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Review

Beating the cold: the functional evolution of troponin C in teleost fish☆

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Abstract

The sensitivity of the cardiac myocyte contractile element for Ca^{2+} decreases with temperature. As myocyte contractility is regulated by changes in cytosolic [Ca^{2+}], this desensitizing effect represents a challenge for temperate fish such as the rainbow trout, *Oncorhynchus mykiss*, living in environments where temperatures are low and variable. To allow cardiac function in a temperate environment it is thought that the comparatively high Ca^{2+} sensitivity of trout cardiac myocytes compensates for the effects of low temperature on myocyte contractility. The high Ca^{2+} sensitivity of the trout myocyte is due, at least in part, to changes in the amino acid sequence of the thin filament protein, cardiac troponin C (cTnC). cTnC is the Ca^{2+}-activated switch that triggers myocyte contraction. The isoform of cTnC cloned from trout ventricle (ScTnC) is 92% identical to mammalian cTnC (McTnC) and is significantly more sensitive to Ca^{2+}. This result suggests that ScTnC has evolved in trout to allow cardiac function at low temperatures. cTnC also appears to play a role in maintaining cardiac function when temperatures change. Increasing myofibrillar pH according to α-stat regulation, as would occur when temperature decreases, increases Ca^{2+} sensitivity. A similar increase in pH also sensitizes cTnC to Ca^{2+}. ScTnC therefore appears critical in maintaining cardiac function in trout at low temperatures as well as during changes in temperature.

Keywords: Temperature; pH; Ca^{2+} affinity; Cardiac myocytes; Contractility; Protein structure; Protein function

1. Introduction

Increases in cytosolic Ca^{2+} concentration ([Ca^{2+}]) are used by cardiac myocytes to initiate contraction and regulate contractility. The sensitivity of the myocyte contractile element to [Ca^{2+}] is an important determinant of contractility. As the heart temperature decreases, cardiac function becomes increasingly impaired as the maximum Ca^{2+}-activated force as well as the sensitivity of the contractile element to [Ca^{2+}] decreases (Brandt and Hibberd, 1976; Harrison and Bers, 1989, 1990b; Sweitzer and Moss, 1990). This effect is reflected in the fact that an increase in [Ca^{2+}] is required to initiate contraction and generate adequate tension. The desensitizing effect of low temperature has been demonstrated in a number of species including a variety of mammals, frogs and rainbow trout, *Oncorhynchus mykiss* (Harrison and Bers, 1990b; Churcott et al., 1994). An interesting phenomenon, in light of this effect of decreasing temperature, is that cardiac function normally occurs in temperate fish species, such as rainbow trout, over a range of temperatures low

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enough to seriously impede a mammalian heart, as well as over a range of low temperatures.

One critical adaptation allowing cardiac function at low temperature in rainbow trout is that the thin filament has an enhanced sensitivity for Ca^{2+}. Churcott et al. (1994) demonstrated that cardiac myofilaments isolated from rainbow trout required significantly less Ca^{2+} to generate half-maximal tension between 7 and 21 °C than those isolated from rabbit, rat and guinea pig. This work suggests that the Ca^{2+}-sensing mechanism is more effective in salmonid cardiac myocytes at low temperatures than in mammalian myocytes. Increased sensitivity for Ca^{2+} would allow the myocytes to remain responsive to changes in $[Ca^{2+}]$ at low temperatures.

In addition to working at comparatively low temperatures, cardiac function in temperate fishes is maintained over a range of temperatures. As ectotherms, the body temperature of these animals is subject to change as the fish move through their thermally heterogeneous environment. Such changes in temperature occur on a very short time scale thereby precluding significant alteration to cellular structure. One mechanism by which function may be maintained in salmonid cardiac myocytes under such circumstances is through the resultant alteration of pH as occurs in most poikilothermic tissues with temperature change. Such change in blood and tissue pH occurs to keep relative alkalinity (OH^-/H^+) approximately constant. The observed relationship, called α-stat regulation, is from -0.016 to -0.019 pH units/°C and has the effect of keeping the fractional dissociation of the α-imidazole groups of protein histidine residues approximately constant. Churcott et al. (1994) have demonstrated that the Ca^{2+} sensitivity of actin–myosin ATPase in isolated trout cardiac myofibrils is significantly increased at 7 °C when pH is increased from 7.0 to 7.2. These results suggest that an increase in pH, as would occur due to α-stat regulation when cardiac temperature decreases, would help compensate for a decrease in Ca^{2+} sensitivity. This effect of pH on cardiac myocyte Ca^{2+} sensitivity may also be important in maintaining contractility in trout during cold acclimation.

