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Myosin Heavy Chain Expression in the Red, White, and Ventricular Muscle of Juvenile Stages of Rainbow Trout

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ABSTRACT Juvenile stages of rainbow trout, smaller parr and older juveniles, termed smolts, show differences in red muscle contractile properties: parr red muscle has faster kinetics and a faster maximum shortening velocity than smolt red muscle. A developmental reduction in the number of MHC isoforms as detected by SDS-PAGE between parr and smolt has also been observed. To investigate whether this shift in contractile kinetics results from differential gene expression, three different MHC cDNA fragments, one each from red, white, and ventricular muscle, were identified. The red muscle and ventricular forms are novel MHCs, and the white muscle form is identical to a published MHC from adult trout white muscle. Tissue and developmental stage-specific expression patterns of these MHC isoforms were examined using isoform-specific RT-PCR. Ventricular muscle typically showed only the ventricular form; 60% parr and 80% smolts expressed the ventricular form only. Approximately half of the white muscle samples of either parr or smolts, 58% and 50%, respectively, expressed only white muscle MHC. Red muscle samples were the most heterogeneous, with red muscle MHC found in combination with either the white or ventricular form or both. Combining samples from the anterior and posterior, 8% of parr red muscle samples expressed solely the red muscle MHC form, and 30% of smolt red muscle samples expressed the red muscle form alone. Variations in the relative contribution of each MHC to the red muscle of parr and smolt may explain observed differences in protein composition and contractile properties. *J. Exp. Zool. 290:751–758, 2001.* © 2001 Wiley-Liss, Inc.

Salmonids undergo a developmental transition termed the parr-smolt transformation (PST). Parr and smolt are, respectively, smaller (younger) and larger (older) juvenile forms of trout and salmon. During PST, many physiological and morphological changes are observed, including shifts in gill activity, visual capability, body shape and color, and swimming behavior (Hoar, ’88; Hawryshyn et al., ’89; Groot and Margolis, ’91; Coughlin et al., 2001). Changes in swimming behavior include variations in stamina (Hoar, ’88) and swimming speed and tailbeat frequency (Coughlin et al., 2001).

Limited research has examined the mechanistic basis for variations in swimming performance at PST. Using SDS-PAGE analysis of muscle proteins, Martinez et al. (’93) reported that at PST, Atlantic salmon, *Salmo salar*, show a developmental reduction from two forms of myosin heavy chain (MHC) in their red or aerobic musculature in the parr stage to one form in older smolts. There were no reported changes in MHC of the white or anaerobic musculature at PST. One isoform was found in the white muscle of both parr and smolts. Also using SDS-PAGE, Coughlin et al. (2001) reported similar findings for rainbow trout, *Oncorhynchus mykiss*. The red muscle of parr contains two or three forms of MHC, whereas the red muscle of smolts usually contains only one form. Again, in trout, there was no change in the white muscle protein composition at PST.

What are the consequences of developmental changes in MHC composition of swimming musculature? Many different proteins contribute to the machinery of muscle contraction, but the myosin heavy chain protein is the predominant form that is responsible for broad changes in muscle kinetics. Changes in the MHC will commonly be associated with variations in other muscle proteins,

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such as myosin light chain (Schiaffino and Reggiani, '96). Since MHC contains the binding sites for both actin and ATP, a shift in MHC would therefore lead to variations in activation and relaxation kinetics, as well as shortening velocity of muscle (Moss et al., '95; Schiaffino and Reggiani, '96; Fitzhugh and Marden, '97). Based on changes in V_{max}, we predict that a shift in MHC expression will be observed at PST.

Coughlin et al. (2001) were able to relate the shifts in red muscle MHC at PST to changes in red muscle kinetics and fish swimming kinematics. The red muscle of parr had faster rates of activation and relaxation in isometric contractions and had a faster maximum shortening velocity (V_{max}) than red muscle from smolts. Further, parr swam with higher tailbeat frequencies than smolts. For white muscle, no data are available for the effect of PST on contraction kinetics or for swimming kinematics. In other fish species, such as cod, white muscle does slow during ontogeny—older fish have slower white muscle (Altringham and Johnston, '90).

