Cardiac Excitation–Contraction Coupling: Calcium and the Contractile Element

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Glossary

Ca^{2+} activation When a muscle contraction is stimulated by the binding of Ca^{2+} to the cardiac troponin (cTn) complex.

Ca^{2+} affinity The measure of the ability of a protein to bind Ca^{2+}.

Ca^{2+} sensitivity The measure of the ability of a myocyte to contract in response to Ca^{2+}.

Cardiac troponin complex A group of three proteins, cardiac troponin I (cTnI), cardiac troponin C (cTnC), and cardiac troponin T (cTnT), that is attached to the actin thin filament that triggers the formation of cross-bridges during contraction.

Contractile element The components of the sarcomere responsible for the contractile reaction.

Contractile reaction The series of biochemical and biophysical reactions that result in the formation of cross-bridges and the generation of force by the cardiomyocyte.

Cross-bridge A physical connection that forms between the myosin thick filament and the actin thin filament. The formation of cross-bridges causes the sarcomere to shorten during a contraction.

Ectotherm (adj ectothermic) An organism whose body temperature is largely determined by heat exchange with its surroundings. It does not produce and retain enough metabolic heat to elevate its body temperature above ambient temperature, but may use behavioural mechanisms to regulate body temperature.

Endotherm An animal that maintains a set body temperature, regardless of a change in external temperature.

Muscle contraction When a muscle cell shortens and generates force.

Protein conformation The three-dimensional shape of a protein in space.

Protein sequence The specific sequence of amino acids that compose a protein.

Sarcomeres The functional elements of a muscle cell responsible for shortening during a contraction.

Thick filament The structural element of a sarcomere that is composed of the protein myosin.

Thin filament The structural element of a sarcomere that is composed of the protein actin.

α-stat The change in blood and cellular pH that occurs in ectotherms when their body temperature changes.

Cardiomyocyte Contraction: An Overview

The contraction of all vertebrate hearts is powered by the generation of cross-bridges between two very abundant proteins termed thick and thin filaments, which are found in all muscle cells. The thick filaments are composed of the protein myosin and the thin filaments are primarily composed of the protein actin. Globular actin monomers are aligned in a chain, like beads on a string, to create the thin filament (**Figure 1**). The myosin molecule has a head and a tail. The tails are aligned side by side but staggered so that the myosin heads protrude out from the assemblage called the thick filament. Within the cardiomyocytes, the many such myosin thick filaments and actin thin filaments are arranged in a parallel overlapping pattern within structures called 'sarcomeres' (**Figure 1**). During a contraction, the myosin heads rotate out from the thick filament and form temporary connections with the actin thin filament. These physical connections are called cross-bridges. Once attached, the myosin head flexes causing the thin filament (actin) to slide past the thick filament (**Video Clip 1**). This causes
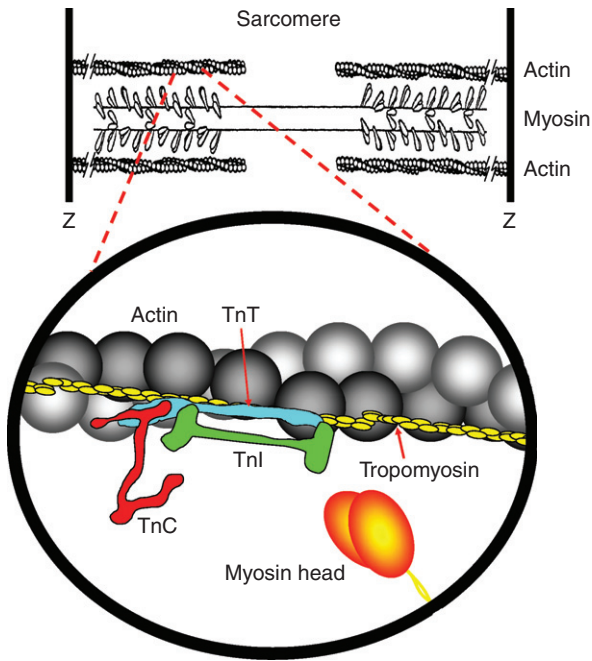
Figure 1 Schematic diagram of the cardiac sarcomere (a) and of the regulatory complex (b) associated with the actin filament responsible for making the contractile reaction activated by Ca^{2+}. Adapted part of figure 1 from Gordon AM, Regnier M, and Homsher E (2001) Skeletal and cardiac muscle contractile activation: Tropomyosin "rocks and rolls". *News in Physiological Science* 16, 49–55, used with permission from the Amerian Physiological Society.

