

Regulatory Proteins

Tropomyosin

Bailey discovered tropomyosin (TM) in 1946. Its solubility and structural properties show a similarity to those of myosin, hence its name. Tropomyosin is a rod-shaped molecule (about 400 Å long and 20 Å wide) with a molecular weight of 65,000-70,000. It consists of two α -helical chains arranged in a paralleled coiled-coil configuration. Tropomyosin molecules are bonded head to tail (Fig. TM1). In skeletal muscle, TM accounts to about 3% of the total muscle proteins.

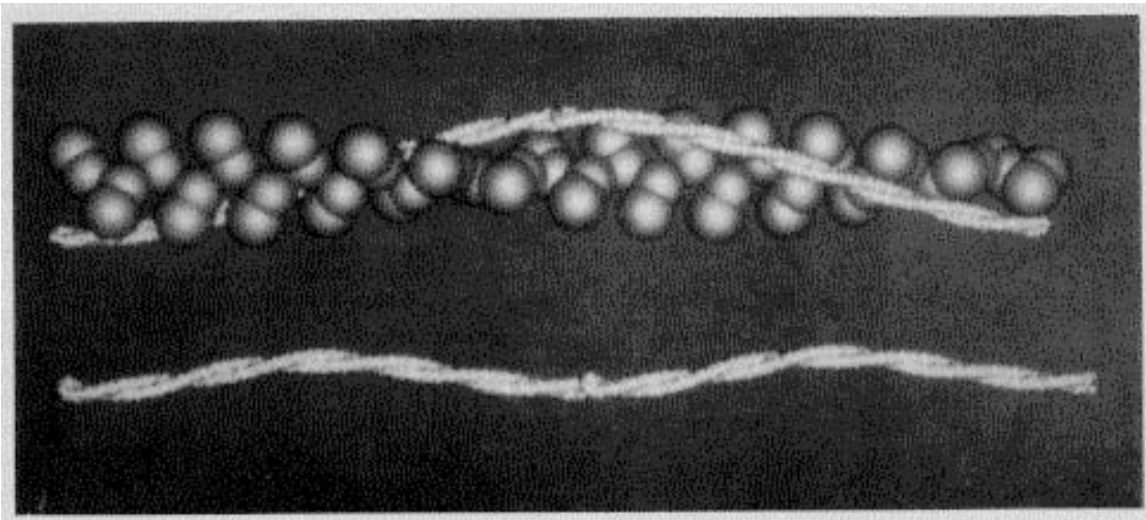


Fig. TM1. Head to tail joint in TM (lower part) and the interaction of TM with each of seven actin monomers (upper part). (Smillie, 1996, with permission from *Biochemistry of Smooth Muscle Contraction*, 1996, Academic Press).

Tropomyosin isoforms: On SDS-PAGE, TM shows two bands, corresponding to the α (fast) and β (slow) chains. The molecular weight of the chains does not differ significantly; it is in the range of 33,000-35,000. Both chains have 284 amino acid residues, but they differ in 39 residues. The amino acid sequence shows a repeating pattern of nonpolar and polar residues, totaling of 7 residues.

The molecular length of a chain calculated from sequence, $284 \times 1.49 \text{ \AA} = 423 \text{ \AA}$, (number of residues multiplied by the effective residue translation in a coiled coil) is larger than the length of 400 Å calculated from X-ray studies. This difference can be accounted for by assuming an overlap of 8-9 residues between the ends of TM molecules.

The ratio of the concentration of alpha and beta subunit varies with the muscle type. Slow (red) skeletal muscle and fetal muscle contain a larger portion of the beta subunit than fast (white) skeletal muscle, whereas rabbit and avian cardiac muscle contain only the alpha subunit.

Two dimensional gel electrophoresis revealed several isoforms of TM in different muscles. Furthermore, some of the TM isoforms can be phosphorylated. Changes in the distribution of TM isoforms were observed during development.

Structure of tropomyosin. The amino acid sequences of the α - and β - chains of rabbit skeletal muscle TM showed a repeating pattern of nonpolar and polar amino acids (reviewed by Smillie, 1996). This is characteristic for a coiled-coil structure in which two α -helices interact along their length by "knobs into holes" packing of nonpolar residues to form a hydrophobic core. Polar and ionic side chains are directed toward the exterior of the two-stranded ropelike arrangement, where they can interact with solvent and/or other protein molecules. Figure TM2 visualizes how the two helices in a coiled-coil interact.

