Structure of the Core Domain of Human Cardiac Troponin in the Ca^{2+}-Saturated Form

Muscle contraction is caused by interactions between two major contractile proteins, myosin and actin, which are polymerized to form the muscle thick filament and the thin filament, respectively. In the skeletal and cardiac muscles, contraction is controlled by intracellular Ca^{2+} concentration, and troponin, the sole Ca^{2+}-receptive protein in the myofilament, plays the key role in the regulation of the sliding action between these two filaments. Troponin (Tn), with a relative molecular mass of approximately 80 kDa, is composed of three polypeptide chains, TnT, TnI and TnC, and together with tropomyosin (Tm), is located in the polymerized-actin at a Tn:Tm: actin ratio of 1:1:7 (Fig. 1) [1]. The molecular basis of Ca^{2+} regulation was initially established by S. Ebashi and co-workers in the 1960's, although the mechanism by which troponin works has remained elusive because high resolution structural information have not been available. Since troponin is fairly flexible, the molecule evades packing in a crystalline lattice for a long time.

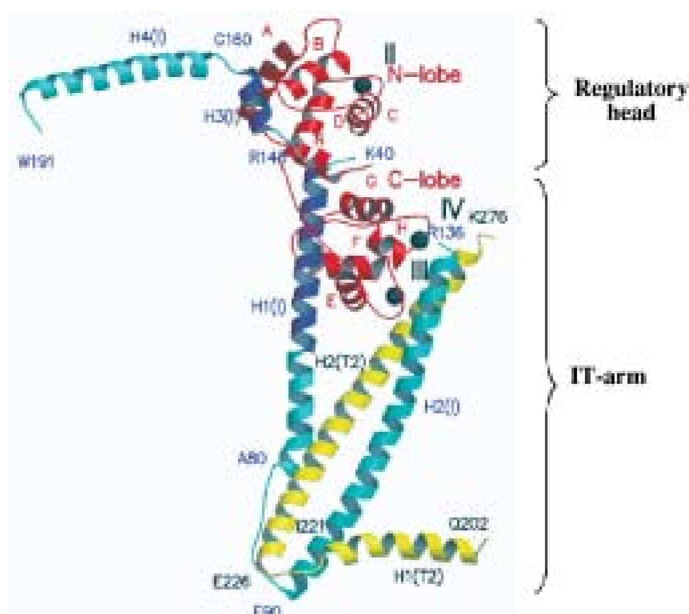

Fig. 2. Ribbon representation of the crystal structure of the core domain of human cardiac troponin. TnC and TnT are in red and yellow, respectively. TnI is in cyan, except for the two stretches of amphiphilic helices (TnC-binding sites) that are in blue. The three Ca^{2+} ions bound to the Ca^{2+}-binding sites (II to IV) are represented by black spheres [2].

We have determined the crystal structure of the core domain of troponin [2], which was reconstituted from *Escherichia coli*-expressed recombinant human cardiac Tn subunits, after dozens of crystallization trials for more than 10 years. Diffraction data were collected at beamlines **BL41XU**, **BL44B2** and **BL45PX** and the structure was determined by the multiwavelength anomalous dispersion (MAD) method. The overall architecture of the core domain of troponin is shown in Fig. 2. Notably, the core domain is further divided into structurally distinct subdomains, denoted as the regulatory head and the IT arm, that are connected by linkers, making the entire molecule highly flexible. The IT arm has an asymmetric and elongated structure (~ 80 Å) including a parallel α-helical coiled-coil formed between TnT (H2(T2)) and TnI (H2(I)). On the other hand, as suggested previously [3,4], the amphiphilic α-helix, H3(I), binds specifically to a conserved hydrophobic patch of the Ca^{2+}-saturated amino-terminal lobe of TnC, forming the regulatory head. The segment H3(I) is located between the two putative actin/Tm-binding sites, the inhibitory region (IR, residues 137 - 148) and the carboxyl terminus of TnI (C-TnI, residues 169 - 210), which are both essential for the inhibitory binding of TnI at a low sarcoplasmic Ca^{2+} concentration. Thus this segment works as a molecular switch that transmits the initial signal of Ca^{2+} binding to TnC and to the other components of the thin filament .

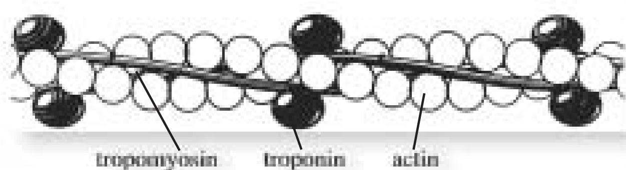

Fig. 1. Molecular arrangement of the proteins within the muscle thin filament.