A likely candidate for conferring enhanced Ca^{2+} sensitivity to salmonid cardiac myocytes and for being the instrument through which pH affects myocyte contractility is the thin filament protein cardiac troponin C (cTnC), the Ca^{2+} activated trigger that initiates myocyte concentration. This review will focus on how the structure and function of a single protein, cTnC, has evolved in temperate fishes to help maintain cardiac function at low and variable temperatures. In addition to providing insight into how myocyte contractility is maintained at low temperatures, it is hoped that this review will stimulate the reader to consider the role of individual proteins when studying how environmental pressures have directed the evolution of physiological processes.

2. Myocyte contraction and its regulation

In order to explore the molecular mechanisms responsible for the higher Ca^{2+} sensitivity of the salmonid cardiac myofilaments it is imperative to briefly review the events involved in contraction. Myocyte contraction is initiated when cytosolic $[Ca^{2+}]$ increases following membrane depolarization and Ca^{2+} binds to troponin C (TnC). The binding of Ca^{2+} to TnC triggers a cascade of reactions through the other components of the thin filament resulting in cross-bridge cycling between actin and myosin. Cardiac troponin C differs in structure from the skeletal muscle isoform of TnC (sTnC) as will be discussed later. cTnC is composed of 161 amino acids and can be broken into three parts: the high and low affinity Ca^{2+} binding domains, and the alpha helical linker (E-linker) connecting the two domains (Fig. 1). Each Ca^{2+} binding domain contains two EF hand structural motifs common to other Ca^{2+} binding molecules such as calmodulin. Usually, each EF hand is able to bind one Ca^{2+}, however, in cTnC the first EF hand (site I) is non-functional due to sequence substitutions. Therefore cTnC only possesses one low affinity Ca^{2+} binding site (site II) (Babu et al., 1987) whereas two are found in sTnC. Site II is referred to as the regulatory site cTnC because it is the binding of Ca^{2+} to this site that triggers cardiac myocyte contraction.

Ca^{2+} binding to site II initiates a complex change in the interrelations of the troponin molecules on the thin filament. In diastole only the high affinity binding sites (sites III and IV) of cTnC are occupied with Ca^{2+}. The near N-terminus of the cardiac troponin I molecule (cTnI) is bound to the C terminus of both cTnC and cardiac troponin T (cTnT) (Krudy et al., 1994; Kleerekoper et al., 1995). A section of the cTnI molecule known as the inhibitory peptide (residues 136–

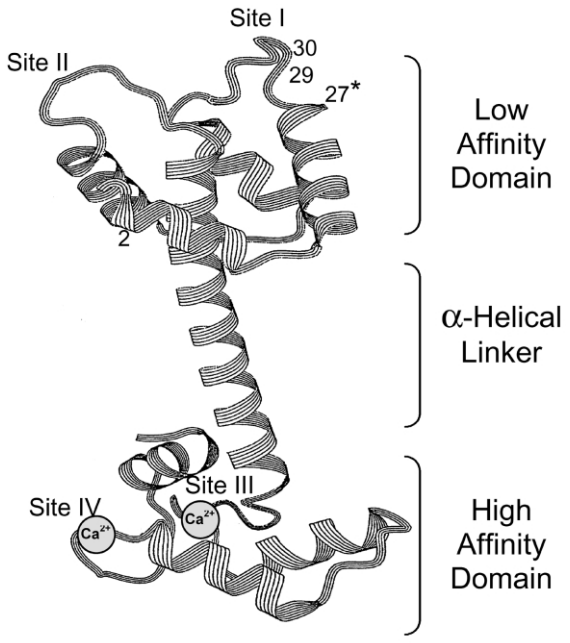

Fig. 1. Ribbon diagram of the tertiary structure of cTnC. Sites I–IV indicate potential Ca^{2+} binding sites with sites III and IV occupied. Locations of amino acid residues that may be responsible for the higher Ca^{2+} sensitivity of salmonid cTnC are indicated by their sequence number. These are asparagine 2, glutamine 29, and aspartate 30 in salmonid cTnC replacing aspartate, leucine and glycine, respectively, in mammalian cTnC. The 27* indicates where tryptophan replaced phenylanine at residue 27 to insert a fluorescent reporter. This ribbon diagram is a modification of the model of skeletal troponin C based on the crystal structure of this molecule by Satyshur et al. (1988).