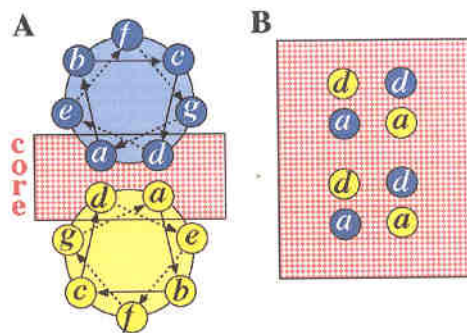


Fig. TM2. Interaction between the two α helices of TM coiled-coil. Each α helix is shown with seven residues (*a-g*) in two turns. **(A)** End of view looking from N-terminus. The interface between the alpha helices derives primarily from hydrophobic residues in core positions *a* and *d*. **(B)** The core interface viewed parallel to the coiled-coil axis shows how residues from one chain occupy the spaces between the corresponding residues from the second chain to give "knobs in holes" packing. (Reprinted with permission from Stewart, M., Structural basis for bending tropomyosin around actin in muscle thin filaments. Proc. Natl. Acad. Sci. USA, 98, 8165-8166, 2001. Copyright, 2001, National Academy of Sciences, USA).

X-ray diffraction studies of TM suffered from the 90% water content of the crystals yielding low resolution structures which identified only the general outline of the molecule, such as the coiled coil. Recently, the crystal structure of an 81 residue N-terminal fragment, comprising almost a third of TM, was determined at 2-Å- resolution (Brown et al., 2001). This refined analysis revealed the two-stranded alpha helical coiled structure with an axial sliding (about 1.2 Å) of the two chains at clusters of alanine residues. The joining of these regions with neighboring segments, where the helices were in axial register, gave rise to specific bends in the molecular axis. This asymmetric design allows the TM molecule to adopt multiple bent conformations (there are seven alanine clusters) which may be involved in the regulatory role of TM in muscle contraction.

Continuation of this work by the same principal investigators (Brown et al., 2005) yielded crystals of the middle three of TM's seven periods. The interaction of this small TM unit with F-actin helix was determined at 2.3-Å- resolution. Based on the distribution of apolar and charged residues on the surface of the interacting proteins a new model for the thin filament was constructed.

Figure TM3 presents a model for the interaction between TM and F-actin filament. The interaction is mainly electrostatic in nature. The axial alignment of TM on actin positions residues 169-172 of TM over residues 329-333 of actin, and it is supported by a very favorable electrostatic interaction (Part c in Fig. TM3). At the barbed end of subdomain 3 (and subdomain 1) of actin, a positively charged patch, consisting of Lys-326 and Lys-328 along with Arg-147 face a stripe of negatively charged residues (177, 181, and 184) of TM.

