscle cell constant. X-ray diffraction studies verified this assumption (Irving et al., 2000). Accurate measurements of interfilament spacing, by synchrotron X-ray diffraction, as a function of sarcomere length, using skinned and intact rat trabeculae demonstrated that the lattice spacing was decreased as sarcomere length increased. Thus, the Starling law can be explained by the enhanced actin and myosin interaction as the result of moving the actin and myosin filaments closer to each other at the longer muscle length.

The Starling law is also characterized by an increase in  $\text{Ca}^{2+}$  sensitivity of the cardiac myofilaments as the sarcomere length increases (Solaro, 1999). At a longer sarcomere length the distance between thick and thin filaments decreases and hence the length of the diffusion pathway for  $\text{Ca}^{2+}$ , in the interfilament space, to reach TN-C decreases. One can assume that saturation of TN-C with  $\text{Ca}^{2+}$  and subsequent activation of the thin filaments is easier and faster at smaller interfilament spacing than at the larger spacing. However, the explanation is more complex because tension development induced by  $\text{Ca}^{2+}$  is not a linear but a cooperative activation of the actin-myosin combination.

Fig. H11 illustrates a current method to measure force-length relation in cardiac muscle. A small strip of cardiac muscle is mounted in an experimental setup. Sarcomere length is measured by laser diffraction techniques. Force is measured by a sensitive force transducer. Muscle length is controlled by a high speed device.



**Fig. H11.** Force-length measurement with small heart strips (Courtesy of Dr. Pieter de Tombe).

## Energetics

The energy that the heart uses, to pump the blood, is generated through the hydrolysis of ATP to ADP and  $\text{P}_i$ . ATP is constantly generated by the mitochondria that are abundant in heart muscle cells. Since the outer mitochondrial membrane is impermeable to adenine nucleotides there is need for "energy carriers" to transport the energy into the

cytosol. This is achieved by the phosphorylcreatine (PCr) shuttle (Bessman and Geiger, 1981), that is, excess ATP is transformed to PCr within the mitochondrial inner membrane through creatine kinase isoforms, located in the mitochondria. The PCr formed diffuses into the cytoplasm to saturate the myofibrillar water. When ATP is hydrolyzed by actomyosin, during heart beat, the ADP formed will be immediately regenerated by PCr with aid of specific creatine kinase isoforms.

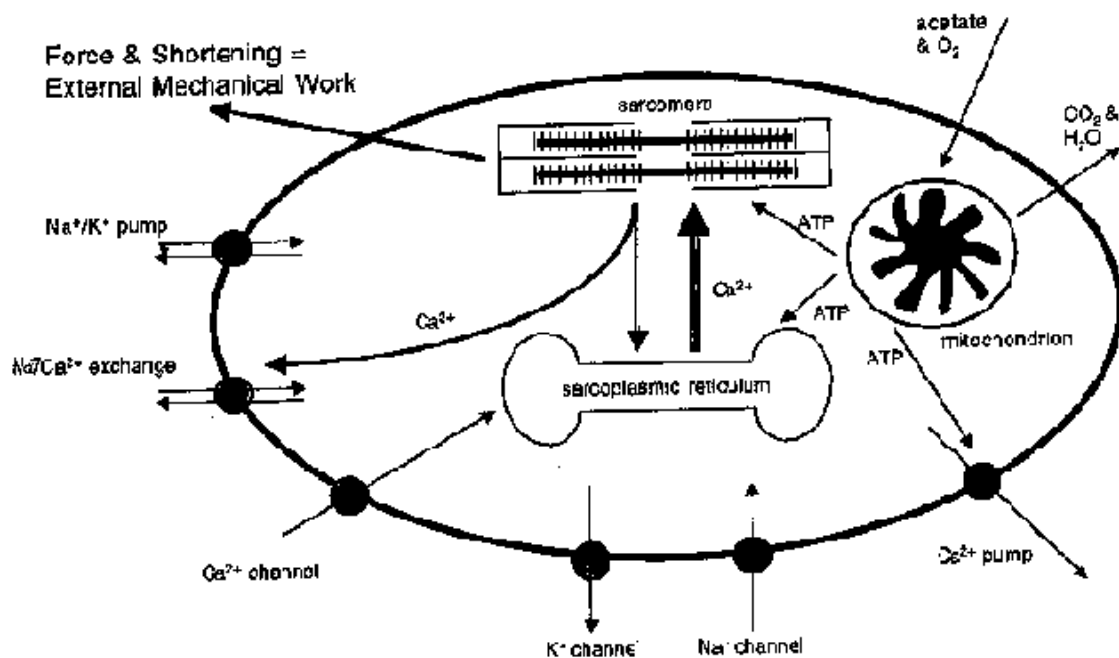
In addition to the contractile apparatus, ATP is also used by the  $\text{Ca}^{2+}$ -ATPase and  $\text{Na}^+/\text{K}^+$ -ATPase of the sarcolemma, by the  $\text{Ca}^{2+}$ -ATPase of SR to store  $\text{Ca}^{2+}$ , and by biosynthetic processes.