A number of fish species show variations in contraction kinetics along their length. In rainbow trout, scup, and largemouth bass, anterior muscle has faster rates of activation and relaxation than posterior muscle (Coughlin, 2000; Rome et al., 2000). However, Coughlin et al. (2001) showed that V_{max} does not vary from anterior to posterior in red muscle of rainbow trout. This indicates that, unlike the prediction of a developmental shift in MHC, no change in MHC is expected along the length of trout. At present there are no data on the tissue- or temporal-specific expression of red muscle (slow) myosins in posthatching (juvenile) trout.

Spatial and temporal variations in MHC expression do occur in fish, however. Fish genomes, like those of other vertebrates, encode multiple myosin genes (Schiaffino and Reggiani, '96); for example, as many as 28 MHC genes are present in carp (Gerlach et al., '90). Expression of individual myosin isoforms during fish life cycles has been shown to be under temporal, spatial (tissue-specific), and/or environmental control. In carp, two MHC genes with identical temporal expression patterns during larval and embryonic development have been identified (Ennion et al., '99). Spatially, expression of these genes showed a rostral-caudal progression in the developing trunk musculature. Both transcripts were expressed laterally in the developing pectoral fins. Only one of these genes (Eggs 22) was expressed in the developing lower jaw, suggesting similar but not identical uses of these MHC in myofibril construction.

Gerlach et al. ('90) provided the first evidence for temperature-dependent expression of different MHC isoforms in carp. Subsequently, Imai et al. ('97) identified three different MHC isoform cDNAs from libraries constructed from white muscle of carp acclimated at different temperatures. Although transcripts from all three genes were detected in adult fish acclimated at all three temperatures studied, the level of transcript of the 10°C isoform was much higher (6-fold) in 10°C acclimated carp, and the level of the 30°C transcript was 11-fold higher in 30°C acclimated fish. Interestingly, 20°C acclimated fish did not show a preponderance of the intermediate isoform, but significantly more of this transcript was present in fish acclimated to 10°C or 20°C than at 30°C. New MHC transcription and translation are likely in temperature acclimation, which requires several weeks to stabilize and is associated with alterations in force production, contraction speed in isolated muscles, and myofibrilar ATPase activity (for review see Imai et al., '97). Differential MHC isoform expression is expected to have some influence on swimming performance, since carp are able to swim faster at low temperatures following low temperature acclimation (and at higher temperatures after high temperature acclimation).

Although two trout white muscle (fast) myosins have been identified (Gauvry and Fauconneau, '96), these had identical expression patterns in developing myotubes through adult white muscle on Northern blots. The authors also detected weak expression of fast myosin in embryonic and juvenile red (or slow) muscle. Subsequent immunohistochemical observations (Bobe et al., 2000) showed a sequential expression of trout MHC proteins during embryonic development. Fast myosin protein was first detected in the deep somites at stage 20 and was found throughout the somites by the time of hatching. Slow myosin protein expression, as detected by antibodies originally raised against rat slow myosin, came on later and was restricted to a layer of superficial somitic tissue at stage 24 (Vernier stages) and then to a few layers of red muscle cell precursors at hatching. It is not clear whether red muscle precursors first express the fast myosin and an isoform switch is taking place as these cells are produced. Both red and white muscle building is still going on at hatching and into juvenile development. MHC isoform switching is also seen in other vertebrates in development, aging, or regeneration (Hughes

et al., '93; Moss et al., '95; Schiaffino and Reggiani, '96; Sachs et al., '97). At present there is no published information on MHC expression in juvenile rainbow trout.

The specific objectives of this study were (1) to identify the MHC transcripts of the red, white, and ventricular muscle of juvenile rainbow trout and (2) to explain developmental shifts in MHC expression in juvenile trout red muscle. cDNAs for three MHC isoforms, one each from red, white, and ventricular muscle of juvenile rainbow trout, were isolated. DNA sequence information was used to generate isoform-specific primers, and the temporal and spatial patterns of MHC expression in the red and white muscle of the two juvenile stages, parr and smolts, were examined.

