3.15: Troponin C

The contraction of striated muscle is triggered by Ca^{2+} ions. Muscle cells are highly specialized, and contain two types of filaments that may slide past each other in an energy-consuming process. One of the filaments, the thin filament, is built up by actin molecules ($M_r \approx 42$ kDa) polymerized end-to-end in a double helix. In the grooves of this helix runs a long rod-like molecule, tropomyosin; and located on this molecule at every seventh actin, is a complex of three proteins, *troponin*. The three proteins in the troponin complex are *troponin I* (TnI), *troponin T* (TnT), and *troponin C* (TnC). A schematic picture of the organization of the thin filament is shown in Figure 3.20.

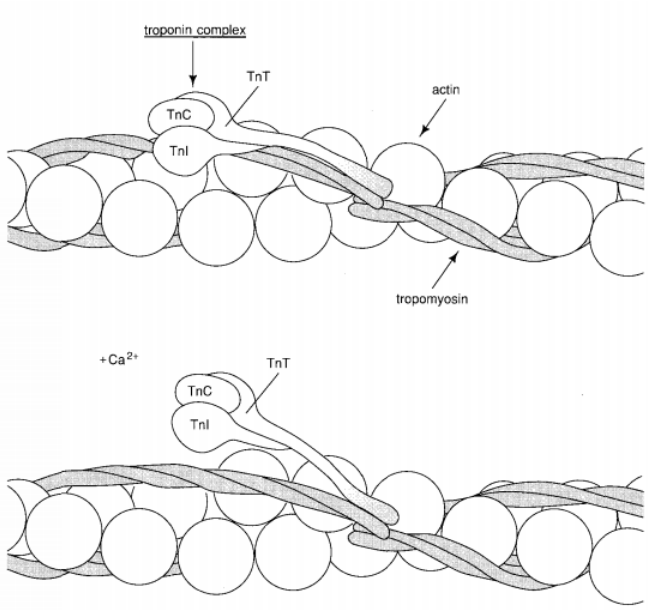

Figure 3.20 - Schematic diagram of the organization of skeletal muscle thin filament, showing the position of tropo-myosin and the troponin complex on the actin filament. The binding of Ca^{2+} to TnC, the calcium-binding subunit of the troponin complex, removes TnI, the inhibitory subunit, from actin and thus permits an interaction with a specialized protein, myosin, on neighboring thick muscle filaments (not shown). An ATP-driven conformation change in the myosin head group makes the thick and thin filaments move relative to one another, so that muscle contraction occurs.

Troponin C is the Ca^2 -binding subunit of troponin, and it is structurally highly homologous to calmodulin. Skeletal-muscle troponin C (sTnC; $M_r \approx 18$ kDa) can bind four Ca^{2+} ions, but cardiac-muscle troponin C (cTnC) has one of the four calcium sites modified, so that it binds only three Ca^{2+} ions. The x-ray structures of sTnC from turkey and chicken skeletal muscle have been determined to resolutions of 2.8 and 3.0 Å, respectively.[102,103] The structure of turkey sTnC is shown in Figure 3.21.

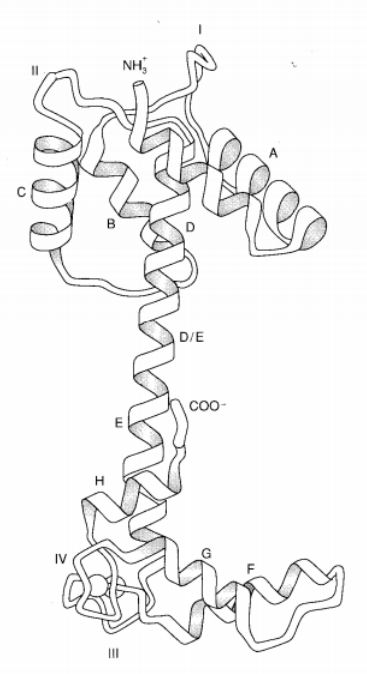

Figure 3.17).

The similarity between the structures of CaM (Figure 3.17) and sTnC is obvious. In sTnC we again find two domains, each with two potential Ca^{2+} sites, separated by a 9-turn α-helix. The crystals were grown in the presence of Ca^{2+} at a low pH (pH = 5), and only two Ca^{2+} ions are found in the C-terminal domain. The two Ca^{2+}-binding sites in this domain have the same helix-loop-helix motif that is found in CaM, and they both conform to the archetypal EF-hand structure. The interhelix angles between helices E and F and between G and H are close to 110°. By contrast, the helices in the N-terminal domain, where no Ca^{2+} ions are bound, are closer to being antiparallel, with interhelix angles of 133° (helices A and B) and 151° (helices C and D).

Both sTnC and cTnC have two high-affinity Ca^{2+}-binding sites (see Table 3.2) that also bind Mg^{2+} ions competitively, although with a much lower affinity. These two sites are usually called "*the Ca^{2+}-Mg^{2+} sites*." *76,104* In sTnC there are also two (in cTnC, only one) Ca^{2+}-binding sites of lower affinity ($K_B^{Ca2+} \approx 105\ M^{-1}$) that bind Mg^{2+} weakly and therefore have been called "*the Ca^{2+}-specific sites*." Since Ca^{2+} binding to the latter sites is assumed to be the crucial step in the contractile event, they are often referred to as "*the regulatory sites*" (see below). The existence of additional weak Mg^{2+} sites ($K_B \approx 300\ M^{-1}$) on sTnC, not in direct competition with Ca^{2+}, has also been inferred.[76,104,105] Spectroscopic studies have shown that the two strong Ca^{2+}- Mg^{2+} sites are located in the C-terminal domain, and the weaker Ca^{2+}-specific sites in the N-terminal domain of sTnC.[106] This pattern is similar to that observed with CaM. NMR spectroscopic studies strongly suggest that binding of Ca^{2+} to both sTnC and cTnC is cooperative.[107] In sTnC the C-terminal domain binds Mg^{2+} much more strongly than the N-terminal domain, by contrast to CaM, where the reverse is true.