the length of the sarcomere to shorten by about 10%, which is termed a 'contraction'. During relaxation, the cross-bridges release and the myosin and actin filaments return to their original positions. This mechanism of muscle contraction is described as the 'sliding-filament model'. Thus, each time the heart contracts, every cardiomyocyte undergoes this contractile reaction almost in unison for each cardiac chamber. Therefore, for a heart that is beating 20 times per minute, it repeats every 3 s. The number of cross bridges that are formed is proportional to the amount of force that is developed and the rate of cross-bridge cycling is related to the rate at which force is developed. The number of cross-bridges that can be formed is directly related to the amount of calcium released into the cytoplasm after the cell is excited by the action potential. In this manner, calcium is not only critical in determining the force of contraction, but it also initiates the contraction–relaxation cycle of the cardiomyocyte.

The Activation of a Cardiomyocyte Contraction by Ca^{2+}

When a muscle is relaxed, actin and myosin are not interacting and force cannot be generated. This is because the sites where myosin binds to actin are

blocked by a rod-shaped protein called 'tropomyosin' (TM). This protein lies along the actin filament (Figure 1) and is locked in place by a group of proteins called 'the cardiac troponin (cTn) complex'. The cTn complex is made up of three different proteins: cardiac troponin I (cTnI) (I for inhibitory), which binds directly to actin; cardiac troponin T (cTnT) (T for tropomyosin), which binds the cTn complex to TM; and cardiac troponin C (cTnC) (C for Ca^{2+}), which binds Ca^{2+} to trigger cardiomyocyte contraction. Thus, the cTn complex is bound directly to the thin filament but acts as a latch mechanism to initiate the binding of actin and myosin.

Muscle contraction is initiated when intracellular Ca^{2+} rises as a result of the action potential and binds to cTnC. The cTnC protein then changes its shape (a conformational change), which exposes a number of hydrophobic, charged amino acids within the core of cTnC. These amino acids then attract and pull cTnI away from the actin filament (the latch mechanism), allowing TM to move freely across the surface of actin, which uncovers the myosin-binding sites on the actin molecules. Myosin heads can now attach to actin, which is termed a cross-bridge (Video Clip 1).

The cross-bridges release when the intracellular Ca^{2+} decreases (pumped back into the sarcoplasmic reticulum (SR) or out of the cell), causing Ca^{2+} to be released from cTnC. As a result, cTnC returns to its original conformational shape and releases cTnI, which reattaches to the actin filament. Thus, the latch mechanism that locks TM in a blocking position over the myosin binding sites is reestablished. As no new cross-bridges can form, the myocyte relaxes.

The Influence of Temperature on Ca^{2+} Binding by the Contractile Element

As is the case with most physiological systems, a change in temperature alters the ability of the heart to contract. Reducing temperature a few degrees causes the human heart to beat slower and with weaker contractions. This compromises the heart's ability to circulate blood. One reason for the weaker contraction is that the heart has become less sensitive to Ca^{2+} as temperature decreases. As a result, more Ca^{2+} is required to generate the same strength of contraction. However, the maximum amount of Ca^{2+} that can be released to trigger a myocyte contraction is limited. Even so, animals that routinely function at a lower temperature than humans cannot survive such a loss of Ca^{2+} sensitivity and cardiac function. Something must be different in the fish heart because the human heart would literally stop beating at 5 °C, whereas rainbow trout (*Oncorhynchus mykiss*) heart functions fine at 5 °C. Hearts in polar fishes operate at even colder

temperatures. So, the fish heart must compensate for the effect of low temperature on Ca^{2+} sensitivity.

