Rostral-caudal variation in troponin T and parvalbumin correlates with differences in relaxation rates of cod axial muscle

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Summary

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Key words: axial muscle, relaxation, Atlantic cod, Gadus morhua, contractile protein, parvalbumin, troponin T, regional variation.
ROSTRAL–CAUDAL VARIATION IN TROPONIN T AND PARVALBUMIN CORRELATES WITH DIFFERENCES IN RELAXATION RATES OF COD AXIAL MUSCLE

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Introduction

Relaxation rate has been hypothesized to be an important determinant of contractile frequency (Altringham and Johnston, 1990) and power generation in the axial muscles of fish (Moon et al. 1991; Rome and Swank, 1992; Rome et al. 1993; Johnson et al. 1994). After completing the shortening phase of its contraction cycle, a muscle must relax rapidly if it is to be extended with little resistance (Josephson, 1981). Hypothetically, maximum power can be achieved if muscle is ‘turned on’ instantaneously at the start of shortening and ‘turned off’ instantaneously just before lengthening (Marsh, 1990). A longer causal relaxation rate can increase differences in the phase relationship between muscle strain and excitation. Differences in this phase relationship can have profound effects on the mechanical behavior of skeletal muscle (Josephson, 1985; Altringham and Johnston, 1990; Johnson and Johnston, 1991; Altringham et al. 1993). The resistance of active caudal muscles to being stretched (negative work) can act to stiffen the tail region, which can increase tail thrust. Thus, the rate at which caudal muscle relaxes can play a vital role, not only in the amount of negative work the muscle performs but also in determining the overall thrust produced by the tail region, which ultimately affects the performance of the whole organism (Swank et al. 1997).

Recent work by Davies et al. (1995), comparing rostral versus caudal mechanical properties of Atlantic cod Gadus morhua L. axial muscle fibers, documents a significant rostral–caudal difference in relaxation rates: the time required to relax to half peak isometric tension averaged 54.0±2.9 ms (mean ± S.E.M.) for rostral single fibers and 88.3±8.0 ms for caudal fibers. Similar differences have been reported for the white muscle fibers of pollack Pollachius virens (Altringham et al. 1993) and the red muscle fibers of scup Stenotomus chrysops (Rome et al. 1993). The present study explores the biochemical basis of rostral–caudal variation in cod axial muscle relaxation rate.

Relaxation of skeletal muscle after a single twitch or tetanic contraction is initiated by a reduction in myoplasmic Ca$^{2+}$ concentration. Four major mechanisms are implicated in controlling relaxation. The first mechanism involves dissociation of Ca$^{2+}$ from troponin C. Troponin T is believed to affect this rate of dissociation, as has been shown in frogs (Baylor et al. 1983; Gillis, 1985) and rabbits (Schachat et al. 1987). The second mechanism involves facilitated diffusion of Ca$^{2+}$ from the myofibrils to regions near the sarcoplasmic reticulum. Parvalbumin, a low-molecular-mass Ca$^{2+}$-binding protein, has been shown to play a key role in this process in both frogs (Hou et al. 1991) and mice (Müntener et al. 1995). A third mechanism involves uptake of Ca$^{2+}$ into the sarcoplasmic reticulum by the Ca$^{2+}$-ATPase pump (e.g. frog, Baylor et al. 1983; numerous vertebrates, Gillis, 1985; teleosts,
Rome et al. 1996; Swank et al. 1997). The number and/or efficiency of Ca\textsuperscript{2+}-ATPase pumps determines the rate of Ca\textsuperscript{2+} uptake. A fourth mechanism involves changes in the actomyosin crossbridge detachment kinetics (rabbit, Greaser et al. 1988; Sweeney and Stull, 1990). Myosin isoforms can be a strong determinant of detachment kinetics.

The present study implicates the first two of these regulatory mechanisms in controlling rostral–caudal variation in the relaxation rate of cod axial muscle.

Materials and methods

Experimental animals

Three specimens of fresh Atlantic cod Gadus morhua L. (cod GM01, 653 mm; cod GM02, 635 mm; cod GM03, 775 mm standard length) were obtained commercially from Maine waters during the months of May and June 1997 and March 1998. A fourth specimen (cod GM04, 470 mm standard length) was captured off the Massachusetts coast, maintained in captivity for several days at Northeastern University’s Marine Science Laboratory in Nahant, MA, USA, and killed by pithing prior to axial muscle dissection.