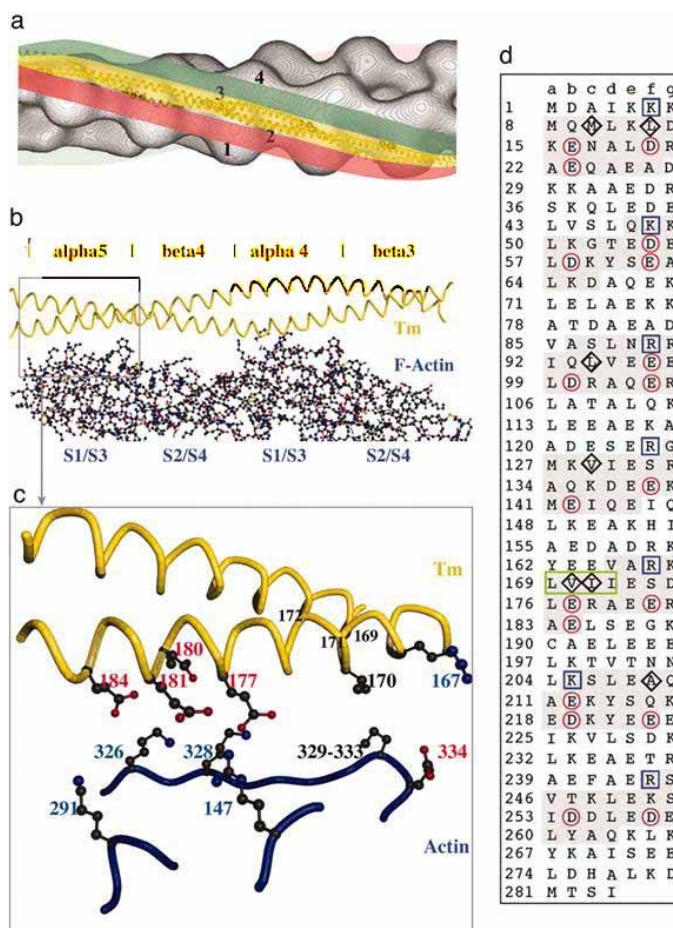


Fig. TM3. Model for the interaction between actin and TM. (a) The azimuthal positions of TM, in the off (red), Ca^{2+} -activated (yellow) and fully activated (green) states, on F-actin (gray). The four subdomains of actin are labeled. (b) Proposed axial model of the thin filament in the Ca^{2+} -activated state aligns the α zones of TM (yellow) over subdomain3 (S3) and subdomain1 (S1) of F-actin. (c) A close up view of TM α zone 5 and actin showing the chemical basis for the model. The axial and rotational alignments are based on positioning apolar (black numbering) as well as oppositely charged (red, acid; blue, base) clusters of residues across from one another. (d) Sequence of rat striated α -TM, emphasizing the outer surface (b, c, and f) residues from the α -zones (shaded) that are proposed to form close contacts with subdomain3 (and subdomain1) of actin. (Reprinted with permission from Brown, J.H., Zhou, Z., Reshetnikova, L., Robinson, H., Yammani, R.D., Tobacman, L.S., and Cohen, C., Structure of the mid region of tropomyosin: Bending and binding sites for actin. Proc. Natl. Acad. Sci. USA, 102, 18,878 -18,883, 2005. Copyright, 2005, National Academy of Sciences, USA).

Binding properties: Tropomyosin has high affinity to actin, as evidenced by the difficulty in removing TM in course of actin purification. However, in the presence of 0.6 M NaCl, pH 7.5, TM dissociates from F-actin indicating that electrostatic interactions play a major role in the binding of TM to actin. Each TM molecule binds 7 actin monomers in F-actin. When bound to actin, each TM is believed to be supercoiled with a radius of about 40 Å and the molecular length is reduced to about 385 Å.

Electron microscopic image reconstruction and X-ray diffraction studies suggest that during muscle activation TM moves from its lateral position on the actin filament by a distance of 10-15 Å toward the center of the groove in the actin double helix. It is postulated that in the resting muscle TM occupies the site of actin necessary for combination with the myosin head. The movement of TM, at the beginning of contraction, liberates the myosin-binding site, thus actomyosin can be formed and the muscle can contract. (This TM movement is discussed in the chapter of Regulation of Muscle Contraction).

The tropomyosin-binding component of troponin (TN-T) binds to TM; this will be described under Troponin. The encyclopaedic review of Perry (2001), about the distribution, properties, and function of vertebrate tropomyosin is a special learning experience.

Troponin

Ebashi discovered troponin (TN) in 1963. Subsequently, Greaser and Gergely (1971) showed that troponin consists of three components. These protein subunits differ in their molecular weights (based on early electrophoretic studies), and each possesses a specific function (Fig. TN1).

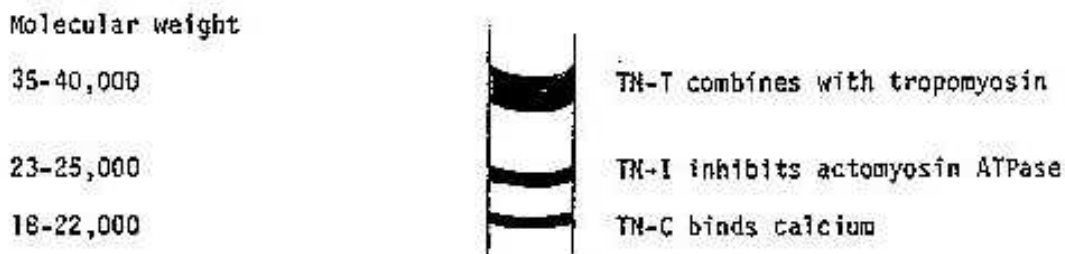


Fig. TN1. SDS-PAGE of troponin.