One way the rainbow trout heart does this is by being more sensitive to Ca^{2+} than the mammalian heart. When compared at the same temperature, the trout heart is approximately 10 times more sensitive to Ca^{2+} than the mammalian heart. This means that it takes 1/10th the amount of Ca^{2+} to generate a contraction in the rainbow trout heart when compared with a mammalian heart at the same temperature. The ability for cardiac tissue from rainbow trout, frog (*Rana pipiens*), and rat (*Rattus norvegicus*) to generate force in response to Ca^{2+} over a range of temperatures is shown in **Figure 2**. The Ca^{2+} concentration (μM) required to generate half-maximal force ($K_{F1/2}Ca$) is plotted on a logarithmic scale. This figure clearly shows, for all three species, the inverse relationship between temperature and the Ca^{2+} concentration required to generate half-maximum force. The difference among species is evident when a comparison is made at the same temperature. For example, at $7\,^{\circ}C$, it takes less Ca^{2+} to generate half-maximal force in the rainbow trout heart (2.8 μM) compared with either the

frog (5.6 μM) or the rat (28.2 μM) – a 10-times difference between rainbow trout and rat.

An equally important observation is possible, if the line for the rat is extrapolated to $37\,^{\circ}C$. The $K_{F1/2}Ca$ for rat is similar to that of the rainbow trout at $7\,^{\circ}C$. Thus, when compared at their respective physiological temperatures, the Ca^{2+} sensitivities of the rat and rainbow trout hearts are approximately the same. This suggests that the comparatively high Ca^{2+} sensitivity of the rainbow trout heart acts to compensate for the desensitizing effect of low temperature on Ca^{2+} sensitivity.

Adaptation of the Trout Contractile Element to Low Temperature

What is responsible for the comparatively high Ca^{2+} sensitivity of the rainbow trout heart? To begin answering this question, a series of studies were undertaken to compare the structure and function of rainbow trout and human cTnC. The logical place to start with is cTnC because it is a Ca^{2+}-binding protein. The comparison of rainbow trout and human cTnC structure and function is revealing in this regard.

Of the 161 amino acids that comprise cTnC, rainbow trout cTnC has 13 unique residues compared with the amino acid sequence of human cTnC (**Figure 3**). Since the amino acid sequence determines protein function, such differences in amino acid sequence could be important to Ca^{2+}-binding ability. Thus, the ability of these two proteins to bind Ca^{2+} was compared by monitoring the protein as it bound Ca^{2+} at $37\,^{\circ}C$, $21\,^{\circ}C$, and $7\,^{\circ}C$. At all temperatures, Ca^{2+} affinity of rainbow trout cTnC is ~2.3 times that of human cTnC (**Figure 4**). In addition, the Ca^{2+} affinity of both proteins decreased as temperature decreased. Therefore, rainbow trout cTnC activates the contractile reaction at a lower Ca^{2+} concentration and the Ca^{2+} affinity of cTnC is directly related to temperature. Thus, the effect of low temperature on the Ca^{2+} affinity of cTnC is at least partially responsible for desensitizing the heart to Ca^{2+}.