147) is bound to the actin–tropornyosin complex and inhibits the force generating reaction between actin and myosin (Syska et al., 1976; Talbot and Hodges, 1981). The binding of Ca^{2+} to the single activation site of cTnC alters the structure of the molecule causing it to 'open', exposing a hydrophobic patch that then binds to cTnI near the inhibitory peptide. This interaction pulls cTnI away from the actin–tropomyosin complex, releasing its inhibitory effect on the cross-bridge cycling reaction, allowing force generation.

The contractile reaction is dependent on complex protein–protein interactions between the components of the thin filament as well as conformational changes in a number of functional proteins. A protein's structure and function are determined by its amino acid sequence. Therefore, by modifying the sequence of a key protein, such as cTnC, the entire contractile reaction can be altered. An example of this, as demonstrated by Harrison and Bers (1990a), is that the temperature dependence of cardiac myocyte Ca^{2+} sensitivity can be relieved in rat ventricular myocytes by replacing native cTnC with sTnC. This result suggests that isoform (or structural) differences in TnC mediate the differential effects of temperature on myofilament Ca^{2+} sensitivity (Stephenson and Williams, 1981; Goldman et al., 1987).

Cardiac and skeletal muscle exhibit physiologically important differences in the length–tension relationship that underlies the Starling effect. In cardiac muscle, active tension development demonstrates a steeper dependence on length and this is due in part to the fact that the contractile element has an increased sensitivity for Ca^{2+} at longer lengths. There is experimental evidence to suggest that this difference between cardiac and skeletal muscle is also attributable to differences between cTnC and sTnC (Babu et al., 1988; Gulati et al., 1991; Akella et al., 1997) although this remains a controversial subject (Moss et al., 1991; McDonald et al., 1995). Thus, although TnC is relatively conserved and the structural differences between cTnC and sTnC are not great, important physiological properties have been ascribed to them.

Mammalian cTnC (McTnC) is one residue longer than mammalian sTnC (MsTnC) and 65% identical at the amino acid level. These sequence differences translate into differences in protein function that, as illustrated by the Harrison and Bers (1990a) study, have obvious consequences on certain aspects of myocyte contractility. In addition, as discussed earlier, cTnC contains one less functional low affinity Ca^{2+} binding site than sTnC. An EF hand binds Ca^{2+} through the alignment of the metal ion with six charged residues in a 12-residue loop region. When aligned with the Ca^{2+} ion the six residues approximate the axes of a three-dimensional Cartesian co-ordinating system. These residues form a pentagonal bipyramidal arrangement around the Ca^{2+} ion and are at positions $1(x)$, $3(y)$, $5(z)$, $7(-y)$, $9(-x)$, and $12(-z)$ of the 12 residue canonical loop. In site I of McTnC a valine has been inserted preceding residue x while x and y have been replaced with uncharged residues. This change in protein sequence disrupts the functional characteristics responsible for the Ca^{2+} binding abilities and clearly demonstrates that small changes in sequence can have major effects on protein function.

3. Salmonid and mammalian cTnC, a comparison of structure and function

The higher Ca^{2+} sensitivity observed in the cardiac fibers of salmonid fish compared to that of mammalian cardiac fibers could be a result of differences in protein structure of cTnC. There is almost complete conservation of the amino acid sequence of cTnC cloned from endothermic animals, with 100% sequence identity between human, pig, and cow cTnC (McTnC) and 99% identity between these and that from chicken heart. Salmonid cTnC cloned from the heart of rainbow trout (ScTnC) is 92% identical to McTnC (Moyes et al., 1996). This is equal to 13 differences out of a sequence of 161 amino acids (Moyes et al., 1996). Of these, four are conservative replacements in which an amino acid with similar physical properties has been substituted, such as isoleucine replacing valine at residue 28 in ScTnC. Such a conservative replacement should have little effect on protein tertiary structure and therefore function. The remaining nine non-conservative substitutions have a much higher potential for affecting protein structure and function. This conservation of sequence is remarkable considering teleosts and mammals diverged over 400 million years ago. When comparing the sequence of ScTnC with McTnC, a striking finding is the complete sequence identity at the region that forms site II, the regulatory site. As there is no difference in the sequence of site II, any variation in the affinity of this site for Ca^{2+} between ScTnC and McTnC is as a result of sequence difference in other regions of a protein having an allosteric effect.