Figure TN1a visualizes the three TN subunits interacting with tropomyosin and actin in the thin filament.

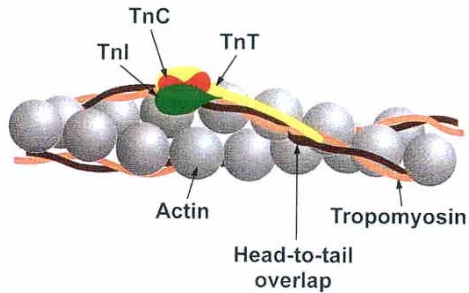


Fig. TN1a. A model of the molecular arrangement of TN subunits, TM, and actin in skeletal muscle thin filament (From Gordon et al., 2000). TN-I green, TN-C red, TN-T yellow. TM alpha subunit brown, beta subunit orange. Actin gray. Note the adjacent TM molecules overlap head to tail with the N-terminus of TN-T lying along the overlap region. The C-terminus of TN-T interacts with TN-C and TN-I, and TN-I also interacts with actin.

Figure TN1b shows the detailed interactions of the thin filament proteins pictured in Figure TN1a.

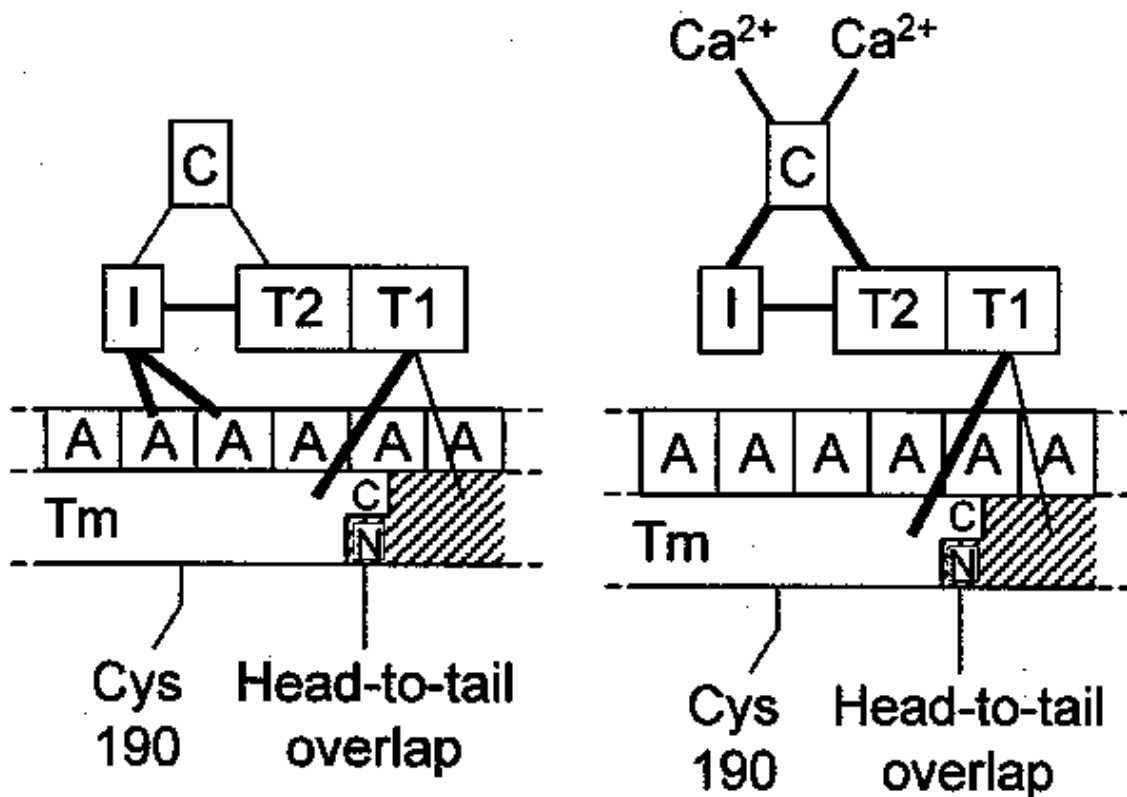


Fig. TN1b. Diagram indicating the effect of Ca^{2+} -binding to TN-C on the interactions of the proteins shown in Fig. TN1a (From Gordon et al., 2000). A, actin; I, TN-I; C, TN-C; T1, N-terminus of TN-T; T2, C-terminus of TN-T; Tm, tropomyosin with the N- and C-terminals indicated in the Head-to-tail overlap. Thicker lines imply stronger binding and weaker lines imply weaker binding. *Left* without bound Ca^{2+} , *right* with bound Ca^{2+} . Note, Ca^{2+} -binding to TN-C enhances the TN-C--TN-I and TN-C--TN-T2 interactions and weakens the TN-I--A interactions.