Mutant human cTnC proteins have been used to identify the specific amino acids responsible for the comparatively high Ca^{2+} affinity of trout cTnC. These proteins contained different combinations of the amino acids found in rainbow trout cTnC. Four amino acid residues in rainbow trout cTnC were responsible for its comparatively high Ca^{2+} affinity: asparagine (N), isoleucine (I), glutamine (Q), and aspartate (D) at positions 2, 28, 29, and 30, respectively, in cTnC (NIQD HcTnC). Furthermore, when NIQD HcTnC was incorporated into rabbit cardiomyocytes, the Ca^{2+} sensitivity of force generation by these cardiomyocytes was increased by around twofold (**Figure 5**). This confirms that a cTnC molecule with a higher Ca^{2+} affinity does increase the Ca^{2+} sensitivity of cardiac
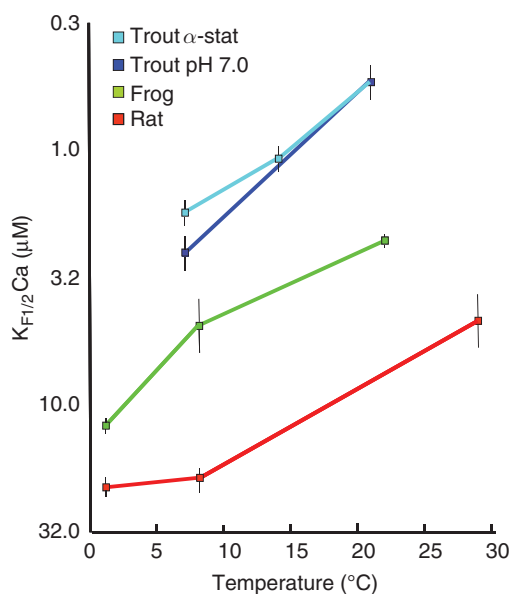

Figure 2 Comparison of the Ca^{2+} sensitivity of force generation by ventricular fibers isolated from hearts of rainbow trout, frog, and over a range of temperatures. Ca^{2+} sensitivity was measured as $K_{F1/2}Ca$, which is the Ca^{2+} concentration required to generate half-maximum force. When compared at the same temperature, the trout preparations required 10-fold less Ca^{2+} to generate the same measure of force than those from the mammalian species. Adapted from Churcott CS, Moyes CD, Bressler BH, Baldwin KM, and Tibbits GF (1994) Temperature and pH effects on Ca^{2+} sensitivity of cardiac myofibrils: A comparison of trout with mammals. *American Journal of Physiology* 267: R62–R70, used with permission from the American Physiological Society.

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Salmonid    mndiykaaveqltdeqknefkaafdifiqdaedgcistkelgk
Mammalian   ,d,,,,,,,,,,e,,,,,,,,,,,,,vlg,,,,,,,,,,,,,

            vmrmlgqnptpeelqemidevdedgsgtvdfdeflvmmvrcmkddskgk
            ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,

            teeeladlfcmfdknadgyidlqelkvmleatgeaiteddieel
            s,,,,s,,,r,,,,,,,,,,,,,e,,,i,,q,,,,t,,,,,,,,,

            mkdgdknndgkidydeflefmkgve
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Figure 3 Alignment of rainbow trout cardiac troponin C and human cardiac troponin C amino acid sequences. The differences in amino acid sequence between the two proteins are shown in bold print within the human sequence.
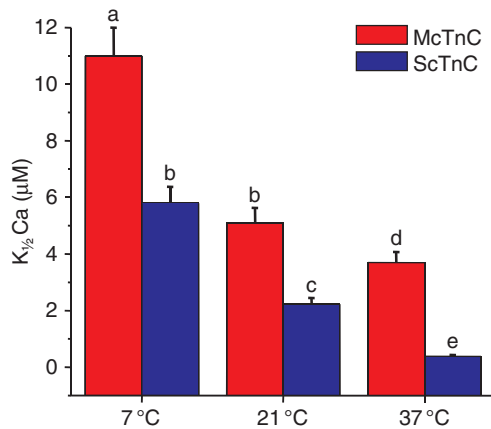

Figure 4 Comparison of the Ca^{2+} affinity of human cardiac troponin C and rainbow trout cardiac troponin C at different temperatures. Ca^{2+} affinity is shown as the $K_{1/2}Ca$, which is the Ca^{2+} concentration (in μM) required to half-saturate the molecule. Ca^{2+} affinity was measured at 37 °C, 21 °C, and 7 °C. All values are shown as mean ± SE. The Ca^{2+} affinity of rainbow trout cTnC was greater than that of human cTnC at each temperature. Adapted from Gillis TE and Tibbits GF (2002) Beating the cold: The functional evolution of troponin C in teleost fish. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 132: 763–772, with permission from Elsevier.