Myofibril preparation

Fresh white muscle samples (0.5–1.0 g) were dissected from the epaxial arm and cone region of every sixth myomere and from one rostral and one caudal hypaxial cone site from cod GM01 (Fig. 1). Each muscle sample was minced on a chilled glass plate, transferred to an Eppendorf tube and suspended in 1 ml of buffer A, containing 15 mmol l\textsuperscript{-1} Tris, pH 7.6, 100 mmol l\textsuperscript{-1} NaCl, 4 mmol l\textsuperscript{-1} EGTA, 2 mmol l\textsuperscript{-1} MgCl\textsubscript{2}, 7 mmol l\textsuperscript{-1} β-mercaptoethanol, 0.1 mmol l\textsuperscript{-1} phenylmethylsulfonylfluoride (PMSF) and 75 ng ml\textsuperscript{-1} each of antipain, leupeptin and pepstatin A. Samples were vortexed and centrifuged at 14,000 revs min\textsuperscript{-1} (12,500g) for 12 s in an Eppendorf 5415 microcentrifuge, and the resulting pellets were resuspended in 1 ml of buffer B (composition as for buffer A but containing only 0.3 μl ml\textsuperscript{-1} leupeptin, antipain and pepstatin). The samples were vortexed and centrifuged (at 12,500g), and the resulting pellets were incubated in 1 ml of buffer C (buffer B plus 2% Triton X-100) for 10 min. After recentrifugation at (12,500g), the pellets were washed twice more with buffer B and resuspended in 1 vol of buffer B.

To prepare myofibrillar homogenates, fresh superficial white muscle samples were dissected from the epaxial arm region of every sixth myomere from cod GM02 and GM03. All samples were minced as described above and suspended in approximately 5 vols of buffer A supplemented with 0.6 mol l\textsuperscript{-1} NaCl. Samples were incubated on ice for 10 min and centrifuged at room temperature (21°C) for 2 min at 12,500g, and the supernatants were reserved for further characterization.

SDS–PAGE

Sample preparation

Gel samples were prepared by 1:1 dilutions with 2× Laemmli reducing buffer (2% SDS, 50 mmol l\textsuperscript{-1} Tris, pH 6.8, 20% glycerol, 1% β-mercaptoethanol, Bromophenol Blue). Samples were then heated for 3 min at 100°C, frozen until solid, reheated for 2 min at 100°C, and centrifuged. Supernatants were diluted as necessary in Laemmli reducing buffer (Laemmli, 1970).

Preparation of gels

SDS–PAGE was performed according to Laemmli (1970) using 10.5%, 12.5% and 15% polyacrylamide–SDS gels as indicated. Mini-gels were run at room temperature on a BioRad Protean II cell, and full-sized gels were electrophoresed at 8°C using Hoefer Scientific Instruments SE500 apparatus. Unless otherwise specified, all gels were fixed and stained with Coomassie Brilliant Blue G250 as described by Neuhoff et al. (1988).

Gels were scanned into Adobe Photoshop v. 3.05 with an Agfa Arcus II Scanner and quantification was performed with NIH Image v. 1.61. When necessary, intensity plots were fitted.
using Jandel PeakFit v. 4. The scanner was calibrated with a Kodak density step tablet (CAT 1523406), and dilutions of myofibril samples were run to ensure linearity of staining.

Relative molecular mass (Mr) was determined by comparing the relative mobility of the cod myofibrillar proteins with that of proteins from rabbit psoas myofibrils, whose molecular masses are known from direct sequencing or cDNA analysis.

Immunoblotting (western blotting)

Gel samples were prepared from homogenates, myofibrils or supernatants as indicated. Based on the method of Davies et al. (1995), four sample sites were selected; one epaxial rostral site, one epaxial caudal site, one hypaxial rostral site and one hypaxial caudal site from the right side of two individuals (cod GM02 and GM03). To normalize to levels of actin, two lanes were run for each sample: one for Coomassie staining, and the second for immunoblotting.

Parvalbumin was identified following transfer to nitrocellulose using monoclonal anti-parvalbumin mouse ascites fluid PA-235 (Sigma 3171) and the protocol outlined in the Vectastain ABC kit. The blot intensities were quantified densitometrically and normalized to actin using NIH Image.