Troponin C is the Ca^{2+} receptor in the thin filament. It has been crystallized and its three-dimensional structure determined (Herzberg and James, 1985; Houdusse et al., 1997). Fig. TN2. illustrates the structure.

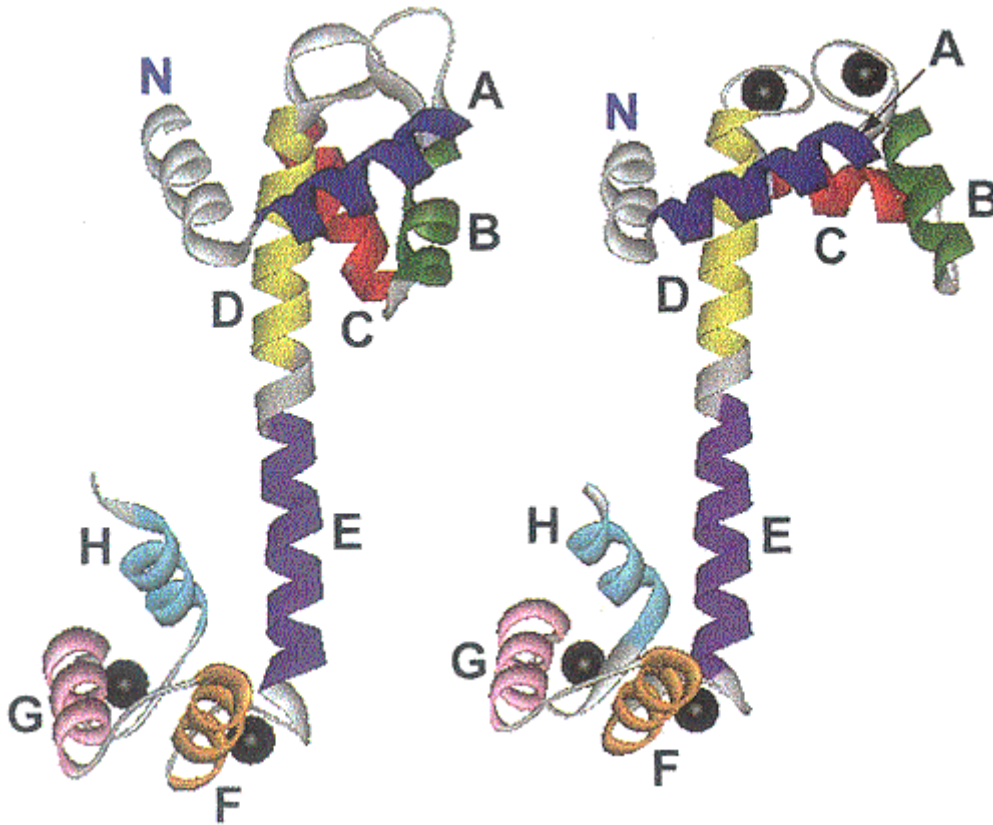


Fig. TN2. Ribbon diagram of skeletal muscle TN-C, with one molecule of Ca^{2+} bound (*left*) and two molecules of Ca^{2+} bound (*right*). (From Gordon et al., 2000). Solid circles represent Ca^{2+} , the letters A-H correspond to the helices, N is the amino terminal end and C is the carboxyl terminal end.

TN-C belongs to the Ca^{2+} -binding protein family. Its Ca^{2+} -binding domains comprise a short α helix, a Ca^{2+} -binding loop and a second short α helix. The domain folds around the Ca^{2+} ion in a manner of a clenched right hand, with the extended forefinger and thumb representing the helices and the middle finger forming the loop structure. This domain is termed an E-F hand. Two E-F hand domains form a stable pair in many Ca^{2+} -binding proteins. In TN-C, the two E-F hand domains are connected by a long α helix resulting in four Ca^{2+} -binding sites per molecule. Two of the four sites have high affinity to Ca^{2+} and are located in the carboxyl-terminal domain, whereas the other two sites have low affinity to Ca^{2+} and are located in the amino-terminal domain. Only the low affinity Ca^{2+} -binding sites of TN-C are involved in the regulation of muscle contraction.