About 90% of the ATP is synthesized by oxidative phosphorylation in the mitochondria and about 10% by glycolysis, that take place in the cytosol. Mitochondria are strictly dependent on  $\text{O}_2$ , they mainly oxidize fatty acids (note: of all the food we eat fat has the highest calorie value) and pyruvate, arising from the glycolysis of glucose.

Since PCr plays an important role in cardiac energetics, creatine depletion and creatine supplementation studies were carried out in order to gain more insight into muscle energetics. Feeding rats with the creatine analogue  $\beta$ guanidinopropionate ( $\beta$ -GP) reduced myocardial PCr and Cr by about 80%, the velocity of the creatine kinase reaction decreased by 90%, but the level of ATP remained unchanged (Neubauer et al., 1999). The same biochemical alterations were found in isolated rat hearts perfused with  $\beta$ -GP; this was accompanied by reduced contractile performance *in vitro*. However, in intact rats only a minimal functional impairment was observed. Thus, in intact rat heart cardiac and/or humoral compensatory mechanisms are sufficient to maintain normal hemodynamics in spite of the greatly reduced PCr concentration. The same conclusion could be drawn from studies on creatine kinase knock-out animals, which exhibited normal muscle activity suggesting that neither creatine kinase nor PCr are central to cellular energy metabolism. However, both creatine kinase knock out and creatine analogue fed animals showed marked myofibrillar and mitochondrial remodeling, suggesting that energy transduction was altered.

Five days high dose creatine feeding enhanced creatine disposal and glycogen storage in rat skeletal muscles (Op't et al., 2001). The creatine and glycogen response was markedly greater in oxidative than in glycolytic muscles. This investigation contributes to the understanding of how the increased use of creatine by athletes, as a dietary supplement, improves their physical performance.

**Summary:** Fig. H12 shows the factors which determine contractility in the heart.



**Fig. H12.** An overview of the chemical events taking place in the working heart (Courtesy of Dr. Pieter de Tombe).

The movements of Na<sup>+</sup> and K<sup>+</sup> determine the electrical properties of the heart membrane. The Ca<sup>2+</sup> homeostasis is established by the Ca<sup>2+</sup> channel which lets the Ca<sup>2+</sup> in and the Ca<sup>2+</sup> pump and Na<sup>+</sup>/Ca<sup>2+</sup> exchanger which remove the excess Ca<sup>2+</sup> from the heart cell. The intracellular Ca<sup>2+</sup> is partially stored in the SR, but its main function is to activate the sarcomere to produce force and shortening. The energy cost for the external mechanical work and Ca<sup>2+</sup> storage is covered by ATP, produced by the mitochondrion. The mitochondria burn glucose, acetate and other fatty acids to CO<sub>2</sub> and H<sub>2</sub>O, which leave the cell by diffusion.

Suggested readings: The following books (Solaro, 1986; Katz, 1992; Bers, 2001) and reviews (Solaro and Van Eyk, 1996; Tobacman, 1996; Janssen, 1997; Solaro, 1999; Maier and Bers, 2002; Kobayashi and Solaro, 2005) can help to increase knowledge in the biochemistry of cardiac contractility

## References

- Bárány, M., Barron, J.T., Gu, L., and Bárány, K. (2001). Exchange of the actin-bound nucleotide in intact arterial smooth muscle. *J. Biol. Chem.*, **276**, 48398-48403.
- Bárány, M., and de Tombe, P.P. (2004). Rapid exchange of actin-bound nucleotide in perfused rat heart. *Am. J. Physiol. Heart Circ. Physiol.*, **286**, H1394-H1401.