Ca$^{2+}$-binding proteins

Two-dimensional electrophoresis was performed using standard 12.5% SDS—PAGE with all solutions supplemented with 1 mmol l$^{-1}$ EDTA in the first dimension and with 12.5% SDS—PAGE with all solutions supplemented with 1 mmol l$^{-1}$ CaCl$_2$ in the second dimension. Lanes from the first dimension were excised and mounted horizontally on the stacker for the second dimension using an agarose solution containing 0.125 mol l$^{-1}$ Tris, pH 6.8, 1.25% low-melting-point agarose (FMC Corp.), 0.1% SDS, 1:4000 β-mercaptoethanol and 1 mmol l$^{-1}$ CaCl$_2$.

The gel was stained with Coomassie Brilliant Blue as described above. A right shift of the proteins away from the diagonal is an indicator of Ca$^{2+}$ binding. Ca$^{2+}$ binding to proteins displaces SDS, reducing the charge and mobility of Ca$^{2+}$-binding proteins in the second dimension and resulting in an upward shift in position in the second dimension (Burgess et al. 1980).

Protein purifications: myosin and troponin

Owing to the inherent thermal instability of gadoid myosins (Connell, 1960; Castell et al. 1973; Laird and Mackie, 1981), special precautions were taken to ensure successful purification of the myosin and troponin myofibrillar subfractions. Axial muscle tissue samples were dissected from cod GM04, immediately frozen in liquid nitrogen and stored at −80°C for 2 days.

Approximately 2 g of muscle from the arm region of site 3 (see Fig. 1) were pulverized in liquid nitrogen. The resulting powder was washed in 10 vols of chilled solution 1 [50 mmol l$^{-1}$ Tris/HCl, 30 mmol l$^{-1}$ Tris base, 50 mmol l$^{-1}$ LiCl, 15% glycerol (BRL Molecular Biology Grade), 7 mmol l$^{-1}$ β-mercaptoethanol, 0.1 mmol l$^{-1}$ PMSF, 1/10000 (25 mg ml$^{-1}$) leupeptin] and centrifuged for 5 min at 3000 g in a Jouan CR412 refrigerated centrifuge. LiCl was incorporated into solution 1 since it has been shown to be more effective at extracting protein from fish muscle than either sodium chloride or potassium chloride under most conditions (Kelleher and Hultin, 1991). Glycerol was added to further stabilize against denaturation (Gekko and Timasheff, 1981). The supernatant from the first centrifugation was saved for the analysis of soluble proteins, and the pellet was washed a second time in 10 vols of solution 1. Myosin, troponin and tropomyosin were prepared by incubating the pellet in 3 vols of chilled extraction buffer (1 mol l$^{-1}$ LiCl, 25 mmol l$^{-1}$ Tris/HCl, 7.5 mmol l$^{-1}$ Tris base, 15% glycerol, 7 mmol l$^{-1}$ β-mercaptoethanol, 0.1 mmol l$^{-1}$ PMSF) for 10 min at 0°C. Following incubation, the sample was centrifuged for 75 min at 250,000 g at 2°C in a 100Ti rotor in a Beckman tabletop ultracentrifuge. The supernatant, containing solubilized myofibrillar proteins, was dialyzed overnight at 4°C against a low-salt solution (15% glycerol, 25 mmol l$^{-1}$ LiCl, 30 mmol l$^{-1}$ Tris/HCl, 10 mmol l$^{-1}$ Tris base, 2 mmol l$^{-1}$ MgCl$_2$, 1 mmol l$^{-1}$ EGTA, 14 mmol l$^{-1}$ β-mercaptoethanol, 0.1 mmol l$^{-1}$ PMSF) to precipitate the myosin. The dialysis solution was changed after the first 4 h and replaced with a fresh solution devoid of EGTA. Following dialysis, the sample was centrifuged at 4°C, and the pelleted myosin and the supernatant (containing mainly actin, tropomyosin and troponin) were separated. The supernatant was supplemented with 5 mmol l$^{-1}$ phosphate buffer and loaded onto a 5 ml hydroxyapatite column. The column was washed with 2 vols of 5 mmol l$^{-1}$ phosphate buffer (5 mmol l$^{-1}$ phosphate, 0.6 mol l$^{-1}$ LiCl, 1 mmol l$^{-1}$ MgCl$_2$, 14 mmol l$^{-1}$ β-mercaptoethanol, 0.1 mmol l$^{-1}$ PMSF, pH 7) and eluted with a linear 5 mmol l$^{-1}$ to 200 mmol l$^{-1}$ phosphate gradient in the same salt at 4°C. Fractions (1 ml) were collected and analyzed using SDS—PAGE.