Figure 5 Ca^{2+} titration of force generation in rabbit cardiac cardiomyocytes containing either native rabbit cTnC ($n = 19$); human cardiac troponin C ($n = 12$); or NIQD human cardiac troponin C ($n = 7$), at 15 °C, pH 7.0. Data are normalized with respect to the maximal force generated during each $Ca^{2+←}$ titration and presented as mean ± SE. The Ca^{2+} concentration required to generate half-maximum force ($K_{F1/2}Ca$) are shown as mean ± SE. Means indicated with the same superscript are not significantly different from each other ($p > 0.05$). Adapted from Gillis TE, Marshall CR, and Tibbits GF (2007) Functional and evolutionary relationships of troponin C. *Physiological Genomics* 32: 16–27, used with permission from the American Physiological Socity.

tissue. Together, these results demonstrated that trout cTnC is partially responsible for the high Ca^{2+} sensitivity of the rainbow trout heart. However, further work is needed to explain why the rainbow trout heart is ∼10-fold more sensitive to Ca^{2+} than the mammalian heart and this may involve other proteins such as cTnI and cTnT.

Phylogenetic Analysis of cTnC from a Variety of Vertebrate Species

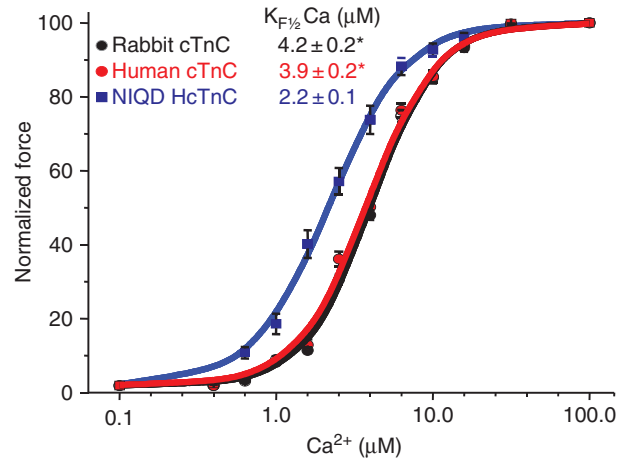Clearly, the sequence and function of cTnC have changed during the evolution of the vertebrate heart. By compiling all known amino acid sequences of cTnC, a phylogenetic tree can be created, based on how similar the proteins are to each other. This type of analysis aligns the protein sequences to identify amino acid substitutions. The more similar two proteins are, the closer they are on a phylogenetic tree. This type of analysis also helps to identify specific amino acids that are critical to the function of a protein – for example, if a specific amino acid is always at the same position in the protein from a variety of unrelated animals. In addition, if there are specific amino acids that are unique to animals with similar physiological conditions – for example, body temperature – this helps to identify which amino acids have evolved for that specific condition.