MATERIALS AND METHODS

Fish handling

Rainbow trout, *Oncorhynchus mykiss*, were obtained from the Huntsdale Fish Culture Station (Carlisle, PA) of the Fish and Boat Commission of the Commonwealth of Pennsylvania. Fish were maintained on a diet of prepared fish feed (Zeigler Trout Grower, Millersville, PA) in a recirculating aquarium system at 10°C. The parr used in this research had obvious parr marks and a mean length of 15.3 ± 0.9 cm (n = 6); the smolts had lost their parr marks, displayed a more silvery appearance, and had a mean total length of 25.2 ± 2 cm (n = 5). In nonanadromous salmonids, there is not a true PST. Older juveniles are referred to as smolts here, but they remain freshwater fish. All handling of experimental animals was reviewed by the Widener University Institutional Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council.

RNA isolation, cDNA synthesis, and cloning

Trout smolts (n = 2) and trout parr (n = 2) were sacrificed by spinal transection, and tissue was dissected from red, white, or ventricular muscle. Samples were quick-frozen in liquid nitrogen and weighed prior to RNA isolation. Total RNA was isolated from trout muscle using the TRIzol Reagent (Life Technologies, Grand Island, NY). First-strand cDNA was synthesized using the SUPERSCRIPT (Reverse Transcription, or RT) Preamplification System from GIBCO (Grand Island, NY). MHC-specific degenerate primers EB and TW2 for the 3′ end of the 5-kd myosin message (sequences given in Lutz et al., '98), obtained from CyberSyn (Aston, PA), were used to amplify myosin-specific cDNA by polymerase chain reaction (PCR). PCR was performed using the Perkin Elmer (Branchburg, NJ) reagent kit and Taq polymerase as supplied by the manufacturer in 1× buffer, 0.2 mM dNTP, and 4.5 mM $MgCl_2$, and at a 48°C annealing temperature. cDNA products were subcloned using the p-GEM T-Easy Vector System from Promega (Madison, WI).

Purification of cDNAs, sequencing, and sequence comparisons

Plasmids were purified from potential clones using the Promega Wizard Plus Mini-Prep Purification System, and the presence of inserts was verified by BstZ1 digestion. When necessary, larger-scale plasmid preparations were made using either Qiagen (Valencia, CA) or Promega Wizard Mini-Prep kits. DNA sequences of MHC cDNAs were determined at the University of Delaware DNA Sequencing Core Facility using an ABI 377 and the dye terminator chemistry, and were processed using the program Chromas (ver. 1.45; http://www.technelysium.com.au/chromas.html; Southpark, Queensland, Australia). DNA sequence information was entered into BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) for comparison with known MHC sequences. DNA sequences were aligned using the program Pile Up from the Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, WI. Predicted protein sequences, estimated molecular weights, and isoelectric points were also determined using the Wisconsin Package.

Isolation of myofibrils

To study MHC expression as a function of longitudinal position (anterior vs. posterior) or developmental stage, an additional 5 smolts and 6 parr (sizes given above) were sacrificed and white and red muscle samples were isolated from anterior and posterior positions. Anterior position was defined as the muscle from 25% of total length behind the snout to 45% total length. The posterior position ranged from 65% to 85% total length. Tissue samples were also taken from ventricular muscle from each fish, and both sets were quick-frozen in liquid nitrogen.

Isoform-specific RT-PCR

The program Oligos (www.biocenter.helsinki.fi/bi/) was used to generate oligonucleotide pairs (shown in Table 1) specific for each MHC isoform with matched annealing temperatures. Primer sequences were obtained from CyberSyn. Total RNA

TABLE 1. *Isoform-specific primers*[1]

Primer pair	Sequence 5′ to 3′	Expected product length
Wh 1	CCAGCACATGTCTAAGTTC	224 bp
Wh 2	AACGCTCAACATTTCAC	
Rd 1	AAACTGCAGCTGAAGGTC	280 bp
Rd 2	TTTTCAGAAAGGTCCATCC	
Vn 1	CGGAAATATGAGAGGC	354 bp
Vn 2	CTTAGGGCTTTGGATAGAC	