Results

Identification of the myosin, troponin and tropomyosin subunits

The abundance of myofibrillar actin and myosin heavy chain makes them readily identifiable on denaturing polyacrylamide gels (Fig. 2). Unambiguous identification of the remaining myofibrillar proteins requires purified or enriched preparations. Myosin, troponin and tropomyosin were purified from the arm region of site 3 (Fig. 1), a site where all the major muscle proteins and their isoforms are expressed. Initial attempts at protein purification using standard techniques, however, resulted in aggregated and denatured proteins. Both formylation and thermal denaturation have been implicated in inducing aggregation of gadoid muscle proteins (Connell, 1960; Tokunaga, 1964; Castell et al. 1973; Laird and Mackie, 1981). To combat potential formylation, muscle samples were dissected immediately after the fish had been killed, flash-frozen in liquid nitrogen and stored at −80°C. The samples were then pulverized in liquid nitrogen and placed in a homogenization buffer supplemented with Tris, a primary
mhc c ci
Actm
—
—
—

Fig. 2. Electrophoresis of purified cod contractile proteins. 12.5% SDS-PAGE was used to identify the subunits of cod axial muscle proteins. Myofibrils (lane a) were run for comparison with an enriched myosin preparation (lane b) and purified fractions of troponin (lane c) and tropomyosin (lane d). The three fast myosin light chains in the purified myosin sample are labeled mlc-1f, mlc-2f and mlc-3f, and the two fast troponin T species are labeled TnT-1 and TnT-2, where the numbers reflect the relative mobility of the proteins in ascending order. mhc, myosin heavy chain; TnI, troponin I; TnC, troponin C; Tm, tropomyosin.

Arm versus cone

Investigation of both myofibrils (Fig. 3) and homogenates (Fig. 4) along the rostral-caudal axis by SDS-PAGE revealed a marked difference in the ratio of the TnT isoforms. The faster-migrating rostral TnT isoform, TnT-2, is gradually replaced by the slower-migrating caudal isoform, TnT-1. Fig. 5 quantifies the shift in TnT isoforms as a function of myomere position in the three cod sampled. Each animal exhibits a rostral to caudal shift from TnT-2 to TnT-1 expression; at the level of the third dorsal fin, site A6 (Fig. 1) (approximately two-thirds of the way down the body), all the TnT in both the arm and cone regions is composed of the slower-migrating TnT-1 isoform.

While the difference in TnT expression was the only

Fig. 3. Comparison of arm and cone myofibrillar proteins in cod myomeres. A 10.5% SDS gel stained with Coomassie Brilliant Blue G250, comparing samples from arm (A1–A8) and corresponding cone (C1–C8) myofibrils from eight sequential myomeres of cod GM01 (standard length 653 mm). Note that the arm and cone samples show similar muscle protein expression. Both regions show a rostral-caudal shift in two troponin T isoforms. A, arm; C, cone; Myo, myosin heavy chain; Tm, tropomyosin; TnT, troponin T. Note that the faster-migrating rostral troponin T, TnT-2, is gradually replaced by the slower-migrating caudal isoform TnT-1. At the level of the third dorsal fin (site A6, approximately two-thirds of the way down the body), all myofibrillar troponin T in both the arm and cone regions is of the slower-migrating TnT-1 isoform.
myofibrillar variation, three additional rostral–caudal differences were characterized in the muscle homogenates (Fig. 4A). These differences appear to involve differential expression of low-molecular-mass cytosolic 
Ca\(^{2+}\)-binding proteins in the rostral axial white muscle.

Of these three proteins, the protein with an intermediate \(M_r\) was identified as parvalbumin by western blotting. Greater amounts of parvalbumin (\(M_r = 10,320\)) are present rostrally than caudally, as demonstrated by western blotting (Fig. 6) and gels stained with Coomassie Brilliant Blue G250 (Fig. 4; Table 1). The relative abundance of parvalbumin was assessed by determining its concentration relative to that of actin. In cod GMO3, the epaxial rostral site, A1, contains 8.3 times more parvalbumin than the caudal site A8. In cod GMO2, a similar bias in rostral parvalbumin expression was observed. In the hypaxial muscle samples (HR and HC), both cod GMO2 and GMO3 show a similar elevation of rostral parvalbumin
expression with exceedingly low caudal parvalbumin levels (Table 1).