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                 Trout      M-NDIYKAAVEQLTDEQKNEFKAAFDIFIQDAEDGCISTKELGKV...
                 Puffer     ,-N,,,,,,,,,,,E,,,,,,,,,,,,,IQD,,,,,,,,,,,,...
                 Zebrafish  ,-N,,,,,,,,,,,,,,R,,,,,,VQD,,,,,,,,,,,,...
                 Mummichog  ,-N,,,,,,,,,,,,,,,,,,,,,,VQD,,,,,,,,,,,,...
                 Icefish    X-XXXXX,,,,,,,,D,,,,,,,,,,,VQD,,,,,,,,,,,,...
Ectothermic      Sucker     X-XXXXX,,,,,,,,,,R,,,,,,VQD,,,,,,,,,,,,...
                 Goldfish   X-XXXXX,,A,,,,,,,,,,R,,,,,VQD,,,,,,,,,,,,...
                 Tuna       X-XXXXX,,,,,,,,,,,,,,,,,,VQD,,,,,,,,,,,,...
                 Bichir     X-XXXXX,,,,N,,E,,,,,,,,,,VQD,,,,,,,,,,,,...
                 Perch      X-XXXXX,,,,,,,E,,,,,,,,,,VQD,,,,,,,,,,,,...
                 Lamprey 1  ,AE,VDR,,,,,,,E,,,K,,R,,,,VQD,,,,,,,,,,,,...
                 Lamprey 2  ,AE,VDR,,,,,,,E,,,K,,R,,,,VQD,,,,,,,,,,,,...
                 Clawed frog ,-D,,,,,,,,,,,E,,,,,,R,,,,,VQD,,,,,,,,,,,,...

                 Human      ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Cow        ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Pig        ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Rabbit     ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Monkey     ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
Endothermic      Mouse      ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Rat        ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Squirrel   ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Chicken    ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Quail 1    ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
                 Quail 2    ,-D,,,,,,,,,,,E,,,,,,,,,,,VLG,,,,,,,,,,,...
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Figure 6 Comparison of the N-terminus of all known cardiac troponin C homologs. Residues identified as being responsible for the comparatively high Ca^{2+} affinity of trout cTnC are indicated in mauve. Sequences are organized to indicate if they were cloned from ectothermic or endothermic species. The X in place of an amino acid indicates that the residue is not known. Adapted from Gillis TE, Marshall CR, Tibbits GF (2007) Functional and evolutionary relationships of troponin C. *Physiological Genomics* 32: 16–27, used with permission from the American Physiolocical Society.

Interestingly, all ectothermic species (this includes teleost fish, lamprey, and frogs) contain at least two of the four amino acids identified in rainbow trout as being responsible for high Ca^{2+} affinity of cTnC (**Figure 6**). In contrast, cTnC from endothermic species (birds and mammals) do not have these four amino acids at these same positions. As lampreys are the earliest known vertebrates, and fish and amphibians evolved earlier than either mammals or birds, it is suggested that these identified residues (N, I, Q, and D at positions 2, 28, 29, and 30) were present in the earliest forms of cTnC. These residues were then lost through natural selection as the body temperature of the ancestor species of mammals and birds started to increase and become constant.

This suggests that the lower Ca^{2+} affinity of mammalian cTnC is an adaptation to high temperature (37 °C) and high heart rate. Consider a human heart at 37 °C; if it contained a cTnC with the same Ca^{2+} affinity as rainbow trout cTnC, it would likely remain in contracture or beat very slowly as Ca^{2+} would be released at a very slow rate. Remember that rainbow trout cTnC has twice the Ca^{2+} affinity as human cTnC and that as temperature increases the Ca^{2+} affinity increases (**Figure 4**). The heart would remain in contracture as the Ca^{2+} affinity of the protein would be so high that Ca^{2+} would remain bound to the protein. This would result in the continuous formation of cross-bridges between actin and myosin. If a heart is in contracture, the animal dies. It

is no surprise, therefore, that the four residues identified as being responsible for the high Ca^{2+} affinity of rainbow trout cTnC are not found in human cTnC.

Cardiac Contractility in Changing Environmental Temperatures

Fish, unlike mammals, expose their heart to a highly variable temperature. Rapid temperature changes can be >10 °C. In addition to Ca^{2+} sensitivity, temperature changes blood and tissue pH. This change in pH is automatic and acts to keep the relative alkalinity ($[OH_2]/[H]$) approximately constant. This relationship, called α-stat regulation, is a change of −0.016 to −0.019 pH units °C^{-1}. Therefore, a 10 °C decrease in temperature would cause a ∼0.2-unit increase in cellular pH.