[1]Primer pairs for the white (Wh), red (Rd), and ventricular (Vn) isoforms of trout MHC were designed. Each pair includes an upstream (1) and downstream (2) primer. PCR with these primer pairs led to single-product amplification when the individual primer pairs were tested on their respective parent sequences in RT-PCR and to no amplification with the other parent sequences.

was isolated from the individual tissue samples using TRIzol Reagent (Life Technologies). First-strand cDNA was synthesized using the SUPERSCRIPT (RT) Preamplification System from GIBCO. Polymerase chain reaction was performed using the Perkin Elmer reagent kit and Taq polymerase as supplied by the manufacturer in 1× buffer, 0.2 mM dNTP, and 4.5 mM $MgCl_2$ with an annealing temperature of 55°C. These conditions resulted in single product amplification when the individual primer pairs were tested on their parent sequences in RT-PCR and no amplification of the other parent sequences. Attempts at multiplexing primers at these conditions in RT-PCR resulted in smears of products that could not be interpreted; therefore, a single reaction was run for each primer pair. The resulting products were analyzed on 1.6% agarose-Tris Acetate EDTA gels (Sambrook et al., '89), where all reactions from each fish were run at the same time. To control for intra-assay variability, the same sample was analyzed using the red, white, or ventricular primer pairs at the same time. All samples were assayed a minimum of twice to control for *inter*-assay variability.

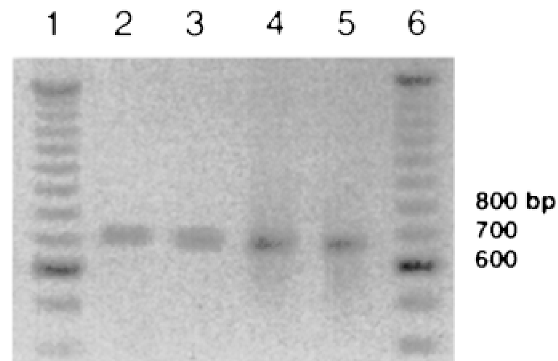

Fig. 1. RT-PCR analysis of RNA isolated from trout muscle. To obtain cDNA clones of the different myosin isoforms we conducted RT-PCR reactions on total RNA from red, white, and ventricular muscle from parr and smolts. A generalized myosin specific primer pair was used (Lutz et al., 1998). PCR products were separated on agarose gels and visualized by staining with ethidium bromide. Lanes 1 and 6 are Life Technologies 100 base pair ladder. Polaroid photographs of stained gels were scanned using a Hewlett Packard Scanjet 4c/T scanner. Images were contrast enhanced using Adobe Photoshop. In this negative image, lane 2 contains the MHC RT-PCR product for a smolt red muscle sample, lane 3 for a parr red muscle sample, lane 4 for a parr white muscle sample, and lane 5 for a parr ventricular muscle sample. One product band was observed for each muscle sample. The ventricular and white muscle products typically appeared to be of a slightly smaller size than the red muscle products.

RESULTS

RT-PCR from parr and smolt red, white, and ventricular muscle typically gave a single product or MHC isoform (Fig. 1). The white and ventricular PCR products appeared to be slightly smaller than those from red muscle. Three distinct MHC fragments were cloned from the RT-PCR products, two of which are novel cDNAs. The sequences of these cDNAs have been submitted to GenBank (accession numbers AY009125 for the red muscle form, and AY009126 for the ventricular form.). Figure 2 shows the deduced protein sequences of these MHC and their predicted molecular weights and isoelectric points (pI). These proteins, although distinct both at nucleotide and at peptide levels, are extremely similar to one another. Comparison of the deduced protein sequences of the three myosins revealed similar amino acid sequences and estimated molecular weights. The ventricular form has the lowest estimated molecular weight and migrates farthest on SDS-PAGE (Coughlin et al., 2001). We were able to obtain cDNA clones of the white muscle form from both white and red muscle, but not from ventricular muscle. Our red muscle form was cloned only from red muscle but could not be cloned from white or ventricular muscle. The ventricle form could only be cloned from ventricular muscle.