To determine if the differences in protein sequence between ScTnC and McTnC result in differences in the Ca^{2+} binding capabilities of these two proteins, we (Gillis et al., 2000) have utilized F27W cTnC mutants to measure Ca^{2+} sensitivity. In these proteins, phenylalanine at residue 27 was replaced with a tryptophan in both ScTnC and McTnC through site-directed mutagenesis. We have established that the tryptophan acts as a fluorescent reporter monitoring Ca^{2+} binding to site II without significantly affecting the tertiary structure of McTnC and ScTnC (Moyes et al., 1996). As the proteins in solution are titrated with Ca^{2+}, fluorescence increases as Ca^{2+} is bound and protein conformation changes and reaches a maximum when saturation occurs. Ca^{2+} sensitivity was determined by measuring the $p\mathrm{Ca}_{50}$, which is

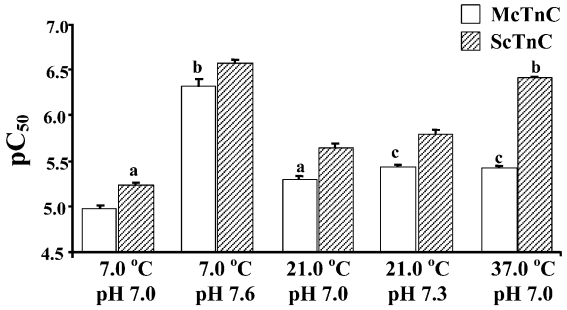

Fig. 2. $p\mathrm{Ca}_{50}$ values, the pCa at half-maximal fluorescence, of Ca^{2+} binding to McTnC and ScTnC at: 7.0 °C, pH 7.0 and 7.6; 21.0 °C, pH 7.0 and 7.3; and at 37.0 °C, pH 7.0. Values presented as mean ± S.E.M. All $p\mathrm{Ca}_{50}$ values were significantly different from all others ($P < 0.05$) unless indicated by a superscript 'a', 'b', or 'c'. Values that are indicated with the same letter are not significantly different from each other.

the pCa ($-\log[Ca^{2+}]$) required to generate half-maximal fluorescence. This study demonstrated, first, that when temperature was decreased and pH was kept constant at 7.0, the Ca^{2+} sensitivity of both isoforms decreased. This result indicates that cTnC is at least partially responsible for the desensitizing effect of low temperature on myocyte contractility. This study also demonstrated that when measured under identical conditions, ScTnC was always more sensitive to Ca than McTnC (Fig. 2). For example, when measured at 21 °C, pH 7.0, ScTnC was 2.3 times more sensitive to Ca^{2+} than McTnC (Fig. 3). This result clearly demonstrates that the differences in sequence between ScTnC and McTnC have functional consequences and suggest that the higher Ca^{2+} sensitivity of salmonid cardiac myofilaments is due, at least in part, to the enhanced Ca^{2+} sensitivity of ScTnC.

The next step is to identify the sequence differences specifically responsible. The most promising substitutions are those that occur in the N terminus (residues 1–41) which encompasses two separate regions. The first section (residues 1–10) is the N-terminal α-helical overhang and the second component is Ca^{2+} binding site I. It has been established that these two regions, while not directly involved in binding Ca^{2+} in the cardiac isoform, have a significant effect on protein function (Gulati et al., 1992; Gulati and Rao, 1994; Rao et al., 1995). Through the construction of chimeric proteins from skeletal TnC and cardiac TnC, it was demonstrated that both of these regions must be