This pH change is relevant to cardiac function because an increase in pH increases the Ca^{2+} sensitivity of the heart. **Figure 1** compares the Ca^{2+} sensitivity of rainbow trout cardiac tissue over a range of temperatures when pH is kept constant at 7.0 versus when pH is allowed to change according to α-stat regulation. At 7 °C, the pH difference between the two treatments is 0.2 pH units. The $K_{F1/2}Ca$ of the rainbow trout cardiac tissue at pH 7.2 is 1.6 μM versus 2.8 μM at pH 7.0, which corresponds to a ∼1.6
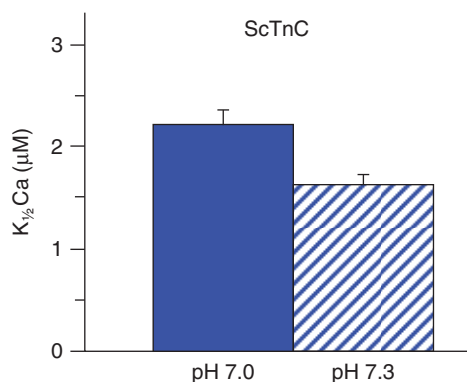
Figure 7 Effect of a 0.3 pH unit increase on the Ca^{2+} affinity of trout cTnC. Ca^{2+} affinity is shown as the $K_{1/2}Ca$, which is the Ca^{2+} concentration (in μM) required to half-saturate the molecule. Measurements were made at 21 °C. The Ca^{2+} affinity of trout cTnC was greater at pH 7.3 than at pH 7.0. Adapted from Gillis TE, Marshall CR, Xue XH, Borgford TJ, and Tibbits GF (2000) Ca^{2+} binding to cardiac troponin C: Effects of temperature and pH on mammalian and salmonid isoforms. *American Journal of Physiology* 279: R1707–R1715.

difference. Therefore, as pH increases with decreasing temperature, the Ca^{2+} sensitivity of the contractile element increases as a result of the pH change, allowing the heart to maintain contractile ability despite the decrease in temperature.

The effect of pH on the Ca^{2+} sensitivity of cardiac tissue is due, at least in part, to a pH effect on the Ca^{2+} affinity of cTnC. A 0.3-pH increase significantly increases the Ca^{2+} affinity of rainbow trout cTnC by ~1.4-fold (**Figure 7**). This increase in Ca^{2+} affinity would make it possible for cTnC to be activated by Ca^{2+} and help the heart to continue beating if a fish swims from warm surface water into colder waters after prey.

Future Directions

There is much to be learned about the molecular mechanisms that regulate cardiac contraction in fish and how these have been adapted to enable cardiac function in the variety of environments in which different fish are found. One current area of interest is the signaling pathways that are used to rapidly change the rate and strength of cardiac contraction. This includes the α- and β-adrenergic pathways. These pathways turned on by the stress response (also called the fight-

or-flight response) activate functional proteins called 'kinases' that phosphorylate a number of the cardiac proteins, including cTnI and cTnT. Phosphorylation takes place when a phosphate group is attached to a specific amino acid, usually a serine or tyrosine, thereby adding an additional positive charge to the protein. This acts to change how the amino acids around the phosphate group interact with each other and, as a result, affect the function of the protein. The phosphorylation of cTnI and cTnT in the mammalian heart acts to increase the rate of cardiac contraction. Little is known of how phosphorylation of fish contractile proteins regulates the function of the fish heart.

See also: **Design and Physiology of the Heart:** Cardiac Excitation-Contraction Coupling: Routes of Cellular Calcium Flux; Physiology of Cardiac Pumping.
Integrated Response of the Circulatory System: Integrated Responses of the Circulatory System to Temperature.

Further Reading

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