Deduced protein sequences were compared to known MHC using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The white muscle form was identical to a previously published sequence of trout white muscle MHC (Gauvry and Fauconneau, '96). These white muscle MHC are most similar to other so-called fast, white muscle myosins from other fish, frogs, and chickens. The ventricular form shares 81% identity with a recently published sequence of ventricular myosin isolated

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Trout Red      1 KAKKAITDAAMMAEELKKEQDTSAHLERMKKNMEQTIKDLQHRLDEAEQIAMKGGKKQIQ
Ventricle      1 KPKKAITDAAMMPEELKKEQDTSSHLERMKKTMEQPIKDLQHRLDEADQIAMKGGKKQVQ
Trout White    1 KAKKAITDAAMMAEELKKEQDTSSHLERMKKNLEVTVKDLQHRLDEAENLAMKGGKKQLQ
Consensus      1 *.**********.**********.*******..*...**********...********.*

Trout Red     61 KLEARVRELETEVELEQRRSSDSVKGVRKYERRIKELTYQTEEDRKNLSRLQDLVDKLQL
Ventricle     61 KLENRVRELENEVEGEQKKGADAIKGIRKYERRIKELTYQTDEDRKNMTRLQDLVDKLQL
Trout White   61 KLESRVRELETEVEAEQRRGVDAVKGVRKYERRVKELTYQTEEDRKNVGRLQDLVDKLQM
Consensus     61 *** ******.***.**... *..**.******.*******.**.**..**********.

Trout Red    121 KVKSYKRTSEEAEEQANSNLGKFRKIQHELDEAEERADIAESQVNKMRAKSRDAGSKKGK
Ventricle    121 KVKAYKRSAEEAEEQANNHLGKFRKIQHELDEAEERADIAESQVNKLRAKSRDVGSKKGH
Trout White  121 KVKAYKRHSEEAEEAANQHMSKFRKVQHELEEAEERADIAETQVNKLRAKTRDSGKGKEV
Consensus    121 ***.***..*****.**....****.****.**********.****.***.** *..*..

Trout Red    181 DEE*          Theoretical pI/MW       8.56 / 21348.13
Ventricle    181 DEE~          Theoretical pI/MW       8.86 / 21127.94
Trout White  181 AE~~          Theoretical pI/MW       8.86 / 21374.12
Consensus    181 .*.
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Fig. 2. Deduced protein sequences for three MHC identified from juvenile trout muscle. All three forms were extremely similar in length and in isoelectric points. Differences are shown by a dot in the consensus sequence and the relevant amino acids are indicated in bold face type. Among the three proteins, the ventricular form appears to be the most distinct, but this limited amount of information does not explain the apparent difference in molecular weights seen in the protein isoform analysis.

from Zebra fish (Yelon et al., '99). The red muscle form is also very similar to Zebra fish ventricular myosin (78% identity). The red and ventricular MHC we isolated are 81% identical to one another and share considerable similarities to alpha cardiac myosins (so-called slow myosins) from a number of vertebrate species.

Isoform-specific RT-PCR

Both developmental stage and muscle type (Fig. 3) influenced the patterns of myosin messages detected in RT-PCR analysis. As can be seen in Table 2, RNA isolated from ventricular muscle of parr and smolt typically showed only the ventricular form (60–80% of samples had ventricle form only), consistent with previous SDS-PAGE analysis (Coughlin et al., 2001). The white muscle form was the predominate one detected in white muscle samples; 100% of these samples had the white muscle form. Combining data from anterior and posterior, 58% of parr and 50% of smolts had only the white form, also consistent with one or two isoforms being observed on SDS-PAGE from the same fish. Red muscle samples were the least uniform; typically the red muscle form was detected in combination with either the white or the ventricular form or both. Combining data from both longitudinal positions, only 8% of parr and 30% of smolts expressed solely the red muscle RT-PCR product.

We were unable to detect any longitudinal variation in RT–PCR products in red muscle samples taken from either parr or smolts, which was consistent with the lack of longitudinal variation in MHC isoforms detected in SDS-PAGE (data not shown). Only 1 parr (out of 6) and 1 smolt (out of 5) showed differences in anterior vs. posterior MHC expression in red muscle (Table 2).