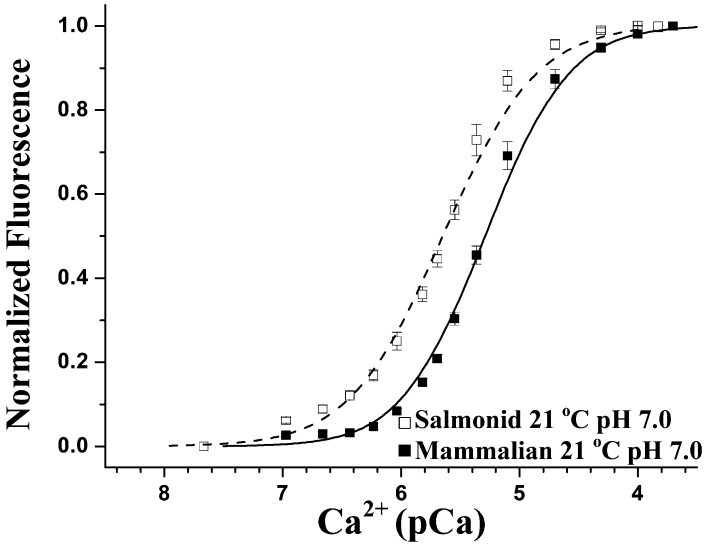

Fig. 3. Difference in Ca^{2+} sensitivity between ScTnC ($n=9$) and McTnC ($n=10$) at 21 °C, pH 7.0. The curves generated by fitting the data with the Hill equation have been added for comparison against data points. Data are normalized with respect to the maximal fluorescence of each Ca^{2+} titration and presented as means ± S.E.M. The $K_{1/2}$, the *p*Ca at half-maximal fluorescence, of each curve are significantly different from each other ($P<0.05$). Figure modified from Gillis et al. (2000).

present in the protein construct for sTnC to behave as cTnC when reconstituted into skinned ventricular trabeculae (Gulati et al., 1992). These authors suggest that a strong interaction between the N-terminal overhang and site I is imperative in establishing the cTnC phenotype. Of the five differences in sequence between ScTnC and McTnC in the N terminus, three may have functional consequences. The first is at residue 2 where ScTnC has an asparagine instead of an aspartate (Fig. 1). The absence of a negatively charged side chain in the N-terminal overhang could alter the tertiary structure of this region and/or how it interacts with the rest of the protein, thereby affecting its function. The other two significant differences between ScTnC and McTnC in the N terminus occur at residues 29 and 30 where ScTnC has glutamine and aspartate instead of leucine and glycine, respectively (Fig. 1). The absence of leucine, a hydrophobic amino acid as well as the presence of an additional negatively charged amino acid, aspartate, have the potential to impact the function of the protein. The net effect of the differences in protein sequence result in ScTnC being able to be activated by Ca^{2+} at lower concentrations. This could be as a result of a number of possibilities discussed below.

The first possible explanation as to why ScTnC is more sensitive to Ca^{2+} than McTnC is that site II, the activation site, has a higher affinity for Ca^{2+} and therefore is able to trigger the change in protein confirmation at lower $[Ca^{2+}]$. This could be the result of the differences in the N terminal region of ScTnC having an allosteric effect on site II influencing how Ca^{2+} is bound and/or off loaded by the protein.

The second possibility is that ScTnC is more 'flexible' and therefore more reactive at low temperatures. It has been suggested that cold-adapted proteins function better at low temperatures because they exhibit an appropriate flexibility for the temperature to which they are adapted (Hochachka and Somero, 1984). Common traits of these proteins are a reduced thermal stability and lower activation energy compared to a warmer adapted isoform (Genicot et al., 1996). To achieve lower thermal stability, so as to remain flexible at low temperature, cold-adapted enzymes have fewer salt bridges, have fewer weakly polar (aromatic) interactions and are less hydrophobic than warm adapted isoforms (Davail et al., 1994; Genicot et al., 1996). Preliminary indications are that ScTnC may be less thermally stable and therefore more flexible than McTnC at low temperatures (Gillis et al., 2000). At 37 °C, pH 7.0 this protein behaved aberrantly as it loaded Ca^{2+} at very low concentrations, continued to load Ca^{2+} at concentrations greater than *p*Ca 3.5, and never achieved saturation