In addition to parvalbumin, two cytoplasmic proteins, designated Cal and Ca2 (Mr 14800 and 9900, respectively), are present in rostral samples (A1-A4) but are absent from caudal samples (A5-A8). Their identification as Ca2-binding proteins is based on a mobility-shift assay. As shown in the two-dimensional gel in Fig. 7, proteins Cal and Ca2 shift to the right of the diagonal when electrophoresed in the presence of 1 mmol L−1 CaCl2. Such Ca2-dependent mobility shifts are indicative of Ca2-binding proteins (Burgess et al. 1980). Like parvalbumin, both proteins are expressed at high levels in rostral sites and are undetectable in the most caudal sites (Fig. 4A).

In contrast to the systematic rostral—caudal variation in TnT and Ca2-binding proteins, SDS—PAGE revealed no rostral—caudal differences in the expression of tropomyosin or alkali myosin light chains (Fig. 3A). The ratio of myosin light chains is virtually invariant rostrocaudally. Low-percentage polyacrylamide gel electrophoresis also failed to detect differences in the rostrocaudal distribution of the myosin heavy chain (data not presented). Given the observations of Davies et al. (1995), who report rostral—caudal differences in maximal contraction velocity, it is likely that higher-resolution techniques (e.g. cDNA sequencing) will reveal rostral—caudal variation in myosin heavy chain expression.

**Discussion**

The results of this study implicate several cod axial muscle proteins in the regulation of relaxation rates along the length of the body. Changes in the proportions of these proteins are likely to alter the dynamics of Ca2-dissociation from the thin filament and affect the rate at which Ca2 is removed from the myoplasm. The differences detected include (1) a rostral—caudal shift in isofrom expression of troponin T, a subunit of the thin filament Ca2-regulatory complex, (2) a significant decrease in the amount of the Ca2-binding protein parvalbumin in caudal axial muscle sites and (3) the presence of two soluble Ca2-binding proteins in rostral muscle. Together, these factors provide compelling molecular correlates to measured differences in relaxation rates along the length of cod white axial muscle.

**TnT isoforms**

Although the properties of the two identified TnT isoforms have not been measured, differences in troponin T have been found to correlate with differences in Ca2-sensitivity of muscle activation in numerous vertebrates, including rabbits (Schachat et al. 1987; Greaser et al. 1988; Nassar et al. 1991), chickens (Reiser et al. 1992), rats (Akella et al. 1995) and humans (Mesnard et al. 1995). Studies in which mammalian myofibrils were reconstituted with specific TnT isoforms (e.g. bovids, Tobacman and Lee, 1987; human, Wu et al. 1995) provide experimental support for a role in determining the Ca2 concentration needed for half-maximal activation of
the thin filament. Different combinations of TnT isoforms in rabbit axial and limb muscle have also been found to influence thin filament cooperativity, i.e. the ease with which the thin filament switches between the active and inactive states in response to the binding and release of Ca\(^{2+}\) (Schachat et al. 1987).

Several researchers have attempted to make simple correlations between the sensitivity and cooperativity of Ca\(^{2+}\) activation and the length and charge of the variable N-terminal region that defines TnT isoforms (e.g. rabbit skeletal muscle, Greaser et al. 1988; dragonfly flight muscle, Fitzhugh and Marden, 1997). Others have found that length and charge are not the critical variables influencing muscle fiber physiology (Schachat et al. 1987; Briggs et al. 1987). Using rabbit skeletal muscle, they find that what distinguishes fibers with the lowest cooperativity is not a difference in molecular mass or in the charge structure of the muscle fiber TnT isoforms, but rather the regulated expression of TnTs that include a specific amino acid sequence encoded by mammalian exon 4. Similar specificity has been reported for chicken skeletal muscle (Reiser et al. 1992; Schachat et al. 1995) and would probably hold true for fish skeletal muscle as well.

To establish a direct molecular explanation for how TnT isoform variation affects the Ca\(^{2+}\) responsiveness and relaxation rates of cod axial muscle, sequence data coupled with substitution of specific TnT isoforms into myofibrils from which pCa/tension curves can be generated, is necessary.

**Parvalbumin**

In the case of parvalbumin, direct correlations between parvalbumin levels and rates of muscle relaxation have been modeled and measured experimentally (Kretschmer and Nockholds, 1973; Müntener et al. 1995). Parvalbumin has been shown to affect relaxation rates in a dose-dependent manner in both frogs (Hou et al. 1991) and mammals (Müntener et al. 1995), and it appears to affect regional relaxation rates in cod axial muscle in a similar manner. The greater the amount of parvalbumin, the faster the muscle relaxes.