TN-C forms specific complexes with both TN-I and TN-T. The crystal structure of TN-C complexed with the N-terminal fragment of TN-I has been determined (Vassilyev et al., 1998). In the complex TN-C has a compact globular shape, in contrast to the elongated

dumb-bell shaped molecule of uncomplexed TN-C. The TN-I fragment binds to the N-terminal domain of TN-C in its Ca^{2+} -bound conformation. Apparently, the complex formation between the two proteins requires a specific sterical configuration and, therefore, the complex has a functional significance.

Troponin I inhibits the Mg^{2+} -activated ATPase of actomyosin (identical with the actin and Mg^{2+} -activated myosin ATPase). TN-I is a basic protein that readily complexes with the acidic TN-C; importantly Ca^{2+} strengthens the complex formation. Thus, upon muscle stimulation TN-C binds Ca^{2+} and then complexes with TN-I; this provides a simple mechanism to relieve the inhibition of the actomyosin Mg^{2+} -ATPase by TN-I. Studies with deletion mutants suggest that the C-terminal residues of TN-I are involved in the Ca^{2+} -sensitive molecular switch during muscle contraction (Ramos, 1999; Szczesna et al., 1999) Several isoforms of TN-I are known in the molecular mass range of 21-23 kDa.

Troponin T is an asymmetric molecule that interacts with TM. Evidence obtained from cocrystals of TN-T with TM suggests that TN-T is an asymmetric molecule that contacts the C-terminal region of TM along much of its length (Perry, 2001). Skeletal TN-T extends 160 Å along the TM molecule whereas the cardiac isoform with a slightly longer polypeptide chain occupies 180 Å.

The TM -TN-T interaction serves to fix the position of the entire TN complex within the thin filament, so that subtle changes in the conformation of these proteins may regulate contraction. In this respect, it is important that TN-T also binds TN-C, thus the Ca^{2+} -induced conformational changes in TN-C are transmitted through TN-T to TM. A highly conserved protein domain was described in the amino acid sequence of TN-T (Stefancsik et al., 1998) that is characterized by a heptad repeat motif with a potential for α helical coiled coil formation. A similar, potentially coiled coil forming domain is also conserved in all known TN-I sequences, suggesting that these protein domains play a role in TN-T - TN-I interaction. This is another example of how specific interactions of the TN subunits build the entire TN molecule.

Troponin-T, similarly to TN-C and TN-I, has several isoforms in the molecular mass range of 30-35 kDa. Variations in TN isoforms may modulate muscle performance.

Recent X-ray Studies on Troponin Crystals

Vinogradova et al. (2005) described the 3.0-Å crystal structure of skeletal muscle troponin complex in the Ca^{2+} -activated state and a 7.0-Å crystal structure in the Ca^{2+} -free state.

The structure of TN reveals a non-compact molecule (Fig. TN3) that looks like two pairs of chopsticks holding a dumbbell. There is a two-fold symmetry of TN-I and TNT2 subunits (Part A of Fig. TN3). The long helices of TN-I and TNT2 interact with each other through the IT coiled coil. Part B of Fig. TN3 shows the central linker of TN-C, a long α helix that runs almost perpendicular to the IT coiled coil and defines the extended dumbbell shape of the TN-C subunit. Part C of Fig. TN3 shows the inhibitory segment of

TN-I, which is an ordered loop that interacts with TN-C. This loop is stabilized by electrostatic interactions with TN-C and hydrophobic interactions with TN-I.