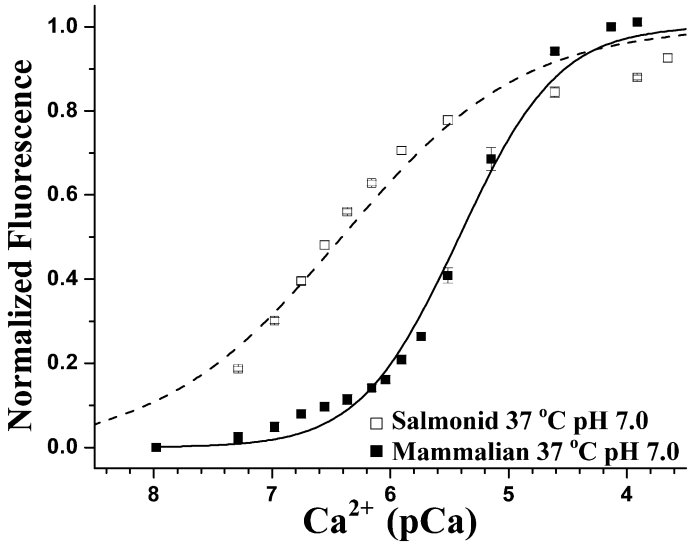

Fig. 4. Difference in Ca^{2+} sensitivity between ScTnC ($n=8$) and McTnC ($n=8$) at 37 °C, pH 7.0. The curves generated by fitting the data with the Hill equation have been added for comparison against the data points. Data are normalized with respect to the maximal fluorescence of each Ca^{2+} titration and presented as mean ± S.E.M. The $K_{1/2}$, the *p*Ca at half-maximal fluorescence, of each curve are significantly different from each other ($P<0.05$).

under the experimental conditions (Fig. 4). This dramatic change in Ca^{2+} binding properties is likely due to a loss of tertiary structure due to temperature. As the upper lethal temperature for rainbow trout is 23–25 °C (Black, 1950) it is not unexpected that temperatures as high as 37 °C affect protein integrity. How the lower thermal stability of ScTnC affects its function within its physiological temperature range is not known at this time. However, an increased flexibility would make it easier for the protein to change confirmation, as less activation energy is required (Genicot et al., 1996). It is also currently not possible to predict which sequence difference(s) between ScTnC and McTnC is/are responsible for the decrease in thermal stability of ScTnC.

The third possible explanation for the higher Ca^{2+} sensitivity of ScTnC is that the presence of glutamine and aspartate at residues 29 and 30 restores functionality to site I. Residues 29 and 30 are located within site I, which in all cardiac isoforms examined to date is non-functional as a Ca^{2+} binding site. Residue 29 corresponds to the *x* of the Cartesian co-ordinating system and residue 30 to the non-coordinating residue between *x* and *y* (Fig. 5). The replacement of a negatively charged amino acid in this area of the loop could allow for Ca^{2+} binding by correcting the alignment of the co-ordinating residues around the Ca^{2+} ion. If site

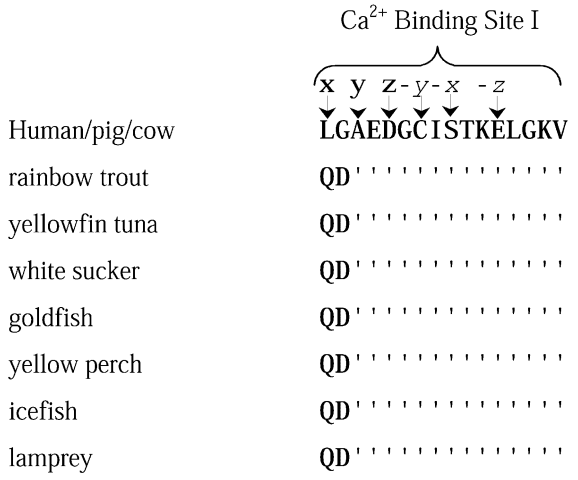

Fig. 5. Comparison of the amino acid sequences encoding for Ca^{2+} binding site I in: mammalian cardiac troponin C; rainbow trout, *Oncorhynchus mykiss* (Moyes et al., 1996); yellowfin tuna, *Thunnus albacares* (Yang et al., 2000); white sucker, *Catostomus commersoni* (Yang et al., 2000); goldfish, *Carassius auratus* (Yang et al., 2000); yellow perch, *Perca flavescens* (Yang et al., 2000); icefish, *Chaenocephalus aceratus* (Yang et al., 2000); and lamprey, *Entosphenus japonicus* (Yuasa et al., 1998). The six residues predicted to co-ordinate and form the Ca^{2+} binding site are indicated by *x*, *y*, *z*, $-y$, $-x$, $-z$.