- Bárány, M., Gaetjens, E., Bárány, K. and Karp, E. (1964). Comparative studies of rabbit cardiac and skeletal myosins. *Arch. Biochem. Biophys.* **106**, 280-293.
- Bers, D.M. (2001). Excitation-contraction coupling and cardiac contractile force. Kulwer Academic Publishers, Dordrecht.
- Bessman, S. P., and Geiger, P.J. (1981). Transport of energy in muscle: the phosphoryl creatine shuttle. *Science*, **211**, 448-452.
- Brown, J.H., and Cohen, C. (2005). Regulation of muscle contraction by tropomyosin and troponin: How structure illuminates function. *In Advances in Protein Chemistry. Fibrous Proteins: Muscle and Molecular Motors*, vol. 71 (J.M. Squire and D.A.D. Parry, eds.), pp 121 – 159, Academic Press, San Diego.
- Chandra, M., Kim, J.J., and Solaro, R. (1999). An improved method for exchanging troponin subunits in detergent skinned rat cardiac fiber bundles. *Biochim. Biophys. Res. Commun.* **263**, 219-223.
- Chandra, M., Tschirgi, M.L., Rajapakse, I., and Campbell, K.B. (2006). Troponin T modulates sarcomere length-dependent recruitment of cross-bridges in cardiac muscle. *Biophys. J.*, **90**, 2867-2876.
- Dong, W-J., Xing, J., Robinson, J.M., and Cheung, H.C. (1991).  $Ca^{2+}$  induces an extended conformation of the inhibitory region of troponin I in cardiac muscle troponin. *J. Mol. Biol.* **314**, 51-61.
- Franzini-Armstrong, C., Protasi, F., and Tijskens, P. (2005). The assembly of calcium release units in cardiac muscle. *Ann. N.Y. Acad. Sci.*, **1047**, 76-85.
- Gaponenko, V., Abusamhadneh, E., Abbot, M.B., Finley, N., Gasmi-Seabrook, G., Solaro, R.J., Rance, M., and Rosevear, P.R. (1999). Effects of troponin I phosphorylation on conformational exchange in the regulatory domain of cardiac troponin C. *J. Biol. Chem.* **274**, 16881-16884.
- Irving, T.C., Konhilas, J., Perry, D., Fischetti, R., and De Tombe, P.P. (2000). Myofilament lattice spacing as a function of sarcomere length in isolated rat myocardium. *Am. J. Physiol.* **279**, H2568-H2573.
- Janssen, P.M.L. (1997). Determinants of contraction and relaxation in mammalian myocardium: Effects of calcium and sarcomere length. *Ph.D. Thesis, University of Utrecht, The Netherlands*, ISBN 90-393-1120-X
- Katz, A. M. (1992). Physiology of the heart. Raven Press, New York.
- Kobayashi, T., and Solaro, R.J. (2005). Calcium, thin filaments, and the integrative biology of cardiac contractility. *Annu. Rev. Physiol.*, **67**, 39-67.

- Kobayashi, T., and Solaro, R.J. (2006). Increased  $\text{Ca}^{2+}$  affinity of cardiac thin filaments reconstituted with cardiomyopathy-related mutant cardiac troponin I. *J. Biol. Chem.*, **281**, 13471-13477.
- Kopp, S.J., and Bárány, M. (1979). Phosphorylation of the 19,000-Dalton light chain of myosin in perfused rat heart under the Influence of negative and positive inotropic agents. *J. Biol. Chem.* **254**, 12007-12012.
- Layland, J., Solaro, R.J., and Shah, A.M. (2004). Regulation of cardiac contractile function by troponin I phosphorylation. *Cardiovascular Research*, **66**, 12-21.
- Maier, L.S., and Bers, D.M. (2002). Calcium, calmodulin, and calcium-calmodulin kinase II: Heartbeat to heartbeat and beyond. *J Mol. Cell Cardiol.*, **34**, 919-939.
- McAuliffe, J.J., Gao, L., and Solaro, R.J. (1990). Changes in myofibrillar activation and troponin C  $\text{Ca}^{2+}$  binding associated with troponin T isoform switching in developing rabbit heart. *Circulation Research* **66**, 1204-1216.
- Mittmann, K., Jaquet, K., and Heilmeyer Jr., L.M.G. (1992). Ordered phosphorylation of a duplicated minimal recognition motif for cAMP -dependent protein kinase present in cardiac troponin I. *FEBS Letters*, **302**, 133-137.
- Morita, H., Seidman, J., and Seidman, C.E. (2005). Genetic causes of human heart failure. *J. Clin. Invest.*, **115**, 518-526.
- Neubauer, S., Hu, K., Horn, M., Remkes, H., Hoffmann, K. D., Schmidt, C., Schmidt, T.J., Schnakerz, K., and Ertl, G. (1999). Functional and energetic consequences of chronic myocardial creatine depletion by beta-guanidinopropionate in perfused hearts and in intact rats. *J. Mol. Cell. Cardiol.* **31**, 1845-1855.
- Op't, E.B., Richter, E.A., Henquin, J.-C., Kiens, B., and Hespel, P. (2001). Effect of creatine supplementation on creatine and glycogen content in rat skeletal muscle. *Acta Physiologica Scandinavica*, **171**, 169-176..
- Palm, T., Graboski, S., Hitchcock-DeGregori, S.E., and Greenfield, N.J. (2001). Disease-causing mutations in cardiac troponin-T: Identification of a critical tropomyosin-binding region. *Biophys. J.*, **81**, 2827-2837.
- Palmiter, K.A., Kitada, Y., Muthuchamy, M., Wieczorek, D.F., and Solaro, R.J. (1996). Exchange of beta for alpha tropomyosin in hearts of transgenic mice induces changes in thin filament response to  $\text{Ca}^{2+}$ , strong cross-bridge binding, and protein phosphorylation. *J. Biol. Chem.* **271**, 11611-11614.
- Park, H., Park, I.Y., Kim, E.J., Youn, B., Fields, K., Dunker, A.K., and Kang, C.H. (2004). Comparing skeletal and cardiac calsequestrin structures and their calcium binding. *J. Biol. Chem.*, **279**, 18026-18033.