The rates of dissociation of Ca^{2+} and Mg^{2+} from sTnC have been measured by both stopped-flow and ^{43}Ca NMR techniques.[76,108] As with CaM, the actual numbers depend on the solution conditions, ionic strength, presence of Mg^{2+}, etc. (see Table 3.4). On the rate of Mg^{2+} dissociation from the Ca^{2+}- Mg^{2+} sites, quite different results have been obtained by stopped-flow studies[76] of fluorescence-labeled sTnC ($k_{off}^{Mg2+} \approx 8\ s^{-1}$) and by ^{25}Mg NMR ($k_{off}^{Mg2+} \simeq 800\text{-}1000\ s^{-1}$).[109] This apparent discrepancy seems to have been resolved by the observation that both binding and release of Mg^{2+} ions to the Ca^{2+}-Mg^{2+} sites occur *stepwise*, with $k_{off}^{Mg2+} < 20\ s^{-1}$ for one of the ions, and $k_{off}^{Mg2+} \geq 800\ s^{-1}$ for the other.[110] The rates of dissociation of the Mg^{2+} ions are important, since under physiological conditions the Ca^{2+}-Mg^{2+} sites of sTnC are likely to be predominantly occupied by Mg^{2+} ions, release of which determines the rate at which Ca^{2+} can enter into these sites.

Spectroscopic and biochemical data[111] indicate that upon binding Ca^{2+}, sTnC and cTnC undergo significant conformation changes. Comparisons of NMR spectroscopic changes on Ca^{2+} binding to intact sTnC, as well as to the two fragments produced by tryptic cleavage (essentially the N-terminal and C-terminal halves of the molecule, just as was the case with CaM), have shown that the conformation changes induced are mainly localized within the domain that is binding added ions.[110,112] Thus the central α-helix connecting the domains seems unable to propagate structural changes from one domain to the other. It has been suggested that the structural differences found in the x-ray structure of turkey sTnC between the C-terminal domain, which in the crystal contains two

bound Ca^{2+} ions, and the N-terminal domain, in which no Ca^{2+} ions were found, may represent these conformational changes.[113] This rather substantial conformational change is schematically depicted in Figure 3.22.

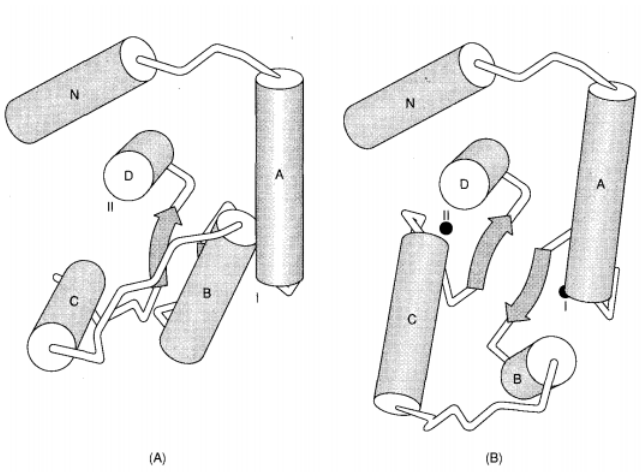

Figure kindly provided by N. C. J. Strynadka and M. N. G. James.

However, preliminary structure calculations[114] of the calcium-saturated and calcium-free forms of calbindin D_{9k} indicate that much more subtle conformational changes take place upon binding Ca^{2+} in calbindin D_{9k}. Interestingly, ^1H NMR spectroscopy has provided evidence for the concept that the structural change induced by Mg^{2+} binding to the C-terminal domain of sTnC must be very similar to that induced by Ca^{2+} ions. Another result obtained by ^{113}Cd NMR studies[108] is that the cadmium-loaded N-terminal domain of sTnC in solution undergoes a rapid interchange between two or more conformations, with an exchange rate on the order of 10^3-10^4 s^{-1}.

Just as CaM exerts its biological function in complexes with other proteins, TnC participates in the three-protein troponin complex. It presently appears that TnC and TnI form a primary complex that is anchored by TnT to a binding site on tropomyosin.[115] In the troponin complex the Ca^{2+} affinity is increased by a factor of about ten over that in isolated sTnC, both at the Ca^{2+}-Mg^{2+} sites and at the Ca^{2+}-specific sites. A similar increase in affinity is found for Mg^{2+}. Given the amounts of "free" Mg^{2+} inside muscle cells (1 to 3 mM), it seems likely that the Ca^{2+}-Mg^{2+} sites in the resting state of troponin are filled with Mg^{2+}, so that a transitory release of Ca^{2+} leads primarily to rapid Ca^{2+} binding to the Ca^{2+}-specific sites, and subsequently to conformation change and contraction.