I is functional, in the salmonid cTnC it may have substantial impact on the Ca^{2+} binding dynamics of activation and perhaps enhances co-opertivity of Ca^{2+} binding. This hypothesis is currently being tested.

The amino acid sequence encoding for site I in ScTnC has been found to be conserved in cTnC sequenced from the following fishes: yellowfin tuna, *Thunnus albacares* (Yang et al., 2000); white sucker, *Catostomus commersoni* (Yang et al., 2000); goldfish, *Carassius auratus* (Yang et al., 2000); yellow perch, *Perca flavescens* (Yang et al., 2000); icefish, *Chaenocephalus aceratus* (Yang et al., 2000); and lamprey, *Entosphenus japonicus* (Yuasa et al., 1998) (Fig. 5). This means that glutamine and aspartate are also present in all these cTnC isoforms at residues 29 and 30. No other isoforms of fish cTnC have been cloned than those mentioned above. The maintenance of these specific residues in site I of cTnC from such a variety of phylogenetically and environmentally dispartate fishes emphasizes its potential importance in regulating the functional characteristics of the protein. The number of amino acid substitutions between ScTnC and cTnC sequenced from the other species of fish ranged from 7 with tuna cTnC to 34 with lamprey cTnC, most of which are observed in areas of the protein outside of the Ca^{2+} binding domains (Yang et al., 2000). It is not known at this time if any of the differences in sequence between the different fish cTnCs have functional consequences.

4. A role for cTnC in thermal acclimation

A number of morphological and functional changes have been measured in the hearts of active cold acclimated fish including trout and yellow perch. First, with cold acclimation, there is a proliferation of sarcoplasmic reticulum (SR) in perch cardiac myocytes (Bowler and Tirri, 1990), while in trout heart there is an increase in the SR Ca^{2+} transport capability with cold acclimation (Keen et al., 1994; Aho and Vornanen, 1998). Second, the activity of Ca^{2+}-sensitive myofibriller ATPase was 60% higher in trout acclimated to 3 °C than to 18 °C (Yang et al., 2000). An increased capacity to deliver Ca^{2+} to the contractile element and the up-regulation of the enzyme involved in cross-bridge cycling suggests that attempts are being made by the cardiac myocyte to defend contractility against the effects of low temperature.

The Ca^{2+} sensitivity of the contractile element is yet another mechanism that must remain functional at low temperature in order to maintain cardiac function. One possible strategy is to begin expressing sTnC instead of cTnC, as the skeletal isoform is less temperature sensitive. Previous studies have also shown that there are shifts in the isoforms of TnC and TnI expressed during tissue development (Toyota and Shimada, 1983; Obinata, 1985; Gahlmann et al., 1988; Hunkeler et al., 1991; Sasse et al., 1993) and the replacement of cTnC with sTnC relieves the temperature dependence of cardiac myocyte contractility (Harrison and Bers, 1990a). As well, previous studies have illustrated that cold acclimation affects the expression of other myofilament proteins in teleost muscle (Crockford and Johnston, 1990; Gerlach et al., 1990; Hwang et al., 1991; Ball and Johnston, 1996). Yang et al. (2000) used Northern blot and RT–PCR analyses to determine if, during cold acclimation, there was a change in TnC isoform expression in the rainbow trout heart. It was found that there were no differences in the expression of cTnC between acclimation groups, implying that that only one cTnC isoform is required for cardiac function at this animal's upper and lower thermal limits.