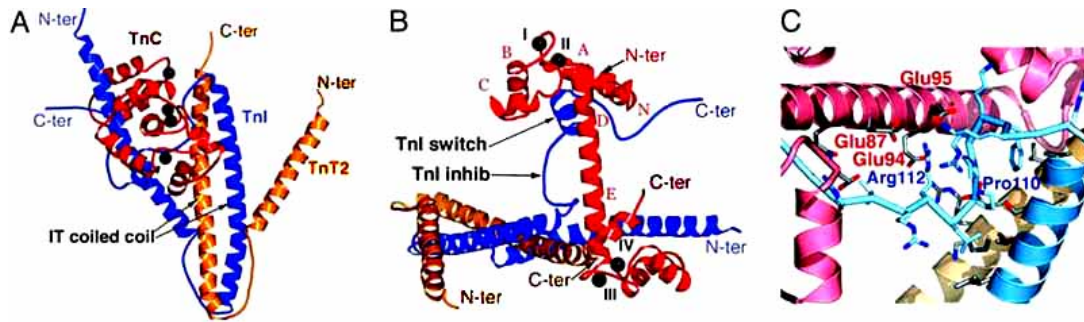


Fig. TN3. Structure of skeletal troponin complex presented in two orientations. The TN subunits are color coded: orange, TNT2; blue, TN-I; red, TN-C. Black spheres are Ca. (A) The nearly twofold symmetrical assembly of TN-I and TNT2 subunits. (B) The TN-C central helix is oriented perpendicular to the plane of TN-I and TNT2. (C) Close up view of the TN-I inhibitory segment. The residues determining the position of the inhibitory segment are shown as stick models. (Reprinted with permission from Vinogradova, M.V., Stone, D.B., Malanina, G.G., Karatzaferi, C., Cooke, R., Mendelson, R.A., and Fletterick, R.J., *Ca²⁺-regulated structural changes in troponin*, Proc. Natl. Acad. Sci. USA, 102, 5,038 -5,043, 2005. Copyright, 2005, National Academy of Sciences, USA).

The 7-Å resolution x-ray diffraction data allowed the visualization of the skeletal TN complex in the Ca²⁺-free state. The central linker of TN-C has lost its helical conformation and became disordered. This caused the Ca²⁺ regulatory N-terminal domain of TN-C to rotate 38° relative to its position in the Ca²⁺-activated state.

From the data a model for TN activation and relaxation was proposed (Fig. TN4). During muscle activation, Ca²⁺ binding would induce opening of the hydrophobic pocket in the N-lobe of TN-C and the TN-I switch segment would bind there. This would restore the helical conformation of the central linker of TN-C and remove the TN-I inhibitory segment from actin. These reactions would be reversed during muscle relaxation in Ca²⁺ free media: The central linker of TN-C would lose its helical conformation, consequently the TN-I inhibitory segment would be released and combine with actin.

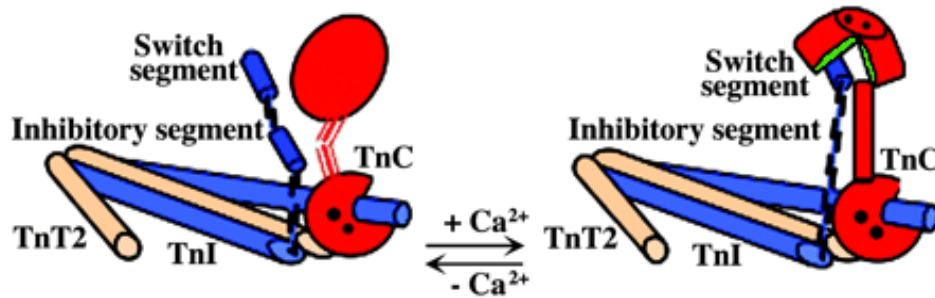


Fig. TN4. Cartoon representation of the conformational changes occurring in troponin during muscle contraction. For color coding see the legend to Fig. TN3. Ca ions are shown as black circles. The hydrophobic pocket opened in the N-terminal lobe of TN-C is colored in green. The switch segment and the inhibitory segment of TN-I are indicated. (Reprinted with permission from Vinogradova, M.V., Stone, D.B., Malanina, G.G., Karatzaferi, C., Cooke, R., Mendelson, R.A., and Fletterick, R.J., Ca²⁺-regulated structural changes in troponin. *Proc. Natl. Acad. Sci. USA*, 102, 5,038 -5,043, 2005. Copyright, 2005, National Academy of Sciences, USA).

The book of Perry (1996) is a good source for learning about the structure and properties of the troponin-tropomyosin system. Perry (1998 and 1999) also authored specific reviews on TN-T and TN-I. The review of Gordon et al., (2000) on the Regulation of Contraction in Striated Muscle includes a detailed discussion on the regulatory proteins. Recent advances on the structure-function relationship of TM and TN are outlined by Brown and Cohen (2005).