- Perry, S.V. (1996). Molecular mechanisms in striated muscle. *Cambridge University Press*, Cambridge, UK.
- Rarick, H.M., Tu, X-H., Solaro, R.J., and Martin, A.F. (1997). The C terminus of cardiac troponin I is essential for full inhibitory activity and  $\text{Ca}^{2+}$  sensitivity of rat myofibrils. *J. Biol. Chem.*, **272**, 26887-26892.
- Robinson, P., Mirza, M., Knott, A., Abdulrazzak, H., Wilott, R., Marston, S., Watkins, H., and Redwood, C. (2002). Alterations in thin filament regulation induced by a human cardiac troponin T mutant that causes dilated cardiomyopathy are distinct from those induced by troponin T mutants that cause hypertrophic cardiomyopathy. *J. Biol. Chem.*, **277**, 40710-40716.
- Rundell, V.L.M., Geenen, D.L., Buttrick, P.M., and de Tombe, P.P. (2004). Depressed cardiac tension in experimental diabetes is due to altered myosin heavy chain isoform expression. *Am. J. Physiol. Heart Circ. Physiol.*, **287**, H408-H413.
- Solaro, R.J., Pang, D.C., and Briggs, N. (1971). The purification of cardiac myofibrils with Triton X-100. *Biochim. Biophys. Acta.*, **245**, 259-262.
- Solaro, R.J., Moir, A.J.G., and Perry S.V. (1976). Phosphorylation of troponin I and the inotropic effect of adrenaline in the perfused rabbit heart. *Nature*, **262**, 615-617.
- Solaro, R.J. (1986) *In Protein Phosphorylation in the Heart Muscle* (R.J. Solaro, Ed.) pp. 129-156, CRC Press Inc., Boca Raton, FL.
- Solaro, R.J., and Van Eyk, J. (1996) Altered interactions among thin filament proteins modulate cardiac function. *J. Mol. Cell. Cardiol.*, **28**, 217-230.
- Solaro, R.J. (1999). Integration of Myofilament response to  $\text{Ca}^{2+}$  with cardiac pump regulation and pump dynamics. *Advances in Physiology Education*, **22**, S155-S163.
- Takeda, S., Yamashita, A., Maeda, K., and Maéda, Y. (2003). Structure of the core domain of human cardiac troponin in the  $\text{Ca}^{2+}$ -saturated form. *Nature*. **424**, 35-41.
- Tobacman, L.S. (1996). Thin filament-mediated regulation of cardiac contraction. *Annu. Rev. Physiol.* **58**, 447-481.
- Vinogradova, M.V., Stone, D.B., Malanina, G.G., Karatzaferi, C., Cooke, R., Mendelson, R.A., and Fletterick, R.J. (2004).  $\text{Ca}^{2+}$ -regulated structural changes in troponin. *Proc. Natl. Acad. Sci. USA*, **102**, 5038-5043.
- Wolska, B. M., Stojanovic, M.O., Luo, W., Kranias, E.G., and Solaro, R.J. (1996). Effect of ablation of phospholamban on dynamics of cardiac myocyte contraction and intracellular  $\text{Ca}^{2+}$ . *Am. J. Physiol.* **271**, C391-C397.
- Yumoto, F., Lu, Q.W., Morimoto, S., Tanaka, H., Kono, N., Nagata, K., Ojima, T., Takahashi-Yanaga, F., Miwa, Y., Sasaguri, T., Nishita, K., Tanokura, M., and Ohtsuki, I.

(2005). Drastic  $\text{Ca}^{2+}$  sensitization of myofilament associated with a small structural change in troponin I in inherited restrictive cardiomyopathy. *Biochem. Biophys. Res. Com.*, **338**, 1519-1526.