The Ca^{2+} sensitivity of trout myofibrils increases when temperature is kept constant and pH is increased. It has been known for a long time that in mammalian hearts Ca^{2+} sensitivity is highly dependent on pH. (Fabiato and Fabiato, 1978; Solaro et al., 1988; Gulati and Babu, 1989; Hofmann et al., 1993; Komukai et al. 1998). Churcott et al. (1994) have suggested that the sensitization of the cardiac myocytes to Ca^{2+}, as would occur as cytosolic pH increases when temperature decreases due to α-stat regulation, may be a mechanism by which contractility is maintained as temperatures decrease. We have demonstrated when maintaining constant temperature and increasing pH according to α-stat, that Ca^{2+} sensitivity of both salmonid and mammalian cTnC increases (Gillis et al., 2000). The pCa_{50} of ScTnC and McTnC increased by 0.14 when pH was increased from 7.0 to 7.3 at 21 °C and by 1.35 when pH was increased from 7.0 to 7.6 at 7 °C (Fig. 2). If the effects of pH on myofibrils and on isolated cTnC are compared, it is apparent that the effect of pH on the former is due mostly to the latter (Gillis et al., 2000). It is interesting to note that while the sensitivity of ScTnC decreased when

pH was maintained at 7.0 and temperature was decreased from 21 °C to 7 °C, its sensitivity for Ca^{2+} was higher at 7.0 °C, pH 7.6 than at 21 °C, pH 7.3 (Fig. 2) (Gillis et al., 2000). Therefore, without having to invoke differential isoform expression it is possible to increase cTnC sensitivity for Ca^{2+} even at a lower temperature. This is a considerably energetically more efficient alternative to changing the structure of the thin filament during cold acclimation. The sensitizing effect of increased pH on ScTnC may, therefore, be critical in maintaining myocyte Ca^{2+} sensitivity during cold acclimation.

5. Conclusions and perspective

The conservation of sequence between salmonid and mammalian cTnC is dramatic considering that there are over 400 million years separating teleost fish from mammals. Such high sequence identity between isoforms from animals of widely disparate phyla suggests that only small sequence modifications are required to alter its function. The higher Ca^{2+} sensitivity of ScTnC, as well as its apparent reduced thermal stability, represents the effect of such sequence manipulation. The enhanced ability of ScTnC to respond to $[Ca^{2+}]$ is at least partially responsible for the higher Ca^{2+} sensitivity observed in the intact trout cardiac myocyte. This finding again illustrates the fact that by changing a few amino acids in a single protein, functional characteristics of a cell can be dramatically altered allowing, in this case, for trout cardiac myocytes to be sensitive to changes in $[Ca^{2+}]$ at low temperature. In mammals functioning at 37 °C the high sensitivity observed in salmonid cTnC would be problematic as it would seriously interfere with the off loading of Ca^{2+} from cTnC resulting in dramatically impaired relaxation.

The sensitizing effect of increased pH on ScTnC may be important in maintaining cardiac myocyte contractility during cold acclimation as well as during acute changes in temperature. Without having to make structural changes to the contractile element, Ca^{2+} sensitivity may be defended as pH increases, due to α-stat regulation, sensitizing ScTnC to Ca^{2+}. This influence of cTnC on myocyte contractility is supported by the fact that the same effect of temperature and pH on the Ca^{2+} sensitivity of intact cardiac myocytes was seen on the Ca^{2+} sensitivity of isolated cTnC (Churcott et al., 1994; Gillis et al., 2000). As well, the contractility of skinned cardiac myocytes can be manipulated by cTnC replacement (Harrison and Bers, 1990a). The functional characteristics of ScTnC, due to its unique protein sequence, appears to be important to both maintaining the Ca^{2+} sensitivity of the contractile element at low temperatures as well as over a range of temperatures.

A significant amount of work remains to characterize the difference in Ca^{2+} sensitivity between ScTnC and McTnC. The residues responsible for the higher Ca^{2+} sensitivity need to be identified as well as the mechanisms by which this occurs. Studies in which native ScTnC is replaced with McTnC in salmonid cardiac myocytes are also required to establish if the differences in Ca^{2+} sensitivity measured in solution are as dramatic in a functioning contractile element.

In conclusion, by comparing the structure and function of proteins cloned from animals that have evolved in different environments, great insight can be gained into how environmental pressures have influenced protein design. With the sequences of more proteins becoming known every day, the disciplines of comparative physiology and environmental physiology will benefit from the ability to explore physiological processes at the most fundamental level of structure and function, the protein.

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