DISCUSSION

Examination of MHC isoform expression does indicate that differential gene expression may be the basis for developmental changes in red muscle kinetics and swimming performance. Developmental stage affected the patterns of myosin expression detected by isoform-specific RT-PCR, supporting the idea that the developmental reduction in the number of MHC isoforms seen on SDS-PAGE of smolt red muscle as compared to parr red muscle (reported by Coughlin et al., 2001) is due to differential gene expression. Protein and RT-PCR analysis of parr and smolt red and white muscle revealed that these tissues contain mixtures of all three myosins in varying ratios, depending on the individual fish. Parr ventricular muscle was the most homogenous, followed by

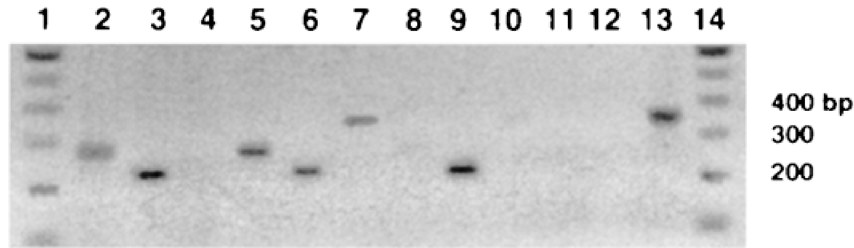

Fig. 3. Isoform specific RT-PCR analysis of RNA isolated from parr and smolt muscle samples. Isoform specific primer pairs (Table 1) were used to detect the presence of each MHC isoform in red, white, and ventricular muscle samples. PCR products were separated on agarose gels and visualized by staining with ethidium bromide. Polaroid photographs of stained gels were scanned using a Hewlett Packard Scanjet 4c/T scanner. Images were contrast enhanced using Adobe Photoshop. Lanes 1 and 14 are Life Technologies 100 base pair ladder. Expression was determined by the presence of the appropriate size product for a given primer pair. In this negative image, lanes 2–4 represent a smolt red muscle sample analyzed with, respectively, the Rd, Wh, and Vn primer pairs. Lanes 5–7 represent a parr red muscle sample, lanes 8-10 represent a parr white muscle sample and lanes 11–13 represent a parr ventricular muscle sample, each analyzed with PCR and the same series of primer pairs. The smolt red muscle sample shows the expression of the red (lane 2) and white (lane 3) MHC isoforms, but not the ventricular form (lane 4). The parr red muscle sample shows the expression of all three forms. The parr white sample shows expression of only the white isoform (lane 9) and the parr ventricular sample shows the expression of only the ventricular isoform (1 and 13).

smolt ventricular muscle. Although white muscle is more likely to express only the white muscle MHC by RT-PCR, the red and ventricular forms are also present. Red muscle is much more likely to express all three forms, with parr red muscle being the most heterogeneous. Importantly, there may be additional MHC isoforms not detected by our probes.

The diverse pattern of transcripts detected may be due to the higher sensitivity of RT-PCR as compared to protein isoform analysis or to the persistence of MHC expression as fish mature. The fish used in this study were natural parr and smolts, and thus represent individuals at different stages of maturation. The kinetic differences previously reported (Coughlin et al., 2001) between parr and smolt red muscle appear to be influenced by a suite of MHC proteins, the relative fractions of which shift in predominance as the fish move through these juvenile stages. Although the precise contribution of any single MHC in a mixture to the V_{max} of trout red muscle cannot yet be determined, this particular kinetic parameter is known to be strongly influenced by MHC composition. In model studies, any addition of a slow MHC to a fast-twitch fiber is predicted to result in a “disproportionately large” reduction in V_{max}, and production of fast fibers (Type II) in slow muscle tissue is known to increase V_{max} (Moss et al., ’95). These observations are consistent with

TABLE 2. PCR products using isoform specific primers[1]