Troponin is anchored to the thin filament mainly through Tm binding to two distinct portions of TnT, specifically the amino terminus (TnT1, residues 1 -1 82) and the carboxyl terminus (C-TnT, residues 272 - 288), irrespective of sarcoplasmic Ca^{2+} concentration [1]. On the basis of the present structures, the separation between TnT1 and C-TnT is estimated to be about 60 Å (Fig. 3), although the structures of

these components are currently unknown. During Ca^{2+} regulation, the carboxyl terminal portion of TnI (denoted as TnI_{reg}, residues 137 - 210) undergoes major changes both with regard to position and conformation. At high Ca^{2+} concentrations, TnI_{reg} associated with the N lobe of TnC detaches from actin/Tm, as observed from the present crystal structure. On the other hand, at low Ca^{2+} concentrations, TnI_{reg} must form an extra attachment to actin/Tm, so that the Tm strand is anchored to the actin filament. The IT arm may have an important role in the regulatory process. This subdomain is large, rigid and conserved between the species and bridges the two Tm-anchoring regions without directly interacting with actin/Tm. Moreover, the location is interesting. The IT arm resides immediately upstream of the Tm-anchoring site (C-TnT) and the mobile TnI_{reg}. An intriguing possibility is that the formation of a third attachment by TnI_{reg} could cause a minute rotation of the IT arm around the pivotal point; that is, the carboxyl terminal of the coiled-coil. The formation of the third attachment itself, as well as the rotation of the IT arm, may alter the properties of the Tm strand in the actin filament.

The nature of the changes remains to be elucidated. A plausible explanation is that the strain imposed on the Tm strand is altered depending on Ca^{2+} concentrations; thereby the mobility and/or the flexibility of the Tm strand may be changed. Changes in the properties of the tropomyosin strand may modify the accessibility of the myosin head to actin. In the present work we firstly propose a hypothesis that explains the mechanism by which troponin works on the basis of its atomic structure. The crystal structure additionally provides an opportunity to visualize sites of genetic disorders in the troponin molecule that are associated with cardiac dysfunctions [5].

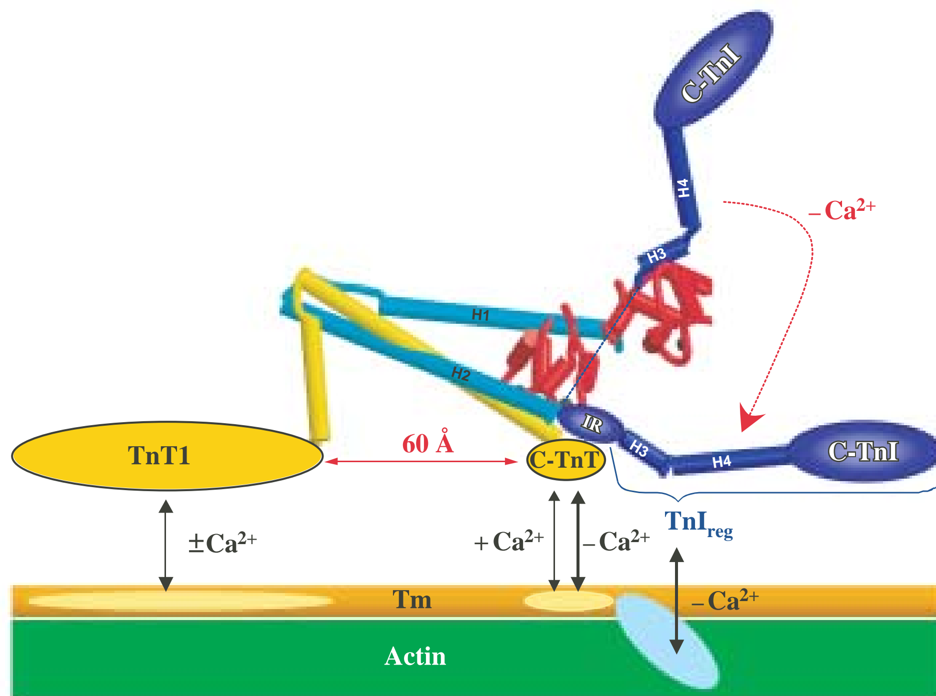

Fig. 3. Schematic representation of interactions between Tn and other thin filament components [2].

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