Suggested readings: Brown and Cohen., 2005; Vinogradova et al., 2004.

References

Bailey, K. (1946). Tropomyosin a new asymmetric protein component of muscle. *Nature*, **157**, 368.

Bown, 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.

Brown, J.H., Kim, K-H., Jun, G., Greenfield, N.J., Dominguez, R., Volkman, N., Hitchcock-DeGregori, S.E., and Cohen, C. (2001). Deciphering the design of the tropomyosin molecule. *Proc. Natl. Acad. Sci. USA*, **98**, 8496-8501.

Brown, J.H., Zhou, Z., Reshetnikova, L., Robinson, H., Yammani, R.D., Tobacman, L.S., and Cohen, C. (2005). Structure of the mid-region of tropomyosin: Bending and binding sites for actin. *Proc. Natl. Acad. Sci. USA*, **102**, 18878-18883.

Ebashi, S. (1963). Third component participating in the superprecipitation of actomyosin. *Nature*, **200**, 1010.

Garrett, R.H., and Grisham, C.M. (1995). *Molecular Aspects of Cell Biology*. Saunders College Publishing.

Gordon, A.M., Homsher, E., and Regnier, M. (2000). Regulation of contraction in striated muscle. *Physiological Reviews*, **80**, 853-924.

Greaser, M.L., and Gergely, J. (1971). Reconstitution of troponin activity from three protein components. *J. Biol. Chem.*, **246**, 4226-4233.

Herzberg, O., and James, M.N. (1985). Structure of the calcium regulatory protein troponin-C at 2.8 Å resolution. *Nature*, **313**, 653-659.

Houdusse, A., Love, M.L., Dominguez, R., Grabarek, Z., and Cohen, C. (1997). Structures of four Ca²⁺-bound troponin C at 2.0 Å resolution; further insights into the Ca²⁺-switch in the calmodulin superfamily. *Structure* **5**, 1695-1711.

Perry, S.V. (1996). *Molecular Mechanisms in Striated Muscle*. Cambridge University Press.

Perry, S.V. (1998). Troponin T: genetics, properties, and function. *Journal of Muscle Research and Cell Motility*, **19**, 575-602.

Perry, S.V. (1999). Troponin I: Inhibitor or facilitator. *Mol. Cell. Biochem.* **190**, 9-32.

Perry, S.V. (2001). Vertebrate tropomyosin: distribution, properties and function. *Journal of Muscle Research and Cell Motility*, **22**, 5-49.

Ramos, C.H.I. (1999). Mapping subdomains in the C-terminal region of troponin I involved in its binding to troponin C and to thin filament. *J. Biol. Chem.*, **274**, 18189-18195.

Smillie, L.B. (1996). Tropomyosin. *In Biochemistry of Smooth Muscle Contraction* (M. Bárány, ed.), pp. 63-90. Academic Press.

Stefancsik, R, Jha, P.K., and Sarkar, S. (1998). Identification and mutagenesis of a highly conserved domain in troponin T responsible for troponin I binding: Potential role for coiled coil interaction. *Proc. Natl. Acad. Sci. USA*, **95**, 957-962.

Stewart, M. (2001). Structural basis for bending tropomyosin around actin in muscle thin filaments. *Proc. Natl. Acad. Sci. USA*, **98**, 8165-8166.

Szczesna, D., Zhang, R., Zhan, J., Jones, M., and Potter, J.D. (1999). The role of the NH₂- and COOH domains of the inhibitory region of troponin I in the regulation of skeletal muscle contraction. *J. Biol. Chem.*, **274**, 29536-29542.

Vassylyev, D.G., Takeda, S., Vakatsuki, S. Maeda, K., and Maeda, Y. (1998). Crystal structure of troponin C in complex with troponin I fragments at 2.3-Å resolution. *Proc. Natl. Acad. Sci. USA*, **95**, 4847-4852.

Vinogradova, M.V., Stone, D.B., Malanina, G.G., Karatzaferi, C., Cooke, R., Mendelson, R.A., and Fletterick, R.J. (2004). Ca²⁺-regulated structural changes in troponin. *Proc. Natl. Acad. Sci. USA*, **102**, 5038-5043.