Muscle type	Position	Rd primers	Wh primers	Vn primers		Combined for each stage
Red					% Rd only	% Rd only
Parr	Anterior (n=6)	100%	83%	33%	17% (1/6)	8% (1/12)
	Posterior (n=6)	83%	100%	33%	0% (0/6)	
Smolt	Anterior (n=5)	100%	60%	20%	40% (2/5)	30% (3/10)
	Posterior (n=5)	100%	60%	60%	20% (1/5)	
White					% Wh only	% Wh only
Parr	Anterior (n=6)	17%	100%	17%	67% (4/6)	58% (7/12)
	Posterior (n =6)	50%	100%	50%	50% (3/6)	
Smolt	Anterior (n=5)	40%	100%	20%	40% (2/5)	50% (5/10)
	Posterior (n=5)	80%	100%	60%	60% (3/5)	
Ventricular					% Vn only	
Parr	N/A (n=5)	0%	20%	100%	80% (4/5)	
Smolt	N/A (n=5)	0%	40%	80%	60% (3/5)	

[1]Five smolts and six parr were used in this study. For red and white muscle, samples from the anterior and posterior region of each fish are presented. Percentages represent the number of tissue samples of a given developmental stage that showed product amplification for each isoform-specific primer pair. Rd = red; Wh = white; Vn = ventricular.

the kinetically faster red muscle of parr being less likely to express only the slow (red) muscle MHC, and the slower red muscle of smolts being more likely to contain the red (slow) muscle MHC. Juvenile trout white muscle would be predicted to have a faster V_{max} because of the higher incidence of tissue expressing the fast (white) form only. Among the fish sampled here, 58% and 50% of parr and smolt samples, respectively, had the white muscle form only. At present there are no kinetic data available to compare parr white to smolt or adult white muscle in trout, although in other fish, e.g., cod, a maturation-dependent slowing of relaxation rate has been observed (Altringham and Johnston, '90).

We have identified only the tail fragment of the large ~220-kDa myosin protein. An important contributor to force generation is the myosin head, which is essential for actin and ATP binding (Irving and Piazzesi, '97; review). Crossbridge formation and ATPase activity are also known to influence V_{max}. As yet undetermined sequence and/or conformational alterations between the myosin heads may affect the observed kinetic differences between parr and smolt red muscle. We are presently working to obtain full-length cDNAs of all three myosins identified here.

Our analysis did not reveal sufficient differences in MHC to account for longitudinal variations in activation and relaxation kinetics *within a developmental stage*. The lack of variation in MHC expression between anterior and posterior red muscle regions of the animal does correlate with the uniform V_{max} of this muscle (Coughlin et al., 2001). It is likely that other sarcomeric proteins have a greater effect on shifts in activation and relaxation along the length of fish. One candidate is troponin T (Tn-T), because differences in Ca^{2+} sensitivity by isoforms of Tn-T result in variations in activation time (Schachat et al., '87; Fitzhugh and Marden, '97). Thys et al. ('98, 2001) showed that changes in white muscle kinetics along the length of cod and bass are correlated with variations in Tn-T expression. To date, no data are available for patterns of Tn-T expression in the red muscle of any fish. A second candidate is the regulatory myosin light chain. Changes in myosin light chain express could also lead to the longitudinal variations in kinetics observed in red muscle (Moss et al., '95). Future work will examine the influence of Tn-T and myosin light chain on the observed longitudinal variations in contraction kinetics of rainbow trout.

Of the three MHCs presented here, the ventricular form is the most distinct. Since we do not have any kinetic data on trout ventricular tissue, it is not possible to correlate the presence of this MHC with kinetic parameters. Our studies did not extend successfully to atrial tissue, so we can only hypothesize the presence of an atrial-specific MHC, as has been reported for Zebra fish (Yelon et al., '99). The limited amount of information obtained from the deduced protein sequences does not explain the apparent difference in molecular weights seen in protein isoform analysis of parr and smolt red or white muscle. Migration in SDS-PAGE is affected by a number of other factors, such as differential SDS affinity, differences elsewhere in the proteins, such as the head regions, or post-translational modifications, as has been suggested for murine MHCs (Maggs et al., 2000). Although we cannot rule out these effects, that the number of MHC bands on SDS-PAGE was consistent with the number of RT-PCR products from the same longitudinal position on the opposite side of the same fish strongly suggests that differential MHC expression *is* taking place as the fish mature.

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