Regional expression of parvalbumin, the major component of the soluble fraction of fast teleost muscle (Hamoir, 1974; Zawadowska and Supikova, 1992; Perry, 1996), has been studied by other researchers (Huriaux et al. 1996, 1997). In the adult barbel Barbus barbus, total parvalbumin concentration decreases caudally, as in cod. Huriaux et al. (1992) propose that temporal and spatial variations in total parvalbumin concentration and differential expression of parvalbumin isoforms in barbel reflect the functional requirements of the fish axial musculature according to fish size and myomeric location. To date, however, the present investigation is the first to correlate regional changes in physiology with regional differences in parvalbumin concentration.

We also performed a preliminary investigation of axial muscle proteins from a short-horned sculpin Myoxocephalus scorpius (standard length 312 mm). Unlike the cod, the relaxation rate of short-horned sculpin axial muscle remains constant along the body (Johnston et al. 1993). Consistent with the effects of parvalbumin in mammals, no significant differences were detected in the parvalbumin content of rostral versus caudal white muscle samples in short-horned sculpin axial muscle.

**Other Ca\(^{2+}\)-binding proteins**

The final difference detected between rostral and caudal muscle fibers is the rostral presence of two Ca\(^{2+}\)-binding soluble proteins: Ca1 and Ca2 (Figs 4, 6). If and how these proteins affect muscle performance is unknown. Given their relative molecular mass, a possible candidate for the higher-molecular-mass protein, Ca1, is calmodulin, a member of the troponin C family, with four Ca\(^{2+}\)-binding sites. Calmodulin plays a pivotal role in many cellular processes controlled by Ca\(^{2+}\) (Cheung, 1980); however, its concentration in mammalian skeletal muscle is typically so low (5 \(\mu\)mol L\(^{-1}\)) that it cannot usually alter the cytosolic [Ca\(^{2+}\)] by the mere binding of Ca\(^{2+}\) (Gillis, 1985).

On the basis of its low relative molecular mass, a possible candidate for the Ca2 protein is another isoform of parvalbumin. While Ca2 does not react with the parvalbumin antibody, it may be parvalbumin-like and have similar effects in relaxation. Multiple parvalbumin isoforms have been reported in numerous species of fish (Gerday, 1982).

To determine the specific identity of both Ca1 and Ca2, micro-sequence data establishing amino acid sequence homologies with other known Ca\(^{2+}\)-binding proteins would be informative.

**Other mechanisms controlling relaxation**

Two other mechanisms may play a part in governing rostral–caudal differences in the relaxation rate of white axial muscle in fishes. These include (1) a difference in the concentration or activity of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (as shown to be the case in the red axial muscle of scup Stenotomus chrysops; Swank et al. 1997) and/or (2) differences in myosin (as reviewed in Gillis, 1985). Whereas neither the myosin nor the volume and surface densities of T-tubules and sarcoplasmic reticulum (Davies et al. 1995) appear to vary significantly from rostral to caudal in cod white axial muscle, it is possible that the activity of the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase may differ rostrocaudally. Experiments similar to those conducted on red muscle by Swank et al. (1997) could address this possibility.

Compared with the red axial muscle system of the scup, however, the white muscle system of the cod appears to regulate regional differences in relaxation rate through distinctly different mechanisms. Interestingly, while the red and white muscle systems of the scup and cod, respectively, may utilize different molecular mechanisms, the end result is similar. Both systems appear to increase the rate at which Ca\(^{2+}\) is removed from the myoplasm of rostral muscles. The scup appears to increase the efficiency of the sarcoplasmic reticulum Ca\(^{2+}\) pump in its rostral red muscle (Swank et al. 1997), while in rostral cod white muscle, increased expression of parvalbumin and differential expression of other...
myofibrillar and myoplasmic proteins appear to facilitate the rate of Ca\(^{2+}\) transport from the thin filament to the sarcoplasmic reticulum.

In conclusion, few studies provide direct links between molecular differences in Ca\(^{2+}\) regulatory proteins and their physiological function in whole muscles and whole-organism performance. This study describes a suite of rostral-caudal biochemical variations that provide compelling molecular correlates to the significantly slower relaxation rates of caudal axial white muscle fibers compared with rostral white muscle fibers.

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Contractile proteins in cod axial muscle


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